Graduate School for Cellular and Biomedical Sciences

University of Bern

Genetic properties of extended cephalosporin-resistant *Escherichia coli* in humans and animals

PhD Thesis submitted by

Salome Nadja Seiffert

from **Riehen BS**

for the degree of

PhD of Science in Biochemistry and Molecular Biology

Supervisor

Prof. Dr. Vincent Perreten

Institute of Veterinary Bacteriology

Vetsuisse Faculty of the University of Bern

Co-advisor

Prof. Dr. med. Andrea Endimiani

Institute for Infectious Diseases

Faculty of Medicine of the University of Bern

Accepted by the Faculty of Medicine, the Faculty of Science and the Vetsuisse Faculty of the University of Bern at the request of the Graduate School for Cellular and Biomedical Sciences

Bern, Dean of the Faculty of Medicine

Bern, Dean of the Faculty of Science

Bern, Dean of the Vetsuisse Faculty Bern

ACKNOWLEDGEMENTS

Thanks to Vincent Perreten and Andrea Endimiani for giving me the opportunity to work on this project. Moreover, I appreciate their support and guidance during the time of my doctoral degree.

Thanks to the entire teams at the Institute for Infectious Diseases as well as the Institute for Veterinary Bacteriology at the University of Bern for their technical support and their team spirit.

Special thanks to Prof. Alessandra Carattoli, Regula Tinguely, Sybille Schwendener, Agnese Lupo, João Pires, Lea Hirtzberger, Yuvia Guilarte, Alexandra Collaud, Valentina Doná and all the other people from the research and diagnostic unit(s) for their help and assistance.

Last but for sure not least, I thank my family and friends for their support during my studies.

This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and of the Institute of Veterinary Bacteriology (IVB), and by Grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office (BVET).

Salome N. Seiffert was a PhD student working at both the Institute of Infectious Diseases of Bern (IFIK) and of the Institute of Veterinary Bacteriology (IVB).

CONTENTS

SUMMARY 6
AIM OF THE THESIS 7
INTRODUCTION 8
OVERVIEW: an introductory review of the situation 14
Chapter I 40
Chapter II 47
Chapter III 54
Chapter IV 60
Chapter V 80
Chapter VI 86
Chapter VII 91
CONCLUSION 94
OUTLOOK 103
CURRICULUM VITAE 104

SUMMARY

Highly-drug resistant bacteria are becoming an increasing threat to the public health systems. This development can also be observed, although to a minor extent, in the veterinary health systems. The assumption that horizontal transmission could be a driving force for the spread of resistance genes between the animal and human reservoirs has been mentioned manifold however firm evidences are still scarce.

In this thesis we focused on the accomplishment of two parts. The first part was to investigate potential links between the human and animal reservoirs that could indicate active transmission. To fulfil this goal basic information was needed regarding the molecular characteristics of extended-spectrum cephalosporin resistant *Enterobacteriaceae* (ESC-R *Ent*) from the various reservoirs. Hence molecular analysis was performed on ESC-R *Ent* in hospitalized humans, in raw poultry-meat, and in pet food. Analysing isolates from hospitalized patients an overall prevalence of 6.5% ESC-R *Ent* has been reported. Examining the poultry-meat as well as the pet food collections 70% of the samples were contaminated with ESC-R *Ent* in both of the studies. The prevalence and molecular data from the poultry-meat were comparable to other studies from the Netherlands. Noteworthy was a discovery of a *P. mirabilis* strain carrying a ~17 kb class 1 integron which was previously reported in a human patient from France.

The pet food samples were heavily contaminated with acquired β-lactamase (*bla*) genes. Interestingly, the majority of the detected *bla* genes were usually more related to the human reservoir. The comparison of the three studies showed that the ST types as well as the major *bla* genes are distinct in the human and animal reservoirs, however one *bla* gene (bla_{CMY-2}) that was prevalent in both settings. Investigating the genetic background of bla_{CMY-2} carrying strains showed that some of them were carried on IncK plasmids. Full sequencing of the $bla_{CMY-2/4}$-carrying plasmids resulted in the identification of a novel plasmid incompatibility variant of the IncK group, named IncK2. This finding provides strong evidence that there is transmission between the animal and human reservoirs.

The second part of the thesis was focused on the identification of emerging resistance genes in new genetic backgrounds. The first study describes the first case of an OXA-48-producing *S. enterica* outside Egypt as well as the first report of *Salmonella* co-producing OXA-48 and VEB-8. In another study a double-carbapenemase producing XDR *Klebsiella pneumonia* was detected in a patient who got transferred back from Belgrade to Bern. In the last report a MDR double-carbapenemase producing *Citrobacter freundii* was identified in a sample from a patient who got also transferred back from Belgrade to Basel. Interestingly, the bla_{NDM-1} gene could be identified in a novel IncHI2 plasmid background.

To sum up, the thesis gives firm evidence of horizontal gene transfer between the human and animal reservoirs mainly by the identification of the identical class 1 integron and the detection of the same plasmids in both settings. It also illustrates the impressive spread of multi-drug resistant plasmids inside the bacterial community as well as to the various reservoirs. To prevent the further spread of such highly drug-resistant pathogens active surveillance is needed.

AIM OF THE THESIS

The objectives of the thesis were to determine if a link exists between extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R *Ent*) from animal and human origin, examine different possible reservoirs for ESC-R *Ent*, and characterize novel genetic elements containing ESC-R genes. The global and actual knowledge of the situation was presented in an introductory review written at the beginning of the PhD thesis (see **overview: an introductory review of the situation**).

The PhD projects were then divided into two parts A and B. The aim of part A was to assess if there is the potential risk for the transmission of ESC-R *Ent* from humans to animals (or vice versa). To achieve this goal comparison of molecular characteristics was performed on ESC-R *Ent* of human and veterinary origin collected in Switzerland.

Part A was split into four studies each represented in a separate chapter:

- **Chapter 1:** Investigation of the molecular characteristics of ESC-R *E.coli* isolated from hospitalized patients in Bern, Switzerland (p35-41).
- **Chapter 2:** Analysis of raw poultry-meat as a potential source for ESC-R *Ent* (p42-48).
- **Chapter 3:** Examination of the contamination of pet food with ESC-R *Ent* (p49-54).
- **Chapter 4:** Detection of a new plasmid incompatibility group variant (IncK2) that is was found in samples of human, poultry, and poultry-meat (p55-73).

The aim of part B was to characterize unknown resistance mechanisms in *Enterobacteriaceae*. This was accomplished by analysing samples with an exceptional, previously unreported, resistance phenotype.

Part B resulted in three different studies each represented in a separate chapter:

- **Chapter 5:** Description of for the first case of an MDR *Salmonella enterica* Kentucky harbouring $bla_{\text{VEB-8}}$ and $bla_{\text{OXA-48}}$ (p75-80).
- **Chapter 6:** Characterization of a XDR *Klebsiella pneumoniae* co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, *qnrA*, and *armA* methyltransferase (p81-85).
- **Chapter 7** : Detection of an MDR *Citrobacter freundii* harbouring $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$, $bla_{\text{SHV-12}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{TEM-1}}$, and *armA*. Moreover, $bla_{\text{NDM-1}}$ gene was captured in a novel IncHI2 plasmid background (p86-87).

INTRODUCTION

Since the discovery of penicillin in the 1920s by Prof. Dr. Fleming, β-lactam antibiotics became of the most important antibiotic classes for the treatment of infections caused by Gram-negative and Gram-positive bacteria.[1, 2] Meanwhile, many penicillin-based derivatives have been developed all having the same mechanism of action. The β-lactam antibiotics impede the cell wall biogenesis of bacteria by inhibiting the penicillin-binding proteins (PBPs). In order to maintain the cell shape as well as to resist the osmotic pressure, PBPs are needed for crosslinking the single peptidoglycan strands into a rigid cell wall. Hence, by blocking the PBPs, β-lactam antibiotics are disabling the cell wall maintenance which is leading to a weakening of the cell wall and consequently cell death.[3]

Bacteria have developed several resistance mechanisms to circumvent the action of the β-lactam antibiotics amongst them porin mutations, efflux-pumps, alternative PBPs and β-lactam degradation enzymes so called β-lactamases. Methicillin-resistant *Staphylococcus aureus* (MRSA) on one hand has acquired the *mecA* gene which encodes for the PBP2A, a penicillin-insensitive PBP variant that renders the bacteria resistant against penicillin-like antibiotics.[3] Several Gram-negative bacteria on the other hand have acquired β-lactamases that inactivate the antibiotic by hydrolysing the active site, the so called β-lactam-ring, in the periplasmic space.[2]

These β-lactamases consist of a structurally highly diverse group of enzymes, which also differ in their hydrolytic activity against the diverse β-lactam classes. They can be sub classified according to two major schemes depending on their hydrolytic activity (Bush-Jacoby-Medeiros System) and based on their structural similarities (Ambler classification System).[4] The most widespread β-lactamases are the Extended-Spectrum β-lactamases (ESBLs) that belong to the Bush-Jacoby-Medeiros Group 2be and the Ambler class A. ESBLs are hydrolysing 3rd- and 4th- generation cephalosporins but are ineffective against carbapenems.[4] With increasing numbers of reports worldwide carbapenemases are becoming an emerging public health problem as they are hydrolysing all classes of β-lactam antibiotics from cephalosporins to carbapenems. Carbapenemases are highly diverse enzymes that belongs to three of the Bush-Jacoby-Medeiros Groups (2f, 2d, and 3a) and are represented in three of the four Ambler classes (A, B, and D).[4]

ESBLs and carbapenemases are located on mobile genetic elements (e.g., IS-elements, transposons, and plasmids) whereby they can spread inside the bacterial community and even to other bacterial species.[2] Moreover, especially plasmids and class 1 integrons can co-carry many additional resistance genes against other classes of antibiotics (e.g., quinolones and aminoglycosides) and at times along with multiple classes of β-lactamases that render the bacteria multi-drug resistant (MDR), extensively-drug resistant (XDR), or even pan-drug-resistant (PDR).[5]

In the times of globalization where animals, goods, and people travel quickly all around the world, such highly drug-resistant bacteria can be easily spread and cause epidemics. The hyperepidemic clone *Escherichia coli* (*E.coli*) ST131 carrying the ESBL CTX-M-15 has certainly taken advantage of this modern lifestyle.[6] Within a few years this *E.coli* has managed to spread all around the globe and to become the major clone in the human setting in almost all of the countries. Nowadays, many countries set up surveillance systems in order to prevent outbreaks of difficult-to-treat pathogens.

Such highly drug-resistant bacteria are imposing an increasing threat to the public health systems worldwide. In 2013, the Centers for Disease Control and Prevention (CDC) have classified carbapenem-resistant *Enterobacteriaceae* as an urgent public health threat with the need of immediate interventions in order to limit the further spread of such worrisome pathogens (CDC, Antibiotic Resistance Threats in the United States, 2013). In 2014, the World Health Organization (WHO) has published their global antimicrobial resistance report where they state that the post-antibiotic area is approaching if not immediate actions are undertaken (WHO, Antimicrobial resistance: global report on surveillance, 2014). The two reports suggest various interventions including the reduction and prevention of the spread of highly drug-resistant bacteria by better hygiene and improved access to clean water and sanitation, the development of rapid diagnostic tests, new antibiotics, and vaccines as well as a responsible antibiotic prescription in humans and animals. Another task is to limit the usage of human antibiotics in non-human settings (e.g., animals, environment) which can lead to the selection and spread of difficult-to-treat pathogens that can eventually cause severe infections in humans.

In Switzerland, authorities have mandated the Federal Office of Public Health, together with the Federal Food Safety and Veterinary Office and the Federal Office for Agriculture to develop by 2015 a national strategic action plan to control antimicrobial resistance (StAR) (see http://www.bag.admin.ch/themen/medizin/14226/index.html?lang=de).

These reports are based on a globally emerging problematic where increasing numbers of MDR and even XDR pathogens are being identified and the antibiotic armamentarium is starting to become ineffective. This development is even more dramatic when taking into consideration that there are almost no new antibiotic class in the pipeline, a situation that WHO referrers to as "discovery void". As the antibiotic pipeline is virtually empty, future strategies must: *i)* contain the spread of highly drug-resistant bacteria, and *ii)* limit the use of the so called last-resort antibiotics and preserve them for the therapy of cases with nearly untreatable pathogens (CDC, Antibiotic Resistance Threats in the United States, 2013; WHO, Antimicrobial resistance: global report on surveillance, 2014).

With increasing numbers of difficult-to-treat or even untreatable pathogens we are approaching a post-antibiotic area where previously treatable infections can once again lead to an increase of morbidity and mortality in humans and animals (WHO, Antimicrobial resistance: global report on surveillance, 2014). Furthermore, such highly drug-resistant bacteria are not exclusively detected in hospitals and other locations where the antibiotic consumption is high, but they are also more and more detected in settings where the consumption and/or use of antibiotics is low like for example in healthy people from the community, environment, food animals, as well as food chain. [7-11] The review paper in the following section (Overview) describes in detail the role and zoonotic potential of livestock for public health.[2]

In order to limit the spread of antibiotic-resistant zoonotic bacteria between animals and humans, it is of particular importance: *i)* to determine potential reservoirs of such highly drug-resistant bacteria, and *ii)* to identify the transmission pathways linking the various settings.

This thesis determined whether there is transmission of extended cephalosporin-resistant bacteria, especially *E. coli*, between the human and animal reservoirs. These two settings are of special importance as the antibiotic usage is high, particularly in hospitals and food-producing animals, and as food may contribute as vehicle between both settings. [2] Beside hospitals, the food producing animal industry is using an enormous amount of antibiotics for the prevention and treatment of infection diseases. [2] This load of antibiotics creates an important selective pressure, which consequently gives rise to resistant bacteria that can spread within the animal reservoir, into the environment as well as to humans, primarily via contaminated food or via aerosols.[9-11] Even more worrisome is the usage of the same or similar antibiotic classes in both reservoirs (e.g., 3rd generation cephalosporins), which can lead to the transmission and subsequent spread of similar resistance genes throughout the bacterial community in both settings.[2]

Switzerland represents a good setting for such a study as the prevalence rates of highly drug resistant *Enterobacteriaceae* in hospitalized humans are among the lowest in whole Europe, which makes it an ideal area to investigate the transmission pathways between the human and animal reservoirs.

Another focus of the thesis was to study β-lactamases in new genetic elements and/or new bacterial species. Such strains were mainly collected from patients that were hospitalized in foreign countries and were later on transferred to Switzerland. In order to maintain the low resistance rates and to prevent the spread of highly drug-resistant pathogens, Switzerland is following strict isolation rules for patients coming from foreign countries. Part of this surveillance is to characterize the strains for the detection of highly drug-resistant bacteria. Several of those isolates were further characterized in this thesis.

Knowing the potential transmission pathways would help in limiting the quick expansion of MDR and XDR pathogens and give more time to research to develop new antibiotics, vaccines and/or other therapeutics.

The several different reservoirs described above have been studied during this thesis and are presented in the following chapters providing novel and important findings on the global spread of antibiotic resistance.

References:

1. Fleming A. Classics in infectious diseases: on the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae* by Alexander Fleming, Reprinted from the British Journal of Experimental Pathology 10:226-236, 1929. *Reviews of infectious diseases* 1980; **2**: 129-39.
2. Seiffert SN, Hilty M, Perreten V et al. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* 2013; **16**: 22-45.
3. Stapleton PD, Taylor PW. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. *Science progress* 2002; **85**: 57-72.
4. Bush K, Fisher JF. Epidemiological expansion, structural studies, and clinical challenges of new beta-lactamases from gram-negative bacteria. *Annual review of microbiology* 2011; **65**: 455-78.
5. Carattoli A. Plasmids and the spread of resistance. *International journal of medical microbiology : IJMM* 2013; **303**: 298-304.
6. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. *Clinical microbiology reviews* 2015; **28**: 565-91.
7. van Hoek AH, Schouls L, van Santen MG et al. Molecular characteristics of extended-spectrum cephalosporin-resistant *enterobacteriaceae* from humans in the community. *PloS one* 2015; **10**: e0129085.
8. Blaak H, Lynch G, Italiaander R et al. Multidrug-Resistant and Extended Spectrum Beta-Lactamase-Producing *Escherichia coli* in Dutch Surface Water and Wastewater. *PloS one* 2015; **10**: e0127752.
9. Seiffert SN, Tinguely R, Lupo A et al. High prevalence of extended-spectrum-cephalosporin-resistant *enterobacteriaceae* in poultry meat in Switzerland: emergence of CMY-2- and VEB-6-possessing *Proteus mirabilis*. *Antimicrobial agents and chemotherapy* 2013; **57**: 6406-8.
10. Zurfluh K, Nuesch-Inderbinen M, Morach M et al. Extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. *Applied and environmental microbiology* 2015; **81**: 3115-20.
11. Gao L, Tan Y, Zhang X et al. Emissions of *Escherichia coli* carrying extended-spectrum beta-lactamase resistance from pig farms to the surrounding environment. *International journal of environmental research and public health* 2015; **12**: 4203-13.

OVERVIEW: an introductory review of the situation

OVERVIEW: an introductory review of the situation

In order to get a precise overview of the situation regarding the risk of transmission of ESC-R *Ent* between the animal and human reservoirs, a review including all relevant papers from the previous years has been written.

The review was entitled "**Extended-Spectrum Cephalosporin-Resistant Gram-negative Organisms in Livestock: an Emerging Problem for Human Health?**" and was published in Drug Resistance Updates in 2013 (Seiffert et al., Drug Resistance Updates; 16(1-2):22-45, 2013).

Drug Resistance Updates 16 (2013) 22–45

Contents lists available at SciVerse ScienceDirect

Drug Resistance Updates

journal homepage: www.elsevier.com/locate/drup

Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: An emerging problem for human health?

Salome N. Seiffert[a,b,c], Markus Hilty[a,d], Vincent Perreten[b], Andrea Endimiani[a,*]

[a] Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Bern, Switzerland
[b] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[c] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland
[d] Department of Infectious Diseases, University Hospital of Bern, Switzerland

ARTICLE INFO

Article history:
Received 17 November 2012
Accepted 22 December 2012

Keywords:
ESBL
AmpC
E. coli
Salmonella
Acinetobacter
Cattle
Pig
Poultry

ABSTRACT

Escherichia coli, *Salmonella* spp. and *Acinetobacter* spp. are important human pathogens. Serious infections due to these organisms are usually treated with extended-spectrum cephalosporins (ESCs). However, in the past two decades we have faced a rapid increasing of infections and colonization caused by ESC-resistant (ESC-R) isolates due to production of extended-spectrum-β-lactamases (ESBLs), plasmid-mediated AmpCs (pAmpCs) and/or carbapenemase enzymes. This situation limits drastically our therapeutic armamentarium and puts under peril the human health. Animals are considered as potential reservoirs of multidrug-resistant (MDR) Gram-negative organisms. The massive and indiscriminate use of antibiotics in veterinary medicine has contributed to the selection of ESC-R *E. coli*, ESC-R *Salmonella* spp. and, to less extent, MDR *Acinetobacter* spp. among animals, food, and environment. This complex scenario is responsible for the expansion of these MDR organisms which may have life-threatening clinical significance. Nowadays, the prevalence of food-producing animals carrying ESC-R *E. coli* and ESC-R *Salmonella* (especially those producing CTX-M-type ESBLs and the CMY-2 pAmpC) has reached worryingly high values. More recently, the appearance of carbapenem-resistant isolates (i.e., VIM-1-producing *Enterobacteriaceae* and NDM-1 or OXA-23-producing *Acinetobacter* spp.) in livestock has even drawn greater concerns. In this review, we describe the aspects related to the spread of the above MDR organisms among pigs, cattle, and poultry, focusing on epidemiology, molecular mechanisms of resistance, impact of antibiotic use, and strategies to contain the overall problem. The link and the impact of ESC-R organisms of livestock origin for the human scenario are also discussed.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Several gram-negative organisms (GNOs) that have a high clinical and economic impact in human medicine (i.e., *Escherichia coli*, *Salmonella* spp. and *Acinetobacter* spp.) are also colonizing or causing infections in animals.

In humans, *E. coli* is responsible for a variety of intestinal and extra-intestinal infections. These pathogenic *E. coli* harbor different virulence and adhesion factors which allow them to cause specific diseases. Enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, verotoxigenic, and enteroaggregative *E. coli* isolates are relevant agents of diarrhea (Croxen and Finlay, 2010), whereas the others are frequent causes of urinary tract infections (UTIs), abdominal and bloodstream infections (BSIs) in both community and hospitalized patients (Chen et al., 2011; Fluit et al., 2001; Lu et al., 2012).

Salmonella spp. are common cause of zoonotic disease acquired by oral ingestion of water and/or food of animal origin or via contact with carriers (Foley and Lynne, 2008). These pathogens can be responsible for self-limiting episodes of gastroenteritis but can also spread beyond the intestinal mucosa causing systemic infections (e.g., BSI, meningitis, bone and joint infections) that necessitate antibiotic treatment (Gordon, 2011; Guerrant et al., 2001). Moreover, the ability of host-adapted strains to cause a persistent infection/colonization is important for transmission, as these patients or animals act as reservoirs (Ruby et al., 2012).

Acinetobacter spp. is a non-fermenting GNO responsible for nosocomial infections of severely ill patients who undergo extended medical procedures. This genus is attracting a lot of interest because it is difficult to treat due to its ability to express multiple mechanisms that make the organism multidrug-resistant (MDR) to

* Corresponding author at: Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Friedbühlstrasse 51, Postfach 61, CH-3010, Switzerland. Tel.: +41 31 632 8632; fax: +41 31 632 8766.
E-mail addresses: aendimiani@gmail.com, andrea.endimiani@ifik.unibe.ch (A. Endimiani).

1368-7646/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.drup.2012.12.001

antibiotics. High morbidity and mortality rates have been reported in hospitals and long-term care facilities (Perez et al., 2008, 2007, 2011; Vila and Pachon, 2012).

Serious hospital infections due to the above GNOs (e.g., BSI, pneumonia, intra-abdominal infections, complicated UTIs) are usually treated with extended-spectrum cephalosporins (ESCs) included in the third and fourth generations (Endimiani and Paterson, 2007; Michalopoulos and Falagas, 2010). Like other β-lactams, these antibiotics interfere with the metabolism of the cell wall by binding the penicillin-binding proteins (PBPs), the enzymes involved in the synthesis of the peptidoglycan. The common chemical structure of cephalosporins (i.e., the β-lactam and the six-membered dihydrothiazide rings) confer to these antibiotics a broad-spectrum of target organisms, less toxicity than other antimicrobials, good penetration in many body sites, and good manageability and versatility in the clinic (Grayson, 2010). This makes their use very attractive, especially when the causative pathogen is not known (i.e., for empirical treatment). However, in the past two decades there has been a rapid increase of infections due to extended-spectrum cephalosporin-resistant (ESC-R) GNOs (Meyer et al., 2010; Pitout and Laupland, 2008). In this context, quinolones (e.g., ciprofloxacin) and aminoglycosides (e.g., gentamicin and amikacin) are the alternative antibiotics taken into account but high resistance rates to them are also co-associated (Giamarellou and Poulakou, 2009; Hawser et al., 2010). Therefore, the spread of ESC-R GNOs represents a serious threat to the health-care systems because it is challenging antibiotic options (Giamarellou and Poulakou, 2009; Paterson et al., 2001; Schwaber and Carmeli, 2007). To overcome infections caused by these MDR pathogens, clinicians often turn to carbapenem antibiotics contributing to the rapid selection of carbapenem-resistant GNOs that we are observing worldwide (Nordmann et al., 2009; Perez et al., 2010; Walsh, 2010).

Animals are considered as reservoirs of antibiotic-resistant GNOs and their impact on human health have drawn considerable global attention. The massive and indiscriminate use of different classes of antibiotics in the veterinary context has contributed to the selection and spread of MDR GNOs (EMEA, 2009; Marshall and Levy, 2011). In particular, ESC-R *E. coli* (ESC-R-*Ec*), ESC-R *Salmonella* (ESC-R-*Sal*) and MDR *Acinetobacter* spp. have been isolated from farm, wild, companion animals, and also in food and the environment (Endimiani et al., 2011; Guardabassi et al., 2004; Guenther et al., 2011; Hamouda et al., 2011; Mesa et al., 2006; Poirel et al., 2012a; Wieler et al., 2011a). This complex multi-setting scenario is certainly responsible for the amplification and the expansion of these clinically significant life-threatening organisms and, more importantly, is driving a further transmission to humans via fecal-oral route (Fig. 1). In this context, pathogens can be directly transferred from animals to humans, as well described for the zoonotic agent *Salmonella*. However, as observed for *E. coli*, animals may also harbor commensal flora which contains resistance genes that can be transferred horizontally from one bacterium to another via mobile genetic elements (e.g., plasmids).

In the present review, we focus on the aspects related to the impressive spread of ESC-R-*Ec* that we are facing worldwide among food-producing animals (i.e., pigs, cattle, and poultry) and its link with the human scenario; we also discuss ESC-R-*Sal* and the emerging problem of MDR *Acinetobacter* spp. isolates. The main molecular mechanisms conferring resistance to ESCs, the epidemiology of ESC-R GNOs and the impact of antibiotic use in livestock are discussed along with the possible strategies that can be implemented to limit this growing public health problem.

2. Mechanisms of resistance possessed by ESC-R GNOs

Resistance to β-lactams in GNOs may be due to three different mechanisms: mutations in the PBPs, reduced permeability of the cell wall (i.e., disruption of porin proteins, efflux systems), and production of β-lactamase enzymes able to hydrolyze and inactivate the β-lactam ring. This last mechanism is the most frequent in the family of *Enterobacteriaceae*. To date, >1000 β-lactamases have been described (Bush and Fisher, 2011) (http://www.lahey.org/Studies/). These periplasmic enzymes can be grouped into four main classes (i.e., A–D) based upon amino acid sequence homology (Bush and Jacoby, 2009). Class A and C β-lactamases are the most commonly found in *Enterobacteriaceae* in humans and confer resistance to different β-lactam classes with various degrees (Bush and Fisher, 2011).

2.1. Extended-spectrum β-lactamases (ESBLs)

The most clinically important class A enzymes are named ESBLs. TEM-, SHV-, and CTX-M-types are the three main families of ESBLs described (Bush and Jacoby, 2009; Paterson and Bonomo, 2005). While TEM- and SHV-type ESBLs arise via substitutions in strategically positioned amino acids from the natural narrow-spectrum TEM-1/-2, or SHV-1 β-lactamase, all CTX-M enzymes demonstrate an ESBL phenotype (Gniadkowski, 2008). *Enterobacteriaceae* producing narrow-spectrum enzymes are resistant to penicillins (e.g., ampicillin), first- (e.g., cephalothin, cefazolin) and second-generation cephalosporins (e.g., cefuroxime, cefotetan), whereas those producing ESBLs are also resistant to third- (e.g., ceftriaxone, ceftazidime, ceftiofur), fourth-generation cephalosporins (e.g., cefepime, cefpirome, cefquinome) and aztreonam. However, cephamycins (e.g., cefoxitin) and carbapenems (e.g., imipenem, meropenem, ertapenem, and doripenem) are not hydrolyzed by ESBLs. Moreover, class A β-lactamases are usually inhibited by the commercially available β-lactamase inhibitors (i.e., clavulanate, sulbactam, and tazobactam) (Paterson and Bonomo, 2005). Until the 1990s, most ESBLs identified in humans were of SHV-/TEM-types (Paterson and Bonomo, 2005). Nowadays, the CTX-M enzymes (especially CTX-M-15) have become the most prevalent type of ESBLs (Livermore et al., 2007; Rossolini et al., 2008). Moreover, the analysis of clonality by using the Multilocus Sequence Typing (MLST) has indicated that most $bla_{\text{CTX-M-15}}$ are internationally carried by an "hyper-epidemic" *E. coli* isolate of sequence type (ST) 131; this lineage has been associated with rates of ciprofloxacin resistance of 100%, whereas those of aminoglycosides are around 70–80% (Johnson et al., 2010; Peirano and Pitout, 2010).

2.2. Chromosomal (cAmpCs) and plasmid-mediated AmpC (pAmpCs) β-lactamases

Several GNOs possess genes encoding for class C cAmpCs (e.g., *Citrobacter freundii*, *Enterobacter* spp.). Such β-lactamases confer resistance to third-generation cephalosporins and β-lactam/β-lactamase inhibitor combinations (e.g., amoxicillin-clavulanate), but not to carbapenems (Bush et al., 1995; Harris and Ferguson, 2012). It should be noted that *E. coli* possess a chromosomal bla_{AmpC} that is normally repressed or only weakly expressed. However, mutations in the promoter region can lead to constitutive hyper-expression of the gene resulting in ESCs resistance (Jorgensen et al., 2010).

In the past 5 years, an increasing number of pAmpCs β-lactamase genes have been discovered on plasmids that can easily spread by horizontal transfer among *Enterobacteriaceae* (e.g., *E. coli* and *Salmonella*). These enzymes derived from those possessed by the chromosomal producers and belong to several families (i.e., CMY-, FOX-, LAT-, MIR-, ACT-, DHA-, ACC-, MOX-types) (Endimiani

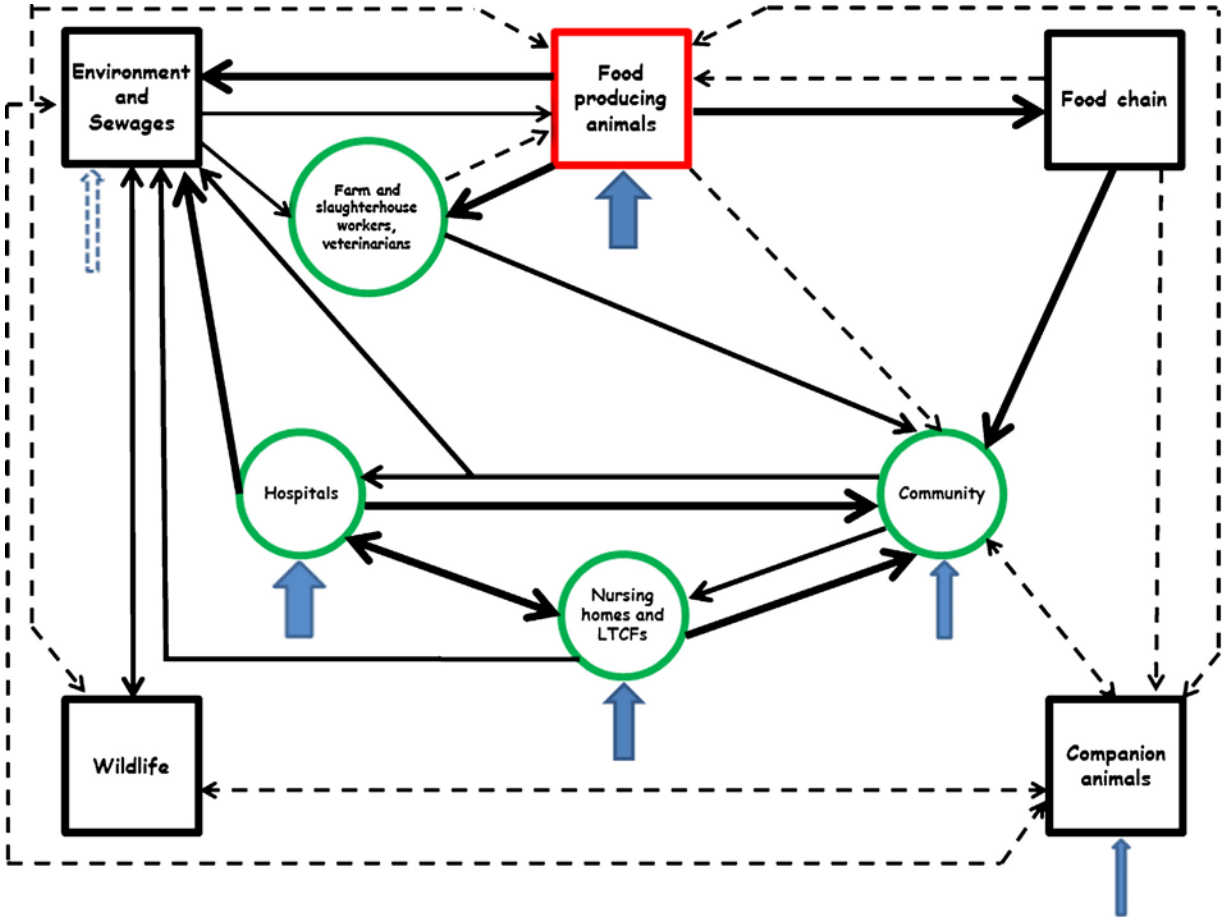


Fig. 1. Settings contributing to the pool of antimicrobial resistance and transmission of MDR GNOs. The human settings are represented in green, whereas that of food-producing animals in red. Blue arrows indicate the use or presence of antibiotics in each specific setting. The size of arrows is proportional to the selective pressure of the drugs (blue) or to the relevance of studies demonstrating transmission of MDR GNOs (black). Segmented arrows indicate a possible transmission of resistant bacteria between two settings but this is not yet well demonstrated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

et al., 2009; Jacoby, 2009). Unlike class A enzymes, cAmpCs and pAmpCs are poorly inhibited by the standard β-lactamase inhibitors but the fourth-generation cephalosporins usually remain in the susceptible ranges (Endimiani et al., 2008).

2.3. Carbapenemases

Carbapenemases are β-lactamases able to hydrolyze carbapenems. Since their discovery in Japan in the early 1990s, there has been a substantial rise in reporting of carbapenemases, especially in the last 10 years. Carbapenemases have been identified in each of the four Ambler molecular classes, though those of class A, B and D have major epidemiological impact in humans (Canton et al., 2012; Nordmann et al., 2011a; Walsh, 2010). Class A carbapenemases can be chromosomally or plasmid-encoded (e.g., KPC-, GES-types). KPC-types are the most clinically common carbapenemases and are found in *Enterobacteriaceae*, *Pseudomonas* spp. and *Acinetobacter* spp. (Rapp and Urban, 2012; Walther-Rasmussen and Hoiby, 2007). Class B carbapenemases (also called metallo-β-lactamases, MBLs) are usually of VIM- and IMP-types, but the recently emerged NDM-types are becoming the most threatening carbapenemases. MBLs are found worldwide and like the KPCs have spread rapidly (especially NDM-1), presenting a serious threat. Most MBL producers are hospital-acquired and involve *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. (Nordmann et al., 2011b; Queenan and Bush, 2007). Class D enzymes are mainly represented by OXA-48-like producers (e.g., OXA-48, -162, and -181). These genes are extensively reported among *E. coli* and *Klebsiella pneumoniae* isolates in the European and African Mediterranean countries (Poirel et al., 2012b).

2.4. Mobile genetic elements carrying the β-lactamase genes (bla)

The *bla* genes encoding for ESBL, pAmpCs, or carbapenemase enzymes are usually associated with highly mobile genetic elements such as transposons, insertion sequences, integrons, and plasmids.

Transposons are small, mobile DNA sequences that can replicate and insert copies of themselves within chromosome and plasmids. They have nearly identical sequences at each end, oppositely oriented (inverted) repeats and contain enzymes (i.e., transposases which include excisases and integrases) that catalyzes their insertion and further genes such as those conferring antibiotic resistance. Insertion sequences (ISs) are short DNA sequences (i.e., 700–2500 bp) that act as simple transposable elements which do not have accessory genes. Both transposons and ISs can be mobilized from chromosome to plasmid(s) and vice versa within the same bacterial cell. Some transposons are conjugative, whereas others necessitate mobile elements (e.g., plasmids) to be exchanged between different bacterial cells (Toleman and Walsh, 2011).

All bla_{TEM} genes are carried on transposons (i.e., Tn*1*, Tn*2*, or Tn*3*). These elements are genetically very similar to each other and possess 38 bp inverted repeats. For instance, bla_{TEM-52} is located on Tn*3*, whereas bla_{TEM-10} and bla_{TEM-12} are on Tn*2* transposons (Cloeckaert et al., 2007; Partridge and Hall, 2005). In contrast to the bla_{TEM}, the bla_{SHV} genes originated from the chromosome of *K. pneumoniae* and spread to plasmids carried by the same bacterial cell following IS*26* dependent mobilization. For instance, the bla_{SHV-5} gene is flanked by two IS*26* (Miriagou et al., 2005). A similar strategy was followed by the bla_{CTX-M} genes that originated from the chromosome of the natural carrier *Kluyvera* spp. One copy of specific ISs (i.e., IS*Ecp-1*-like) positioned upstream of different bla_{CTX-M} genes were found to be responsible for transposition (Naseer and Sundsfjord, 2011). The same ISs are responsible for the mobilization of $bla_{CMY-types}$ (especially bla_{CMY-2}) (Giles et al., 2004). More intriguingly, another IS (i.e., IS*CR1*) located upstream the bla_{CTX-M} and several bla_{pAmpCs} can mobilize the gene via rolling-circle transposition and insertion into a class 1 integrons (Naseer and Sundsfjord, 2011). Once transferred on plasmid(s) and/or integron(s), the above bla_{ESBLs} and bla_{pAmpCs} genes have broader opportunities for horizontal spread among different Gram-negative organisms.

Integrons are genetic elements divided into three classes found in plasmids and/or chromosomes that are able to capture single genes and integrate them in resistance cassettes. An integron commonly contains an integrase (Int1), followed by an *attI* site for integration of cassettes and recognition of the integrase, and a promoter to drive expression. An *attC* sequence is a repeat that flanks the cassette and enables it to be integrated at the *attI* site, excised, and undergo horizontal gene transfer (Toleman and Walsh, 2011). The integrons with the bla_{CTX-M} genes are mostly of class I and co-carry other structurally unrelated genes conferring resistance to non-β-lactam antibiotics (e.g., aminoglycosides, sulphonamides) and quaternary ammonium compounds (Naseer and Sundsfjord, 2011). For this reasons, co-resistance to aminoglycosides, tetracyclines and sulphonamides is very frequent among ESC-R *Enterobacteriaceae* (ESC-R-*Ent*).

Plasmids are circular DNA molecule that can replicate independently from the chromosome and promote lateral transfer among different species of bacteria through the conjugation process. Plasmids can be classified analyzing the replicon control system, a genetic trait constantly present. This system determines the plasmid incompatibility group (Inc) that is the inability of two correlated plasmids to spread stably in the same bacterial cell (Carattoli, 2009). A well-established PCR-based replicon typing methodology is available since 2005 and has been implemented extensively to study the Inc groups of plasmids carried by ESC-R-*Ent* (Carattoli et al., 2005). More recently, a deeper characterization of plasmids has been made implementing the plasmid MLST (pMLST) (Carattoli, 2011).

2.5. Resistance traits associated to the bla genes

Quinolones resistance among *Enterobacteriaceae* is usually mediated by chromosomal mutations in the quinolone-resistance determining region (QRDR) that encode DNA gyrase (*gyrA* and *gyrB*) genes (Hooper, 2001). Nevertheless, plasmid-mediated quinolone resistance (PMQR) can also arise from the expression of proteins encoded by: (1) *qnrA*, *-B*, *-S*, *-C*, *-D* genes that are able to protect the DNA gyrase from the quinolones action; (2) an aminoglycoside acetyltransferase encoded by the *aac(6′)-Ib-cr* gene; and (3) plasmid-mediated quinolone efflux-pumps (qepA-like). While two mutations in the QRDR genes are able to confer high-level resistance to quinolones, the PMQR elements only confer low-level resistance (Strahilevitz et al., 2009). The prevalence of *qnr* genes in ESBL-producing *E. coli* of human origin is estimated around 10% (Karah et al., 2010), whereas that of *aac(6′)-Ib-cr* is much more higher (15–50%) (Ambrozic Avgustin et al., 2007; Pitout et al., 2008). It is of great concern that this gene is spreading along with the pandemic CTX-M-15-producing *E. coli* of ST131 (Coque et al., 2008; Johnson et al., 2010).

Aminoglycosides resistance in *Enterobacteriaceae* is generally due to enzymatic inactivation, which is mediated by 3 classes of aminoglycoside-modifying enzymes (AMEs): acetyltransferases, nucleotidyltransferases, and phosphotransferases (Magnet and Blanchard, 2005; Shaw et al., 1993). More recently, a new aminoglycosides resistance mechanism has been described. It consists of ribosomal protection through enzymatic methylation of specific nucleotides within the A-site of 16S rRNA which impedes binding of aminoglycosides to the 30S ribosomal subunits. These 16S rRNA methylases (ArmA, RmtA, RmtB, RmtC, RmtD, and NpmA) confer extraordinarily high-levels of resistance to aminoglycosides and can be mobilized among different species (Doi and Arakawa, 2007; Wachino et al., 2007). For instance, the *armA* gene (the most prevalent 16S rRNA methylase gene) is located on a composite transposon (Tn*1548*) on a transferable plasmid and is frequently associated with the $bla_{CTX-M-9}$ ESBL gene (Galimand et al., 2005). Furthermore, production of CTX-M-9 ESBLs is seen in many strains with *rmtB* gene (Yan et al., 2004). Overall, data on the human prevalence of 16S rRNA methylases among *Enterobacteriaceae* is scarce. A recent work performed in China indicates that the prevalence of *rmtB* genes among ESBL-producing *E. coli* from humans is increasing (Yu et al., 2010).

2.6. Mechanisms of resistance in Acinetobacter spp.

Acinetobacter baumannii and the other species of the genus can express a very complex combination of resistance mechanisms. Production of acquired ESBLs (e.g., CTX-M-15 and TEM-92) (Endimiani et al., 2007; Potron et al., 2011), class B and/or class D carbapenemases (e.g., IMP- and VIM-types; OXA-23, -24/40 and -58), over-expression of chromosomal enzymes (i.e., ADCs and OXA-58-like) and efflux pumps, loss of outer membrane proteins, altered PBPs, production of AMEs or 16S RNA methylases and mutations in *gyrA* and *parC* genes are common mechanisms of resistance found in MDR clinical isolates (Perez et al., 2007; Poirel et al., 2011, 2010).

3. Prevalence and characteristics of ESC-R GNOs in livestock

While the first ESBL in humans was described in *K. pneumoniae* in 1983 in Germany (Knothe et al., 1983), the earliest reports of ESC-R-*Ent* in food-producing animals were at the beginning of the new millennium. The first isolates in cattle and pigs were reported in 1999–2000 in United States (US) where several authors described CMY-2-producing *Salmonella* spp. (Fey et al., 2000; Winokur et al., 2000). In Spain (2003), CMY-2-, CTX-M-14-, and SHV-12-producing *E. coli* isolates were found in fecal-samples of healthy chickens; the authors also described two ESC-R-*Ec* due to mutations in the promoter region of the bla_{cAmpC} (Brinas et al., 2003). Similarly, in France (2005), Girlich et al. reported non-clonally related CTX-M-1-producing *E. coli* in poultry (Girlich et al., 2007). In another Spanish study (2006), eight ESBL-producing *E. coli* isolates were found in swine but molecular identification of the *bla* genes was not performed (Mesa et al., 2006).

In the last 5 years, the number of publications referring to commensal colonization of livestock with ESC-R GNOs has exponentially increased. In particular, numerous studies have underlined the remarkable spread of ESC-R-*Ent* isolates among food-producing animals, indicating that ESC-R-*Ec* and, to a less extent, ESC-R-*Sal* isolates are the major players in this context. Most

of these studies are performed in the European Union (EU), while data regarding other countries are scarce.

3.1. Cattle

According to the most recent (2009) European Food Safety Authority (EFSA) survey, the overall prevalence of ESC-R-*Ec* isolates in cattle in the EU was 1.6%, with a range of 0% (Austria, Denmark, Finland, Sweden) to 6.5% (Hungary); in 2007 and 2008, ESC-R strains were found only in Estonia, France, the Netherlands, and Germany (range 0.8–4%). ESC-R-*Sal* in 2009 were only reported in Germany (prevalence of 1%), whereas in 2007–2008 none of the countries detected resistant isolates (EFSA, 2011). In US, the prevalence of ESC-R-*Ec* was 6% (Wittum et al., 2010), whereas that of ESC-R-*Sal* ranged from 2.4% to 14.5% (Frye et al., 2008; USDA, 2011). During the US National Antimicrobial Resistance Monitoring System study (NARMS, 1999–2003), the prevalence of ESC-R-*Sal* was 17.6% (Frye and Fedorka-Cray, 2007). Recently, in the same country, ESC-R-*Ec* and ESC-R-*Sal* had an impressive prevalence of 95% and 37.9%, respectively (Mollenkopf et al., 2012). In Asia, ESC-R-*Ec* is ranging between 1 and 33% (Asai et al., 2011; Hiroi et al., 2011; Ho et al., 2011; Zheng et al., 2012). During the first nationwide surveillance in Switzerland (2010–2011), we observed a prevalence of 3.9% for ESC-R-*Ec* (Endimiani et al., 2012b).

3.2. Pigs

In the EU (2009), the prevalence of ESC-R-*Ec* in pigs was 2.3%, with a range of 0% (Denmark and Estonia) to 3.8% (Hungary and Poland); in 2007–2008 ESC-R strains were found with low prevalence (i.e., range of 0.6–1.2%) in Austria, Denmark, France, Italy, the Netherlands, and Spain. ESC-R-*Sal* in 2009 were only reported in Spain and Germany (prevalence of 1% and 2%, respectively), whereas in 2007 ESC-R isolates were also observed in Estonia (5.3%), Ireland (1.5%), and Italy (0.7%) (EFSA, 2011). During the US NARMS study (1999–2003), a prevalence of 4.6% for ESC-R-*Sal* was reported (Frye and Fedorka-Cray, 2007), whereas in 2009 the prevalence was 4.2% (USDA, 2011). In Asia, ESC-R-*Ec* range between 1 and 64% (Asai et al., 2011; Hiroi et al., 2011; Ho et al., 2011; Rayamajhi et al., 2008; Tian et al., 2012; Zheng et al., 2012). In Switzerland (2010–2011), the prevalence for ESC-R-*Ec* was 3.3% (Endimiani et al., 2012b). We also recently reported a prevalence of 12.5% for ESC-R-*Ec* in the nose of pigs (Endimiani et al., 2012a).

3.3. Poultry

The most numerous reports of ESC-R-*Ent* in livestock concern poultry. In the EU (2009), the mean prevalence of ESC-R-*Ec* isolates was 8.5%, with a range of 0% (Denmark) to 26.4% (Spain); in 2007 ESC-R strains were found in Denmark (1.8%), France (2%), Italy (11.1%), the Netherlands (20.9%), and Sweden (1%). The overall prevalence of ESC-R-*Sal* in 2009 was 2%, with a range of 0% (Austria, Finland, Greece, Latvia, Portugal, Slovakia, Slovenia, and UK) to 12% (the Netherlands). In 2007, ESC-R-*Sal* were described in Italy (2.9%), the Netherlands (13.4%), and Spain (7.8%) (EFSA, 2011). In US (1999–2003), the prevalence of ESC-R-*Sal* was 6.8–7.1% (Frye and Fedorka-Cray, 2007). In Asia, ESC-R-*Ec* range between 8 and 60% (Asai et al., 2011; Hiroi et al., 2011, 2012; Ho et al., 2011; Li et al., 2010; Zheng et al., 2012). The prevalence of ESC-R-*Ec* in Switzerland was of 25% in 2011 (Endimiani et al., 2012b).

3.4. Molecular characteristics of ESC-R-Ec and ESC-R-Sal detected in livestock

The majority of large national and international surveillances have taken in consideration only the phenotype (i.e., resistance to ESCs) of the *Enterobacteriaceae* analyzed. In contrast, molecular data regarding *bla* genes possessed by the organisms, the clonality of isolates (e.g., the ST of *E. coli*), and the characteristics of plasmids (e.g., the Inc group) or other mobile genetic elements are limited to small and local studies. However, this information is essential to comprehend the epidemiology and spread of ESC-R GNOs and its link with the human setting. It should also be noted that the impact of other ESC-R-*Ent* (e.g., *K. pneumoniae*, *Enterobacter* spp., *Citrobacter* spp.) has been taken into account only very rarely (Geser et al., 2012a; Hiroi et al., 2012; Poirel et al., 2012a). These species can contribute to the pool of transmission of MDR mobile genetic elements like *E. coli* and *Salmonella* (Fig. 1).

A summary of the distribution of ESC-R-*Ec* (the most studied bacterial organism) in the three main livestock animals with respect to geographic origin, prevalence, and molecular characteristics is shown in Table 1. In these studies the specific bla_{ESBL} genes responsible for ESC-R phenotype were analyzed and their relative prevalence was usually calculated. However, the impact of pAmpCs and/or cAmpCs was frequently not considered because the authors did not implement the adequate phenotypic and molecular tests (Doi and Paterson, 2007; Endimiani et al., 2012b). Moreover, compared to the human studies, information regarding STs and Inc group of plasmids are still insufficient to drive solid global conclusions.

In general, most of the available studies are from European countries and indicate prevalence of ESBL- and AmpC-producing *E. coli* ranging between 0–94% and 0–13%, respectively. Data from Asia and America are limited (range for ESBLs and AmpCs of 0–64% and 0–95%, respectively), whereas those from Africa and Australia are lacking. Overall, the most frequent bla_{ESBL} genes associated with ESC-R-*Ec* in food-producing animals encode for several CTX-M-types (i.e., CTX-M-1, -2, -9, -14, -15, -32, and -55), followed by SHV-12 and TEM-52 ESBLs. In particular, the CTX-M-1 is disseminated in EU in all food-producing animals but is rarely reported in other regions and settings. This ESBL is carried by Inc*N*, Inc*FII*, Inc*FIB*, and Inc*I1* plasmids in heterogeneous STs. The CTX-M-14 and CTX-M-55 are the most prevalent ESBL in Asia, mainly involving poultry and, to less extent, cattle and pigs. Although data are scarce, the $bla_{\text{CTX-M-14}}$ seems to be carried by Inc*FII*/*FIB* and Inc*K* plasmids. In livestock, CTX-M-15 has less impact than CTX-M-1/-14 and is associated to Inc*I1* plasmids. SHV-12 and TEM-52 are mainly reported in poultry from EU countries: $bla_{\text{SHV-12}}$ is carried by Inc*FIB*, Inc*N*, and Inc*I1* plasmids, whereas $bla_{\text{TEM-52}}$ is generally associated with Inc*I1* plasmids. Interestingly, in some regions (e.g., North America and Asia) the CMY-2 pAmpC has a very high prevalence (sometimes higher than that of ESBLs) among ESC-R-*Ec* isolated from livestock. This was also observed in EU in poultry (i.e., prevalence of 38–78% of the ESC-R-*Ec*) when the authors analyzed the presence of AmpC producers. Overall, $bla_{\text{CMY-2}}$ is usually carried by Inc*I1* and Inc*A*/*C* plasmids (see also section 6.3. for plasmids) (Carattoli, 2009).

With regard to the ESC-R-*Sal*, the following bla_{ESBLs} and/or bla_{pAmpCs} have been detected in food-producing animals from different countries: Belgium (poultry: CTX-M-2, TEM-52) (Bertrand et al., 2006; Cloeckaert et al., 2007), Brazil (poultry: CTX-M-2) (Fernandes et al., 2009); Canada (cattle: CMY-2) (Martin et al., 2012); France (poultry: CTX-M-1/-9; cattle: CTX-M-1) (Cloeckaert et al., 2010; Madec et al., 2011; Weill et al., 2004), Germany (poultry: CTX-M-1, TEM-20/-52, CMY-2; pigs and cattle: CTX-M-1) (Rodriguez et al., 2009); Ireland (poultry: SHV-12, CMY-2) (Boyle et al., 2010); Italy (poultry: SHV-12) (Chiaretto et al., 2008); Japan (poultry: TEM-52) (Shahada et al., 2010); the Netherlands (poultry: CTX-M-1/-2, TEM-20/-52, ACC-1) (Dierikx et al., 2010); Spain (poultry: CTX-M-9; pigs: SHV-12) (Riano et al., 2006); UK (poultry: CMY-2) (Liebana et al., 2004); USA (cattle: CMY-2, CTX-M-1; pigs: CTX-M-1) (Frye et al., 2008; Wittum et al., 2012; Zhang and LeJeune, 2008). Based on the above studies, the following main plasmid Inc

Table 1
Recent representative studies analyzing the molecular characteristics of extended-spectrum cephalosporins resistant *E. coli* (ESC-R-*Ec*) detected in the three main food-producing animals.

Area	Pigs[a]				Cattle[a]				Poultry[b]			
	bla genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)	*bla* genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)	*bla* genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)
Europe	Czech Republic: CTX-M-1 (100%) (Bardon et al., 2012)	ESBLs (6%) AmpCs (NT)	NT	NT	Czech Republic: CTX-M-1 (100%) (Dolejska et al., 2011)	ESBLs (1–39%) AmpCs (NT)	NT	N (CTX-M-1)	Denmark: CTX-M-2 (61%), CTX-M-1 (28%), CTX-M-9/gr-9 (11%) (Bortolaia et al., 2011)	Not calculated AmpCs (NT)	ST10 (CTX-M-2), ST48 (CTX-M-1/-2)	N (CTX-M-gr-9), I1 (CTX-M-1)
	Denmark: CTX-M-1 (80%) (Moodley and Guardabassi, 2009)	Not calculated AmpCs (NT)	NT	N (CTX-M-1)	France: CTX-M-gr-1 (66%), CTX-M-gr-9 (27%) (Haenni et al., 2012; Valat et al., 2012)	ESBL (NT) AmpCs (NT)	ST36 (TEM-52), ST98 (TEM-52)	I1 (TEM-52)	Germany: SHV-12 (44%), CTX-M-1 (41%) (Kola et al., 2012)	ESBLs (44%) AmpCs (NT)	NT	NT
	Denmark: CTX-M-1 (66%), cAmpC (15%), CTX-M-14 (7%) (Agerso et al., 2012)	ESBLs + AmpCs (11%)	NT	NT	France: CTX-M-1 (84%), SHV-12 (12%) (Madec et al., 2008)	ESBLs (4%) AmpCs (2%)	NT	NT	Holland: CTX-M-1 (49%), TEM-52 (26%) (Leverstein-van Hall et al., 2011)	ESBLs (94%) AmpCs (NT)	ST10 (CTX-M-1,TEM-52), ST58 and ST117 (CTX-M-1)	I1 (CTX-M-1, TEM-52)
	Poland: CTX-M-1 (33%), CMY-2 (11%) (Wasyl et al., 2012)	ESBLs + AmpCs (33%)	NT	NT	France: CTX-M-15 (100%), OXA-1 (22%) (Madec et al., 2012)	ESBLs (NT) AmpCs (NT)	ST88 (CTX-M-15), ST2210-2215 (CTX-M-15)	FII, FIA, FIB (CTX-M-15), I1 (CTX-M-15)	Holland: CMY-2 (38%), CTX-M-1 (31%), SHV-12 (16%) (Dierikx et al., 2012)	ESBLs + AmpCs (>80%)	ST93 (CTX-M-1), ST770 (CTX-M-1, SHV-12), ST2309 and ST115 (CMY-2)	I1 (CTX-M-1), K (CMY-2), N (SHV-12)
	Spain: CTX-M-1 (69%), SHV-12 (21%) (Blanc et al., 2006)	ESBLs (30%) AmpCs (0%)	NT	NT	France: CTX-M-1 (62%), TEM-71 (38%) (Hartmann et al., 2012)	ESBLs (5%) AmpCs (0%)	ST2487, ST2498 (CTX-M-1), ST178 (TEM-71)	NT	Italy: SHV-12 (64%), CTX-M-32 (19%) (Bortolaia et al., 2010)	Not calculated AmpCs (NT)	NT	FIB (SHV-12), I1 (SHV-12), N (CTX-M-32)
	Spain: SHV-12 (57%), CTX-M-1, -9, and -14 (14%) (Escudero et al., 2010)	ESBLs (13%) AmpCs (4%)	NT	NT	France: CTX-M-14 (60%), CTX-M-1 (40%) (Dahmen et al., 2012)	ESBLs (<1%) AmpCs (NT)	ST23, ST58 (CTX-M-1/-14), ST10 (CTX-M-14)	F (CTX-M-14), I1 (CTX-M-1)	Italy: CTX-M-32 (47%), CTX-M-1 (33%) (Bortolaia et al., 2011)	Not calculated AmpCs (NT)	ST93 (CTX-M-32), ST115 (CTX-M-32/-1), ST1628 (CTX-M-32)	N (CTX-M-32), FIB (CTX-M-1)
	Spain: CTX-M-gr-1 (69%), SHV-5/12 (21%), CMY-2 (0%) (Cortes et al., 2010)	ESBLs (NT) AmpCs (NT)	ST10 (SHV-5), ST1286 (CTX-M-1)	NT	Germany: CTX-M-1 (86%), CTX-M-15 and TEM-52 (7%) (Wieler et al., 2011b)	ESBLs (1%) AmpCs (NT)	ST297, ST718	NT	Poland: CMY-2 (78%), CTX-M-1 (6%) (Wasyl et al., 2012)	ESBLs + AmpCs (55%)	NT	NT
	Portugal: CTX-M-1(100%) (Goncalves et al., 2010)	ESBLs (25%) AmpCs (NT)	NT	N, FII, FIB, P (CTX-M-1)	Switzerland: CTX-M-1 (56%), CTX-M-15 (25%), CTX-M-14 (12%) (Geser et al., 2012a)	ESBLs (14%) AmpCs (NT)	NT	NT	Switzerland: CTX-M-1/gr-1 (90%), SHV-12 (7%) (Geser et al., 2012a)	ESBLs (63%) AmpCs (NT)	NT	NT
	Switzerland: CTX-M-1 (89%), CTX-M-14 (11%) (Geser et al., 2012a)	ESBLs (15%) AmpCs (NT)	NT	NT	Switzerland: CTX-M-1 (50%), CTX-M-15 (50%) (Endimiani et al., 2012b)	ESBLs (4%) AmpCs (0%)	ST537 (CTX-M-15), ST528 (CTX-M-1)	NT	Switzerland: CMY-2 (40%), CTX-M-1 (30%), SHV-12 (17%) (Endimiani et al., 2012b)	ESBLs (25%) AmpCs (2%)	ST86 (TEM-52), ST61 (CMY-2), ST536 (CTX-M-1), ST21 (SHV-12)	NT

Table 1 (*Continued*)

Area	Pigs[a]				Cattle[a]				Poultry[b]			
	bla genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)	*bla* genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)	*bla* genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)
	Switzerland: CTX-M-1 (50%), CTX-M-3(50%) (Endimiani et al., 2012b)	ESBLs (3%) AmpCs (0%)	ST3 (CTX-M-3), ST529 (CTX-M-1)	NT	UK: CTX-M-15 (50%), CTX-M-1 (37%), CTX-M-14 (32%) (Hunter et al., 2010)	ESBL (1%) AmpCs (NT)	NT	NT	Spain: CTX-M-gr-9 (65%), CMY-2 (18%) (Cortes et al., 2010)	ESBLs (NT) AmpCs (NT)	ST131 (CTX-M-9), ST648 (CTX-M-32), ST362 (CTX-M-1), ST115 (CMY-2)	NT
	Switzerland: CMY-2 (13%) (Endimiani et al., 2012a)	ESBLs (0%) AmpCs (13%)	ST2, ST532, ST539	I1 (CMY-2)					UK: CTX-M-1(78%), CTX-M-15 (15%) (Randall et al., 2011)	ESBLs (54.5%) AmpCs (NT)	ST57 (CTX-M-1/-15), ST156 (CTX-M-15), ST88 (CTX-M-1)	I2 (CTX-M-1)
America	US: CMY-2 (80%) (Chander et al., 2011)	ESBLs (NT) AmpCs (NT)	NT	NT	US: CMY-2 (95%), TEM-types (3%), cAmpC (3%) (Donaldson et al., 2006)	ESBLs (NT) AmpCs (NT)	NT	NT	Data not available	Data not available	Data not available	Data not available
					US: CMY-2 (95%), CTX-M-1/-14/-15 (4%) (Mollenkopf et al., 2012)	ESBL (NT) AmpC (NT)	NT	II (CTX-M-15), F (CTX-M-14), N (CTX-M-1), A/C, F (CMY-2)				
Asia	China: CMY-2 (41%), CTX-M-22 (29%) (Tian et al., 2012)	ESBLs (6%) AmpCs (4%)	NT	NT	China: CTX-M-14 (80%), CTX-M-55 (40%) (Zheng et al., 2012)	ESBLs (6%) AmpCs (0%)	NT	NT	China: CTX-M-55 (38%), CTX-M-14 (28%) (Zheng et al., 2012)	ESBLs (12%) AmpCs (0%)	NT	NT
	China: CTX-M-14 (33%), CTX-M-65 (28%), CMY-2 (6%) (Zheng et al., 2012)	ESBLs (10%) AmpCs (1%)	NT	NT	Hong Kong: CTX-M-14 (47%), CTX-M-55 (41%) (Ho et al., 2011)	ESBLs (33%) AmpCs (NT)	NT	NT	China: CTX-M-14 (43%), CTX-M-65 (23%), CTX-M-55 (18%) (Li et al., 2010)	ESBLs (25%) AmpCs (NT)	NT	NT
	Hong Kong: CTX-M-14 (46%), CTX-M-3 (14%) (Ho et al., 2011)	ESBLs (64%) AmpCs (NT)	NT	NT	Japan: CMY-2 (50%) (Hiroi et al., 2011)	ESBLs (0%) AmpCs (1%)	NT	NT	Hong Kong: CTX-M-14 (38%), CTX-M-55 (31%) (Ho et al., 2011)	ESBLs (33%) AmpCs (NT)	NT	NT
	Japan: CMY-2 (50%) (Hiroi et al., 2011)	ESBLs (0%) AmpCs (1%)	NT	NT	Japan: CTX-M-2 (33%), CMY-2 (33%) (Asai et al., 2011)	ESBLs (3%) AmpCs (3%)	NT	NT	Japan: CTX-M-2 (27%), CMY-2 (27%) (Hiroi et al., 2011)	ESBLs (8%) AmpCs (5%)	NT	NT
	Japan: CMY-2 (33%) (Asai et al., 2011)	ESBLs (0%) AmpCs (1%)	NT	NT	Korea: CTX-M-14 (100%) (Lim et al., 2009)	ESBLs (1%) AmpCs (NT)	NT	NT	Japan: CMY-2 (40%), CTX-M-25 (14%) (Asai et al., 2011)	ESBLs (10%) AmpCs (13%)	NT	NT
	Korea: TEM-20 (40%), DHA-1 (40%) (Rayamajhi et al., 2008)	ESBLs (2%) AmpCs (2%)	NT	NT					Japan: CTX-M-2 (50%), SHV-12 (10%) (Hiroi et al., 2012)	ESBLs (60%) AmpCs (NT)	NT	NT

Note: NT, not tested; AmpCs, includes both chromosomal AmpCs (cAmpCs) and plasmid-mediated AmpCs (pAmpCs).

[a] Data for pigs and cattle includes papers from the year 2006.

[b] Data for poultry includes papers from the year 2010.

groups were identified: $bla_{CTX\text{-}1\text{-}M}$ (Inc*N*, Inc*I1*, Inc*B*/*O*, and Inc*HI1*), $bla_{CTX\text{-}2\text{-}M}$ (Inc*HI2*), $bla_{CTX\text{-}9\text{-}M}$ (Inc*HI2*), $bla_{SHV\text{-}12}$ (Inc*I1*), $bla_{TEM\text{-}52}$ (Inc*I1*), and $bla_{CMY\text{-}2}$ (Inc*I1*, Inc*A*/*C*). The main *Salmonella* serovars identified in the above studies were Typhimurium, Virchow, and Kentucky.

Only a few data on the prevalence of PMQR, AMEs and 16S rRNA methylases among ESC-R strains detected in animals are available. Ma et al. reported a PMQR prevalence of 35% among ESBL producers (mostly *E. coli*) of animal origin in China (Ma et al., 2009). However, more recent European studies indicate very low prevalence in pigs, cattle and poultry (Endimiani et al., 2012b; Randall et al., 2011). Plasmid-mediated *armA* and *rmtB* genes have been identified from *E. coli* in swine from Spain and China, respectively (Chen et al., 2007; Gonzalez-Zorn et al., 2005). Other genes conferring resistance to tetracyclines (*tet* genes), sulphonamides (*sul* genes), trimethoprim (*dfr* genes), and phenicols (*cmlA1*, *catA1*, *catIII*, *catB3*, *floR*) are frequently associated to the bla_{ESBL} and bla_{pAmpC} genes (Blanc et al., 2006; Endimiani et al., 2012b; Smet et al., 2010b).

3.5. *Acinetobacter spp. in livestock*

In several studies involving pets, MDR *A. baumannii* isolates of international clones I, II and III have been reported (Endimiani et al., 2011; Zordan et al., 2011). These lineages are the same of those frequently associated to hospital outbreaks in humans (Perez et al., 2010, 2007). In contrast, information regarding the impact of *Acinetobacter* spp. in livestock is almost lacking. Hamouda et al. have recently described several *A. baumannii* isolates from cattle and pigs included in the above three international clones (Hamouda et al., 2011). It should also be noted that *Acinetobacter* spp. is part of the intestinal microbiota of cattle and can be isolated from clinical samples (Nam et al., 2009; Rudi et al., 2012).

3.6. *The emergence of carbapenemase-producers*

The methodologies implemented to identify the ESC-R GNOs (e.g., screening of feces with selective agar plates) during the numerous surveys conducted in food-producing animals should also be able to detect most carbapenemase-producing organisms (EFSA, 2011). However, only very recently the emergence of these life-threatening isolates has been reported.

During a longitudinal study in 2011 from a German pig farm, Fischer et al. found a VIM-1-producing *E. coli* from the corridor of a fattening unit with 5-month-old pigs. After that, the authors also detected the $bla_{VIM\text{-}1}$ in another *E. coli* from the feces of a pig residing in the same farm (Fischer et al., 2012a). These authors also reported three VIM-1-producing *Salmonella* isolates detected in one poultry and two pig farms located in the same German federal region (of which one was the same farm of the VIM-1-producing *E. coli*) (Fischer et al., 2012b).

In August 2010, Poirel et al. analyzed the rectal swabs collected from 50 dairy cattle located in a farm near Paris. Of the 50 samples, 9 contained carbapenem-resistant *Acinetobacter genomospecies* 15TU. In particular, these isolates harbored the OXA-23 carbapenemase. Most animals from which $bla_{OXA\text{-}23}$-possessing isolates were identified received antimicrobial drugs in the previous weeks for the treatment of mastitis (i.e., amoxicillin-clavulanate, oxytetracycline, and neomycin) (Poirel et al., 2012a).

During October–December 2010, Wang et al. analyzed 396 rectal swabs collected from food animal farms and one slaughterhouse located in eastern China. One sample from a chicken was positive for an NDM-1-possessing *Acinetobacter lwoffii*. The antibiotic usage records for the chicken farm were the isolate was found indicated that penicillin, cefotaxime, cefradine, doxycycline, tilmicosin and neomycin were usually implemented for curing or preventing bacterial infections (Wang et al., 2012).

Overall, these recent findings should be taken in serious consideration because may represent "the tip of the iceberg" for the future spread of untreatable human pathogens among food animals (Fig. 1).

4. Use of antimicrobial agents in livestock

Since the advent of the "antibiotic era" in the 1950s, antimicrobial agents have been largely implemented in the livestock production for the following main reasons: (i) treatment of sick animals; (ii) prophylaxis to prevent infection in specific situations at risk (e.g., contact with other animals with infection, transportation in limited spaces); (iii) growth promotion to increase the rate of weight gain or feed efficiency and, therefore, to improve commercial production (Schwarz et al., 2001).

Although with several differences depending on the country, numerous classes of antimicrobial agents with diverse molecular targets are approved for use in food-producing animals in the different countries. A summary of these antimicrobials is shown in Table 2. Interestingly, many antibiotics with remarkable clinical importance in human medicine are also used in food animals. In particular, several classes of β-lactams, quinolones, aminoglycosides, and macrolides are available for use in livestock. In this section, we mainly focus on the aspects of β-lactams use.

4.1. *Use of β-lactams*

Like in humans, the overall characteristics of β-lactams make their use also very appealing in veterinary medicine. The most frequently used β-lactams for the treatment of infections are the following: penicillins (e.g., benzylpenicillins, ampicillin, amoxicillin), first- (cefadroxil, cephapirin, cephalexin, cefalonium, cefazolin, cefacetrile), third- (e.g., cefovecin, cefpodoxime, ceftriaxone, cefoperazone, ceftiofur), fourth-generation cephalosporins (e.g., cefquinome), and β-lactam/β-lactamase inhibitor combinations (e.g., amoxicillin-clavulanate) (Hammerum and Heuer, 2009). In the past, besides their use in clinical therapy, β-lactams (especially penicillins) have also been implemented as feed additives to improve growth. In EU they have been banned, whereas they are still used at sub-therapeutic dosages for growth promotion in the US (EMEA, 2009; EU, 2003; Smet et al., 2010b).

4.1.1. *Cattle*

The following β-lactams are implemented in cattle for specific clinical conditions: mastitis (penicillin, various ESCs, including ceftiofur and cefquinome), lameness (ampicillin), interdigital necrobacillosis (ceftiofur, cefquinome), calf diarrhea (ampicillin, amoxicillin, amoxicillin-clavulanate), metritis (penicillin, ampicillin, ceftiofur), septic arthritis (ampicillin, amoxicillin, various ESCs), salmonellosis (ceftriaxone). In particular, amino-penicillins are often used, whereas ESCs are usually approved as second-line treatment options for specific clinical conditions (EMEA, 2009; Smet et al., 2010b).

In the dairy cattle setting, antibiotics are generally implemented to treat or prevent specific infections in both weaned heifers and adult cows. In US (2007), cows treated with antibiotics were 16.4% for mastitis, 7.4% for reproduction, 7.1% for lameness, 2.8% for respiratory infections, and 1.9% for diarrhea. Furthermore, almost all farms used intramammary antimicrobials for prevention of diseases following the last milking of lactation. For the bovine mastitis (the most common diseases), the following antibiotics were implemented through intramuscular or intramammary routes: cephalosporins 53.2%, lincosamide 19.4%, and other

Table 2
Antimicrobials approved for use in food-producing animals.

Antimicrobial families/classes	Antimicrobial	USA			Europe[b]			Switzerland (CliniPharm-CliniTox, 2012)[b]
		Used in C, P, S	Used in feed	Human use	Used in C, P, S	Used in feed	Human use	Use in C, P, S
Penicillins	Amoxicillin	C, P, S	No	Yes	S, C	No	Yes	C, S
	Ampicillin	C, P, S	No	Yes	S, C	No	Yes	C, S, P
	Cloxacillin	C	No	Yes	NL	NL	Yes	C
	Penicillin (Procaine)	C, P, S	Yes	Yes	Withdrawn	Withdrawn	Yes	C, S, P
I generation cephalosporins[a]	Cephalexin	C, P, S	No	Yes	C, P, S	No	Yes	C
	Cefalonium	C, P	No	No	C, P	No	No	NA
	Cephapirin	C	No	No	C	No	No	C
	Cefazolin	C, P	No	Yes	C, P	No	Yes	C
	Cefacetrile	C	No	No	C	No	No	C
II generation cephalosporins[a]	Cefuroxime	C	No	Yes	C	No	Yes	Withdrawn
III generation cephalosporins[a]	Cefoperazone	C, P	No	Yes	C, P	No	Yes	C
	Ceftiofur	C, P, S	No	No	S, C	No	No	C, S, P
	Ceftriaxone	C, P, S	No	Yes	C, P, S	No	Yes	NA
IV gen. cephalosporin[a]	Cefquinome	C, P, S	No	No	C, S	No	No	C, S
Quinolones	Danofloxicin	C	No	No	C	No	No	C, S, P
	Enrofloxacin	C	No	No	S, C	No	No	C, S, P
Aminoglycosides	Apramycin	S	Yes	No	NL	NL	No	Withdrawn
	Gentamicin	C, P, S	No	Yes	S, P	Yes	Yes	NA
	Neomycin	C, P, S	Yes	Yes	S, P, C	Yes	Yes	NA
	Hygromycin	P, S	Yes	No	NL	NL	No	NA
Tetracyclines	Chlortetracycline	C, P, S	Yes	No	Withdrawn	Withdrawn	No	C, S, P
	Oxytetracycline	C, P, S	Yes	Yes	Withdrawn	Withdrawn	Yes	C, S
	Tetracycline	C, P, S	No	Yes	Withdrawn	Withdrawn	Yes	C, S, P
Macrolides	Oleandomycin	C	No	No	P, S	Yes	Yes	Withdrawn
	Tilmicosin	P, S	Yes	No	NL	NL	No	C, S
	Tylosin	C, P, S	Yes	No	S, C	Withdrawn	No	C, S, P
	Erythromycin	C, P, S	No	Yes	P, C, S	Yes	Yes	C, P
Bacitracin	Bacitracin	C, P, S	Yes	Yes	Withdrawn	Withdrawn	Yes	C, S, P
Arsenicals	Arsanilic acid	P	Yes	No	NL	NL	No	NA
	Roxarsone	C, P, S	Yes	No	P, S	Yes	No	NA
Orthosomycin	Avilamycin	S	Yes	No	Withdrawn	Withdrawn	No	NA
Bambermycin	Bambermycin	C, P, S	Yes	No	Withdrawn	Withdrawn	No	NA
Quinoxaline	Carbadox	P, S	Yes	No	Withdrawn	Withdrawn	No	NA
Polypeptides	Colistin/Polymyxin B	C, P	Yes	Yes	P, S, C	Yes	Yes	C, S, P
Elfamycin	Efrotomycin	S	No	No	S	Yes	No	NA
Phenicol	Florfenicol	C, P	No	No	C, S	No	No	C, S, P
Lincosamines	Lincomycin	C, P, S	Yes	Yes	P, S	Yes	No	C, S, P
	Pirlimycin	C	No	No	NL	NL	No	C
Novobiocin	Novobiocin	C, P	Yes	No	C, P	No	Yes	NA
Aminocyclitol	Spectinomycin	C, P, S	No	Yes	NL	NL	Yes	NA
Diterpene	Tiamulin	S	Yes	No	S, P	Yes	No	C, S, P
Triamilide	Tulathromycin	C, S	No	No	NL	NL	No	C, S
Streptogrammin	Virginiamycin	P, S	Yes	No	Withdrawn	Withdrawn	No	NA
Sulfonamides	Sulfachlorpyridizine	C, S	No	No	NL	NL	No	C, S, P
	Sulfadimethoxine	C, P, S	No	No	P	Yes	No	C, S, P
	Sulfaethoxypyridazine	C, P, S	No	No	NL	NL	No	C, S, P
	Sulfamethazine	C, P, S	Yes	No	S	Yes	No	C, S, P
	Sulfathiazole	C, S	Yes	No	NL	NL	No	C, S
Fosfomycin	Fosfomycin	NA	NA	Yes	NL	NL	Yes	NA

Adapted from Mathew et al. (2007), Guardabassi and Courvalin (2006), and Marshall and Levy (2011).
Note: C, cattle; P, poultry; S, swine, NA, not available; NL, not listed in Guardabassi and Courvalin (2006), Marshall and Levy (2011), and Mathew et al. (2007).
[a] Although extensively implemented in the past, FDA and EU have now banned the off-label and unapproved use of cephalosporins (especially, ESCs) in poultry, cattle, and pigs (EMEA, 2009; FDA, 2012a,b).
[b] Although withdrawn, several antimicrobial might be still implemented (see Section 4.2).

β-lactams 19.1% (of which penicillin G/streptomycin and cephapirin were the most used) (USDA-APHIS, 2008a,b).

It should be noted that the milk of cattle under antibiotic treatment (especially with ESCs) for acute of sub-acute mastitis must be withheld until the infection issue is resolved and following residue recommendations. However, the milk of cows recently treated is frequently used by farmers to feed calves before the withdrawal time is elapsed (EMEA, 2009). For instance, as the withholding period for ceftiofur and cefquinome is not existent or only 12 h (Trott, 2012), there is a high risk that these ESCs are used in preference to other alternative antimicrobials with longer withholding periods.

4.1.2. Pigs

In pigs, necrotic enteritis is usually treated with penicillins, whereas ceftiofur, and to less extent cefquinome, are implemented for respiratory, septicemia, polyarthritis and polyserositis infections. Amino-penicillins are often used, whereas ESCs should be implemented as second-line treatment options for different clinical conditions (EMEA, 2009; Smet et al., 2010b). However, ESCs seem to be used more frequently than expected. For instance, in a national survey in Australia, ceftiofur use was reported in 25% of herds (Jordan et al., 2009). In Canada (2006–2008), macrolides and lincosamides were the most used drugs for disease prevention, growth promotion and treatment of enteric disease, whereas

ceftiofur was not used. However, the drug was implemented via parenteral route for treatment of lameness, respiratory, and enteric diseases in 21–29% of herds (Deckert et al., 2010).

4.1.3. Poultry

Several β-lactams are commonly implemented for the following clinical conditions in poultry: colibacillosis, fowl cholera, respiratory infections due to *Ornithobacterium rhinotracheale*, septicemia due to *Riemerella antipestifer* (ampicillin, amoxicillin), dysbacteriosis (benzylpenicillins), and erysipelas (penicillins). Ampicillin, amoxicillin and amoxicillin-clavulanate are the antibiotics of choice in poultry medicine in many countries, whereas the use of ESCs is usually not allowed (EMEA, 2009; Smet et al., 2010b).

4.2. Off-label use of antibiotics

Another important problem that makes difficult monitoring the use of antibiotics in animals is their off-label implementation for non-authorized indications. In some countries, veterinarians can prescribe an antimicrobial registered for a precise disease and in a specific species to another animal with the same or different infection, but only if no other active therapeutic choices are available (EMEA, 2009; EU, 2001; Passantino, 2007). However, some veterinarians and producers have abused of the extra-label use for situations that violates the specific brand instructions. For instance, off-label use of ceftiofur in US and EU has been frequently implemented to prevent early mortality due to septicemia in 1-day old chickens. This cephalosporin has also been used as spray or by subcutaneous injection in poultry hatcheries and directly in eggs (Bertrand et al., 2006; Dutil et al., 2010; EFSA, 2011). In Denmark, ceftiofur was commonly implemented in pig farms for the treatment of diarrhea and for prophylaxis of systemic infection in piglets (DANMAP, 2007; Jorgensen et al., 2007). Recently, there has also been an increased concern about the illegal use of this drug after purchasing via internet (EFSA, 2011). These overall practices must be discouraged and prevented because clearly linked to the development of ESC resistance in commensal *E. coli* and *Salmonella* spp. (see below).

4.3. Overall quantity of antibiotics used in livestock

The overall quantity of antibiotics used in the modern food-producing animal industry is not clearly known because of the numerous confounding factors in the provided data (Anderson et al., 2003). For instance, quantities of antimicrobial agents can be reported as: (i) weight of active ingredients; (ii) total weight of feed additives (therefore including other complexes); or (iii) total weight of feed supplements that may contains further antimicrobials. In addition, data regarding veterinary use for companion animals and livestock are usually not turned apart and information regarding the specific purpose for the antibiotic implementation (e.g., for feed or for treatment) is frequently unreported. Moreover, data for penicillins and various cephalosporins classes are often reported all together as "β-lactams", making difficult an adequate interpretation of the available documents. It should also be noted that data are not provided in daily doses, as systematically done for humans (EMEA, 2009, 2012).

As shown in Table 3, official data reports indicate that tetracyclines, sulfonamides, and penicillins are the most frequently used antibiotic classes in the various countries. In general, the overall quantities of drugs used in the last few years are slightly decreasing in all countries. However, those of β-lactams, and more specifically those of cephalosporins, are significantly increasing. More appropriate analyses indicate that the systemic use of antibiotics in food-producing animals is dominated by ESCs (EMEA, 2009). For instance, in Switzerland (period 2007–2011) the consumption has diminished for the majority of antimicrobial classes (e.g., tetracyclines, macrolides, trimethoprim, polymyxins) but that of penicillins and cephalosporins has increased (Table 3).

5. Impact of antibiotic use on antimicrobial resistance in livestock

The first observation that antibiotic use in food-producing animals selects for resistant organisms was described in 1951 in California (Starr and Reynolds, 1951). The authors noted that streptomycin-resistant coliform isolates were drastically increasing among turkeys fed with such antibiotic. Since that time, debates regarding the use of antibiotics in food-producing animals (both for feeding and for treatment) have been raised and questioned by international, professional, and governmental organizations.

A significant body of the scientific literature has now supported the link between antimicrobial use in food animals and increased prevalence of resistant organisms, especially for commensal fecal *E. coli* and *Salmonella* isolates. The spread of these organisms follows two strategies: (i) selection of resistant bacteria (usually at intestinal level) under the pressure of the antibiotic usage; and (ii) dissemination of such resistant bacteria by cross-contamination of fecal material among animals (especially those that are part of the intensive industrial livestock production) (EFSA, 2011). With regard to the potential effects of antibiotics on resistance in bacteria, systemic use is probably inducing a major impact than local use (e.g., intramammary injection) because different microbial populations located in different body sites are exposed, increasing the risk to select for resistant organisms.

5.1. Effects of using non-ESCs antibiotics

The use of chlortetracycline and/or sulfamethazine is clearly linked to the increase prevalence of cattle colonized with tetracycline-resistant *E. coli* isolates (Alexander et al., 2010, 2008; Checkley et al., 2010; Platt et al., 2008; Sharma et al., 2008). The administration of chlortetracycline, even in absence of sulfamethazine, can lead to the emergence of resistance to other classes of antibiotics not in the administered regimen, including ampicillin and chloramphenicol (Alexander et al., 2008; Mirzaagha et al., 2011). A direct association between chlortetracycline consumption and probability of resistance to tetracycline and/or not administered antibiotics (e.g., ampicillin, cephalotin) has also been observed in fecal *Salmonella* spp. and *E. coli* isolates in pigs (Varga et al., 2009; Vieira et al., 2009; Wagner et al., 2008). In this setting, administration of a common combination of three antibiotics (i.e., chlortetracycline, sulfamethazine, and penicillin) increased the prevalence of *E. coli* isolates resistance to aminoglycosides (Looft et al., 2012).

However, the main question of the scientific community is: "*Can non-ESCs antibiotics select for ESC-R GNOs?*" As illustrated, most ESBL/pAmpC producers carry additional genes (e.g., those to sulphonamides, tetracyclines, and aminoglycosides) conferring resistance to commonly used veterinary antibiotics (Blanc et al., 2006; Endimiani et al., 2012b; Machado et al., 2008). Therefore, though this aspect has been analyzed in a few studies, the risk to select for ESC-R isolates is theoretically not only restricted to the use of ESCs (EFSA, 2011). Persoons et al. demonstrated that amoxicillin is significantly associated to the emergence of ESC -R-*Ec* in poultry; the authors also showed that poor hygienic condition of the medicinal treatment reservoir, no acidification of drinking water, more than three feed changes during the production cycle, hatchery of origin, breed, and litter material

Table 3
Trend of veterinary consumption for different classes of antimicrobials in representative countries.

Antibiotic classes	Countries and tones of active ingredients														
	Czech Republic (Grave et al., 2012)			Denmark (DANMAP, 2011, 2007, 1998)			Finland (FINRES-Vet, 2011)			France (ANSES, 2011)			The Netherlands (Bondt et al., 2012)		
	2005	2007	2009	1998	2007	2011	2001	2007	2009	1999	2007	2010	1999	2007	2011
β-Lactams (total)	27.54	14.55	19.70	20.95	36.32	36.66	8.07	9.70	10.11	97.25	102.80	98.85	35	61	66
Penicillins	27.20	14.13	19.23	20.90	35.66	36.22	6.92	8.67	9.12	91.08	93.64	90.63	–	–	–
Cephalosporins (total)	0.34	0.42	0.47	0.05	0.66	0.44	1.15	1.03	0.99	6.17	9.16	8.22	–	–	–
I–II generation	–	0.29	0.31	–	–	–	–	–	0.99	5.25	7.16	5.94	–	–	–
III–IV generation	–	0.13	0.17	–	–	–	–	–	0.002	0.92	2.00	2.28	–	–	–
Tetracyclines	35.87	44.30	36.17	12.10	38.25	29.45	1.94	1.71	2.28	625.14	677.79	471.98	162	321	157
Aminoglycosides	0.90	0.49	2.42	7.80	8.13	5.67	0.63	0.18	0.18	98.71	74.25	62.49	13	12	7
Macrolides	8.87	6.51	4.67	4.00	13.30	10.98	0.49[a]	0.75[a]	0.43	79.82	97.60	81.37	10	55	34
Quinolones (total)	1.27	1.07	1.26	0.40	0.38	0.38	0.10	0.09	0.10	22.07	15.59	13.22	7	9	5
Amphenicols	0.44	0.53	0.52	–	0.47	0.92	0.0	0.0	0.006	4.31	5.88	5.12	–	–	–
Sulfonamides	10.62	14.49	12.28	8.7[c]	14.65[c]	13.36[c]	2.49[c]	2.66[c]	2.64	259.69	224.59	174.00	72[c]	99[c]	58[c]
Trimethoprim	–	–	–	8.7[c]	14.65[c]	13.36[c]	2.49[c]	2.66[c]	0.53	37.48	33.84	26.29	72 [c]	99 [c]	58 [c]
Lincosamides	1.14	1.01	0.25	–	3.24	2.54	0.49[d]	0.75[d]	0.17	5.87	9.07	6.72	–	–	–
Polymyxins	0.54	0.44	0.47	–	–	–	–	–	0.0	63.04	72.44	64.05	–	–	–
Pleuromutilins	4.07	4.55	3.62	–	7.46	7.55	–	–	0.08	31.14	10.32	7.62	–	–	–
Other	0.05	0.03	0.65	3.40	0.31	0.44	0.10	0.08	0.0	5.08	2.78	2.52	11	8	10
Total	91.33	87.97	82.02	57.30	122.51	107.94	13.82	15.16	16.51	1329.58	1326.95	1014.24	310	565	338

Antibiotic classes	Countries and tones of active ingredients																
	New Zealand (NZFSA, 2010)			Norway (Grave et al., 2012; NORM-VET, 1999, 2007)			Sweden (SVARM, 2011)			Switzerland (SwissMedic, 2011, 2007)			United Kingdom (VMD, 2011, 2005)			USA (FDA, 2011, 2010)	
	2005/06	2006/07	2008/09	1999	2007	2009[1]	2002	2007	2010	2004	2007	2011	1998	2007	2010	2009	2010
β-Lactams (total)	16.50	16.12	17.08	2.32	2.73	3.09	9.63	9.46	9.03	12.37	13.66	14.53	63	72	93	651.84	875.54
Penicillins	14.98	14.68	15.55	–	–	3.09	8.95	8.51	8.45	–	13.18	13.96	61	66	86	610.51	870.95
Cephalosporins (total)	1.52	1.44	1.53	–	–	0.001	0.68	0.95	0.58	–	0.48	0.57	2	6	7	41.33	24.59
I–II generation	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
III–IV generation	–	–	–	–	–	0.001	–	–	–	–	–	–	–	–	–	–	–
Tetracyclines	3.92	4.79	4.49	0.22	0.32	0.22	1.42	1.85	1.12	10.27	16.71	13.74	228	174	200	4612	5592
Aminoglycosides	1.66	1.62	1.22	0.20	0.17	0.83	0.75[b]	0.72[b]	0.56[b]	3.85	3.72	3.32	21	20	22	339.68	200.79
Macrolides	5.24 [a]	4.68[a]	5.44[a]	0.02[d]	–	0.0	1.41[a]	1.52[a]	0.74[a]	2.38	4.02	3.48	23	33	35	861.99	553.23
Quinolones (total)	0.034	0.033	0.041	0.02	0.03	0.03	0.19	0.18	0.15	0.30	0.39	0.39	1.88	1.95	2.23	–	–
Amphenicols	–	–	–	–	–	0.024	–	–	–	–	0.23	0.28	–	–	–	–	–
Sulfonamides	5.25[c]	5.22[c]	5.19[c]	1.65[c]	1.63[c]	1.6	2.48	2.43	2.00	26.84	29.13	23.12	85	61	63	517.88	506.22
Trimethoprim	[e]	[e]	[e]	1.65[c]	1.63[c]	0.2	0.41	0.44	0.36	2.78	2.02	1.55	14	12	12	–	–
Lincosamides	[e]	[e]	[e]	0.02[d]	0.02	0.02	1.41[a]	1.52[a]	0.74[a]	–	0.11	0.07	–	–	–	115.84	154.65
Polymyxins	–	–	–	–	–	–	[e]	[e]	[e]	2.10	1.67	1.45	–	–	–	–	–
Pleuromutilins	–	–	–	–	–	0.10	1.00	0.51	0.17	–	–	–	–	–	–	–	–
Other	30.28	23.74	22.35	1.90	1.46	–	–	–	–	0.45	0.18	0.41	14	14	20	5967	5338
Total	62.88	56.20	55.81	6.30	6.36	6.13	17.27	17.11	14.12	61.34	71.82	62.35	449	387	447	13066	13220

Note: –, data not available.
[a] Includes lincosamides.
[b] Includes polymyxins.
[c] Sulfonamides and trimethoprim were taken together.
[d] Includes macrolides.
[e] Reported with another class.

used can favor the spread of ESC-R-*Ec* (Persoons et al., 2011). The importance of amoxicillin treatment (and to less extent trimethoprim-sulfadimethoxine) in selecting ESC-R-*Ec* and favoring plasmid exchange was also observed by Dheilly et al. using an experimental model with chicks (Dheilly et al., 2012).

5.2. Effects of using ESCs

The extensive use of ESCs in the livestock and the increased prevalence of resistant isolates have recently stimulated the researchers to investigate their specific impact on the livestock setting. This is based on the knowledge that ESCs (but also quinolones and aminoglycosides) are antibiotics clearly linked to the risk of selection for ESBL, cAmpC, and pAmpCs producers in humans (Ben-Ami et al., 2009).

During parenteral therapy with ceftiofur, ESC-R-*Ec* expanded in absolute number and relative frequency (Volkova et al., 2012). Dolejska et al. recorded a statistically significant correlation between ESCs use and prevalence of CTX-M-1-producing *E. coli* after comparing the epidemiological data of a conventional cattle farm with high consumption of parenteral/intramammary cephalosporins to those of an organic farm without use of antibiotics (i.e., prevalence of 39% vs. 0%) (Dolejska et al., 2011). In a recent study, 11% of Danish slaughter pigs had fecal sample positive for ESC-R-*Ec*; a significantly higher prevalence was observed among pigs originating from farms with registered ESCs consumption (26.3% vs. 10.8%; $P = 0.034$) (Agerso et al., 2012). A strong correlation between off-label use of ceftiofur and high prevalence of ESC-R-*Ec*/-*Sal* has also been reported (Dutil et al., 2010; Jorgensen et al., 2007). Lowrance et al. showed that administration of a single dose of ceftiofur favored transient expansion of MDR fecal *E. coli* (e.g., resistance to ceftiofur, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline) in steers; the flora returned to its initial susceptibility approximately 2 weeks after the antibiotic administration (Lowrance et al., 2007). In an experimental model, administration of a single ceftiofur dose to turkey not colonized with resistant strains did not result in the emergence of ESC-R species. However, if the turkeys were previously colonized with both susceptible *Salmonella* and pAmpC-producing *E. coli* isolates, the plasmid was readily exchanged (Poppe et al., 2005). In another study, the use of amoxicillin, ceftiofur, or cefquinome increased the count of a CTX-M-1-producing *E. coli* previously inoculated intragastrically in pigs. In particular, ceftiofur and cefquinome had larger selective consequences than amoxicillin and the effects persisted beyond the withdrawal times suggested for these ESCs. The increase in the number of ESC-R-*Ec* was mainly due to the proliferation of indigenous isolates that probably acquired via conjugation the plasmid carrying the $bla_{\text{CTX-M-1}}$ gene (Cavaco et al., 2008). Similar results were also observed in cattle inoculated with CMY-2-producing *E. coli* and treated with ceftiofur (Alali et al., 2009). To understand the dynamics of plasmid-mediated resistance to ESCs in enteric commensals of cattle, Volkova et al. developed a mathematical model to study ESC-R and -susceptible commensal *E. coli* in absence or during parenteral therapy with ceftiofur. The results suggested that ESC-R-*Ec* could persist in the absence of immediate ceftiofur pressure because a low and stable fraction of them can be maintained (even if they grow slower than that of the sensitive ones) by horizontal and vertical transfers of plasmids with the indigenous flora and/or ingestion of additional resistant *E. coli* isolates. The latter could occur if the conditions on the farm allow for a close circulation of isolates (including those that are ESC-R) between cattle and their environment (Volkova et al., 2012).

The above studies support the link between ESCs use and selection and spread of ESC-R-*Ent*. As ESCs (e.g., ceftiofur and cefquinome) are mainly eliminated through the urines, we emphasize that very low concentrations in the intestines of treated animals contribute to the selection and transfer of ESCs resistance (EMEA, 2009; Hornish and Kotarski, 2002). This phenomenon might be the "perfect storm" to select for ESC-R-*Ent* in the gut of animals.

5.3. Controversial studies and different point of views

Although with a less extent, points of view that differ from the above studies have also been reported. In a Canadian study, Checkley et al. conducted a prospective observational study to examine antimicrobial resistance patterns of fecal *E. coli* of calves on arrival at the feedlot, and then evaluate the associations between the antimicrobials used for treatment (i.e., ampicillin, sulphamethoxazole, tetracycline, trimethoprim/sulfanilamide, or trimethoprim) and changes in antimicrobial resistance during the feeding period. As a result, a statistically significant association between antimicrobial use and antimicrobial resistance was not found (Checkley et al., 2010). Platt et al. evaluated the impact of the administration of chlortetracycline in feed of cattle as a method to select for tetracycline-resistant enteric bacteria in feedlot settings. As expected, proportion of tetracycline-resistant *E. coli* was significantly greater in exposed than in unexposed animals. However, though co-resistant to tetracycline, exposure to the antibiotic led to a significant decrease in the amount of ESC-R-*Ec* (Platt et al., 2008).

Consistently with other studies, Tragesser et al. showed that dairy cow herds in which ceftiofur was administered were more likely to have animals colonized with ESC-R-*Ec* than herds where ceftiofur was not implemented. However, a linear relationship between the percentage of cows with ESC-R-*Ec* and the percentage of cows in the herds recently treated with ceftiofur was not found. Therefore, the authors suggested that interventions to reduce the spread of these pathogens would be most effective at the herd level rather than at individual cow-level (Tragesser et al., 2006). Singer et al. observed that CMY-2-producing *E. coli* was isolated only from dairy cows receiving ceftiofur because there was a significant drop down of the antibiotic-susceptible *E. coli* strains part of the intrinsic flora ($P < 0.027$). Actually, the resistant population did not increase in quantity within the treated cows; levels stayed low and were overtaken by a returning of the susceptible population. There was no difference in the genetic diversities of the *E. coli* between the treated and untreated cows prior to ceftiofur administration or after the susceptible population recovered in the treated cows. Therefore, the authors concluded that ceftiofur provided only a window to detect the presence of ESC-R-*Ec* but did not appear to cause its acquisition. The finding of resistant isolates following antibiotic treatment is not sufficient to estimate the strength of the selection pressure nor it is sufficient to demonstrate a causal link between antibiotic use and the emergence or amplification of resistance (Singer et al., 2008). Combining an in vivo and an observational study, Daniels *et al.* assessed the potential effects of ceftiofur use in dairy cattle on transfer and dissemination of a $bla_{\text{CMY-2}}$-bearing plasmid in *Salmonella* spp. and commensal *E. coli*. The authors concluded that plasmid transfer and frequency of occurrences of ESC-R isolates were not associated to ceftiofur treatment (Daniels et al., 2009). Notably, occurrence and persistence of ESBL- and/or pAmpC-producing *E. coli* in the apparent absence of ESCs use have been reported in poultry and cattle (Liebana et al., 2006; MARAN, 2005).

6. Similarities between human and livestock epidemiologies

Different efforts at national and local levels have been performed to establish the prevalence of ESC-R-*Ent* in humans. In general, the following prevalence of ESBL-producing *E. coli* by

Table 4
Recent representative studies analyzing the molecular characteristics of extended-spectrum cephalosporins resistant *E. coli* (ESC-R-*Ec*) detected in humans.[a]

	bla genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)	*bla* genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)	*bla* genes (relative prevalence)	Prevalence	STs (main *bla*)	Inc (main *bla*)
Europe	Czech Republic: CTX-M-9 (11%), CTX-M-15 (89%) (Hrabak et al., 2009)	ESBLs (1%) AmpCs (NT)	ST131, ST393 and ST638 (CTX-M-15), ST405 (CTX-M-9)	FII (CTX-M-15), FII, F1A, Y (CTX-M-15), A/C (CTX-M-15)	Czech Republic: CTX-M-types (78%), cAmpCs (11%), pAmpCs (11%) (Husickova et al., 2011)	ESBLs (5%) AmpCs (1%)	NT	NT	Belgium: CTX-M-15 (52%), TEM-52 (17%) (Rodriguez-Villalobos et al., 2011)	ESBLs (7%), AmpCs (NT)	ST88 (CTX-M-2/-15), ST167, ST131 and ST501 (CTX-M-15), ST540 (CTX-M-1)	NT
	Belgium: CTX-M-15 (32%), CTX-M-1 (22%) (Smet et al., 2010a)	ESBLs (6%) AmpCs (NT)	NT	NT	Bulgaria: TEM-139 (46%), CTX-M-15 (42%) (Markovska et al., 2008)	ESBLs (NT) AmpCs (NT)	NT	NT	Denmark: CTX-M-14 (60%), CMY-2 (40%) (Hammerum et al., 2011)	ESBLs (4%) AmpCs (1%)	ST1631 and ST641 (CTX-M-14), ST1800 and ST 1822 (CMY-2)	NT
	[b]Denmark: CTX-M-1 (60%) (Moodley and Guardabassi, 2009)	ESBLs (60%), AmpCs (NT)	NT	N (CTX-M-1)	France: CTX-M-15 (22%), CTX-M-14 (15%), CMY-2 (12%) (Courpon-Claudinon et al., 2011)	ESBLs (2%) AmpCs (2%)	NT	NT	Holland: CTX-M-1 (24%), TEM-52 (6%) (Leverstein-van Hall et al., 2011)	ESBLs (NT) AmpCs (NT)	ST10 (CTX-M-1, TEM-52), ST58 and ST117 (CTX-M-1)	I1 (CTX-M-1, TEM-52), B/O and N (CTX-M-1)
	[b]Holland: CTX-M-1 (55%), CMY-2 and SHV-12 (23%) (Dierikx et al., 2012)	ESBLs (28%) AmpCs (17%)	ST93 (CTX-M-1, CMY-2), ST442 (CTX-M-1), ST359 (CMY-2), ST69 (SHV-12)	I1 (CTX-M-1, CMY-2), K (CMY-2), N (SHV-12)	Holland: CTX-M-15 (54%), CTX-M-14 (15%) (van der Bij et al., 2011)	ESBLs (5%), AmpCs (NT)	ST131 and ST648 (CTX-M-15), ST405 (CTX-M-14/-15)	NT	Italy: CTX-M-15 (93%), CTX-M-14 and SHV-12 (4%) (Carattoli et al., 2008)	ESBLs (17%) AmpCs (NT)	NT	FII, FIA and FIB (CTX-M-15)
	Portugal: CTX-M-14 (40%) CTX-M-1/-15 (30%) (Correia et al., 2012)	ESBLs (8%) AmpCs (NT)	ST131 (CTX-M-15), ST410 (CTX-M-14), ST58 (CTX-M-1/-14)	FIIA (CTX-M-15), I1 (CTX-M-1/-14), A/C (CTX-M-1)	Spain: CTX-M-14 (45%), SHV-12 (20%) (Oteo et al., 2009)	ESBLs (NT) AmpCs (NT)	ST131 (CTX-M-9), ST167 (SHV-12), ST10-like (CTX-M-14, SHV-12), ST23-like (CTX-M-14)	NT	Sweden: CTX-M-15 (54%), CTX-M-14 (28%) (Onnberg et al., 2011)	ESBLs (NT) AmpCs (NT)	NT	NT
	Switzerland: CTX-M-15 (38%), CTX-M-27 (18%), CMY-2 (13%), cAmpCs (13%) (Hilty et al., 2012a)	ESBLs (5%) AmpCs (2%)	ST131 (CTX-M-14/-15/-27), ST648 (CTX-M-15/-27)	F/FII (CTX-M-types), I1 and HI2 (CMY-2)	UK: CTX-M-gr-1 (58%), CTX-M-gr 9 (18%) (Enoch et al., 2012)	ESC-R-*Ent* (1%)	ST131, ST405, ST69 (CTX-M-gr-1)	NT	UK: CTX-M-15 (NT), CTX-M-3 (NT), CTX-M-14 (NT), CTX-M-27 (NT), SHV-12 (NT) (Doumith et al., 2012)	ESBLs (NT) AmpCs (NT)	ST131 (CTX-M-15)	FIA1 (CTX-M-14/-15), FIA2, FIB6, I1, HI2 (CTX-M-14), FIA2 (CTX-M-27), FIB1o (SHV-12)
America	Argentina: CTX-M-15 (47%), CTX-M-2 (20%), CTX-M-14 (20%) (Sennati et al., 2012)	ESBLs (6%) AmpCs (0%)	ST131 (CTX-M-15)	NT	Canada: CTX-M-15 (65%), CTX-M-14 (22%) (Simner et al., 2011)	ESBLs (4%) AmpCs (3%)	ST131 (50% of ESBLs; 21% of AmpCs)	NT	Canada: CTX-M-15 (60%), CTX-14 (29%) (Peirano et al., 2012)	ESBLs (4%), AmpCs (NT)	ST131, ST405, ST10-like, ST648 (CTX-M-15), ST38, ST315, ST393 (CTX-M-14)	NT
	Mexico: CTX-M-15 (67%), SHV-5 (17%) (Garza-Gonzalez et al., 2011)	ESBLs (21%) AmpCs (NT)	NT	NT	US: CTX-M-15 (66%), CTX-M-14 (11%), SHV-5/-7 (14%), CMY-2/-32 (3%) (Sidjabat et al., 2009)	ESBLs (NT) AmpCs (NT)	ST131, ST410 and ST648 (CTX-M-15)	NT	US: CTX-M-15 (73%), CTX-M-14 (13%), SHV-2 (13%) (Peirano et al., 2010)	ESBLs (NT) AmpCs (NT)	ST131 (CTX-M-14/-15)	FII (CTX-M-14/-15)
	US: CTX-M-15 (52%), CTX-M-14 (26%), CMY-2 (16%) (Castanheira et al., 2008)	ESBLs (2%) AmpCs (<1%)	NT	NT	US: CTX-M-15 (64%), CMY-2 (11%), KPCs (2%) (Park et al., 2012)	ESBLs (NT) AmpCs (NT)	NT	NT				

Africa	Nigeria: CTX-M-15 (100%) (Aibinu et al., 2012)	ESBLs (13%) AmpCs (0%)	ST131, ST617, ST448, ST501 (CTX-M-15)	NT	South Africa: CTX-M-15 (59%), CTX-M-14 (32%), CTX-M-3 (5%), SHV-2 (5%) (Peirano et al., 2011)	ESBLs (NT) AmpCs (NT)	ST131 (CTX-M-15)	NT	Tanzania: CTX-M-15 (100%) (Mshana et al., 2011)	ESBLs (NT) AmpCs (NT)	ST131, ST405, ST638, ST648 (CTX-M-15)	FIA, FIB, FII (CTX-M-15)
Asia and Australia	Australia: CTX-M-gr-1 (63%), CTX-M-gr-4 (25%), TEM-116 (13%) (Gundogdu et al., 2012)	ESBLs (3%) AmpCs (0%)	NT	NT	Australia: CTX-M-9 (93%), CTX-M-15 (7%) (Stuart et al., 2011)	ESBLs (12%) AmpCs (NT)	NT	NT	China: DHA-1 (48%), CMY-2 (52%), CTX-M-types (61%), SHV-types (35%) (Li et al., 2008)	ESBLs (NT) AmpCs (2%)	NT	NT
	China: CTX-M-15 (18%), CTX-M-55 (13%) (Tian et al., 2012)	ESBLs (39%) AmpCs (19%)	NT	NT	Japan: CTX-M-gr-9 (56%), CTX-M-gr-1 (26%), CTX-M-gr-2 (14%) (Nakamura et al., 2012)	ESBLs (3%) AmpCs (NT)	NT	FIA, FIB, I1 (CTX-M-gr-9), N (CTX-M-gr-2)	Philippines: CTX-M-15 (50%), CTX-M-14 (33%), SHV-12 (17%) (Kanamori et al., 2011)	ESBLs (18%) AmpCs (3%)	NT	NT

Note: NT, not tested; AmpCs, includes both chromosomal AmpCs (cAmpCs) and plasmid-mediated AmpCs (pAmpCs).

[a] Data includes papers from the year 2008.

[b] These studies refer to colonized people.

continent has been recorded: Europe (1–17%), America (2–21%), Africa (13%), Asia (3–39%), and Australia (3%). Those for the AmpC producers (both plasmid- and chromosomally-mediated) are as follow: Europe (1–2%), America (0.5–3%), and Asia (2–19%). The specific prevalence of ESBL- and AmpC-producing *E. coli* for the main countries is presented in Table 4. Overall, data regarding ESC-R-*Ec* indicate that humans and food animals may share some molecular characteristics, such as *bla* types, clones (STs), and plasmid scaffolds.

6.1. ESBL and pAmpCs bla genes in ESC-R-Ec

The three most frequent *bla* genes reported in ESC-R-*Ec* isolates found in humans are (in rank) $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-14}}$, and $bla_{\text{CMY-2}}$. As deduced from the representative studies presented in Table 4, their relative prevalence according to the continent are as follows: CTX-M-15 (Europe: 22–93%; America: 47–73%; Africa: 59–100%; Asia/Australia: 7–50%), CTX-M-14 (Europe: 4–60%; America: 11–26%; Africa: 32%; Asia/Australia: 33%), and CMY-2 (Europe: 12–40%; America: 3–16%; Asia: 50%). Notably, the $bla_{\text{CMY-2}}$ is the most frequent *bla* gene found in *Salmonella* isolates, especially in North America (Frye et al., 2008; Zhang and LeJeune, 2008).

In contrast to humans (see also Section 3.4), in food animals CTX-M-1, CTX-M-14 and CMY-2 are the most frequent enzymes conferring resistance to ESCs (e.g., prevalence in Europe of 14–100%, 11–60%, 13–78%, respectively). More importantly, CTX-M-15 has much less impact than in people and it is identified only in recent analyses performed in Europe (Table 1). Therefore, humans and livestock share only part of the *bla* genes. This is a general observation that may be not true for small geographic areas where the sharing of genes might be higher (Leverstein-van Hall et al., 2011). Future surveillance of genetic distribution of genes conferring ESC-R phenotype will allow determining if the prevalence of $bla_{\text{CTX-M-1}}$ from animal origin is increasing in the human population. On the other hand, such surveillance will also determine if $bla_{\text{CTX-M-15}}$ is also increasing in the animal population.

6.2. Sequencing types (STs) of ESC-R-Ec

For ESC-R-*Ec* isolates of human origin, the following STs are more frequently observed than others (in rank): ST131, ST648, ST405, ST38, ST101, ST393, ST410, ST117, ST10, ST69, and ST95 (Ewers et al., 2012). In particular, ST131 represents 50–60% of the overall ESC-R-*Ec*, it is globally reported, and it is responsible for infections in both hospital and community (Peirano and Pitout, 2010; Rogers et al., 2011). This lineage usually carries the $bla_{\text{CTX-M-15}}$, but other $bla_{\text{CTX-M-types}}$ can also be harbored. ST131, along with the above listed commonly described human STs (e.g., ST648), is also found in livestock (Cortes et al., 2010). However, ST131 is, so far, more rarely reported than in people. This is in contrast to its high prevalence observed in pets (Ewers et al., 2012). Moreover, most ST131 isolates from food animals (especially poultry) appear to possess specific virulence genes that are absent in human and pet isolates (Platell et al., 2011). Therefore, as concluded for the comparison of the *bla* genes, the above general data indicates a possible ongoing transmission of same STs of ESC-R-*Ec* between humans and food animals but also parallel independent evolution of the two host populations of *E. coli* strains.

6.3. Plasmids spreading among ESC-R-Ent

The following Inc group plasmids have been described for the most important *bla* genes in ESC-R-*Ec* and ESC-R-*Sal* found

in humans (in rank): $bla_{CTX-M-1}$ (Inc*N*, Inc*I1*, Inc*F*, Inc*L*/*M*), $bla_{CTX-M-2}$ (Inc*A*/*C*, Inc*HI2*, Inc*P*, Inc*I1*), $bla_{CTX-M-9}$ (Inc*HI2*, Inc*P*, Inc*F*, Inc*I1*, Inc*Y*, Inc*B*/*O*, Inc*K*), $bla_{CTX-M-14}$ (Inc*K*, Inc*F*, Inc*I1*, Inc*HI2*, Inc*B*, Inc*A*/*C*), $bla_{CTX-M-15}$ (Inc*F*, Inc*I1*, Inc*A*/*C*, Inc*L*/*M*, Inc*N*), bla_{SHV-12} (Inc*I1*, Inc*K*, and Inc*F*, Inc*A*/*C*, Inc*HI2*), bla_{TEM-52} (Inc*I1*), bla_{CMY-2} (Inc*I1*, Inc*A*/*C*, Inc*F*), bla_{VIM-1} (Inc*N*, Inc*HI2*, *Inc*I1, Inc*W*), and bla_{NDM-1} (Inc*N*, Inc*L*/*M*) (Carattoli, 2009; Nordmann et al., 2011b) (Table 4). The comparison of food animals, food products and human data indicate that epidemic plasmids belonging to Inc*F*, *N*, *I1*, *I2*, *A*/*C*, *HI2*, and *K* groups are present in both hosts.

The most frequent plasmids associated to the ESBL and pAmpC *bla* genes in *E. coli* and *Salmonella* strains detected in food animals and humans are of **Inc*F*** group (Johnson et al., 2007). All bla_{CTX-M} and bla_{SHV} genes together with the bla_{CMY-2} may be linked to this type of plasmids. Highly transmissible epidemic Inc*F* plasmids carrying the $bla_{CTX-M-15}$ are of particular global impact among *Enterobacteriaceae* from humans and, with less extent, in food animals. These plasmids are not exclusive to clone ST131, since they are identified in other STs (e.g., ST405, ST354). Inc*F* plasmids contribute to the fitness of the bacterial hosts by providing virulence factors, non-*bla* resistance genes (e.g., AMEs, *aac(6′)-Ib-cr*, *qnr*, and *armA* genes), and addiction systems (toxin-antitoxin, restriction enzymes) that assure their stability in the cells (Carattoli, 2011). It should also be noted that they are detected in >50% of non-ESC-R-*Ec* from healthy people and birds (Johnson et al., 2007). **Inc*N*** plasmids are especially linked to the $bla_{CTX-M-1}$ and the bla_{SHV-12}. These mobile genetic elements are extensively distributed among food animals in both ESC-R-*Ec* and ESC-R-*Sal*, but humans (both colonized and infected) and retail meats are also frequently involved. Inc*N* are recognized as major players for the animal-to-humans transmission of $bla_{CTX-M-1}$ because they are each other highly related regardless of host and setting of isolation (Bortolaia et al., 2010; Cavaco et al., 2008; Moodley and Guardabassi, 2009; Novais et al., 2007; Randall et al., 2011). Plasmids of **Inc*I1* and Inc*I2*** groups are also common in ESC-R-*Ent* of both human and animal origin (Johnson et al., 2007). Both are associated to the bla_{CMY-2}, whereas Inc*I1* is also frequently linked to many different $bla_{CTX-M-type}$ genes (Antunes et al., 2010; Johnson et al., 2011; Mataseje et al., 2009). Genetically related Inc*I1* plasmids carrying $bla_{CTX-M-1}$ have been identified in the Netherlands in ESC-R-*Ec* from poultry meat and clinical human isolates (Leverstein-van Hall et al., 2011). Various serovar of ESC-R-*Sal* responsible for outbreaks in Belgium, France, the Netherlands, Denmark and UK and linked to poultry possess Inc*I1* plasmids carrying the bla_{TEM-52} located on a Tn*3* transposon (Carattoli, 2011; Cloeckaert et al., 2007). An Inc*I1* plasmid carrying the $bla_{CTX-M-15}$ has been identified in the *E. coli* O104:H4 responsible for the recent outbreak in Germany (Brzuszkiewicz et al., 2011). **Inc*A*/*C*** plasmids harboring $bla_{CTX-M-2}$ are recovered from *E. coli* and *Salmonella* strains from poultry and humans, especially in UK. These plasmids are also frequently associated to the dissemination of bla_{CMY-2} in North America and several EU countries (Carattoli, 2011; Giles et al., 2004; Mulvey et al., 2009; Randall et al., 2011). Plasmids of **Inc*HI2*** are mainly responsible for the dissemination of CTX-M-2 and CTX-M-9 among human and animal ESC-R-*Ent* in Europe. These two bla_{CTX-M} genes are located in similar class 1 integrons (along with the IS*CR1*) which are inserted in Tn*21*-like structures (Arduino et al., 2002; Eckert et al., 2006; Garcia Fernandez et al., 2007). **Inc*K*** plasmids are associated to the diffusion of $bla_{CTX-M-14}$ in both people and food animals (Diestra et al., 2009; Navarro et al., 2007; Valverde et al., 2009). These plasmids are even related to the CMY-2-producing *E. coli* found in animals and foods (Dierikx et al., 2010). Overall, the above data indicate that plasmids are probably the main mechanism responsible for the dissemination of common *bla* genes among the different habitats and that may be responsible for the animal-to-human transmission of these resistance traits.

6.4. Carbapenemase-producers

The bla_{VIM-1} gene recently found in *E. coli* in Germany was carried by a class 1 integron along with *aacA4* and *aadA1* AMEs, and *sul1* (sulphonamides resistance). This integron (i.e., In*110*) had been found in other *Enterobacteriaceae* and in *Pseudomonas* spp. of human origin inserted within transposon Tn*21*. Moreover, the integron was located on a 220 kb Inc*HI2* plasmid which also carried the bla_{ACC-1} pAmpC and the *strA/B* (streptomycin resistance) genes. The *E. coli* isolates were of ST88, a strain previously found in Germany among chicken, cattle, turkeys and humans samples (Fischer et al., 2012a). The bla_{VIM-1} gene of the *Salmonella* isolates was located in the same transposon as in *E. coli* and was carried by a 300 kb Inc*HI2*. The strains were identified as *Salmonella* Infantis (i.e., *S. enterica* group C of ST32), a serovar frequently detected in pig and poultry meat and responsible for outbreaks characterized by serious human invasive infections (Fischer et al., 2012b).

OXA-23 is a common source of carbapenem resistance in *A. baumannii* strains isolated in hospitals but not from the community. The bla_{OXA-23} gene found in several *A. genomospecies* 15TU of cattle origin in France was located in transposon Tn*2008* or Tn*2008*-like (Poirel et al., 2012a). This transposon is one of the genetic structures responsible for the spread of the bla_{OXA-23} gene in *A. baumannii* in humans (Mugnier et al., 2010).

The bla_{NDM-1} gene found in *A. lwoffii* in China was carried on a non-typable plasmid that was transferrable to *E. coli*. More remarkably, the expression of NDM-1 was driven by a promoter partly provided by an intact IS*Aba125*. Since this IS has not been found intact in species other than *Acinetobacter* spp. (Nordmann et al., 2011b), it is possible that the bla_{NDM-1} may have originated in these environmental bacteria and then spread to *Enterobacteriaceae* causing infection in humans (Wang et al., 2012).

7. Transmission of ESC-R GNOs from livestock to humans

MDR GNOs can be horizontally transferred from food animals to humans but such bacteria can also transfer their genetic resistance traits (e.g., bla_{ESBLs} and bla_{pAmpCs}) to the commensal intestinal flora (Marshall and Levy, 2011). The levels of evidence supporting these in vivo mechanisms are limited and most conclusions are derived from observational epidemiological studies and experimental models. However, the recent advent of new molecular techniques (e.g., MLST to analyze the clonality of strains; Inc groups and pMLST for plasmids characterization) is offering more chances to study and to demonstrate animal-to-human transmission of MDR GNOs.

7.1. Horizontal transfer of bacteria

This route of transmission is particularly associated to *Salmonella*, but *E. coli* is not excluded from this phenomenon. Humans can acquire ESC-R-*Sal* and ESC-R-*Ec* by direct contact with food-producing animal carriers or contact with contaminated environments, whereas person-to-person transmission is uncommon (Hilty et al., 2012b). However, the main path of transmission seems to be the food chain and this is based on three main observations (Perreten, 2005).

First, there is indubitable evidence that food products from livestock sources may contain ESC-R-*Ent* (Hasman et al., 2005; Mollenkopf et al., 2011). For instance, in Spain, Germany and the Netherlands the prevalence of retail poultry meat colonized by ESC-R-*Ec* has been recently reported between 45 and 90% (Egea et al., 2012; Kola et al., 2012; Overdevest et al., 2011). It should also be noted that Calbo et al. have recently described a foodborne nosocomial outbreak due to an ESBL-producing *K. pneumoniae* isolate

(Calbo et al., 2011). Second, the trends of emergence of ESC-R-*Ent* in livestock and/or its food products are specular to those observed for human infections. This is particularly true for the CMY-2-producing *Salmonella* isolates detected in US (Frye and Fedorka-Cray, 2007; Gupta et al., 2003; Lopes et al., 2006), and for the ESBL producers (e.g., CTX-M-2 and CTX-M-9) responsible for outbreaks in Europe (Bertrand et al., 2006; Weill et al., 2004). In France, clonally-related CMY-2-producing *Salmonella* infections have been linked to the consumption of meat from a common retailer (Espie et al., 2005). A Canadian study showed that *E. coli* from retail chicken (i.e., those of ST131 and ST117) and honeydew melon (i.e., ST95) were indistinguishable from those causing UTIs in women (Vincent et al., 2010). In the Netherlands, 39% of CTX-M-1- and TEM-52-producing *E. coli* found in poultry samples belonged to identical genotypes (i.e., ST10, ST58, ST117) present in human clinical samples (Leverstein-van Hall et al., 2011). Third, in several studies, patients infected with ESC-R-*Sal* had more contact with food-producing animals than subjects with susceptible isolates (Gupta et al., 2003). For instance, Fey et al. described one isolate of CMY-2-producing *Salmonella* detected in a 12-year-old boy who was in contact with the father's cattle where the same clonally-related strain caused severe diarrheal disorder and animal death (Fey et al., 2000).

7.2. Transfer of resistance genes

Humans ingest resistant bacteria on a daily basis and during the passage through the intestinal tract such bacteria may transfer their resistance genes to other host-adapted bacteria. In an experimental model simulating the human intestinal tract, Smet et al. have elegantly demonstrated this phenomenon. The TEM-52-positive Inc*I1* plasmid of an avian *E. coli* previously added to artificial human stools was easily transferred (within 24 h) to the *E. coli* strains part of the commensal microbiota. This occurred without selective pressure of antibiotics but administration of cefotaxime increased the chance of plasmid horizontal transmission and population size of ESC-R-*Ec* (Smet et al., 2011).

As discussed, plasmids with the same Inc group and other genetic elements coding for ESBLs and pAmpCs have been described in both food-producing animals and humans. However, to support the hypothesis that plasmid exchange between livestock and humans can occur, researchers would focus on: (i) analyses involving subjects from the same geographic region and period of time and (ii) clinical cases where the identical plasmid was found in the acquired zoonotic ESC-R isolate and the commensal bacterial flora. Overall, we note that these two kinds of studies are fairly scarce.

In a large analysis of CMY-2-positive plasmids from *E. coli* and *Salmonella* isolates obtained from humans, animals and environment, Mataseje et al. concluded that genetically very similar CMY-2 plasmids of Inc*I1*, Inc*A*/*C*, and Inc*K*/*B* were widely distributed across Canada in the three settings and among the two bacterial hosts (Mataseje et al., 2010). Evidence for transfer of CMY-2 plasmids between *E. coli* and *Salmonella* isolates from food animals and humans was also recorded by Winokur *et al.* (Winokur et al., 2001). Clockaert et al. reported that a common Inc*I1* TEM-52 plasmid spreading among poultry and humans was capable to move among different *Salmonella* serotypes, indicating a possibility for indirect resistance transfer (Cloeckaert et al., 2007). In a very accurate Dutch study, Leverstein-van Hall et al. showed that 19% of ESC-R-*Ec* and ESC-R-*Sal* found in human clinical samples contained bla_{ESBL} genes located on plasmids that were indistinguishable from those obtained from poultry [i.e., $bla_{CTX\text{-}M\text{-}1}$ on Inc*I1*plasmids of clonal complex 7 (ST7); $bla_{TEM\text{-}52}$ on Inc*I1*of clonal complex 5 (ST10 or ST36)] (Leverstein-van Hall et al., 2011). Remarkable proofs of plasmid exchange have also been reported in farmers (see below Section 7.3).

A plasmid-mediated CMY-2 pAmpC was identified in *E. coli* and *Salmonella* strains both from the same patient. Conjugation experiments and molecular analyses indicated that the same $bla_{CMY\text{-}2}$ harboring plasmid was transferred from *E. coli* to *Salmonella* (Yan et al., 2002). In another study, cephalosporin-susceptible *Salmonella* and *E. coli* were initially isolated from a hospitalized patient who, after 2 weeks of ceftriaxone treatment, developed infection with ESC-R-*Sal* and ESC-R-*Ec* genetically indistinguishable from the firsts. Resistant isolates carried a conjugative 95 kb plasmid with the CTX-M-3, the most prevalent ESBL spreading in that hospital (Su et al., 2003).

7.3. Professions at risk for colonization and transmission

Evidences supporting the potential risk of colonization and transmission of antibiotic-resistant *Enterobacteriaceae* (e.g., those resistant to tetracyclines or aminoglycosides) between livestock and humans working in specific settings (e.g., farm and abattoir workers, veterinarians) have been provided in the past and well discussed in a recent review of Marshall and Levy (Marshall and Levy, 2011). With regard to the ESC-R-*Ent*, data are more limited but still supporting the hypothesis that these settings are a hazard for humans.

The first notable point is that the prevalence of ESC-R-*Ec* among workers of meat-processing companies or farmers is higher (e.g., at least 5.8% in Switzerland and 33% in the Netherlands, respectively) than usually recorded for healthy people (Dierikx et al., 2012; Geser et al., 2012b). Several studies also support the notion that transfer of resistance genes may occur between workers and food animals. Moodley and Guardabassi analyzed the ESC-R-*Ec* from pigs, farm personnel and environment at two Danish pig farms where ceftiofur was implemented for prophylaxis. Human, animal, and environmental strains displayed high clonal diversity but harbored indistinguishable Inc*N* plasmids carrying $bla_{CTX\text{-}M\text{-}1}$, indicating that such plasmids were transmitted between pigs and farm workers across multiple *E. coli* lineages (Moodley and Guardabassi, 2009). Dierikx et al. have observed that farmers carried isolates containing bla_{ESBLs} and bla_{pAmpCs} which were also present in the samples from their animals. Frequently, these *bla* genes were carried on identical plasmid families and/or plasmid subtypes [e.g., Inc*I1*(ST7) with $bla_{CTX\text{-}M\text{-}1}$ or Inc*I1*(ST12) with $bla_{CMY\text{-}2}$] (Dierikx et al., 2012).

Overall, the knowledge of this phenomenon is still insufficient and future studies are necessary to address its impact on the overall spread of MDR GNOs and their genetic elements. This is particularly important because occupational workers, and possibly their families, might provide an important reservoir and channel of entry for ESC-R-*Ent* and/or plasmids harboring bla_{ESBL} and bla_{pAmpCs} into the community (Fig. 1).

8. Strategies for controlling the spread of ESC-R GNOs in livestock

Public/animal health and agriculture official institutions should synergistically work on the development of containment strategies to assure preservation of health for the population but still addressing the needs of animals. In particular, the prudent and judicious use of antibiotics (e.g., elimination of the unnecessary use for viral infection, empirical treatment, or too prolonged treatments) coupled with government regulations (e.g., guidelines with antibiotic alternatives with the same capacity to eradicate the infection in the animals) can decrease the opportunities for selection of MDR organisms in the food-producing animal setting. The importance of monitoring programs at national and international level should also be emphasized (EFSA, 2008, 2011).

Moreover, new concepts (e.g., Hazard Analysis and Critical Control Points, HACCP) have to be integrated in the food chain at slaughterhouses, meat process plants and retail markets to limit contamination and spread of ESC-R bacteria from animals to humans (www.fda.gov/food/foodsafety).

The World Health Organization (WHO) highlights the urgent needs for action to limit antibiotic resistance suggesting multidisciplinary programs where the livestock scenario has a key role. The following general strategies should be ideally implemented: (i) to ban the use of antimicrobials as growth promoters; (ii) to require an obligatory veterinarian prescription and supervision when antibiotics are used; and (iii) to drastically limit the use of critically important antibiotics for human medicine, specifically ESCs, quinolones, aminoglycosides, macrolides and sulfonamides (WHO, 2007). To pursue the above strategic points, different official institutions have released directives and/or suggestions. However, implementation is complex and control on its effectiveness is quite limited (EFSA, 2011).

The US Food and Drug Administration (FDA) has recently joined this overall strategy, suggesting voluntary adoption of practices to ensure the appropriate and judicious use of medically important antibiotics in food-producing animals (FDA, 2012c). The effectiveness of cephalosporins in humans is protected by prohibiting their use in certain food-producing animals. Moreover, the FDA bans off-label and unapproved (e.g., administration for prevention of diseases, wrong routes or dosage levels) use of cephalosporins in cattle, pigs, and poultry (FDA, 2012a, b).

The EU abandoned the use of antibiotics for growth promotion in January 2006 (EU, 2003). The European Medicines Agency (EMEA) indicates that ESCs should be reserved for the treatment of clinical conditions which respond poorly to more narrow-spectrum antibiotics. Oral use of ESCs is strongly discouraged and parenteral prophylactic administration should be limited to specific situations (EMEA, 2009). The Federation of Veterinarians of Europe and other associations at country level released general guidelines regarding the prudent use of antibiotics indicating that narrow-spectrum agents should be preferred to those with broad-/extended-spectrum if appropriated. However, the role and scenario where ESCs should be implemented is usually not specifically discussed (F.o.V.o. Europe, 1999; Morley et al., 2005; Passantino, 2007; Ungemach et al., 2006). The Finnish legislation prohibits the use of ESCs (including that off-label) unless a veterinary product containing such compounds is formally authorized and licensed (MAF, 2003). In the Netherlands and Germany, antimicrobials with a "last option" characteristic in humans should be considered as third choice in veterinary medicine and only in critical situations where they are formally indicated (EMEA, 2009).

The specific impact of the above national and international strategies to contain the problem in food-producing animals is difficult to be evaluated because: (i) other settings (e.g., wild-life, companion animals, agriculture, pisciculture, and environment) participate to the pool of antibiotic resistance, exchanging each other MDR organisms and/or their genetic elements (Fig. 1) (Perreten, 2005); (ii) there are many unrecognized sources of antibiotics that are constantly in contact with animals and humans (e.g., milk containing antibiotics to feed other animals); and (iii) antibiotic resistance is also a natural phenomenon independent from antibiotic pressure (Hammerum and Heuer, 2009). Therefore, we can only implement ways to limit the selection and spread of these harmful resistant bacteria, but we will probably never defeat the overall problem of antibiotic resistance. In this context, we should take into consideration that, though antibiotics currently remain the most cost effective strategy to prevent and cure bacterial infections in food animals, a number of alternatives for treatment and prevention of infection are available or under development and investigation. For instance, bacteriophage therapy, implementation of vaccines and probiotics, breading for healthy animals, bio-security on farms, and overall intense hygiene measures have shown excellent results in reducing the impact of MDR bacteria in livestock (Doyle and Erickson, 2012, 2006; Shryock and Richwine, 2010).

9. Conclusions

The intense use of antibiotics, particularly at non-therapeutic level, in the livestock sector has so far been both a blessing and a curse. It is a blessing because it eradicates and prevents animal infections in a very efficient and seemingly cost effective way; it is a curse because it has undoubtedly created a resistance problem in the bacterial populations of food animals by "breeding" antibiotic-resistant GNOs. This now hampers the options of antibiotic treatment of animal infections but, more importantly, also poses a significant hazard to the human health. Life-threatening human pathogens have become resistant to critically important antibiotics (e.g., ESCs) undermining our therapeutic armamentarium. This is coupled by the fact that the discovery of new and more potent antimicrobials against GNOs has faced a significant slowing-down in the last decade. Therefore, it is vital that we develop and implement strategies to limit and regulate the overall use of antibiotics in view to preserve the effectiveness of those that are vital for the human health. This can also have important implications for the containment of the increased health-care costs due to the nosocomial and community acquired bacterial infections.

So far, robust studies proving unquestionable proofs for the food animal-to-human transmission are limited. Clones resistant to ESCs (especially *E. coli*) have been mostly different in the humans compared to the animals. However, there are strong indications that the same plasmids are simultaneously present in the two settings and, with less extent, there are also evidences regarding their bacterial inter-/intra-species exchange and horizontal transmission between human and animal hosts. Therefore, what is really needed in this context, are large scale studies with a combined design which includes the human (i.e., both community and hospitals) and veterinary settings (i.e., pets, wild and main food animals) at the same time and in the same geographic region. Moreover, the molecular approaches implemented to characterize the MDR isolates should be consistent among the different studies to assure a correct comparison and interpretation of the epidemiological results. Finally, to better comprehend the overall problem of antibiotic resistance, the impact of the use of different antibiotic classes and other risk factors (e.g., profession, environment) should be taken into account for both humans and animals.

Acknowledgements

Salome N. Seiffert, Vincent Perreten, and Andrea Endimiani are supported by Grant 1.12.06 from the Swiss Veterinary Federal Office.

References

Agerso, Y., Aarestrup, F.M., Pedersen, K., Seyfarth, A.M., Struve, T., Hasman, H., 2012. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. The Journal of Antimicrobial Chemotherapy 67, 582–588.

Aibinu, I., Odugbemi, T., Koenig, W., Ghebremedhin, B., 2012. Sequence type ST131 and ST10 complex (ST617) predominant among CTX-M-15-producing *Escherichia coli* isolates from Nigeria. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 18, E49–E51.

Alali, W.Q., Scott, H.M., Norby, B., Gebreyes, W., Loneragan, G.H., 2009. Quantification of the *bla*(CMY-2) in feces from beef feedlot cattle administered three different

doses of ceftiofur in a longitudinal controlled field trial. Foodborne Pathogens and Disease 6, 917–924.

Alexander, T.W., Inglis, G.D., Yanke, L.J., Topp, E., Read, R.R., Reuter, T., McAllister, T.A., 2010. Farm-to-fork characterization of *Escherichia coli* associated with feedlot cattle with a known history of antimicrobial use. International Journal of Food Microbiology 137, 40–48.

Alexander, T.W., Yanke, L.J., Topp, E., Olson, M.E., Read, R.R., Morck, D.W., McAllister, T.A., 2008. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. Applied and Environment Microbiology 74, 4405–4416.

Ambrozic Avgustin, J., Keber, R., Zerjavic, K., Orazem, T., Grabnar, M., 2007. Emergence of the quinolone resistance-mediating gene aac(6')-Ib-cr in extended-spectrum-beta-lactamase-producing *Klebsiella* isolates collected in Slovenia between 2000 and 2005. Antimicrobial Agents and Chemotherapy 51, 4171–4173.

Anderson, A.D., Nelson, J.M., Rossiter, S., Angulo, F.J., 2003. Public health consequences of use of antimicrobial agents in food animals in the United States. Microbial Drug Resistance 9, 373–379.

ANSES, 2011. French agency for food, environment and occupational health and safety (ANSES). Sales Survey of Veterinary Medicinal Products containing Antimicrobials in France, 2010.

Antunes, P., Coque, T.M., Peixe, L., 2010. Emergence of an IncIgamma plasmid encoding CMY-2 β-lactamase associated with the international ST19 OXA-30-producing β-lactamase *Salmonella typhimurium* multidrug-resistant clone. The Journal of Antimicrobial Chemotherapy 65, 2097–2100.

Arduino, S.M., Roy, P.H., Jacoby, G.A., Orman, B.E., Pineiro, S.A., Centron, D., 2002. *bla*CTX-M-2 is located in an unusual class 1 integron (In35) which includes Orf513. Antimicrobial Agents and Chemotherapy 46, 2303–2306.

Asai, T., Masani, K., Sato, C., Hiki, M., Usui, M., Baba, K., Ozawa, M., Harada, K., Aoki, H., Sawada, T., 2011. Phylogenetic groups and cephalosporin resistance genes of *Escherichia coli* from diseased food-producing animals in Japan. Acta Veterinaria Scandinavica 53, 52.

Bardon, J., Husickova, V., Chroma, M., Kolar, M., 2012. Detection of ESBL-positive strains of *Escherichia coli* in pigs in the Czech Republic. Klin Mikrobiol Infekc Lek 18, 65–67.

Ben-Ami, R., Rodriguez-Bano, J., Arslan, H., Pitout, J.D., Quentin, C., Calbo, E.S., Azap, O.K., Arpin, C., Pascual, A., Livermore, D.M., Garau, J., Carmeli, Y., 2009. A multinational survey of risk factors for infection with extended-spectrum β-lactamase-producing *Enterobacteriaceae* in non-hospitalized patients. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 49, 682–690.

Bertrand, S., Weill, F.X., Cloeckaert, A., Vrints, M., Mairiaux, E., Praud, K., Dierick, K., Wildemauve, C., Godard, C., Butaye, P., Imberechts, H., Grimont, P.A., Collard, J.M., 2006. Clonal emergence of extended-spectrum β-lactamase (CTX-M-2)-producing *Salmonella enterica* serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003). Journal of Clinical Microbiology 44, 2897–2903.

Blanc, V., Mesa, R., Saco, M., Lavilla, S., Prats, G., Miro, E., Navarro, F., Cortes, P., Llagostera, M., 2006. ESBL- and plasmidic class C β-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. Veterinary Microbiology 118, 299–304.

Bondt, N.L., Puister, L., Ge, H., van der Veen, R., Bergevoet, B., Douma, A., van Vliet, W.K., 2012. Trends in Veterinary Antibiotic Use in the Netherlands 2005–2011. LEI, Wageningen, UR.

Bortolaia, V., Guardabassi, L., Trevisani, M., Bisgaard, M., Venturi, L., Bojesen, A.M., 2010. High diversity of extended-spectrum β-lactamases in *Escherichia coli* isolates from Italian broiler flocks. Antimicrobial Agents and Chemotherapy 54, 1623–1626.

Bortolaia, V., Larsen, J., Damborg, P., Guardabassi, L., 2011. Potential pathogenicity and host range of extended-spectrum β-lactamase-producing *Escherichia coli* isolates from healthy poultry. Applied and Environment Microbiology 77, 5830–5833.

Boyle, F., Morris, D., O'Connor, J., Delappe, N., Ward, J., Cormican, M., 2010. First report of extended-spectrum-β-lactamase-producing *Salmonella enterica* serovar Kentucky isolated from poultry in Ireland. Antimicrobial Agents and Chemotherapy 54, 551–553.

Brinas, L., Moreno, M.A., Zarazaga, M., Porrero, C., Saenz, Y., Garcia, M., Dominguez, L., Torres, C., 2003. Detection of CMY-2, CTX-M-14, and SHV-12 β-lactamases in *Escherichia coli* fecal-sample isolates from healthy chickens. Antimicrobial Agents and Chemotherapy 47, 2056–2058.

Brzuszkiewicz, E., Thurmer, A., Schuldes, J., Leimbach, A., Liesegang, H., Meyer, F.D., Boelter, J., Petersen, H., Gottschalk, G., Daniel, R., 2011. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). Archives of Microbiology 193, 883–891.

Bush, K., Fisher, J.F., 2011. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from gram-negative bacteria. Annual Review of Microbiology 65, 455–478.

Bush, K., Jacoby, G.A., 2009. Updated functional classification of β-lactamases. Antimicrobial Agents and Chemotherapy 54, 969–976.

Bush, K., Jacoby, G.A., Medeiros, A.A., 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrobial Agents and Chemotherapy 39, 1211–1233.

Calbo, E., Freixas, N., Xercavins, M., Riera, M., Nicolas, C., Monistrol, O., Sole Mdel, M., Sala, M.R., Vila, J., Garau, J., 2011. Foodborne nosocomial outbreak of SHV-1 and CTX-M-15-producing *Klebsiella pneumoniae*: epidemiology and control. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 52, 743–749.

Canton, R., Akova, M., Carmeli, Y., Giske, C.G., Glupczynski, Y., Gniadkowski, M., Livermore, D.M., Miriagou, V., Naas, T., Rossolini, G.M., Samuelsen, O., Seifert, H., Woodford, N., Nordmann, P., 2012. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 18, 413–431.

Carattoli, A., 2011. Plasmids in Gram negatives: molecular typing of resistance plasmids. International Journal of Medical Microbiology: IJMM 301, 654–658.

Carattoli, A., 2009. Resistance plasmid families in *Enterobacteriaceae*. Antimicrobial Agents and Chemotherapy 53, 2227–2238.

Carattoli, A., Bertini, A., Villa, L., Falbo, V., Hopkins, K.L., Threlfall, E.J., 2005. Identification of plasmids by PCR-based replicon typing. Journal of Microbiological Methods 63, 219–228.

Carattoli, A., Garcia-Fernandez, A., Varesi, P., Fortini, D., Gerardi, S., Penni, A., Mancini, C., Giordano, A., 2008. Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases isolated in Rome, Italy. Journal of Clinical Microbiology 46, 103–108.

Castanheira, M., Mendes, R.E., Rhomberg, P.R., Jones, R.N., 2008. Rapid emergence of *bla*CTX-M among *Enterobacteriaceae* in US Medical Centers: molecular evaluation from the MYSTIC Program (2007). Microbial Drug Resistance 14, 211–216.

Cavaco, L.M., Abatih, E., Aarestrup, F.M., Guardabassi, L., 2008. Selection and persistence of CTX-M-producing *Escherichia coli* in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrobial Agents and Chemotherapy 52, 3612–3616.

Chander, Y., Oliveira, S., Goyal, S.M., 2011. Characterisation of ceftiofur resistance in swine bacterial pathogens. Veterinary Journal 187, 139–141.

Checkley, S.L., Campbell, J.R., Chirino-Trejo, M., Janzen, E.D., Waldner, C.L., 2010. Associations between antimicrobial use and the prevalence of antimicrobial resistance in fecal *Escherichia coli* from feedlot cattle in western Canada. Canadian Veterinary Journal 51, 853–861.

Chen, L., Chen, Z.L., Liu, J.H., Zeng, Z.L., Ma, J.Y., Jiang, H.X., 2007. Emergence of RmtB methylase-producing *Escherichia coli* and *Enterobacter cloacae* isolates from pigs in China. Journal of Antimicrobial Chemotherapy 59, 880–885.

Chen, Y.H., Hsueh, P.R., Badal, R.E., Hawser, S.P., Hoban, D.J., Bouchillon, S.K., Ni, Y., Paterson, D.L., 2011. Antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia-Pacific region according to currently established susceptibility interpretive criteria. Journal of Infection 62, 280–291.

Chiaretto, G., Zavagnin, P., Bettini, F., Mancin, M., Minorello, C., Saccardin, C., Ricci, A., 2008. Extended spectrum β-lactamase SHV-12-producing Salmonella from poultry. Veterinary Microbiology 128, 406–413.

CliniPharm-CliniTox, Institut für Veterinärpharmakologie und toxikologie, 2012. CliniPharm CliniTox. http://www.vetpharm.uzh.ch/perldocs/index.i.htm

Cloeckaert, A., Praud, K., Doublet, B., Bertini, A., Carattoli, A., Butaye, P., Imberechts, H., Bertrand, S., Collard, J.M., Arlet, G., Weill, F.X., 2007. Dissemination of an extended-spectrum-β-lactamase *bla*TEM-52 gene-carrying IncI1 plasmid in various *Salmonella enterica* serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. Antimicrobial Agents and Chemotherapy 51, 1872–1875.

Cloeckaert, A., Praud, K., Lefevre, M., Doublet, B., Pardos, M., Granier, S.A., Brisabois, A., Weill, F.X., 2010. Inc*I1* plasmid carrying extended-spectrum-β-lactamase gene *bla*CTX-M-1 in *Salmonella enterica* isolates from poultry and humans in France, 2003 to 2008. Antimicrobial Agents and Chemotherapy 54, 4484–4486.

Coque, T.M., Novais, A., Carattoli, A., Poirel, L., Pitout, J., Peixe, L., Baquero, F., Canton, R., Nordmann, P., 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerging Infectious Diseases 14, 195–200.

Correia, S., Pacheco, R., Radhouani, H., Diniz, J.C., Ponce, P., Jones-Dias, D., Canica, M., Igrejas, G., Poeta, P., 2012. High prevalence of ESBL-producing *Escherichia coli* isolates among hemodialysis patients in Portugal: appearance of ST410 with the *bla*(CTX-M-14) gene. Diagnostic Microbiology and Infectious Disease 74, 423–425.

Cortes, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J.E., Blanco, M., Lopez, C., Andreu, A., Navarro, F., Alonso, M.P., Bou, G., Blanco, J., Llagostera, M., 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Applied and Environment Microbiology 76, 2799–2805.

Courpon-Claudinon, A., Lefort, A., Panhard, X., Clermont, O., Dornic, Q., Fantin, B., Mentre, F., Wolff, M., Denamur, E., Branger, C., 2011. Bacteraemia caused by third-generation cephalosporin-resistant *Escherichia coli* in France: prevalence, molecular epidemiology and clinical features. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 17, 557–565.

Croxen, M.A., Finlay, B.B., 2010. Molecular mechanisms of *Escherichia coli* pathogenicity. Nature Reviews. Microbiology 8, 26–38.

Dahmen, S., Metayer, V., Gay, E., Madec, J.Y., Haenni, M., 2012. Characterization of extended-spectrum β-lactamase (ESBL)-carrying plasmids and clones of *Enterobacteriaceae* causing cattle mastitis in France. Veterinary Microbiology 162, 793–799.

Daniels, J.B., Call, D.R., Hancock, D., Sischo, W.M., Baker, K., Besser, T.E., 2009. Role of ceftiofur in selection and dissemination of *bla*CMY-2-mediated cephalosporin resistance in *Salmonella enterica* and commensal

Escherichia coli isolates from cattle. Applied and Environment Microbiology 75, 3648–3655.

DANMAP, 1998. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark.

DANMAP, 2007. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark.

DANMAP, 2011. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP). Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark.

Deckert, A., Gow, S., Rosengren, L., Leger, D., Avery, B., Daignault, D., Dutil, L., Reid-Smith, R., Irwin, R., 2010. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) Farm Program: results from finisher pig surveillance. Zoonoses Public Health 57 (Suppl. 1), 71–84.

Dheilly, A., Le Devendec, L., Mourand, G., Bouder, A., Jouy, E., Kempf, I., 2012. Resistance gene transfer during treatments for experimental avian colibacillosis. Antimicrobial Agents and Chemotherapy 56, 189–196.

Dierikx, C., van der Goot, J., Fabri, T., van Essen-Zandbergen, A., Smith, H., Mevius, D., 2012. Extended-spectrum-β-lactamase- and AmpC-β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. The Journal of Antimicrobial Chemotherapy.

Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Smith, H., Mevius, D., 2010. Increased detection of extended spectrum β-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Veterinary Microbiology 145, 273–278.

Diestra, K., Juan, C., Curiao, T., Moya, B., Miro, E., Oteo, J., Coque, T.M., Perez-Vazquez, M., Campos, J., Canton, R., Oliver, A., Navarro, F., 2009. Characterization of plasmids encoding blaESBL and surrounding genes in Spanish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. The Journal of Antimicrobial Chemotherapy 63, 60–66.

Doi, Y., Arakawa, Y., 2007. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clinical Infectious Diseases 45, 88–94.

Doi, Y., Paterson, D.L., 2007. Detection of plasmid-mediated class C β-lactamases. International Journal of Infectious Diseases 11, 191–197.

Dolejska, M., Jurcickova, Z., Literak, I., Pokludova, L., Bures, J., Hera, A., Kohoutova, L., Smola, J., Cizek, A., 2011. Inc*N* plasmids carrying *bla* CTX-M-1 in *Escherichia coli* isolates on a dairy farm. Veterinary Microbiology 149, 513–516.

Donaldson, S.C., Straley, B.A., Hegde, N.V., Sawant, A.A., DebRoy, C., Jayarao, B.M., 2006. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. Applied and Environment Microbiology 72, 3940–3948.

Doumith, M., Dhanji, H., Ellington, M.J., Hawkey, P., Woodford, N., 2012. Characterization of plasmids encoding extended-spectrum β-lactamases and their addiction systems circulating among *Escherichia coli* clinical isolates in the UK. The Journal of Antimicrobial Chemotherapy 67, 878–885.

Doyle, M.P., Erickson, M.C., 2012. Opportunities for mitigating pathogen contamination during on-farm food production. International Journal of Food Microbiology 152, 54–74.

Doyle, M.P., Erickson, M.C., 2006. Reducing the carriage of foodborne pathogens in livestock and poultry. Poultry Science 85, 960–973.

Dutil, L., Irwin, R., Finley, R., Ng, L.K., Avery, B., Boerlin, P., Bourgault, A.M., Cole, L., Daignault, D., Desruisseau, A., Demczuk, W., Hoang, L., Horsman, G.B., Ismail, J., Jamieson, F., Maki, A., Pacagnella, A., Pillai, D.R., 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerging Infectious Diseases 16, 48–54.

Eckert, C., Gautier, V., Arlet, G., 2006. DNA sequence analysis of the genetic environment of various blaCTX-M genes. The Journal of Antimicrobial Chemotherapy 57, 14–23.

EFSA, European Food Safety Authority, 2008. Foodborne antimicrobial resistance as a biological hazard. Scientific Opinion of the Panel on Biological Hazards. EFSA Journal 765, 1–87.

EFSA, European Food Safety Authority, 2011. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum β-lactamases and/or AmpC β-lactamases in food and food-producing animals. EFSA Journal 9, 2322.

Egea, P., Lopez-Cerero, L., Torres, E., Gomez-Sanchez Mdel, C., Serrano, L., Navarro Sanchez-Ortiz, M.D., Rodriguez-Bano, J., Pascual, A., 2012. Increased raw poultry meat colonization by extended spectrum β-lactamase-producing *Escherichia coli* in the south of Spain. International Journal of Food Microbiology 159, 69–73.

EMEA, European Medicines Agency, 2009. Reflection paper on the use of third and fourth generation cephalosporins in food producing animals in the European Union: development of resistance and impact on human and animal health. Journal of Veterinary Pharmacology and Therapeutics 32, 515–533.

EMEA, 2012. European Medicines Agency. Sales of Veterinary Antimicrobial Agents in 19 EU/EEA Countries in 2010. Second ESVAC Report (EMA/88728/2012).

Endimiani, A., Doi, Y., Bethel, C.R., Taracila, M., Adams-Haduch, J.M., O'Keefe, A., Hujer, A.M., Paterson, D.L., Skalweit, M.J., Page, M.G., Drawz, S.M., Bonomo, R.A., 2009. Enhancing resistance to cephalosporins in class C β-lactamases: impact of Gly214Glu in CMY-2. Biochemistry 49, 1014–1023.

Endimiani, A., Hilty, M., Perreten, V., 2012a. CMY-2-producing *Escherichia coli* in the nose of pigs. Antimicrobial Agents and Chemotherapy 56, 4556–4557.

Endimiani, A., Hujer, K.M., Hujer, A.M., Bertschy, I., Rossano, A., Koch, C., Gerber, V., Francey, T., Bonomo, R.A., Perreten, V., 2011. *Acinetobacter baumannii* isolates from pets and horses in Switzerland: molecular characterization and clinical data. The Journal of Antimicrobial Chemotherapy 66, 2248–2254.

Endimiani, A., Luzzaro, F., Migliavacca, R., Mantengoli, E., Hujer, A.M., Hujer, K.M., Pagani, L., Bonomo, R.A., Rossolini, G.M., Toniolo, A., 2007. Spread in an Italian hospital of a clonal *Acinetobacter baumannii* strain producing the TEM-92 extended-spectrum β-lactamase. Antimicrobial Agents and Chemotherapy 51, 2211–2214.

Endimiani, A., Paterson, D.L., 2007. Optimizing therapy for infections caused by *Enterobacteriaceae* producing extended-spectrum β-lactamases. Seminars in Respiratory and Critical Care Medicine 28, 646–655.

Endimiani, A., Perez, F., Bonomo, R.A., 2008. Cefepime: a reappraisal in an era of increasing antimicrobial resistance. Expert Review of Anti-infective Therapy 6, 805–824.

Endimiani, A., Rossano, A., Kunz, D., Overesch, G., Perreten, V., 2012b. First country-wide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. Diagnostic Microbiology and Infectious Disease 73, 31–38.

Enoch, D.A., Brown, F., Sismey, A.W., Mlangeni, D.A., Curran, M.D., Karas, J.A., Cone, D.B., Aliyu, S.H., Dhanji, H., Doumith, M., Maharjan, S., Meunier, D., Woodford, N., 2012. Epidemiology of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in a UK district hospital; an observational study. Journal of Hospital Infection 81, 270–277.

Escudero, E., Vinue, L., Teshager, T., Torres, C., Moreno, M.A., 2010. Resistance mechanisms and farm-level distribution of fecal *Escherichia coli* isolates resistant to extended-spectrum cephalosporins in pigs in Spain. Research in Veterinary Science 88, 83–87.

Espie, E., De Valk, H., Vaillant, V., Quelquejeu, N., Le Querrec, F., Weill, F.X., 2005. An outbreak of multidrug-resistant *Salmonella enterica* serotype Newport infections linked to the consumption of imported horse meat in France. Epidemiology and Infection 133, 373–376.

EU, European Community, 2001. Directive 2001/82/EC of the European parliament and of the council, Official Journal of the European Communities L311/1.

EU, European Union, 2003. Regulation 1831/2003 of the European parliament and of the council on aditives for use in animal nutrition.

F.o.V.o. Europe, 1999. Antibiotic Resistance and Prudent Use of Antibiotics in Veterinary Medicine. http://www.fve.org/news/publications/pdf/antibioen.pdf

Ewers, C., Bethe, A., Semmler, T., Guenther, S., Wieler, L.H., 2012. Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 18, 646–655.

FDA, Food and Drug Administration, 2010. 2009 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals.

FDA, Food and Drug Administration, 2011. 2010 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals.

FDA, Food and Drug Administration, 2012a. New animal drugs; cephalosporin drugs; extralabel animal drug use; order of prohibition. Federal Register 77 (4), 735–745, http://www.gpo.gov

FDA, Food and Drug Administration, 2012b. US Department of Health and Human Services. Center for Veterinary Medicine. FDA Approved Animal Drug Products. http://www.accessdata.fda.gov/scripts/animaldrugsatfda/

FDA, Food and Drug Administration, 2012c. US Department of Health and Human Services. Center for Veterinary Medicine. Guidance for Industry. The judicious use of medically important antimicrobial drugs in food-producing animals. http://www.regulations.gov

Fernandes, S.A., Paterson, D.L., Ghilardi-Rodrigues, A.C., Adams-Haduch, J.M., Tavechio, A.T., Doi, Y., 2009. CTX-M-2-producing *Salmonella typhimurium* isolated from pediatric patients and poultry in Brazil. Microbial Drug Resistance 15, 317–321.

Fey, P.D., Safranek, T.J., Rupp, M.E., Dunne, E.F., Ribot, E., Iwen, P.C., Bradford, P.A., Angulo, F.J., Hinrichs, S.H., 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. New England Journal of Medicine 342, 1242–1249.

FINRES-Vet, 2011. Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents, 2007–2009.

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., Guerra, B., 2012a. *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. The Journal of Antimicrobial Chemotherapy 67, 1793–1795.

Fischer, J., Rodriguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., Guerra, B., 2012b. *Salmonella enterica* subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms. The Journal of Antimicrobial Chemotherapy 68, 478–480.

Fluit, A.C., Schmitz, F.J., Verhoef, J., 2001. Frequency of isolation of pathogens from bloodstream, nosocomial pneumonia, skin and soft tissue, and urinary tract infections occurring in European patients. European Journal of Clinical Microbiology and Infectious Diseases 20, 188–191.

Foley, S.L., Lynne, A.M., 2008. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. Journal of Animal Science 86, E173–E187.

Frye, J.G., Fedorka-Cray, P.J., 2007. Prevalence, distribution and characterisation of ceftiofur resistance in *Salmonella enterica* isolated from animals in the USA from 1999 to 2003. International Journal of Antimicrobial Agents 30, 134–142.

Frye, J.G., Fedorka-Cray, P.J., Jackson, C.R., Rose, M., 2008. Analysis of *Salmonella enterica* with reduced susceptibility to the third-generation cephalosporin

ceftriaxone isolated from US cattle during 2000–2004. Microbial Drug Resistance 14, 251–258.

Galimand, M., Sabtcheva, S., Courvalin, P., Lambert, T., 2005. Worldwide disseminated *armA* aminoglycoside resistance methylase gene is borne by composite transposon Tn*1548*. Antimicrobial Agents and Chemotherapy 49, 2949–2953.

Garcia Fernandez, A., Cloeckaert, A., Bertini, A., Praud, K., Doublet, B., Weill, F.X., Carattoli, A., 2007. Comparative analysis of IncHI2 plasmids carrying *bla*CTX-M-2 or *bla*CTX-M-9 from *Escherichia coli* and *Salmonella enterica* strains isolated from poultry and humans. Antimicrobial Agents and Chemotherapy 51, 4177–4180.

Garza-Gonzalez, E., Mendoza Ibarra, S.I., Llaca-Diaz, J.M., Gonzalez, G.M., 2011. Molecular characterization and antimicrobial susceptibility of extended-spectrum β-lactamase-producing *Enterobacteriaceae* isolates at a tertiary-care centre in Monterrey, Mexico. Journal of Medical Microbiology 60, 84–90.

Geser, N., Stephan, R., Hächler, H., 2012a. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. BMC Veterinary Research 8, 21.

Geser, N., Stephan, R., Korczak, B.M., Beutin, L., Hächler, H., 2012b. Molecular identification of extended-spectrum-β-lactamase genes from *Enterobacteriaceae* isolated from healthy human carriers in Switzerland. Antimicrobial Agents and Chemotherapy 56, 1609–1612.

Giamarellou, H., Poulakou, G., 2009. Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs 69, 1879–1901.

Giles, W.P., Benson, A.K., Olson, M.E., Hutkins, R.W., Whichard, J.M., Winokur, P.L., Fey, P.D., 2004. DNA sequence analysis of regions surrounding blaCMY-2 from multiple *Salmonella* plasmid backbones. Antimicrobial Agents and Chemotherapy 48, 2845–2852.

Girlich, D., Poirel, L., Carattoli, A., Kempf, I., Lartigue, M.F., Bertini, A., Nordmann, P., 2007. Extended-spectrum β-lactamase CTX-M-1 in *Escherichia coli* isolates from healthy poultry in France. Applied and Environment Microbiology 73, 4681–4685.

Gniadkowski, M., 2008. Evolution of extended-spectrum β-lactamases by mutation. Clinical Microbiology and Infection 14 (Suppl. 1), 11–32.

Goncalves, A., Torres, C., Silva, N., Carneiro, C., Radhouani, H., Coelho, C., Araujo, C., Rodrigues, J., Vinue, L., Somalo, S., Poeta, P., Igrejas, G., 2010. Genetic characterization of extended-spectrum β-lactamases in *Escherichia coli* isolates of pigs from a Portuguese intensive swine farm. Foodborne Pathogens and Disease 7, 1569–1573.

Gonzalez-Zorn, B., Teshager, T., Casas, M., Porrero, M.C., Moreno, M.A., Courvalin, P., Dominguez, L., 2005. *armA* and aminoglycoside resistance in *Escherichia coli*. Emerging Infectious Diseases 11, 954–956.

Gordon, M.A., 2011. Invasive nontyphoidal *Salmonella* disease: epidemiology, pathogenesis and diagnosis. Current Opinion in Infectious Diseases 24, 484–489.

Grave, K., Greko, C., Kvaale, M.K., Torren-Edo, J., Mackay, D., Muller, A., Moulin, G., 2012. Sales of veterinary antibacterial agents in nine European countries during 2005–2009: trends and patterns. The Journal of Antimicrobial Chemotherapy 67, 3001–3008.

Grayson, M.L., 2010. Cephalosporins and Related Drugs. In Kocers' the Use of Antibiotics, 6th ed. ASM Press, London.

Guardabassi, L., Courvalin, P., 2006. Modes of antibacterial action and mechanisms of bacterial resistance. In: Aarestrup, F.M. (Ed.), Antimicrobial Resistance in Bacterial of Animal Origin. ASM Press, Washington, DC, pp. 1–18.

Guardabassi, L., Schwarz, S., Lloyd, D.H., 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. The Journal of Antimicrobial Chemotherapy 54, 321–332.

Guenther, S., Ewers, C., Wieler, L.H., 2011. Extended-spectrum β-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Frontiers in Microbiology 2, 246.

Guerrant, R.L., Van Gilder, T., Steiner, T.S., Thielman, N.M., Slutsker, L., Tauxe, R.V., Hennessy, T., Griffin, P.M., DuPont, H., Sack, R.B., Tarr, P., Neill, M., Nachamkin, I., Reller, L.B., Osterholm, M.T., Bennish, M.L., Pickering, L.K., 2001. Practice guidelines for the management of infectious diarrhea. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 32, 331–351.

Gundogdu, A., Long, Y.B., Katouli, M., 2012. Prevalence and pathogenesis of extended-spectrum β-lactamase producing *Escherichia coli* causing urinary tract infection in hospitalized patients. European Journal of Clinical Microbiology and Infectious Diseases 31, 3107–3116.

Gupta, A., Fontana, J., Crowe, C., Bolstorff, B., Stout, A., Van Duyne, S., Hoekstra, M.P., Whichard, J.M., Barrett, T.J., Angulo, F.J., 2003. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporins in the United States. Journal of Infectious Diseases 188, 1707–1716.

Haenni, M., Saras, E., Metayer, V., Doublet, B., Cloeckaert, A., Madec, J.Y., 2012. Spread of the *bla*TEM-52 gene is mainly ensured by IncI1/ST36 plasmids in *Escherichia coli* isolated from cattle in France. The Journal of Antimicrobial Chemotherapy 67, 2774–2776.

Hammerum, A.M., Heuer, O.E., 2009. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 48, 916–921.

Hammerum, A.M., Lester, C.H., Jakobsen, L., Porsbo, L.J., 2011. Faecal carriage of extended-spectrum β-lactamase-producing and AmpC β-lactamase-producing bacteria among Danish army recruits. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 17, 566–568.

Hamouda, A., Findlay, J., Al Hassan, L., Amyes, S.G., 2011. Epidemiology of *Acinetobacter baumannii* of animal origin. International Journal of Antimicrobial Agents 38, 314–318.

Harris, P.N., Ferguson, J.K., 2012. Antibiotic therapy for inducible AmpC β-lactamase-producing Gram-negative bacilli: what are the alternatives to carbapenems, quinolones and aminoglycosides? International Journal of Antimicrobial Agents 40, 297–305.

Hartmann, A., Locatelli, A., Amoureux, L., Depret, G., Jolivet, C., Gueneau, E., Neuwirth, C., 2012. Occurrence of CTX-M Producing *Escherichia coli* in Soils, Cattle, and Farm Environment in France (Burgundy Region). Frontiers in Microbiology 3, 83.

Hasman, H., Mevius, D., Veldman, K., Olesen, I., Aarestrup, F.M., 2005. β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands. The Journal of Antimicrobial Chemotherapy 56, 115–121.

Hawser, S.P., Bouchillon, S.K., Hoban, D.J., Badal, R.E., Canton, R., Baquero, F., 2010. Incidence and antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* with extended-spectrum β-lactamases in community- and hospital-associated intra-abdominal infections in Europe: results of the 2008 Study for Monitoring Antimicrobial Resistance Trends (SMART). Antimicrobial Agents and Chemotherapy 54, 3043–3046.

Hilty, M., Aebi, S., Droz, S., Perreten, V., Mühlemann, K., Endimiani, A., 2012a. Characterization of Extended-Spectrum Cephalosporins-Resistant (ESC-R) *Escherichia coli* (Ec) isolates detected in Bern, Switzerland. In: Poster ICAAC 2012.

Hilty, M., Betsch, B.Y., Bogli-Stuber, K., Heiniger, N., Stadler, M., Kuffer, M., Kronenberg, A., Rohrer, C., Aebi, S., Endimiani, A., Droz, S., Muhlemann, K., 2012b. Transmission Dynamics of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the tertiary care hospital and the household setting. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 55, 967–975.

Hiroi, M., Harada, T., Kawamori, F., Takahashi, N., Kanda, T., Sugiyama, K., Masuda, T., Yoshikawa, Y., Ohashi, N., 2011. A survey of β-lactamase-producing *Escherichia coli* in farm animals and raw retail meat in Shizuoka Prefecture, Japan. Japanese Journal of Infectious Diseases 64, 153–155.

Hiroi, M., Yamazaki, F., Harada, T., Takahashi, N., Iida, N., Noda, Y., Yagi, M., Nishio, T., Kanda, T., Kawamori, F., Sugiyama, K., Masuda, T., Hara-Kudo, Y., Ohashi, N., 2012. Prevalence of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in food-producing animals. Journal of Veterinary Medical Science 74, 189–195.

Ho, P.L., Chow, K.H., Lai, E.L., Lo, W.U., Yeung, M.K., Chan, J., Chan, P.Y., Yuen, K.Y., 2011. Extensive dissemination of CTX-M-producing *Escherichia coli* with multidrug resistance to 'critically important' antibiotics among food animals in Hong Kong, 2008–2010. The Journal of Antimicrobial Chemotherapy 66, 765–768.

Hooper, D.C., 2001. Mechanisms of action of antimicrobials: focus on fluoroquinolones. Clinical Infectious Diseases 32 (Suppl. 1), S9–S15.

Hornish, R.E., Kotarski, S.F., 2002. Cephalosporins in veterinary medicine – ceftiofur use in food animals. Current Topics in Medicinal Chemistry 2, 717–731.

Hrabak, J., Empel, J., Bergerova, T., Fajfrlik, K., Urbaskova, P., Kern-Zdanowicz, I., Hryniewicz, W., Gniadkowski, M., 2009. International clones of *Klebsiella pneumoniae* and *Escherichia coli* with extended-spectrum β-lactamases in a Czech hospital. Journal of Clinical Microbiology 47, 3353–3357.

Hunter, P.A., Dawson, S., French, G.L., Goossens, H., Hawkey, P.M., Kuijper, E.J., Nathwani, D., Taylor, D.J., Teale, C.J., Warren, R.E., Wilcox, M.H., Woodford, N., Wulf, M.W., Piddock, L.J., 2010. Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. The Journal of Antimicrobial Chemotherapy 65 (Suppl. 1), i3–i17.

Husickova, V., Chroma, M., Kolar, M., Hricova, K., Stosova, T., Kantor, L., Dubrava, L., 2011. Analysis of ESBL- and AmpC-positive *Enterobacteriaceae* at the Department of Neonatology, University Hospital Olomouc. Current Microbiology 62, 1664–1670.

Jacoby, G.A., 2009. AmpC β-lactamases. Clinical Microbiology Reviews 22, 161–182.

Johnson, J.R., Johnston, B., Clabots, C., Kuskowski, M.A., Castanheira, M., 2010. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clinical Infectious Diseases 51, 286–294.

Johnson, T.J., Shepard, S.M., Rivet, B., Danzeisen, J.L., Carattoli, A., 2011. Comparative genomics and phylogeny of the Inc*I1* plasmids: a common plasmid type among porcine enterotoxigenic *Escherichia coli*. Plasmid 66, 144–151.

Johnson, T.J., Wannemuehler, Y.M., Johnson, S.J., Logue, C.M., White, D.G., Doetkott, C., Nolan, L.K., 2007. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. Applied and Environment Microbiology 73, 1976–1983.

Jordan, D., Chin, J.J., Fahy, V.A., Barton, M.D., Smith, M.G., Trott, D.J., 2009. Antimicrobial use in the Australian pig industry: results of a national survey. Australian Veterinary Journal 87, 222–229.

Jorgensen, C.J., Cavaco, L.M., Hasman, H., Emborg, H.D., Guardabassi, L., 2007. Occurrence of CTX-M-1-producing *Escherichia coli* in pigs treated with ceftiofur. The Journal of Antimicrobial Chemotherapy 59, 1040–1042.

Jorgensen, R.L., Nielsen, J.B., Friis-Moller, A., Fjeldsoe-Nielsen, H., Schonning, K., 2010. Prevalence and molecular characterization of clinical isolates of *Escherichia coli* expressing an AmpC phenotype. The Journal of Antimicrobial Chemotherapy 65, 460–464.

Kanamori, H., Navarro, R.B., Yano, H., Sombrero, L.T., Capeding, M.R., Lupisan, S.P., Olveda, R.M., Arai, K., Kunishima, H., Hirakata, Y., Kaku, M., 2011. Molecular characteristics of extended-spectrum β-lactamases in clinical isolates of *Enterobacteriaceae* from the Philippines. Acta Tropica 120, 140–145.

Karah, N., Poirel, L., Bengtsson, S., Sundqvist, M., Kahlmeter, G., Nordmann, P., Sundsfjord, A., Samuelsen, O., 2010. Plasmid-mediated quinolone resistance

determinants *qnr* and *aac(6′)-Ib-cr* in *Escherichia coli* and *Klebsiella* spp. from Norway and Sweden. Diagnostic Microbiology and Infectious Disease 66, 425–431.

Knothe, H., Shah, P., Krcmery, V., Antal, M., Mitsuhashi, S., 1983. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection 11, 315–317.

Kola, A., Kohler, C., Pfeifer, Y., Schwab, F., Kuhn, K., Schulz, K., Balau, V., Breitbach, K., Bast, A., Witte, W., Gastmeier, P., Steinmetz, I., 2012. High prevalence of extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in organic and conventional retail chicken meat, Germany. The Journal of Antimicrobial Chemotherapy 67, 2631–2634.

Leverstein-van Hall, M.A., Dierikx, C.M., Cohen Stuart, J., Voets, G.M., van den Munckhof, M.P., van Essen-Zandbergen, A., Platteel, T., Fluit, A.C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M.J., Mevius, D.J., 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 17, 873–880.

Li, J., Ma, Y., Hu, C., Jin, S., Zhang, Q., Ding, H., Ran, L., Cui, S., 2010. Dissemination of cefotaxime-M-producing *Escherichia coli* isolates in poultry farms, but not swine farms, in China. Foodborne Pathogens and Disease 7, 1387–1392.

Li, Y., Li, Q., Du, Y., Jiang, X., Tang, J., Wang, J., Li, G., Jiang, Y., 2008. Prevalence of plasmid-mediated AmpC β-lactamases in a Chinese university hospital from 2003 to 2005: first report of CMY-2-Type AmpC β-lactamase resistance in China. Journal of Clinical Microbiology 46, 1317–1321.

Liebana, E., Batchelor, M., Hopkins, K.L., Clifton-Hadley, F.A., Teale, C.J., Foster, A., Barker, L., Threlfall, E.J., Davies, R.H., 2006. Longitudinal farm study of extended-spectrum β-lactamase-mediated resistance. Journal of Clinical Microbiology 44, 1630–1634.

Liebana, E., Gibbs, M., Clouting, C., Barker, L., Clifton-Hadley, F.A., Pleydell, E., Abdalhamid, B., Hanson, N.D., Martin, L., Poppe, C., Davies, R.H., 2004. Characterization of β-lactamases responsible for resistance to extended-spectrum cephalosporins in *Escherichia coli* and *Salmonella enterica* strains from food-producing animals in the United Kingdom. Microbial Drug Resistance 10, 1–9.

Lim, S.K., Lee, H.S., Nam, H.M., Jung, S.C., Bae, Y.C., 2009. CTX-M-type β-lactamase in *Escherichia coli* isolated from sick animals in Korea. Microbial Drug Resistance 15, 139–142.

Livermore, D.M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G.M., Arlet, G., Ayala, J., Coque, T.M., Kern-Zdanowicz, I., Luzzaro, F., Poirel, L., Woodford, N., 2007. CTX-M: changing the face of ESBLs in Europe. Journal of Antimicrobial Chemotherapy 59, 165–174.

Looft, T., Johnson, T.A., Allen, H.K., Bayles, D.O., Alt, D.P., Stedtfeld, R.D., Sul, W.J., Stedtfeld, T.M., Chai, B., Cole, J.R., Hashsham, S.A., Tiedje, J.M., Stanton, T.B., 2012. In-feed antibiotic effects on the swine intestinal microbiome. Proceedings of the National Academy of Sciences of the United States of America 109, 1691–1696.

Lopes, V.C., Wedel, S.D., Bender, J.B., Smith, K.E., Leano, F.T., Boxrud, D.J., Lauer, D.C., Velayudhan, B.T., Nagaraja, K.V., 2006. Emergence of multidrug-resistant *Salmonella enterica* serotype Newport in Minnesota. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 43, 210–213.

Lowrance, T.C., Loneragan, G.H., Kunze, D.J., Platt, T.M., Ives, S.E., Scott, H.M., Norby, B., Echeverry, A., Brashears, M.M., 2007. Changes in antimicrobial susceptibility in a population of *Escherichia coli* isolated from feedlot cattle administered ceftiofur crystalline-free acid. American Journal of Veterinary Research 68, 501–507.

Lu, P.L., Liu, Y.C., Toh, H.S., Lee, Y.L., Liu, Y.M., Ho, C.M., Huang, C.C., Liu, C.E., Ko, W.C., Wang, J.H., Tang, H.J., Yu, K.W., Chen, Y.S., Chuang, Y.C., Xu, Y., Ni, Y., Chen, Y.H., Hsueh, P.R., 2012. Epidemiology and antimicrobial susceptibility profiles of Gram-negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009–2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). International Journal of Antimicrobial Agents 40 (Suppl), S37–S43.

Ma, J., Zeng, Z., Chen, Z., Xu, X., Wang, X., Deng, Y., Lu, D., Huang, L., Zhang, Y., Liu, J., Wang, M., 2009. High prevalence of plasmid-mediated quinolone resistance determinants *qnr*, *aac(6′)-Ib-cr*, and qepA among ceftiofur-resistant *Enterobacteriaceae* isolates from companion and food-producing animals. Antimicrobial Agents and Chemotherapy 53, 519–524.

Machado, E., Coque, T.M., Canton, R., Sousa, J.C., Peixe, L., 2008. Antibiotic resistance integrons and extended-spectrum β-lactamases among *Enterobacteriaceae* isolates recovered from chickens and swine in Portugal. Journal of Antimicrobial Chemotherapy 62, 296–302.

Madec, J.Y., Doublet, B., Ponsin, C., Cloeckaert, A., Haenni, M., 2011. Extended-spectrum β-lactamase *bla*CTX-M-1 gene carried on an IncI1 plasmid in multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104 in cattle in France. The Journal of Antimicrobial Chemotherapy 66, 942–944.

Madec, J.Y., Lazizzera, C., Chatre, P., Meunier, D., Martin, S., Lepage, G., Menard, M.F., Lebreton, P., Rambaud, T., 2008. Prevalence of fecal carriage of acquired expanded-spectrum cephalosporin resistance in *Enterobacteriaceae* strains from cattle in France. Journal of Clinical Microbiology 46, 1566–1567.

Madec, J.Y., Poirel, L., Saras, E., Gourguechon, A., Girlich, D., Nordmann, P., Haenni, M., 2012. Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*(CTX-M-15)-carrying plasmids. The Journal of Antimicrobial Chemotherapy 67, 578–581.

MAF, 2003. Ministry of Agriculture and Forestry, Helsinky. Recommendations for the use of antimicrobial agents in the treatment of the most significant infectious diseases in animals. http://wwwb.mmm.fi/julkaisut/tyoryhmamuistiot/2003/tr2003_9a.pdf

Magnet, S., Blanchard, J.S., 2005. Molecular insights into aminoglycoside action and resistance. Chemical Reviews 105, 477–498.

MARAN, 2005. Monitoring of Antimicrobial Resistance and Antibiotic Usage in the Netherlands in 2004. http://www.cidc-lelystad.nl

Markovska, R., Schneider, I., Keuleyan, E., Sredkova, M., Ivanova, D., Markova, B., Lazarova, G., Dragijeva, E., Savov, E., Haydouchka, I., Hadjieva, N., Setchanova, L., Mitov, I., Bauernfeind, A., 2008. Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Bulgarian hospitals. Microbial Drug Resistance 14, 119–128.

Marshall, B.M., Levy, S.B., 2011. Food animals and antimicrobials: impacts on human health. Clinical Microbiology Reviews 24, 718–733.

Martin, L.C., Weir, E.K., Poppe, C., Reid-Smith, R.J., Boerlin, P., 2012. Characterization of blaCMY-2 plasmids in Salmonella and *Escherichia coli* isolates from food animals in Canada. Applied and Environment Microbiology 78, 1285–1287.

Mataseje, L.F., Baudry, P.J., Zhanel, G.G., Morck, D.W., Read, R.R., Louie, M., Mulvey, M.R., 2010. Comparison of CMY-2 plasmids isolated from human, animal, and environmental *Escherichia coli* and *Salmonella* spp. from Canada. Diagnostic Microbiology and Infectious Disease 67, 387–391.

Mataseje, L.F., Xiao, J., Kost, S., Ng, L.K., Dore, K., Mulvey, M.R., 2009. Characterization of Canadian cefoxitin-resistant non-typhoidal *Salmonella* isolates, 2005–2006. The Journal of Antimicrobial Chemotherapy 64, 723–730.

Mathew, A.G., Cissell, R., Liamthong, S., 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. Foodborne Pathogens and Disease 4, 115–133.

Mesa, R.J., Blanc, V., Blanch, A.R., Cortes, P., Gonzalez, J.J., Lavilla, S., Miro, E., Muniesa, M., Saco, M., Tortola, M.T., Mirelis, B., Coll, P., Llagostera, M., Prats, G., Navarro, F., 2006. Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). Journal of Antimicrobial Chemotherapy 58, 211–215.

Meyer, E., Schwab, F., Schroeren-Boersch, B., Gastmeier, P., 2010. Dramatic increase of third-generation cephalosporin-resistant *E. coli* in German intensive care units: secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008. Critical Care 14, R113.

Michalopoulos, A., Falagas, M.E., 2010. Treatment of *Acinetobacter* infections. Expert Opinion on Pharmacotherapy 11, 779–788.

Miriagou, V., Carattoli, A., Tzelepi, E., Villa, L., Tzouvelekis, L.S., 2005. IS26-associated In4-type integrons forming multiresistance loci in enterobacterial plasmids. Antimicrobial Agents and Chemotherapy 49, 3541–3543.

Mirzaagha, P., Louie, M., Sharma, R., Yanke, L.J., Topp, E., McAllister, T.A., 2011. Distribution and characterization of ampicillin- and tetracycline-resistant *Escherichia coli* from feedlot cattle fed subtherapeutic antimicrobials. BMC Microbiology 11, 78.

Mollenkopf, D.F., Kleinhenz, K.E., Funk, J.A., Gebreyes, W.A., Wittum, T.E., 2011. *Salmonella enterica* and *Escherichia coli* harboring *bla*CMY in retail beef and pork products. Foodborne Pathogens and Disease 8, 333–336.

Mollenkopf, D.F., Weeman, M.F., Daniels, J.B., Abley, M.J., Mathews, J.L., Gebreyes, W.A., Wittum, T.E., 2012. Variable within- and between-herd diversity of CTX-M cephalosporinase-bearing *Escherichia coli* isolates from dairy cattle. Applied and Environment Microbiology 78, 4552–4560.

Moodley, A., Guardabassi, L., 2009. Transmission of Inc*N* plasmids carrying *bla*CTX-M-1 between commensal *Escherichia coli* in pigs and farm workers. Antimicrobial Agents and Chemotherapy 53, 1709–1711.

Morley, P.S., Apley, M.D., Besser, T.E., Burney, D.P., Fedorka-Cray, P.J., Papich, M.G., Traub-Dargatz, J.L., Weese, J.S., 2005. Antimicrobial drug use in veterinary medicine. Journal of Veterinary Internal Medicine 19, 617–629.

Mshana, S.E., Imirzalioglu, C., Hain, T., Domann, E., Lyamuya, E.F., Chakraborty, T., 2011. Multiple ST clonal complexes, with a predominance of ST131, of *Escherichia coli* harbouring *bla*CTX-M-15 in a tertiary hospital in Tanzania. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 17, 1279–1282.

Mugnier, P.D., Poirel, L., Naas, T., Nordmann, P., 2010. Worldwide dissemination of the *bla*OXA-23 carbapenemase gene of *Acinetobacter baumannii*. Emerging Infectious Diseases 16, 35–40.

Mulvey, M.R., Susky, E., McCracken, M., Morck, D.W., Read, R.R., 2009. Similar cefoxitin-resistance plasmids circulating in *Escherichia coli* from human and animal sources. Veterinary Microbiology 134, 279–287.

Nakamura, T., Komatsu, M., Yamasaki, K., Fukuda, S., Miyamoto, Y., Higuchi, T., Ono, T., Nishio, H., Sueyoshi, N., Kida, K., Satoh, K., Toda, H., Toyokawa, M., Nishi, I., Sakamoto, M., Akagi, M., Nakai, I., Kofuku, T., Orita, T., Wada, Y., Zikimoto, T., Koike, C., Kinoshita, S., Hirai, I., Takahashi, H., Matsuura, N., Yamamoto, Y., 2012. Epidemiology of *Escherichia coli*, *Klebsiella* species, and *Proteus mirabilis* strains producing extended-spectrum β-lactamases from clinical samples in the Kinki Region of Japan. American Journal of Clinical Pathology 137, 620–626.

Nam, H.M., Lim, S.K., Kang, H.M., Kim, J.M., Moon, J.S., Jang, K.C., Joo, Y.S., Jung, S.C., 2009. Prevalence and antimicrobial susceptibility of gram-negative bacteria isolated from bovine mastitis between 2003 and 2008 in Korea. Journal of Dairy Science 92, 2020–2026.

Naseer, U., Sundsfjord, A., 2011. The CTX-M conundrum: dissemination of plasmids and *Escherichia coli* clones. Microbial Drug Resistance 17, 83–97.

Navarro, F., Mesa, R.J., Miro, E., Gomez, L., Mirelis, B., Coll, P., 2007. Evidence for convergent evolution of CTX-M-14 ESBL in *Escherichia coli* and its prevalence. FEMS Microbiology Letters 273, 120–123.

Nordmann, P., Cuzon, G., Naas, T., 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infectious Diseases 9, 228–236.

Nordmann, P., Naas, T., Poirel, L., 2011a. Global spread of Carbapenemase-producing *Enterobacteriaceae*. Emerging Infectious Diseases 17, 1791–1798.

Nordmann, P., Poirel, L., Walsh, T.R., Livermore, D.M., 2011b. The emerging NDM carbapenemases. Trends in Microbiology 19, 588–595.

NORM-VET, 2007. Usage of Antimicrobial Agents and Occurence of Antimicrobial Resistance in Norway. http://www.vetinst.no/eng/Publications/Norm-Norm-Vet-Report

NORM-VET, 1999. Usage of Antimicrobial Agents in Animals and Occurence of Antimicrobial Resistance in Bacteria from Animals, Feed, and Food in Norway 1999. http://www.vetinst.no/eng/Publications/Norm-Norm-Vet-Report

Novais, A., Canton, R., Moreira, R., Peixe, L., Baquero, F., Coque, T.M., 2007. Emergence and dissemination of *Enterobacteriaceae* isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1, -3, and -32) plasmids. Antimicrobial Agents and Chemotherapy 51, 796–799.

NZFSA, 2010. New Zeland Food Safety Authority – Ministry of Agriculture and Forestry. Antibiotic Sales and Use Overview 2004–2009.

Onnberg, A., Molling, P., Zimmermann, J., Soderquist, B., 2011. Molecular and phenotypic characterization of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases with focus on CTX-M in a low-endemic area in Sweden. Apmis 119, 287–295.

Oteo, J., Diestra, K., Juan, C., Bautista, V., Novais, A., Perez-Vazquez, M., Moya, B., Miro, E., Coque, T.M., Oliver, A., Canton, R., Navarro, F., Campos, J., 2009. Extended-spectrum β-lactamase-producing *Escherichia coli* in Spain belong to a large variety of multilocus sequence typing types, including ST10 complex/A, ST23 complex/A and ST131/B2,. International Journal of Antimicrobial Agents 34, 173–176.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., van der Zwaluw, K., Huijsdens, X., Kluytmans, J., 2011. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. Emerging Infectious Diseases 17, 1216–1222.

Park, Y.S., Adams-Haduch, J.M., Shutt, K.A., Yarabinec 3rd, D.M., Johnson, L.E., Hingwe, A., Lewis 2nd, J.S., Jorgensen, J.H., Doi, Y., 2012. Clinical and microbiologic characteristics of cephalosporin-resistant *Escherichia coli* at three centers in the United States. Antimicrobial Agents and Chemotherapy 56, 1870–1876.

Partridge, S.R., Hall, R.M., 2005. Evolution of transposons containing blaTEM genes. Antimicrobial Agents and Chemotherapy 49, 1267–1268.

Passantino, A., 2007. Ethical aspects for veterinarians regarding antimicrobial drug use in Italy. International Journal of Antimicrobial Agents 29, 240–244.

Paterson, D.L., Bonomo, R.A., 2005. Extended-spectrum β-lactamases: a clinical update. Clinical Microbiology Reviews 18, 657–686.

Paterson, D.L., Ko, W.C., Von Gottberg, A., Casellas, J.M., Mulazimoglu, L., Klugman, K.P., Bonomo, R.A., Rice, L.B., McCormack, J.G., Yu, V.L., 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. Journal of Clinical Microbiology 39, 2206–2212.

Peirano, G., Costello, M., Pitout, J.D., 2010. Molecular characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* from the Chicago area: high prevalence of ST131 producing CTX-M-15 in community hospitals. International Journal of Antimicrobial Agents 36, 19–23.

Peirano, G., Pitout, J.D., 2010. Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. International Journal of Antimicrobial Agents 35, 316–321.

Peirano, G., van der Bij, A.K., Gregson, D.B., Pitout, J.D., 2012. Molecular epidemiology over an 11-year period (2000–2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. Journal of Clinical Microbiology 50, 294–299.

Peirano, G., van Greune, C.H., Pitout, J.D., 2011. Characteristics of infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* from community hospitals in South Africa. Diagnostic Microbiology and Infectious Disease 69, 449–453.

Perez, F., Endimiani, A., Bonomo, R.A., 2008. Why are we afraid of *Acinetobacter baumannii*? Expert Review of Anti-infective Therapy 6, 269–271.

Perez, F., Endimiani, A., Ray, A.J., Decker, B.K., Wallace, C.J., Hujer, K.M., Ecker, D.J., Adams, M.D., Toltzis, P., Dul, M.J., Windau, A., Bajaksouzian, S., Jacobs, M.R., Salata, R.A., Bonomo, R.A., 2010. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. Journal of Antimicrobial Chemotherapy 65, 1807–1818.

Perez, F., Hujer, A.M., Hujer, K.M., Decker, B.K., Rather, P.N., Bonomo, R.A., 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrobial Agents and Chemotherapy 51, 3471–3484.

Perez, F., Ponce-Terashima, R., Adams, M.D., Bonomo, R.A., 2011. Are we closing in on an "elusive enemy"? The current status of our battle with *Acinetobacter baumannii*. Virulence 2, 86–90.

Perreten, V., 2005. Resistance in the food chain and in bacteria from animals: relevance to human infections. In: White, D.G., Alekshun, M.N., McDermott, P.F. (Eds.), Frontiers in Antimicrobial Resistance: a Tribute to Stuart B. Levy. ASM Press, Washington, DC.

Persoons, D., Haesebrouck, F., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Berge, A.C., Butaye, P., Dewulf, J., 2011. Risk factors for ceftiofur resistance in *Escherichia coli* from Belgian broilers. Epidemiology and Infection 139, 765–771.

Pitout, J.D., Laupland, K.B., 2008. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infectious Diseases 8, 159–166.

Pitout, J.D., Wei, Y., Church, D.L., Gregson, D.B., 2008. Surveillance for plasmid-mediated quinolone resistance determinants in *Enterobacteriaceae* within the Calgary Health Region, Canada: the emergence of *aac(6′)-Ib-cr*. Journal of Antimicrobial Chemotherapy 61, 999–1002.

Platell, J.L., Johnson, J.R., Cobbold, R.N., Trott, D.J., 2011. Multidrug-resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST131 in animals and foods. Veterinary Microbiology 153, 99–108.

Platt, T.M., Loneragan, G.H., Scott, H.M., Norby, B., Thomson, D.U., Brown, M.S., Ives, S.E., Brashears, M.M., 2008. Antimicrobial susceptibility of enteric bacteria recovered from feedlot cattle administered chlortetracycline in feed. American Journal of Veterinary Research 69, 988–996.

Poirel, L., Bercot, B., Millemann, Y., Bonnin, R.A., Pannaux, G., Nordmann, P., 2012a. Carbapenemase-producing *Acinetobacter* spp. in Cattle, France. Emerging Infectious Diseases 18, 523–525.

Poirel, L., Bonnin, R.A., Nordmann, P., 2011. Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. IUBMB Life 63, 1061–1067.

Poirel, L., Naas, T., Nordmann, P., 2010. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrobial Agents and Chemotherapy 54, 24–38.

Poirel, L., Potron, A., Nordmann, P., 2012b. OXA-48-like carbapenemases: the phantom menace. The Journal of Antimicrobial Chemotherapy 67, 1597–1606.

Poppe, C., Martin, L.C., Gyles, C.L., Reid-Smith, R., Boerlin, P., McEwen, S.A., Prescott, J.F., Forward, K.R., 2005. Acquisition of resistance to extended-spectrum cephalosporins by *Salmonella enterica* subsp. enterica serovar Newport and *Escherichia coli* in the turkey poult intestinal tract. Applied and Environment Microbiology 71, 1184–1192.

Potron, A., Munoz-Price, L.S., Nordmann, P., Cleary, T., Poirel, L., 2011. Genetic features of CTX-M-15-producing *Acinetobacter baumannii* from Haiti. Antimicrobial Agents and Chemotherapy 55, 5946–5948.

Queenan, A.M., Bush, K., 2007. Carbapenemases: the versatile β-lactamases. Clinical Microbiology Reviews 20, 440–458 (table of contents).

Randall, L.P., Clouting, C., Horton, R.A., Coldham, N.G., Wu, G., Clifton-Hadley, F.A., Davies, R.H., Teale, C.J., 2011. Prevalence of *Escherichia coli* carrying extended-spectrum β-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. The Journal of Antimicrobial Chemotherapy 66, 86–95.

Rapp, R.P., Urban, C., 2012. *Klebsiella pneumoniae* carbapenemases in *Enterobacteriaceae*: history, evolution, and microbiology concerns. Pharmacotherapy 32, 399–407.

Rayamajhi, N., Kang, S.G., Lee, D.Y., Kang, M.L., Lee, S.I., Park, K.Y., Lee, H.S., Yoo, H.S., 2008. Characterization of TEM-, SHV- and AmpC-type β-lactamases from cephalosporin-resistant *Enterobacteriaceae* isolated from swine. International Journal of Food Microbiology 124, 183–187.

Riano, I., Moreno, M.A., Teshager, T., Saenz, Y., Dominguez, L., Torres, C., 2006. Detection and characterization of extended-spectrum β-lactamases in *Salmonella enterica* strains of healthy food animals in Spain. The Journal of Antimicrobial Chemotherapy 58, 844–847.

Rodriguez-Villalobos, H., Bogaerts, P., Berhin, C., Bauraing, C., Deplano, A., Montesinos, I., de Mendonca, R., Jans, B., Glupczynski, Y., 2011. Trends in production of extended-spectrum β-lactamases among *Enterobacteriaceae* of clinical interest: results of a nationwide survey in Belgian hospitals. The Journal of Antimicrobial Chemotherapy 66, 37–47.

Rodriguez, I., Barownick, W., Helmuth, R., Mendoza, M.C., Rodicio, M.R., Schroeter, A., Guerra, B., 2009. Extended-spectrum β-lactamases and AmpC β-lactamases in ceftiofur-resistant *Salmonella enterica* isolates from food and livestock obtained in Germany during 2003–2007. The Journal of Antimicrobial Chemotherapy 64, 301–309.

Rogers, B.A., Sidjabat, H.E., Paterson, D.L., 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. The Journal of Antimicrobial Chemotherapy 66, 1–14.

Rossolini, G.M., D'Andrea, M.M., Mugnaioli, C., 2008. The spread of CTX-M-type extended-spectrum β-lactamases. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 14 (Suppl. 1), 33–41.

Ruby, T., McLaughlin, L., Gopinath, S., Monack, D., 2012. *Salmonella*'s long-term relationship with its host. FEMS Microbiology Reviews 36, 600–615.

Rudi, K., Moen, B., Sekelja, M., Frisli, T., Lee, M.R., 2012. An eight-year investigation of bovine livestock fecal microbiota. Veterinary Microbiology 160, 369–377.

Schwaber, M.J., Carmeli, Y., 2007. Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in *Enterobacteriaceae* bacteraemia: a systematic review and meta-analysis. The Journal of Antimicrobial Chemotherapy 60, 913–920.

Schwarz, S., Kehrenberg, C., Walsh, T.R., 2001. Use of antimicrobial agents in veterinary medicine and food animal production. International Journal of Antimicrobial Agents 17, 431–437.

Sennati, S., Santella, G., Di Conza, J., Pallecchi, L., Pino, M., Ghiglione, B., Rossolini, G.M., Radice, M., Gutkind, G., 2012. Changing epidemiology of extended-spectrum β-lactamases in Argentina: emergence of CTX-M-15. Antimicrobial Agents and Chemotherapy 56, 6003–6005.

Shahada, F., Chuma, T., Dahshan, H., Akiba, M., Sueyoshi, M., Okamoto, K., 2010. Detection and characterization of extended-spectrum β-lactamase (TEM-52)-producing *Salmonella* serotype Infantis from broilers in Japan. Foodborne Pathogens and Disease 7, 515–521.

Sharma, R., Munns, K., Alexander, T., Entz, T., Mirzaagha, P., Yanke, L.J., Mulvey, M., Topp, E., McAllister, T., 2008. Diversity and distribution of commensal fecal *Escherichia coli* bacteria in beef cattle administered selected subtherapeutic antimicrobials in a feedlot setting. Applied and Environment Microbiology 74, 6178–6186.

Shaw, K.J., Rather, P.N., Hare, R.S., Miller, G.H., 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiological Reviews 57, 138–163.

Shryock, T.R., Richwine, A., 2010. The interface between veterinary and human antibiotic use. Annals of the New York Academy of Sciences 1213, 92–105.

Sidjabat, H.E., Paterson, D.L., Adams-Haduch, J.M., Ewan, L., Pasculle, A.W., Muto, C.A., Tian, G.B., Doi, Y., 2009. Molecular epidemiology of CTX-M-producing *Escherichia coli* isolates at a tertiary medical center in western Pennsylvania. Antimicrobial Agents and Chemotherapy 53, 4733–4739.

Simner, P.J., Zhanel, G.G., Pitout, J., Tailor, F., McCracken, M., Mulvey, M.R., Lagace-Wiens, P.R., Adam, H.J., Hoban, D.J., 2011. Prevalence and characterization of extended-spectrum β-lactamase- and AmpC β-lactamase-producing *Escherichia coli*: results of the CANWARD 2007–2009 study. Diagnostic Microbiology and Infectious Disease 69, 326–334.

Singer, R.S., Patterson, S.K., Wallace, R.L., 2008. Effects of therapeutic ceftiofur administration to dairy cattle on *Escherichia coli* dynamics in the intestinal tract. Applied and Environment Microbiology 74, 6956–6962.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Claeys, G., Lontie, M., Van Meensel, B., Herman, L., Haesebrouck, F., Butaye, P., 2010a. Characterization of extended-spectrum β-lactamases produced by *Escherichia coli* isolated from hospitalized and nonhospitalized patients: emergence of CTX-M-15-producing strains causing urinary tract infections. Microbial Drug Resistance 16, 129–134.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F., Butaye, P., 2010b. Broad-spectrum β-lactamases among *Enterobacteriaceae* of animal origin: molecular aspects, mobility and impact on public health. FEMS Microbiology Reviews 34, 295–316.

Smet, A., Rasschaert, G., Martel, A., Persoons, D., Dewulf, J., Butaye, P., Catry, B., Haesebrouck, F., Herman, L., Heyndrickx, M., 2011. In situ ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration. Journal of Applied Microbiology 110, 541–549.

Starr, M.P., Reynolds, D.M., 1951. Streptomycin resistance of coliform bacteria from turkeys fed streptomycin. American Journal of Public Health and the Nations Health 41, 1375–1380.

Strahilevitz, J., Jacoby, G.A., Hooper, D.C., Robicsek, A., 2009. Plasmid-mediated quinolone resistance: a multifaceted threat. Clinical Microbiology Reviews 22, 664–689.

Stuart, R.L., Kotsanas, D., Webb, B., Vandergraaf, S., Gillespie, E.E., Hogg, G.G., Korman, T.M., 2011. Prevalence of antimicrobial-resistant organisms in residential aged care facilities. Medical Journal of Australia 195, 530–533.

Su, L.H., Chiu, C.H., Chu, C., Wang, M.H., Chia, J.H., Wu, T.L., 2003. In vivo acquisition of ceftriaxone resistance in *Salmonella enterica* serotype anatum. Antimicrobial Agents and Chemotherapy 47, 563–567.

SVARM, 2011. Swedish Veterinary Antimicrobial Resistance Monitoring 2011. Swedish Veterinary Antimicrobial Resistance Monitoring.

SwissMedic, 2007. Antibiotika für die Veterinärmedizin: Vertriebszahlen der Jahre 2004 bis 2006.

SwissMedic, 2011. Federal Department of Economic Affairs, Federal Veterinary Office, Monitoring ARCH-Vet. Report on the Sale Antibiotic Resistance Monitoring of Livestock in Switzerland. http://www.swissmedic.ch/archvet

Tian, G.B., Wang, H.N., Zhang, A.Y., Zhang, Y., Fan, W.Q., Xu, C.W., Zeng, B., Guan, Z.B., Zou, L.K., 2012. Detection of clinically important β-lactamases in commensal *Escherichia coli* of human and swine origin in western China. Journal of Medical Microbiology 61, 233–238.

Toleman, M.A., Walsh, T.R., 2011. Combinatorial events of insertion sequences and ICE in Gram-negative bacteria. FEMS Microbiology Reviews 35, 912–935.

Tragesser, L.A., Wittum, T.E., Funk, J.A., Winokur, P.L., Rajala-Schultz, P.J., 2006. Association between ceftiofur use and isolation of *Escherichia coli* with reduced susceptibility to ceftriaxone from fecal samples of dairy cows. American Journal of Veterinary Research 67, 1696–1700.

Trott, D., 2012. β-lactam resistance in Gram-negative pathogens isolated from animals. Current Pharmaceutical Design 19, 239–249.

Ungemach, F.R., Muller-Bahrdt, D., Abraham, G., 2006. Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. International Journal of Medical Microbiology: IJMM 296 (Suppl. 41), 33–38.

USDA-APHIS, 2008a. United States Department of Agricolture, Animal Plant Health Inspection Service National Animal Health Monitoring System. Antibiotic use in US dairy operation, 2002 and 2007. http://nahms.aphis.usda.gov

USDA-APHIS, United States Department of Agricolture, Animal Plant Health Inspection Service National Animal Health Monitoring System.

USDA, US Department of Agriculture, 2011. National Antimicrobial resistance Monitoring System for Enteric Bacteria (NARMS): Animal Arm Annual Report, 2009. http://www.ars.usda.gov

Valat, C., Auvray, F., Forest, K., Metayer, V., Gay, E., Peytavin de Garam, C., Madec, J.Y., Haenni, M., 2012. Phylogenetic grouping and virulence potential of extended-spectrum-β-lactamase-producing *Escherichia coli* strains in cattle. Applied and Environment Microbiology 78, 4677–4682.

Valverde, A., Canton, R., Garcillan-Barcia, M.P., Novais, A., Galan, J.C., Alvarado, A., de la Cruz, F., Baquero, F., Coque, T.M., 2009. Spread of *bla*(CTX-M-14) is driven mainly by Inc*K* plasmids disseminated among *Escherichia coli* phylogroups A, B1, and D in Spain. Antimicrobial Agents and Chemotherapy 53, 5204–5212.

van der Bij, A.K., Peirano, G., Goessens, W.H., van der Vorm, E.R., van Westreenen, M., Pitout, J.D., 2011. Clinical and molecular characteristics of extended-spectrum-β-lactamase-producing *Escherichia coli* causing bacteremia in the Rotterdam Area, Netherlands. Antimicrobial Agents and Chemotherapy 55, 3576–3578.

Varga, C., Rajic, A., McFall, M.E., Reid-Smith, R.J., Deckert, A.E., Checkley, S.L., McEwen, S.A., 2009. Associations between reported on-farm antimicrobial use practices and observed antimicrobial resistance in generic fecal *Escherichia coli* isolated from Alberta finishing swine farms. Preventive Veterinary Medicine 88, 185–192.

Vieira, A.R., Houe, H., Wegener, H.C., Lo Fo Wong, D.M., Emborg, H.D., 2009. Association between tetracycline consumption and tetracycline resistance in *Escherichia coli* from healthy Danish slaughter pigs. Foodborne Pathogens and Disease 6, 99–109.

Vila, J., Pachon, J., 2012. Therapeutic options for *Acinetobacter baumannii* infections: an update. Expert Opinion on Pharmacotherapy 13, 2319–2336.

Vincent, C., Boerlin, P., Daignault, D., Dozois, C.M., Dutil, L., Galanakis, C., Reid-Smith, R.J., Tellier, P.P., Tellis, P.A., Ziebell, K., Manges, A.R., 2010. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerging Infectious Diseases 16, 88–95.

VMD, Veterinary Medicines Directorate, 2011. Sales of antimicrobial products authorized for use as veterinary medicines in the UK in 2010.

VMD, Veterinary Medicines Directorate, 2005. Sales of antimicrobial products authorized for use as veterinary medicines, antiprotozoals, antifungals, growth promoters and coccidiostats, in the UK in 2004.

Volkova, V.V., Lanzas, C., Lu, Z., Grohn, Y.T., 2012. Mathematical model of plasmid-mediated resistance to ceftiofur in commensal enteric *Escherichia coli* of cattle. PLoS ONE 7, e36738.

Wachino, J., Shibayama, K., Kurokawa, H., Kimura, K., Yamane, K., Suzuki, S., Shibata, N., Ike, Y., Arakawa, Y., 2007. Novel plasmid-mediated 16S rRNA m1A1408 methyltransferase, *NpmA*, found in a clinically isolated *Escherichia coli* strain resistant to structurally diverse aminoglycosides. Antimicrobial Agents and Chemotherapy 51, 4401–4409.

Wagner, B.A., Straw, B.E., Fedorka-Cray, P.J., Dargatz, D.A., 2008. Effect of antimicrobial dosage regimen on *Salmonella* and *Escherichia coli* isolates from feeder swine. Applied and Environment Microbiology 74, 1731–1739.

Walsh, T.R., 2010. Emerging carbapenemases: a global perspective. International Journal of Antimicrobial Agents 36 (Suppl. 3), S8–S14.

Walther-Rasmussen, J., Hoiby, N., 2007. Class A carbapenemases. The Journal of Antimicrobial Chemotherapy 60, 470–482.

Wang, Y., Wu, C., Zhang, Q., Qi, J., Liu, H., He, T., Ma, L., Lai, J., Shen, Z., Liu, Y., Shen, J., 2012. Identification of New Delhi metallo-beta-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. PLoS ONE 7, pe37152.

Wasyl, D., Hasman, H., Cavaco, L.M., Aarestrup, F.M., 2012. Prevalence and characterization of cephalosporin resistance in nonpathogenic *Escherichia coli* from food-producing animals slaughtered in Poland. Microbial Drug Resistance 18, 79–82.

Weill, F.X., Lailler, R., Praud, K., Kerouanton, A., Fabre, L., Brisabois, A., Grimont, P.A., Cloeckaert, A., 2004. Emergence of extended-spectrum-β-lactamase (CTX-M-9)-producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. Journal of Clinical Microbiology 42, 5767–5773.

WHO, World Health Organization, 2007. Critically important antimicrobials for human medicine: categorization for the development of risk managment strategies to contain antimicrobial resistance due to non-human antimicrobial use. In: Report on 2nd WHO Expert Meeting, Copenhagen, Denmark.

Wieler, L.H., Ewers, C., Guenther, S., Walther, B., Lubke-Becker, A., 2011a. Methicillin-resistant staphylococci (MRS) and extended-spectrum β-lactamases (ESBL)-producing *Enterobacteriaceae* in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. International Journal of Medical Microbiology: IJMM 301, 635–641.

Wieler, L.H., Semmler, T., Eichhorn, I., Antao, E.M., Kinnemann, B., Geue, L., Karch, H., Guenther, S., Bethe, A., 2011b. No evidence of the Shiga toxin-producing *E. coli* O104:H4 outbreak strain or enteroaggregative *E. coli* (EAEC) found in cattle faeces in northern Germany, the hotspot of the 2011 HUS outbreak area. Gut Pathogens 3, 17.

Winokur, P.L., Brueggemann, A., DeSalvo, D.L., Hoffmann, L., Apley, M.D., Uhlenhopp, E.K., Pfaller, M.A., Doern, G.V., 2000. Animal and human multidrug-resistant, cephalosporin-resistant salmonella isolates expressing a plasmid-mediated CMY-2 AmpC β-lactamase. Antimicrobial Agents and Chemotherapy 44, 2777–2783.

Winokur, P.L., Vonstein, D.L., Hoffman, L.J., Uhlenhopp, E.K., Doern, G.V., 2001. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrobial Agents and Chemotherapy 45, 2716–2722.

Wittum, T.E., Mollenkopf, D.F., Daniels, J.B., Parkinson, A.E., Mathews, J.L., Fry, P.R., Abley, M.J., Gebreyes, W.A., 2010. CTX-M-type extended-spectrum β-lactamases present in *Escherichia coli* from the feces of cattle in Ohio, United States. Foodborne Pathogens and Disease 7, 1575–1579.

Wittum, T.E., Mollenkopf, D.F., Erdman, M.M., 2012. Detection of *Salmonella enterica* isolates producing CTX-M cephalosporinase in US livestock populations. Applied and Environment Microbiology 78, 7487–7491.

Yan, J.J., Chiu, C.H., Ko, W.C., Chuang, C.L., Wu, J.J., 2002. Ceftriaxone-resistant *Salmonella enterica* serovar Hadar: evidence for interspecies transfer of *bla*CMY-2 in a Taiwanese university hospital. Journal of the Formosan Medical Association 101, 665–668.

Yan, J.J., Wu, J.J., Ko, W.C., Tsai, S.H., Chuang, C.L., Wu, H.M., Lu, Y.J., Li, J.D., 2004. Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals. Journal of Antimicrobial Chemotherapy 54, 1007–1012.

Yu, F.Y., Yao, D., Pan, J.Y., Chen, C., Qin, Z.Q., Parsons, C., Yang, L.H., Li, Q.Q., Zhang, X.Q., Qu, D., Wang, L.X., 2010. High prevalence of plasmid-mediated 16S rRNA

methylase gene *rmtB* among *Escherichia coli* clinical isolates from a Chinese teaching hospital. BMC Infectious Diseases 10, p184.

Zhang, Y., LeJeune, J.T., 2008. Transduction of bla(CMY-2), tet(A), and tet(B) from *Salmonella enterica* subspecies enterica serovar Heidelberg to *S. typhimurium*. Veterinary Microbiology 129, 418–425.

Zheng, H., Zeng, Z., Chen, S., Liu, Y., Yao, Q., Deng, Y., Chen, X., Lv, L., Zhuo, C., Chen, Z., Liu, J.H., 2012. Prevalence and characterisation of CTX-M β-lactamases amongst *Escherichia coli* isolates from healthy food animals in China. International Journal of Antimicrobial Agents 39, 305–310.

Zordan, S., Prenger-Berninghoff, E., Weiss, R., van der Reijden, T., van den Broek, P., Baljer, G., Dijkshoorn, L., 2011. Multidrug-resistant *Acinetobacter baumannii* in veterinary clinics, Germany. Emerging Infectious Diseases 17, 1751–1754.

PART A

Chapter I

Based on the lack of information regarding the prevalence of ESC-R *E.coli* in Switzerland the first study conducted was a detailed characterization of 611 isolates from hospitalized patients at the University Hospital of Bern isolated between September and December 2011.
The overall prevalence of ECR-R *E.coli* was 6.5%, which can be considerate as low value compared to other countries. The most frequently detected resistances were CTX-M-variants (75%, mainly CTX-M-15 with 35%) and CMY-2 (12.5%). Surprisingly, most AmpC-producers were detected in SOC patients, which could serve as a "vehicle" for the transmission of highly-drug resistant *E.coli* into the community (and vice versa).

The study was published in the **Journal of Antimicrobial Chemotherapy** under the title "Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland" in **2013** (Seiffert et al., Journal of Antimicrobial Chemotherapy; 68(10):2249-2254, 2013).

J Antimicrob Chemother 2013; **68**: 2249–2254
doi:10.1093/jac/dkt208 Advance Access publication 12 June 2013

Journal of Antimicrobial Chemotherapy

Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland

Salome N. Seiffert[1–3], Markus Hilty[1,4], Andreas Kronenberg[1,4], Sara Droz[1], Vincent Perreten[2] and Andrea Endimiani[1]*

[1]*Institute for Infectious Diseases, University of Bern, Bern, Switzerland;* [2]*Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;* [3]*Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland;* [4]*Department of Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland*

*Corresponding author. Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, Postfach 61, CH-3010, Switzerland. Tel: +41-31-632-8632; Fax: +41-31-632-8766; E-mail: aendimiani@gmail.com or andrea.endimiani@ifik.unibe.ch

Received 11 March 2013; returned 30 March 2013; revised 23 April 2013; accepted 1 May 2013

Objectives: Resistance to extended-spectrum cephalosporins (ESCs) in *Escherichia coli* can be due to the production of ESBLs, plasmid-mediated AmpCs (pAmpCs) or chromosomal AmpCs (cAmpCs). Information regarding type and prevalence of β-lactamases, clonal relations and plasmids associated with the *bla* genes for ESC-R *E. coli* (ESC-R-*Ec*) detected in Switzerland is lacking. Moreover, data focusing on patients referred to the specialized outpatient clinics (SOCs) are needed.

Methods: We analysed 611 unique *E. coli* isolated during September–December 2011. ESC-R-*Ec* were studied with microarrays, PCR/DNA sequencing for bla_{ESBLs}, bla_{pAmpCs}, promoter region of bla_{cAmpC}, IS elements, plasmid incompatibility group, and also implementing transformation, aIEF, rep-PCR and MLST.

Results: The highest resistance rates were observed in the SOCs, whereas those in the hospital and community were lower (e.g. quinolone resistance of 22.6%, 17.2% and 9.0%, respectively; $P=0.003$ for SOCs versus community). The prevalence of ESC-R-*Ec* in the three settings was 5.3% ($n=11$), 7.8% ($n=22$) and 5.7% ($n=7$), respectively. Thirty isolates produced CTX-M ESBLs (14 were CTX-M-15), 5 produced CMY-2 pAmpC and 5 hyper-expressed cAmpCs due to promoter mutations. Fourteen isolates were of sequence type 131 (ST131; 10 with CTX-M-15). bla_{CTX-M} and bla_{CMY-2} were associated with an intact or truncated IS*Ecp1* and were mainly carried by IncF, IncFII and IncI1plasmids.

Conclusions: ST131 producing CTX-M-15 is the predominant clone. The prevalence of ESC-R-*Ec* (overall 6.5%) is low, but an unusual relatively high frequency of AmpC producers (25%) was noted. The presence of ESC-R-*Ec* in the SOCs and their potential ability to be exchanged between hospital and community should be taken into serious consideration.

Keywords: ESBL, AmpC, CMY, CTX-M, ST131

Introduction

Extended-spectrum cephalosporin-resistant *Escherichia coli* (ESC-R-*Ec*) isolates are dramatically increasing worldwide. This phenomenon is usually due to the production of extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC (pAmpCs) or, more rarely, chromosomal AmpC (cAmpC).[1]

Several sequence types (STs) of ESC-R-*Ec* (e.g. ST131 producing the CTX-M-15 ESBL) have shown the ability to cause severe infections in both hospitalized and community patients.[1–3] However, data describing the rates and characteristics of ESC-R-*Ec* in the community are still limited and, more importantly, those specifically focusing on the subgroup of patients referring to the specialized outpatient clinics (SOCs) are almost lacking.[2,4,5] In Switzerland, several works have described the prevalence and molecular characteristics of ESC-R-*Ec* detected in healthy people, food and animals.[1,6,7] However, there is a lack of contemporary data regarding the ESC-R-*Ec* causing infections in humans.[8]

Materials and methods

Clinical isolates and definitions

All *E. coli* identified at the Laboratory of Clinical Microbiology of the University of Bern (Switzerland) between 1 September and 31 December 2011 were

© *The Author 2013. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com*

Downloaded from http://jac.oxfordjournals.org/ at World Trade Institute on May 28, 2015

analysed. The laboratory receives samples from outpatients and those hospitalized at the University Hospital of Bern (Inselspital). Data regarding outpatients were further stratified as 'community'—patients referring to primary care general physicians (GPs) and emergency departments (ERs)—and 'SOCs'—patients referring to the specialized departments of Inselspital. We defined 'community acquired' (CA) and 'healthcare associated infections' (HCA) according to the current CDC criteria.[9]

Species identification and antimicrobial susceptibility tests (ASTs)

Species identification was achieved by implementing standard biochemical tests. ASTs were routinely assessed using the disc diffusion method and results were interpreted according to the CLSI criteria.[10] The MICs for different antibiotics were obtained in Mueller–Hinton II broth (BBL, Becton Dickinson) using the microdilution Sensititre ESB1F plates (Trek Diagnostics) only for the ESC-R-*Ec* (Table S1, available as Supplementary data at *JAC* Online).

Characterization of β-lactamases

The CT103 microarray (Check-Points) was used to screen the ESC-R-*Ec* for the presence of β-lactamase genes. PCR/DNA sequence analyses for the *bla* genes found with the microarray were performed as previously reported.[11] $Bla_{\text{OXA-types}}$, the promoter region of bla_{cAmpC} and the presence of IS*Ecp1*-like and IS*CR1* genes were also analysed.[8,12,13] Production of β-lactamases was determined using analytical isoelectric focusing (aIEF).[6]

Analysis of clonality

The ST was obtained using multilocus sequence typing (MLST) Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli). Repetitive extragenic palindromic PCR (rep-PCR) and phylogenetic group characterization were also implemented.[6,11]

Plasmid analysis

The incompatibility group (Inc) of the plasmid(s) was characterized using a multiplex PCR (PBRT Kit, Diatheva). Plasmid extract was electroporated into ElectroMAX *E. coli* DH10B (Invitrogen) and cells were selected on Luria–Bertani plates with ampicillin (20 mg/L) or cefotaxime (1 mg/L) (Sigma).

Results and discussion

Six-hundred-and-eleven unique *E. coli* were analysed. The overall resistance rates to trimethoprim/sulfamethoxazole, quinolones, gentamicin and amoxicillin/clavulanate were 31.5%, 17.4%, 19% and 31.5%, respectively (Table S2, available as Supplementary data at *JAC* Online).

The highest resistance rates were observed in the SOCs, whereas those recorded in hospital and community settings were lower. For instance, rates to quinolones were 22.6%, 17.2% and 9.0%, respectively ($P=0.003$ for SOCs versus community), whereas those for amoxicillin/clavulanate were 35.1%, 32.3% and 23.8%, respectively ($P=0.04$ for SOCs versus community) (Table S3, available as Supplementary data at *JAC* Online). Thus, the resistance rates in SOCs are more similar to those observed in hospital rather than in the community. The specific use of antibiotics in this setting may be one of the reasons related to this phenomenon. Unfortunately, in most institutions like ours, statistics regarding the consumption of antimicrobials in SOCs are merged with those of the corresponding hospital wards, making their interpretation in a context of antibiotic resistance difficult. However, we note that 8 of 11 ESC-R-*Ec* found in SOCs were from patients referring to nephrology or urology SOCs (Figure 1). During the same period of our analysis, these two wards showed the highest consumption of quinolones [10.7 and 13.3 defined daily doses (DDD)/100 occupied bed-days (BDs), respectively] (Table S4, available as Supplementary data at *JAC* Online), a recognized risk factor responsible for selection of ESC-R-*Ec*.[2,3,14]

Impact of ESC-R-Ec in the different settings

The overall prevalence of ESC-R-*Ec* ($n=40$; 6.5%) recorded in our area suggests that the impact of such pathogens is still small compared with other countries.[15] However, the analogous prevalence of ESC-R-*Ec* observed in the community ($n=7$; 5.7%), SOCs ($n=11$; 5.3%) and hospital ($n=22$; 7.8%) settings drives further conclusions (Table S2, available as Supplementary data at *JAC* Online).

The relatively low hospital prevalence observed may be justified by the different standards of hospital infection control programmes in Switzerland. In contrast, available studies with which to compare our results about the community and SOCs are scarce. In fact, most surveys considered invasive nosocomial infections, whereas studies regarding non-hospitalized patients are limited.[3,4,15] A general prevalence of 1%–4% among outpatients was recorded, but only a few analyses addressed the specific impact of ESC-R-*Ec* among GP and ER patients.[5,16,17] The prevalence of ESC-R-*Ec* in women with urinary tract infections (UTIs) referring to GPs was 1% in the Netherlands (2009) and 10% in China (2004), whereas one study from the USA (2008) reported a frequency of ∼1% among ER patients.[5,18,19] In our area (2008–09), ESC-R-*Ec* represented 1.7% of UTIs due to *E. coli* in community.[20] Therefore, the prevalence recorded in the present study (4.2% in urine in 2011; Table S2, available as Supplementary data at *JAC* Online) indicates that there may be a more significant expansion of ESC-R-*Ec* in the community than in hospital.

Data about ESC-R-*Ec* in the SOC setting are essentially unavailable because results are frequently merged with those of GPs and/or ERs.[3,14] However, Qi *et al.*[5] reported ESBL-producing *E. coli* (ESBL-*Ec*) in ∼1% of urine samples collected in SOCs, whereas Doi *et al.*[2] noted that 33.6% of CA and 22.8% of HCA infections due to ESC-R-*Ec* occurred in this setting. In this pilot work we explored this location, suggesting its possible key role in the spread of ESC-R-*Ec*. As shown in Figure 1, 10 of 11 SOC patients (mean age 53.5 years) had a CA infection usually involving the genitourinary tract (these subjects were referring to the ambulatories of urology, nephrology or gynaecology). Of note, seven patients had been hospitalized during the previous year. Moreover, most of them were referred to the SOCs for many years and had clinical samples positive for ESC-R-*Ec* for long periods previously and/or later than those analysed in the present study (data not shown). Overall, these figures indicate that SOC patients may serve as a source amplifying the spread of ESC-R-*Ec* between hospital and community settings. In this context, we note that transmission of ESC-R-*Ec* from colonized people previously hospitalized to healthy subjects can occur in the community.[11]

Molecular characteristics of the ESC-R-Ec

Thirty (75%) ESC-R-*Ec* were CTX-M-15 ($n=14$), CTX-M-27 ($n=7$), CTX-M-1 ($n=5$), CTX-M-14 ($n=3$) or CTX-M-79-like ($n=1$) producers. The $bla_{\text{CTX-M}}$ were carried by IncF/FII plasmids and were

Downloaded from http://jac.oxfordjournals.org/ at World Trade Institute on May 28, 2015

Isolate	ST/main *bla* gene	Age/Sex	Infection	Acquisition	Ward	Previously hospitalized (last year)
4810.28	ST617/CTX-M-27	38/F	UTI	HCA	Urology	Yes (Urology 1 mo before)
4810.14	ST405/CMY-2, CTX-M-15	81/F	UTI	CA	Uro-Gynecology	No
4808.28	ST354/CMY-2	35/F	Nephrostomy	CA	SOC: nephrology	Yes (Nephrology 6 mo before)
4902.33	ST648/CTX-M-27	78/F	UTI	CA	Abdominal surgery	No
4809.08	ST648/CTX-M-15	79/M	Liver drainage	CA	ICU	No
4809.61	ST648/cAmpC	55/M	UTI	HCA	SOC: pneumology	Yes (Pneumology/Medicine several times during last 2 y)
4807.55	ST69/CMY-2	49/F	BSI	CA	Emergency	No
4812.41	ST648/CTX-M-27	71/F	UTI	CA	SOC: nephrology	Yes (Nephrology 5 mo before)
4902.09	ST23/CTX-M-79-like	88/F	UTI	CA	Cardiovascular surgery	No
4807.33	ST156/cAmpC	83/M	UTI	CA	SOC: urology	Yes (Urology/ICU 10 mo before)
4806.54	ST156/CTX-M-15	67/M	UTI	HCA	ICU	No
4904.60	ST101/cAmpC	64/F	UTI	CA	SOC: urology	Yes (Gynecology 2 mo before)
4901.67	ST1431/CMY-2	64/F	UTI	CA	SOC: nephrology	Yes (Nephrology/Urology 5 mo before)
4812.17	ST410/cAmpC	68/F	UTI	HCA	ICU	No
4809.09	ST1196/CTX-M-1	52/F	UTI	CA	SOC: nephrology	Yes (Abdominal surgery 2 mo before)
4904.36	ST744/CTX-M-14	75/M	Peritoneal drainage	CA	ICU	Yes (Oncology 4 mo before)
4812.72	ST744/CMY-2	62/M	Intestinal colonization	CA	Medicine	No
4807.68	ST167/CTX-M-1	84/F	UTI	CA	Plastic surgery	No
4904.50	ST95/CTX-M-27	66/F	UTI	HCA	Cardiovascular surgery	No
4901.68	ST10/CTX-M-1	74/F	Abdominal abscess	CA	Emergency	Yes (Abdominal surgery 2 w before)
4807.21	ST10/CTX-M-15	59/F	Liver drainage	HCA	Abdominal surgery	No
4807.06	ST315/CTX-M-14	<1/F	UTI	CA	Emergency	No
4807.08	ST10/CTX-M-1	34/M	Wound	CA	Plastic surgery	Yes (Emergency/Urology 4 mo before)
4902.72	ST453/CTX-M-1	75/F	UTI	CA	Uro-Gynecology	Yes (Uro-Gynecology 6 and 10 mo before)
4901.66	ST131/CTX-M-15	61/F	UTI	CA	SOC: nephrology	No
4903.14	ST131/CTX-M-15	53/F	UTI	CA	Emergency	No
4808.15	ST131/CTX-M-27	25/F	Cervicitis	CA	SOC: gynecology	No
4808.13	ST131/CTX-M-15	56/M	BSI	HCA	Abdominal surgery	No
4901.28	ST131/CTX-M-15	69/F	UTI	HCA	Gastroenterology	No
4904.07	ST131/CTX-M-27	33/F	UTI	CA	Uro-Gynecology	No
4901.09	ST131/CTX-M-15	66/F	UTI	HCA	Medicine	No
4809.34	ST131/CTX-M-15	33/F	Wound	CA	Abdominal surgery	Yes (Abdominal surgery 2 mo before)
4901.44	ST131/CTX-M-27	18/M	Wound	HCA	Plastic surgery	Yes (Orthopedy 2 mo before)
4809.24	ST131/CTX-M-15	57/M	Abdominal abscess	CA	Abdominal surgery	No
4902.61	ST131/CTX-M-15	68/M	BSI	CA	Abdominal surgery	No
4811.56	ST131/CTX-M-15	21/F	Vulvovaginitis	CA	SOC: gynecology	No
4905.17	ST131/CTX-M-15	47/F	UTI	CA	Emergency	No
4903.36	ST131/CTX-M-14	57/F	UTI	CA	SOC: urology	No
4902.63	ST501/CTX-M-15	3/M	Otitis	CA	Emergency	Yes (Paediatric medicine 6 mo before)
4903.72	ST88/cAmpC	5/F	UTI	CA	Emergency	No

Figure 1. Results of rep-PCR and MLST analyses presented along with demographic and clinical data of patients with infection due to ESC-R-*Ec* ($n=40$). ST, sequence type; cAmpC, chromosomal AmpC; CA, community acquired; HCA, healthcare associated; UTI, urinary tract infection; BSI, bloodstream infection; SOC, specialized outpatient clinic; y, years; mo, months; w, weeks. The dotted line indicates the cut-off value of ≥85% determining clonality between the isolates. The isolates were mainly responsible for CA UTIs ($n=17$) and the major clones were represented by ST131 ($n=14$), ST648 ($n=3$) and ST10 ($n=3$). Notably, 11 of 40 ESC-R-*Ec* were detected in SOC patients (of which 8 were referred to nephrology or urology). Notably, two further patients referred to the emergency room (4807.55 and 4903.72) were actually SOC patients (nephrology).

Downloaded from http://jac.oxfordjournals.org/ at World Trade Institute on May 28, 2015

Table 1. Molecular characteristics of the 40 ESC-R-*Ec* isolates detected during the study period

Isolate	CT103 microarray[a]	Molecular characterization (PCR/DNA sequencing)[b,c,d]	aIEF (±0.2)	Plasmid Inc group(s)	Phylo group	MLST	Plasmid Inc group for transformant
4810.28	CTX-M-gr-9, TEMall	CTX-M-27, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	8.3, 6.0, 5.4	F, FII	A	ST617	NP
4810.14	CMY-II, CTX-M-gr-1, TEMall	CMY-2, CTX-M-15, TEM-1, OXA-1-like, IS*Ecp1* (for both *bla* genes)	8.9, 6.0, 5.4	F, FII, I1, X1	D	ST405	F, FII, I1[e]
4808.28	CMY-II	CMY-2, OXA-1-like, IS*Ecp1*	9.0, 7.0, 6.2	F, FII, I1, X1	D	ST354	F, FII, I1, X1
4902.33	CTX-M-gr-9, TEMall	CTX-M-27, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	8.6, 8.1, 5.4	F, FII, X1	D	ST648	F, FII
4809.08	CTX-M-gr-I	CTX-M-15, OXA-1-like, ΔIS*Ecp1* 3′ end	8.9	F, FII, N, Y	D	ST648	NP
4809.61	—	OXA-1-like, [T/A at -32, G/A at -28, G/A at +34, C/T at +58]	8.6	F, FII, I1, HI2	D	ST648	NP
4807.55	CMY-II, TEMall	CMY-2, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	9.0, 6.2, 5.4	F, I2, N, P, X1	D	ST69	TNO
4812.41	CTX-M-gr-9, TEMall	CTX-M-27, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	8.5, 6.2, 5.4	F, FII, N, X1	D	ST648	F, FII
4902.09	CTX-M-gr-I	CTX-M-79-like, OXA-1-like, IS*Ecp1*	9.0	F, FII, I1	A	ST23	F, FII, I1
4807.33	—	OXA-1-like, [C/T at -42, G/A at -18, C/T at -1, C/T at +58]	9.0	B/O, F, FII, I1, P	B1	ST156	NP
4806.54	CTX-M-gr-I, TEMall	CTX-M-15, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	8.9, 6.0, 5.4	I2	B1	ST156	NP
4904.60	—	OXA-1-like, [C/T -42, G/A -18, C/T -1, C/A +24, A/G +49, C/T +58]	8.6	—	B1	ST101	NP
4901.67	CMY-II, TEMall	CMY-2, TEM-1, OXA-1-like, IS*Ecp1*	9.0, 6.0, 5.4	B/O, F, FII, K	B1	ST1431	F, FII, K
4812.17	—	OXA-1-like, [C/T at -42, G/A at -18, C/T at -1, C/T at +58]	9.0, 6.0	F, FII	A	ST410	NP
4809.09	CTX-M-gr-I, TEMall	CTX-M-1, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	8.5, 8.1, 6.0, 5.4	F, FII, I1, P, R	B1	ST1196	FII, I1
4904.36	CTX-M-gr-9, TEMall	CTX-M-14, TEM-1, OXA-1-like, IS*Ecp1*	8.1, 6.0, 5.4	F, FII, I2, X1	A	ST744	TNO
4812.72	CMY-II, TEMall	CMY-2, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	9.0, 6.0, 5.4	F, FII, I1, X1	A	ST744	F, FII, I1, X1
4807.68	CTX-M-gr-I, TEMall	CTX-M-1, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	8.9, 6.2, 5.4	F, FII, N, X1	A	ST167	F, FII
4904.50	CTX-M-gr-9	CTX-M-27, OXA-1-like, ΔIS*Ecp1* 3′ end	8.4	F, FII, X1	B2	ST95	NP
4901.68	CTX-M-gr-I, TEMall	CTX-M-1, TEM-1, OXA-1-like, IS*Ecp1*	8.6, 6.0, 5.4	F, FII, I1, X1	A	ST10	TNO
4807.21	CTX-M-gr-I	CTX-M-15, OXA-1-like, ΔIS*Ecp1* 3′ end	8.9, 7.9, 6.0	F, FII	A	ST10	F, FII
4807.06	CTX-M-gr-9, TEMall	CTX-M-14, TEM-1, OXA-1-like, IS*Ecp1*	8.1, 7.9, 5.4	F, FII	D	ST315	NP
4807.08	CTX-M-gr-I, TEMall	CTX-M-1, TEM-1, OXA-1-like, IS*Ecp1*	8.4, 6.0	F, FII, I2, X1	A	ST10	TNO
4902.72	CTXM-gr-1, TEMall	CTXM-gr-1, TEMall, TEM-164R CTX-M-1, TEM-1, OXA-1-like, ΔIS*Ecp1* 3′ end	5.4	F, FII, N	B1	ST453	TNO
4901.66	CTX-M-gr-I, TEMall	CTX-M-15, TEM-1, OXA-1-like, IS*Ecp1*	8.6, 5.4	F, FII	B2	ST131	NP
4903.14	CTX-M-gr-I, TEMall	CTX-M-15, TEM-1, OXA-1-like, IS*Ecp1*	8.9, 5.4	F, FII, I2	B2	ST131	TNO
4808.15	CTX-M-gr-9	CTX-M-27, OXA-1-like, ΔIS*Ecp1* 3′ end	8.2, 6.0	B/O, F, FII	B2	ST131	NP
4808.13	CTX-M-gr-I	CTX-M-15, OXA-1-like, IS*Ecp1*	8.6, 6.0	B/O, F, FII, X1	B2	ST131	NP
4901.28	CTX-M-gr-I	CTX-M-15, OXA-1-like, ND	8.9, 7.6, 6.0	F, FII	B2	ST131	NP
4904.07	CTX-M-gr-9	CTX-M-27, OXA-1-like, ND	8.6	F, FII	B2	ST131	NP
4901.09	CTX-M-gr-I, TEMall	CTX-M-15, TEM-1, OXA-1-like, IS*Ecp1*	8.9, 6.2, 5.4	F, FII, N, Y	B2	ST131	NP
4809.34	CTX-M-gr-1	CTX-M-15, OXA-1-like, IS*Ecp1*	9.0, 7.6	F, FII	B2	ST131	NP
4901.44	CTX-M-gr-9	CTX-M-27, OXA-1-like, ND	8.2	F, FII	B2	ST131	F, FII
4809.24	CTX-M-gr-I	CTX-M-15, OXA-1-like, ΔIS*Ecp1* 3′ end	8.9, 7.2, 6.2	F, FII	B2	ST131	NP
4902.61	CTX-M-gr-I	CTX-M-15, OXA-1-like, ΔIS*Ecp1* 3′ end	8.9, 7.2, 6.2	F, FII, I1	B2	ST131	NP
4811.56	CTX-M-gr-I	CTX-M-15, OXA-1-like, IS*Ecp1*	8.9	F, FII	B2	ST131	NP
4905.17	CTX-M-gr-1, TEMall	CTX-M-15, TEM-1, OXA-1-like, IS*Ecp1*	8.9, 5.4	F, FII, I1	B2	ST131	NP
4903.36	CTX-M-gr-9, TEMall	CTX-M-14, TEM-1, OXA-1-like, IS*Ecp1*	8.1, 7.9, 5.4	F, FII, N	B2	ST131	F, FII
4902.63	CTX-M-gr-I, TEMall	CTX-M-15, TEM-1, OXA-1-like, IS*Ecp1*	8.6, 5.4	B/O, F	D	ST501	F
4903.72	TEMall	TEM-1, OXA-1-like, [C/T at -42, G/A at -18, C/T at -1, C/T at +58]	9.0, 6.0, 5.4	F, FII, HI2	A	ST88	NP

NP, transformation in DH10B *E. coli* strain not performed; TNO, transformants not obtained; –, negative.
[a]TEMall indicates narrow-spectrum enzymes (e.g. TEM-1/-2) and that TEM-type ESBLs were not present.
[b]Data in square brackets indicate the nucleotide mutations found in the promoter region of the bla_{cAmpC}; results for this analysis are not shown when the promoter region has a wild-type pattern.
[c]The bla_{OXA} gene was only partially sequenced (~200 bp).
[d]IS*Ecp1*, the element was upstream of the *bla* gene; ΔIS*Ecp1* 3′ end, the element was upstream of the *bla* but partially truncated in its 3′ end (isolates with ΔIS*Ecp1* 5′ were not found); ND, not determined (i.e. an intact IS*Ecp1* was detected but its position was undetermined).
[e]For strain 4810.14, the obtained transformant carried both $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CMY\text{-}2}$.

Downloaded from http://jac.oxfordjournals.org/ at World Trade Institute on May 28, 2015

associated with an intact or truncated IS*Ecp1* (Table 1). *Bla*$_{CTX-M-15/-14/-27}$ are the most reported *bla*$_{ESBLs}$ among humans and their association with IS*Ecp1* and IncF/FII plasmids is also common. In contrast, *bla*$_{CTX-M-1}$ is frequent in animals and food products.[1] Since this specific *bla* has been recurrently recorded in Swiss livestock and in the intestinal tract of healthy people, further analyses to confirm its possible transmission between animals and humans should be made.[1,6]

MLST indicated that ST131, ST648 and ST10 were the most preponderant among the ESBL-*Ec*. In particular, ST131 isolates (10 producing CTX-M-15) were found in community ($n=2$), hospital ($n=8$) and SOC ($n=4$) settings (Figure 1). These pandemic clones were responsible for CA infections in all settings, indicating possible exchange and/or common sources among the different patient populations.[1]

Five (12.5%) ESC-R-*Ec* possessed the *bla*$_{CMY-2}$ associated with an intact or truncated IS*Ecp1* (Table 1). These *E. coli* are important human pathogens in both community and hospital settings.[21] Recently, we also recorded a high prevalence of *bla*$_{CMY-2}$ in poultry and pigs.[6,7] In such analyses, this gene was associated to IncI1 plasmids, whereas in the present study further Inc groups (IncF/FII) were also observed. As for *bla*$_{CTX-M-1}$, this may indicate that human and animal isolates partially share the same plasmids.[1] The remaining five isolates were cAmpC hyperproducers due to mutations (at −32 or −42) located within the promoter region. These strains are occasionally reported in humans and animals.[1,12] However, most previous studies implemented methods to detect only the ESBL-*Ec*, probably underestimating the impact of these specific ESC-R-*Ec*.[5,8,14]

CMY-2- or cAmpC-producing isolates were not clonally related to each other (Figure 1). However, some of these strains were from STs also present among the ESBL-*Ec* (ST648, ST156, ST744). Therefore, identical genotypes have the ability to acquire different genetic backgrounds of resistance and have the potential for dissemination. Indeed, several of them (ST648, ST405, ST410) are well recognized pandemic clones that usually carry CTX-M-15.[1]

More surprising is the fact that most of the AmpC producers were responsible for infection in SOC patients (Figure 1). It is probable that in this population there are some factors that contribute to their emergence. For example, in the urology of our institution there is the highest use of β-lactam/β-lactamase inhibitor combinations (70.0 DDD/100 BDs; Table S4, available as Supplementary data at *JAC* Online); moreover, in our region amoxicillin/clavulanate is more frequently implemented than in other areas.[22] These treatments might have some effect in the selection of non-susceptible strains, such as those producing AmpCs.

Conclusions

This is the first Swiss study analysing contemporary ESC-R-*Ec* causing infection in humans. Most ESBL-*Ec* shared common characteristics with those described in other countries (e.g. high prevalence of ST131 producing CTX-M-15). More intriguing is the observation that common clusters of ESC-R-*Ec* have spread transversally among different settings. In particular, the SOCs may play an important role in the expansion of these pathogens because it involves increasing numbers of at-risk outpatients who are constantly referred to healthcare institutions. Since SOC patients may serve as a 'bridge' that favours the spread of ESC-R-*Ec* between the hospital and community (or vice versa), larger epidemiological studies should be planned to better understand the impact of such pathogens in this setting.[11]

Acknowledgements

We thank Regula Tinguely and Carmen Perroulaz for the technical help and Professor Wolfgang Thormann for providing the aIEF apparatus. We also thank Professor Giorgio Zanetti and Dr Catherine Plüss-Suard for providing the antibiotic consumption data.

Funding

This work was supported by grant 1.12.06 (2012–2014) from the Swiss Veterinary Federal Office (BVET) and partially supported by ANRESIS (www.anresis.ch). S. N. S. is a PhD student supported by BVET and by the Institute of Infectious Diseases (IFIK), Bern, Switzerland.

Transparency declarations

None to declare.

Supplementary data

Tables S1–S4 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

References

1 Seiffert NS, Hilty M, Perreten V *et al*. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? *Drug Resist Updat* 2013; **16**: 22–45.

2 Doi Y, Park YS, Rivera JI *et al*. Community-associated extended-spectrum β-lactamase-producing *Escherichia coli* infection in the United States. *Clin Infect Dis* 2013; **56**: 641–8.

3 Rodriguez-Bano J, Picon E, Gijon P *et al*. Community-onset bacteremia due to extended-spectrum β-lactamase-producing *Escherichia coli*: risk factors and prognosis. *Clin Infect Dis* 2010; **50**: 40–8.

4 Rodriguez-Bano J, Alcala JC, Cisneros JM *et al*. Community infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*. *Arch Intern Med* 2008; **168**: 1897–902.

5 Qi C, Pilla V, Yu JH *et al*. Changing prevalence of *Escherichia coli* with CTX-M-type extended-spectrum β-lactamases in outpatient urinary *E. coli* between 2003 and 2008. *Diagn Microbiol Infect Dis* 2010; **67**: 87–91.

6 Endimiani A, Rossano A, Kunz D *et al*. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. *Diagn Microbiol Infect Dis* 2012; **73**: 31–8.

7 Endimiani A, Hilty M, Perreten V. CMY-2-producing *Escherichia coli* in the nose of pigs. *Antimicrob Agents Chemother* 2012; **56**: 4556–7.

8 Lartigue MF, Zinsius C, Wenger A *et al*. Extended-spectrum β-lactamases of the CTX-M type now in Switzerland. *Antimicrob Agents Chemother* 2007; **51**: 2855–60.

9 Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008; **36**: 309–32.

10 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.

11 Hilty M, Betsch BY, Bogli-Stuber K *et al*. Transmission dynamics of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the

Downloaded from http://jac.oxfordjournals.org/ at World Trade Institute on May 28, 2015

tertiary care hospital and the household setting. *Clin Infect Dis* 2012; **55**: 967–75.

12 Jorgensen RL, Nielsen JB, Friis-Moller A *et al*. Prevalence and molecular characterization of clinical isolates of *Escherichia coli* expressing an AmpC phenotype. *J Antimicrob Chemother* 2010; **65**: 460–4.

13 Voets GM, Fluit AC, Scharringa J *et al*. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. *Int J Antimicrob Agents* 2011; **37**: 356–9.

14 Kang CI, Wi YM, Lee MY *et al*. Epidemiology and risk factors of community onset infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* strains. *J Clin Microbiol* 2012; **50**: 312–7.

15 European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2011. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). www.ecdc.europe.eu (11 March 2013, date last accessed).

16 Arpin C, Quentin C, Grobost F *et al*. Nationwide survey of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the French community setting. *J Antimicrob Chemother* 2009; **63**: 1205–14.

17 Aguilar-Duran S, Horcajada JP, Sorli L *et al*. Community-onset healthcare-related urinary tract infections: comparison with community and hospital-acquired urinary tract infections. *J Infect* 2012; **64**: 478–83.

18 den Heijer CD, Donker GA, Maes J *et al*. Antibiotic susceptibility of unselected uropathogenic *Escherichia coli* from female Dutch general practice patients: a comparison of two surveys with a 5 year interval. *J Antimicrob Chemother* 2010; **65**: 2128–33.

19 Ho PL, Wong RC, Yip KS *et al*. Antimicrobial resistance in *Escherichia coli* outpatient urinary isolates from women: emerging multidrug resistance phenotypes. *Diagn Microbiol Infect Dis* 2007; **59**: 439–45.

20 Kronenberg A, Koenig S, Droz S *et al*. Active surveillance of antibiotic resistance prevalence in urinary tract and skin infections in the outpatient setting. *Clin Microbiol Infect* 2011; **17**: 1845–51.

21 Rodriguez-Bano J, Miro E, Villar M *et al*. Colonisation and infection due to *Enterobacteriaceae* producing plasmid-mediated AmpC β-lactamases. *J Infect* 2012; **64**: 176–83.

22 Pluss-Suard C, Pannatier A, Kronenberg A *et al*. Hospital antibiotic consumption in Switzerland: comparison of a multicultural country with Europe. *J Hosp Infect* 2011; **79**: 166–71.

Downloaded from http://jac.oxfordjournals.org/ at World Trade Institute on May 28, 2015

Chapter II

The second study investigated poultry-meat as potential source for ESC-R *Ent*. A total of fourteen samples were purchased at different grocery stores in Bern between November and December 2012. 70% of the samples turned out to be contaminated with ESC-R *E.coli* and ESC-R *P. mirabilis*. The most interesting discovery was a class 1 integron (~17kb) that harboured $bla_{\text{VEB-6}}$ and has been previously reported in a human isolate from France.

Results were presented at the **53rd Interscience Conference on Antimicrobial Agents and Chemotherapy** (ICAAC) – USA, Denver, CO – September 9-13, **2013** (see Poster presentation at the end of this chapter)

The study was published **Antimicrobial Agents and Chemotherapy** under the title "High Prevalence of Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* in Poultry Meat in Switzerland: Emergence of CMY-2- and VEB-6-possessing *Proteus mirabilis*" in **2013** (Seiffert et al., Antimicrobial Agents and Chemotherapy; 57(12):6406-6408, 2013).

AAC Journals.ASM.org

LETTER TO THE EDITOR

High Prevalence of Extended-Spectrum-Cephalosporin-Resistant *Enterobacteriaceae* in Poultry Meat in Switzerland: Emergence of CMY-2- and VEB-6-Possessing *Proteus mirabilis*

Salome N. Seiffert,[a,b,c] Regula Tinguely,[a] Agnese Lupo,[a] Catherine Neuwirth,[d] Vincent Perreten,[b] Andrea Endimiani[a]

Institute for Infectious Diseases, University of Bern, Bern, Switzerland[a]; Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland[b]; Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland[c]; Laboratory of Bacteriology, University Hospital of Dijon, Plateau Technique de Biologie, Dijon, France[d]

The spread of extended-spectrum-cephalosporin-resistant (ESC-R) *Escherichia coli* in poultry meat is a serious concern (1–3). However, data regarding this problem in Switzerland are lacking. Moreover, the role played in this matter by other *Enterobacteriaceae* remains undetermined.

We explored the prevalence of extended-spectrum-β-lactamase (ESBL)- and plasmid-mediated-AmpC (pAmpC)-possessing *Enterobacteriaceae* as contaminants of raw poultry meat retailed in Bern, Switzerland. Twenty samples were purchased on various days during November and December 2012 in three grocery stores (Table 1). Ten grams of meat was incubated overnight in LB broth, and then 50 μl was plated on plates containing selective chromID ESBL agar, MacConkey agar plus ceftazidime (2 μg/ml), and Drigalski agar plus cefotaxime (1.5 μg/ml) (bioMérieux). Colonies were identified by MALDI-TOF MS (matrix-assisted laser desorption ionization–time of flight mass spectrometry; Bruker). ESC-R *Enterobacteriaceae* were characterized using phenotypic and molecular methods as previously done (4–7) (see Table S1 in the supplemental material).

Fourteen samples (70%) contained ESC-R *E. coli* ($n = 11$), ESC-R *P. mirabilis* ($n = 2$), or both resistant species ($n = 1$) (Table 1). ESC-R *E. coli* strains found in meat originating from Switzerland possessed $bla_{\text{CTX-M-1}}$ and $bla_{\text{CMY-2}}$, whereas those detected in meat imported from other countries harbored $bla_{\text{SHV-12}}$, $bla_{\text{TEM-52}}$, or $bla_{\text{CMY-2}}$. Several ESC-R *E. coli* isolates belonged to the same sequence type (ST), but pandemic clones (e.g., ST131) often responsible for human infections were not found (8, 9). The recorded high prevalence of ESC-R *E. coli* (60%) was consistent with prevalences reported in other countries (1–3). However, we note that in Switzerland, CTX-M-1- and CMY-2-producing *E. coli* strains are common colonizers of food animals and may also cause human infections (4, 6, 10).

More intriguing was the detection of ESC-R *P. mirabilis* that grew in MacConkey-ceftazidime and Drigalski-cefotaxime plates. In particular, one meat sample imported from Austria harbored $bla_{\text{CMY-2}}$, whereas two from an unspecified European country carried $bla_{\text{VEB-6}}$. These samples came from the same store and same packing plant but were wrapped on different dates.

CMY-2-positive *P. mirabilis* isolates are commonly found in humans (11), but only unique VEB-6-possessing *P. mirabilis* isolates had been previously reported as responsible for infection in France, Oman, and Australia (7, 12, 13). In our isolates, $bla_{\text{VEB-6}}$ was situated in an ~17-kb class I integron which also carried *aacA4*, *aadB*, *dfrA1*, *sul1*, *tet*(A), and *qnrA1* resistance genes and had a nucleotide sequence identical to that found in the human VEB-6-positive *P. mirabilis* isolate (VB1248) reported in France (12). Both VEB-6-positive *P. mirabilis* isolates also possessed a previously reported class II integron of ~4.6 kb containing *dfrA1*, *sat2*, and *aadA1* resistance genes (GenBank no. DQ268533) and a single ~50-kb IncP plasmid. Electroporation experiments failed to transfer $bla_{\text{VEB-6}}$, suggesting the chromosomal location of the integron. By repetitive extragenic palindromic PCR (rep-PCR), the two VEB-6-positive *P. mirabilis* isolates were identical to each other and showed 94% genomic identity with VB1248. Based on these results, we investigated the possible presence of VEB-6-positive *P. mirabilis* among 484 human isolates identified in Bern during 2011-2012, and we identified only four CMY-2-positive *P. mirabilis* isolates which were not clonally related to those found in poultry meat (see Fig. S1 in the supplemental material).

This is the first report of bla_{VEB}-possessing *P. mirabilis* in a non-human clinical setting and the first description of ESBL- and pAmpC-positive *P. mirabilis* strains in the food chain. Our findings emphasize that not only ESC-R *E. coli* but also other species are responsible for the spread of multidrug-resistant mobile genetic elements in the raw meat. CMY-2- and VEB-6-positive *P. mirabilis* strains might represent an additional group of life-threatening pathogens that can be transmitted through the food chain to humans (8).

Published ahead of print 30 September 2013

Address correspondence to Andrea Endimiani, aendimiani@gmail.com.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.01773-13.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.01773-13

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

TABLE 1 Phenotypic and molecular characteristics of the bla_{ESBL}- and bla_{pAmpC}-possessing *E. coli* and *P. mirabilis* isolates found in poultry meat retailed in Bern during the study period[a]

Meat (store)	Species	bla_{ESBL}, bla_{pAmpC}	Plasmid Inc group(s)	Phylogenetic group	ST	Antimicrobial agents tested (MICs, μg/ml)[b]	Country[c]	
							Production	Packaging (packing plant)
Turkey slices (A)	*E. coli*	CMY-2	F, A/C	D	ST117	CTX (4), CAZ (4), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	GER	GER (I)
Turkey slices (A)	—	—	—	—	—	—	GER	GER (I)
Chicken slices (A)	*E. coli*	SHV-12, TEM-1	F, X1	B1	ST155	CTX (2), CAZ (8), FEP (≤1), ATM (≥32), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	GER	GER (Un)
Chicken slices (A)	*E. coli*	TEM-52	F, I1, X1	A	ST23	CTX (4), CAZ (4), FEP (≤1), ATM (≤1), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	GER	GER (Un)
Chicken ministeaks (A)	*E. coli*	CTX-M-1	F, I1	D	ST2485	CTX (≥64), CAZ (2), FEP (4), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (II)
Chicken ministeaks (A)	—	—	—	—	—	—	CH	CH (II)
Ground chicken (A)	*E. coli*	CTX-M-1, TEM-1	F, FII, I1	A	ST1141	CTX (16), CAZ (≤0.5), FEP (4), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (II)
Chicken legs (A)	—	—	—	—	—	—	CH	CH (II)
Ground chicken (A)	*E. coli*	CTX-M-1	F, I1	B1	ST602	CTX (8), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (II)
Chicken legs (A)	—	—	—	—	—	—	CH	CH (II)
Chicken wings (B)	*E. coli*	CTX-M-1	F	B1	ST1304	CTX (16), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	CH	CH (III)
Chicken wings (B)	—	—	—	—	—	—	CH	CH (III)
Chicken legs (B)	*E. coli*	CMY-2	F, B/O, A/C, K	D	ST38	CTX (8), CAZ (8), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	CH	CH (III)
Chicken wings (B)	*E. coli*	CTX-M-1	I1, Y	D	ST2485	CTX (16), CAZ (≤0.5), FEP (4), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (III)
Ground chicken breasts (B)	*E. coli*	SHV-12	F, FII, I1	B1	ST155	CTX (2), CAZ (8), FEP (≤1), ATM (16), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	AUT	CH (III)
	P. mirabilis	CMY-2	I1	NA	NA	CTX (2), CAZ (4), FEP (≤1), ATM (≤1), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (2), AMK (8), CIP (≤0.125), SXT (≤0.25), COL (≥8), TGC (2)		
Turkey slices (C)	*P. mirabilis*	VEB-6, TEM-1[d]	P	NA	NA	CTX (16), CAZ (≥32), FEP (8), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≥16), AMK (16), CIP (≥4), SXT (≥8), COL (≥8), TGC (2)	EU	CH (IV)
Turkey slices (C)	*P. mirabilis*	VEB-6, TEM-1[d]	P	NA	NA	CTX (16), CAZ (≥32), FEP (16), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (8), AMK (16), CIP (≥4), SXT (≥8), COL (≥8), TGC (2)	EU	CH (IV)
Ground chicken (C)	*E. coli*	CTX-M-1	F, I1, P, U	A	ST23	CTX (16), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (V)
Chicken ministeaks (C)	—	—	—	—	—	—	CH	CH (V)
Chicken slices (C)	*E. coli*	CTX-M-1	F, I1	A	ST23	CTX (32), CAZ (≤0.5), FEP (4), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH (V)

[a] —, isolates possessing bla_{ESBLs} or bla_{aAmpCs} were not detected. NA, not applicable.

[b] Antibiotics and interpretation of MICs according to EUCAST criteria (14) were as follows: CTX, cefotaxime (susceptible [S], ≤ 1 μg/ml); CAZ (S, ≤ 1 μg/ml); FEP, cefepime (S,≤ 1 μg/ml); ATM, aztreonam (S, ≤ 1 μg/ml); TZP, piperacillin-tazobactam (S, ≤ 8 μg/ml); MEM, meropenem (S, ≤ 2 μg/ml); ERT, ertapenem (S, ≤ 0.5 μg/ml); GEN, gentamicin (S, ≤ 2 μg/ml); AMK, amikacin (S, ≤ 8 μg/ml); CIP, ciprofloxacin (S, ≤ 0.5 μg/ml); SXT (S, ≤ 2 μg/ml); COL, colistin (S, ≤ 2 μg/ml); TGC, tigecycline (S, ≤ 1 μg/ml). MICs were determined by using GNX2F plates (Trek Diagnostics).

[c] GER, Germany; CH, Switzerland; AUT, Austria; EU, European Union (the country was not specified on the package sticker). I, II, III, IV, V, and Un (unknown) indicate the different meat-packing plants.

[d] Both isolates possessed the following non-*bla* resistance genes according to the AMR-ve 0.5-m microarray (Alere): bla_{VEB}, bla_{TEM}, *aadA*, *aadB*, *catA*, *ant2Ia*, *aac6-Ib*, *aphA*, *qnr*, *sul1*, *sul2*, *tetA*, *strB*, *intI*, and *intII*. bla_{VEB-6} was carried by a chromosomally located class I integron. For each isolate, analytical isoelectric focusing showed two β-lactamase bands at pI 5.4 (TEM-1) and 7.2 (VEB-6).

ACKNOWLEDGMENTS

We thank Sara Droz and Alexandra Collaud for advice and technical assistance.

This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and by grant 1.12.06 (2012–2014) from the Swiss Veterinary Federal Office (BVET). Salome N. Seiffert is a Ph.D. student supported by IFIK and BVET.

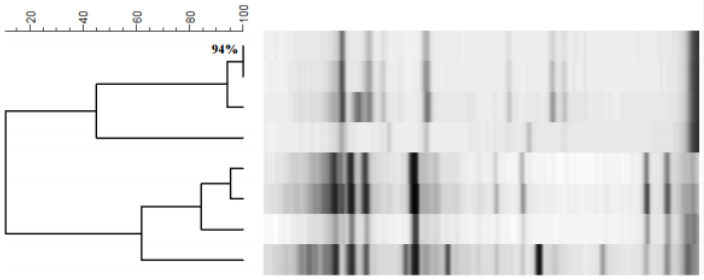
REFERENCES

1. **Kola A, Kohler C, Pfeifer Y, Schwab F, Kuhn K, Schulz K, Balau V, Breitbach K, Bast A, Witte W, Gastmeier P, Steinmetz I.** 2012. High prevalence of extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in organic and conventional retail chicken meat, Germany. J. Antimicrob. Chemother. **67:**2631–2634.
2. **Egea P, Lopez-Cerero L, Torres E, del Gomez-Sanchez MC, Serrano L, Navarro Sanchez-Ortiz MD, Rodriguez-Bano J, Pascual A.** 2012. Increased raw poultry meat colonization by extended spectrum β-lactamase-producing *Escherichia coli* in the south of Spain. Int. J. Food Microbiol. **159:**69–73.
3. **Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ.** 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol. Infect. **17:**873–880.
4. **Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. J. Antimicrob. Chemother. **68:**2249–2254.
5. **Hilty M, Betsch BY, Bögli-Stuber K, Heiniger N, Stadler M, Kuffer M, Kronenberg A, Rohrer C, Aebi S, Endimiani A, Droz S, Mühlemann K.** 2012. Transmission dynamics of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in the tertiary care hospital and the household setting. Clin. Infect. Dis. **55:**967–975.
6. **Endimiani A, Rossano A, Kunz D, Overesch G, Perreten V.** 2012. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. Diagn. Microbiol. Infect. Dis. **73:**31–38.
7. **Zong Z, Partridge SR, Iredell JR.** 2009. A bla_{VEB-1} variant, bla_{VEB-6}, associated with repeated elements in a complex genetic structure. Antimicrob. Agents Chemother. **53:**1693–1697.
8. **Seiffert SN, Hilty M, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant gram-negative organisms in livestock: an emerging problem for human health? Drug Resist. Updat. **16:**22–45.
9. **Woodford N, Turton JF, Livermore DM.** 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev. **35:**736–755.
10. **Endimiani A, Hilty M, Perreten V.** 2012. CMY-2-producing *Escherichia coli* in the nose of pigs. Antimicrob. Agents Chemother. **56:**4556–4557.
11. **D'Andrea MM, Literacka E, Zioga A, Giani T, Baraniak A, Fiett J, Sadowy E, Tassios PT, Rossolini GM, Gniadkowski M, Miriagou V.** 2011. Evolution and spread of a multidrug-resistant *Proteus mirabilis* clone with chromosomal AmpC-type cephalosporinases in Europe. Antimicrob. Agents Chemother. **55:**2735–2742.
12. **Siebor E, Neuwirth C.** 2011. The new variant of *Salmonella* genomic island 1 (SGI1-V) from a *Proteus mirabilis* French clinical isolate harbours bla_{VEB-6} and qnrA1 in the multiple antibiotic resistance region. J. Antimicrob. Chemother. **66:**2513–2520.
13. **Potron A, Poirel L, Elhag K, Al Yaqoubi F, Nordmann P.** 2009. VEB-6 extended-spectrum β-lactamase-producing *Proteus mirabilis* from Sultanate of Oman. Int. J. Antimicrob. Agents **34:**493–494.
14. **EUCAST.** 2013. European Committee on Antimicrobial Susceptibility Testing clinical breakpoints. Version 3.0.

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

Supplementary Materials for Chapter II

Figure 1S. Results of the rep-PCR analysis.



P. mirabilis	Main *bla*	Sample	Country
#16	bla_{VEB-6}	Turkey slices	EU
#17	bla_{VEB-6}	Turkey slices	EU
VB1248	bla_{VEB-6}	Human (blood and stool)	FRA
#15	bla_{CMY-2}	Grounded chicken breast	AUT
4810.05	bla_{CMY-2}	Human (urine)	CH
4810.40	bla_{CMY-2}	Human (blood)	CH
4812.18	bla_{CMY-2}	Human (urine)	CH
5106.42	bla_{CMY-2}	Human (wound)	CH

The two VEB-6-positive P. mirabilis isolates found in poultry meat (#16 and #17) in Bern (Switzerland) are indistinguishable to each other and possess a 94% genetic identity with the VEB-6-positive strain (VB1248) found in a French patient. The CMY-2-positive isolates found in humans in Bern (n=4; i.e., 4810.05, 4810.40, 4812.18, and 5106.42) do not show any genetic relation with those detected in meat.

Table 1S: Primers used for the characterization of the class 2 integron

Number	Primer	Nucleotide sequence (5'-3')	Positions	Designed/Reference
1	Int2-F	CACGGATATGCGACAAAAAGGT	484-505	(2)
2	Int2_F_SSE	TGCACCATACAGCAGCGTAA	142-161	This study
3	Int2_seq1_F	TAAGCAGACAGCTCCTTGGC	1774-1793	This study
4	125`CS	TTTTTGTGCTGCCATATCCGTG	495-505	(5)
5	23`CS	TGGGCTGAGAGAGTGGT	+253--269	(5)
6	dfrA1	AGCTGTTCACCTTTGGC	941-957	(5)
7	hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	723-738	(6)
8	hep51	GATGCCATCGCAAGTACGAG	2913-2932	(6)
9	hep51-RC	CTCGTACTTGCGATGGCATC	2913-2932	This study
10	satR	TCATCCTGTGCTCCCGAG	1937-1954	(5)
11	aadAF	ATGAGGGAAGCGGTGATCGCC	2019-2039	(2)
12	aadA1	TCGATGACGCCAACTAC	2064-2080	(5)
13	Aada1_IntF	ACGTTGTCCCGCATTTGGTA	2583-2602	This study
14	ybfA_F	GTGAGACAACCAGATGGGCA	3832-3851	This study
15	ybfB_R	TGCCCATCTGGTTGTCTCAC	3832-3851	This study
16	ybeB-Fout	TTTCGCTTGCCCTGACAGAT	3328-3347	This study
17	ybgA_R	CAATGAGTTCTTGAGCGCGG	4523-4542	This study

Table 2S: Primers used for the characterization of the class 1 integron

Number	Primer	Nucleotide sequence (5'-3')	Positions	Designed/Reference
1	S026-outR	CAACCTGAAGGGCAACAG	-812-(-794)	(1)
2	IRi	TGTCGTTTTCAGAAGACGG	1-19	(1)
3	Int1-F	GGGTCAAGGATCTGGATTTCG	736-756	(2)
4	Int1-R	ACATGCGTGTAAATCATCGTCG	273-294	(2)
5	5-CS	GGCATCCAAGCAGCAAGC	1256-1273	(3)
6	5-CSINV	GCTCCATAACATCAAACATCG	1290-1310	(3)
7	tnpRTn3-R	TGGAGCGGGCAATACTGA	1727-1744	(1)
8	AACA4-R	TGTTCGCTCGAATGCCTG	2538-2555	(1)
9	aadB-F	CGATCGATGCACGGCTA	2728-2744	(1)
10	AADB-B	CGCATATCGCGACCTGAAAGC	3147-3167	(3)
11	dhfrA1-R	CTTGAACGTGTTACGACCGC	3419-3438	This study
12	QACEΔ1-B	CAAGCTTTTGCCCATGAAGC	4649-4668; 8927-8946; 14075-14094	(3)
13	Sul-F	GACGGTGTTCGGCATTCT	4765-4782; 9043-9060; 14191-14208	(1)
14	Sul1-B	GCAAGGCGGAAACCCGCGCC	5221-5240; 9499-9518; 14647-14666	(3)
15	Sul1-newR	GTGATTGCGCTTCGCAGATC	5513-5532; 9791-9910; 14939-14958	This study
16	tetATn1721-outR	CATGATGGCGTAGTCGAC	5877-5894	(1)
17	tetAintR	CTCCGGCAAAAGGAAACAGC	6154-6173	This study
18	5'of VEB	CAACCACAACGCAAACCCTT	7158-7177	This study
19	VEB-OUT2	GGAATTACTATTTGAATAAATAA	7549-7571	(4)
20	VEBINV1	CAGTTTGAGCATTTGAATACAC	8378-8394	(3)
21	VEBINV2	AGCGTATTTGTTGCAGAGTCC	7627-7647	(3)
22	VEB-OUT1	AATATCCTTTTTACGATTTTCAT	8426-8448	(4)
23	3'of VEB	TTATGACAACGGCGGAAGGG	8789-8808	This study
24	orf513-F	ATGGTTTCATGCGGGTT	10490-10506	(1)
25	orf513-R	CTGAGGGTGTGAGCGAG	10948-10964	(1)
26	3' of Orf513	AACAGAAAACAGCCAAGGCG	11711-11730	This study
27	qnrA1-outF	CCTTTGCCGATCTGGATG	12712-12729	(1)
28	qnrA1-newR	CTTCGAGGTTGACCCGTCTG	12740-12759	This study
29	qnrA1-R	CTAATCCGGCAGCACTAT	12813-12830	(1)
30	PM4-F	CAAGCGCTCTTTGGCAATCA	13009-13028	This study
31	orfPM4-outR	TAAGTGCTTCCCAGAGCGTG	13168-13187	This study
32	PM5-F	CTGCAAACTCACGAGCACAG	13578-13597	This study
33	orf5-R	GATTTCGAGTTCTAGGCG	15633-15650	(1)
34	orf6-mF	CTTCCATCATCAACCCTG	15767-15784	(1)
35	tnpA6100-F	CGATCAGCTATCGCGACCTT	16233-16255	This study
36	tnpA6100-F2	CGCGGTTTCAAATCGATCCC	16715-16734	This study
37	tnpA6100-R2	GCCAGCTCCTGACAGTTCAA	17056-17075	This study
38	IRt	TGTCATTTTCAGAAGACGA	17112-17129	(1)
39	S044-R	TTCTCCCTGATCTTCATC	17620-17637	(1)

REFERENCES

1. Siebor E, Neuwirth C. 2011. The new variant of Salmonella genomic island 1 (SGI1-V) from a Proteus mirabilis French clinical isolate harbours blaVEB-6 and qnrA1 in the multiple antibiotic resistance region. The Journal of antimicrobial chemotherapy 66:2513-2520.
2. Mazel D, Dychinco B, Webb VA, Davies J. 2000. Antibiotic resistance in the ECOR collection: integrons and identification of a novel aad gene. Antimicrob Agents Chemother 44:1568-1574.
3. Naas T, Benaoudia F, Massuard S, Nordmann P. 2000. Integron-located VEB-1 extendedspectrum β-lactamase gene in a Proteus mirabilis clinical isolate from Vietnam. The Journal of antimicrobial chemotherapy 46:703-711.
4. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, Ecker DJ, Massire C, Eshoo MW, Sampath R, Thomson JM, Rather PN, Craft DW, Fishbain JT, Ewell AJ, Jacobs MR, Paterson DL, Bonomo RA. 2006. Analysis of antibiotic resistance genes in multidrug-resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 50:4114-4123.
5. Ramirez MS, Quiroga C, Centron D. 2005. Novel rearrangement of a class 2 integron in two non-epidemiologically related isolates of Acinetobacter baumannii. Antimicrob AgentsChemother 49:5179-5181.
6. White PA, McIver CJ, Rawlinson WD. 2001. Integrons and gene cassettes in theenterobacteriaceae. Antimicrob Agents Chemother 45:2658-2661.

High prevalence of extended-spectrum cephalosporin-resistant *E. coli* and emergence of VEB-6-producing *P. mirabilis* in poultry meat in Switzerland

ICAAC 2013 — Poster: C2-1617

Salome N. Seiffert,[1,2,3*] Regula Tinguely,[1] Sara Droz,[1] Vincent Perreten,[2] and Andrea Endimiani [1#]

[1] Institute for Infectious Diseases, [2]Institute of Veterinary Bacteriology, [3]Graduate School for Cellular and Biomedical Sciences; University of Bern, Switzerland; (*Email: salome.seiffert@ifik.unibe.ch; #aendimiani@gmail.com)

Introduction:

Resistance to extended-spectrum cephalosporins (**ESCs**) in retailed poultry meat is usually due to production of extended-spectrum β-lactamases (**ESBLs**) and/or plasmid-mediated AmpCs (**pAmpCs**).

ESC-R *Escherichia coli* (**ESC-R-*Ec***) are observed at high prevalence in retail poultry meat in many countries which is a matter of concern.

However, **information regarding the prevalence of in retailed poultry meat is still lacking in Switzerland.**

More importantly, **little is known about the role played in this matter by other species of *Enterobacteriaceae*** that might serve as source of transferable bla_{ESBL} and bla_{pAmpC} genes.

Materials and Methods:

We analyzed 20 retailed poultry meat samples purchased during November-December 2012 at different grocery stores located in the Bern (Switzerland).

Ten grams of raw poultry meat were cultured in 20 ml of LB. After overnight incubation, 50 µl of the culture were plated onto selective chromID ESBL (bioMérieux), Drigalski plus cefotoxime, and MacConkey plus ceftazidime (Oxoid) plates.

The subgroup of ESC-R *Enterobacteriaceae* (**ESC-R-*Ent***) were further characterized with:

- Species identification using MALDI-TOF MS (Bruker Daltonik) coupled by standard biochemical tests;
- Antibiotic susceptibility test (MIC) using GNX2F MICs plates (Trek Diagnostics);
- Double disk synergy test with and without cloxacillin [200 µg/l] disk diffusion;
- Microarrays for detection of ESBL, pAmpC, and carbapenemase genes (Check-Points);
- PCR/DNA sequencing for bla_{ESBLs}, bla_{pAmpCs} and promoter region of bla_{AmpC};
- Analytical isoelectric focusing (aIEF);
- Multilocus sequencing typing (MLST) analysis (for *E. coli*; Achtman scheme);
- PCRs for plasmid replicon typing (PBRT; Diatheva);

The subgroup of ESC-R *P. mirabilis* (**ESC-R-*Pm***) were additionally characterized with:

- Microarray for detection of non-*bla* resistance genes (AMR-ve 0.5m, Alere);
- Elecroporation of the plasmid into DH10b *Ec*;
- PCR/DNA sequencing for class I/II integrons;
- Rep-PCR.

Results:

14 out of 20 poultry meat samples **(70%) resulted contaminated with ESC-R-*Ent* (i.e., ESC-R-*Ec*, n=11; ESC-R-*Pm*, n=2; or both resistant species, n=1).**

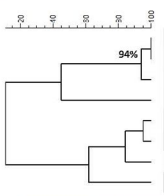
Retailed meat originating from Switzerland contained ESC-R-*Ec* which carried $bla_{CTX-M-1}$ ESBL and bla_{CMY-2} pAmpC genes. In contrast, meat originating from other countries harbored bla_{SHV-12} and bla_{TEM-52} ESBL or bla_{CMY-2} genes (Table 1).

Several ESC-R-*Ec* were of the same sequence type (ST; e.g., ST23) but pandemic clones (e.g., ST131, ST648) often responsible for infection in humans were not found.

Moreover, we also found **three ESC-R-*Pm* strains in poultry meat imported from outside countries**. One harbored the bla_{CMY-2} (Nr. 15), whereas the other two strains (Nr. 16 and Nr. 17) carried the **bla_{VEB-6} ESBL gene in a chromosomally located complex ~17 kb class I integron** co-carrying the *aacA4*, *aadB*, *dfrA1*, *sul1*, *tetA*, and *qnrA1* resistance genes as well as a class II integron (Figure 2).

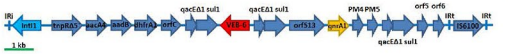
The comparison of these two strains to a VEB-6-carrying ESC-R-*Pm* from a clinical human isolate from France (Dijon) revealed a 94% similarity by rep-PCR (100% similarity of the class 1 integron) (Figure 1).

Figure 1: Results of the rep-PCR analysis for the *P. mirabilis* isolates detected in poultry meat and humans



P. mirabilis	Main *bla*	Source (sample)	Country	
#16	bla_{VEB-6}	Turkey slices	EU	Poultry isolates
#17	bla_{VEB-6}	Turkey slices	EU	
VB1248	bla_{VEB-6}	Human (blood and stool)	FRA	Human isolate from France
#15	bla_{CMY-2}	Grounded chicken breast	AUT	Poultry isolates
4810.05	bla_{CMY-2}	Human (urine)	CH	Clinical isolates from Bern
4810.40	bla_{CMY-2}	Human (blood)	CH	
4812.18	bla_{CMY-2}	Human (urine)	CH	
5106.42	bla_{CMY-2}	Human (wound)	CH	

Figure 2: Class I and class II integrons carried by the ESC-R *P. mirabilis*



Schematic illustration of the class I intergon (~17 kb); Light blue: class 1 integrase; Red: bla_{VEB-6} ESBL gene; Yellow: *qnrA1*



Schematic illustration of the class II integron (~5 kb); Light blue: class 2 integrase; Yellow: *dfrA1*

Table 1: Characteristics of the bla_{ESBL}- and bla_{pAmpC}-possessing *Enterobacteriaceae* found in poultry meat

No	Kind of poultry meat	Species	bla_{ESBL} / bla_{AmpC}	Plasmid Inc group	Phylo-group	ST	Antimicrobial susceptibility tests (MIC values, µg/ml)	Country: Importation	Country: Packaging
1	Turkey slices	*E. coli*	CMY-2	F, A/C	D	ST117	CTX (4), CAZ (4), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	GER	GER
2	Turkey slices	-	-	-	-	-	-	GER	GER
3	Chicken slices	*E. coli*	SHV-12, TEM-1	F, X1	B1	ST155	CTX (2), CAZ (8), FEP (≤1), ATM (≤32), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤8), COL (≤0.125), TGC (≤0.125)	GER	GER
4	Chicken slices	*E. coli*	TEM-52	F, I1, X1	A	ST23	CTX (4), CAZ (4), FEP (≤1), ATM (≤1), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	GER	GER
5	Chicken mini-steaks	*E. coli*	CTX-M-1	F, I1	D	ST2485	CTX (≥64), CAZ (2), FEP (4), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH
6	Chicken mini-steaks	-	-	-	-	-	-	CH	CH
7	Grounded chicken	*E. coli*	CTX-M-1, TEM-1	F, FII, I1	A	ST1141	CTX (16), CAZ (≤0.5), FEP (4), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH
8	Chicken legs	-	-	-	-	-	-	CH	CH
9	Grounded chicken	*E. coli*	CTX-M-1	F, I1	B1	ST602	CTX (8), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH
10	Chicken legs	-	-	-	-	-	-	CH	CH
11	Chicken wings	*E. coli*	CTX-M-1	F	B1	ST1304	CTX (16), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	CH	CH
12	Chicken wings	-	-	-	-	-	-	CH	CH
13	Chicken legs	*E. coli*	CMY-2	F, B/O, A/C, K	D	ST38	CTX (8), CAZ (8), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	CH	CH
14	Chicken wings	*E. coli*	CTX-M-1	I1, Y	D	ST2485	CTX (16), CAZ (≤0.5), FEP (4), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH
15	Grounded chicken breasts	*E. coli*	SHV-12	F, FII, I1	B1	ST155	CTX (2), CAZ (8), FEP (≤1), ATM (16), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≤0.25), COL (≤0.125), TGC (≤0.125)	AUT	CH
		P. mirabilis	CMY-2	I1	NA	NA	CTX (2), CAZ (4), FEP (≤1), ATM (≤1), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (2), AMK (8), CIP (≤0.125), SXT (≤0.25), COL (≥8), TGC (2)		
16	Turkey slices	*P. mirabilis*	VEB-6, TEM-1	P	NA	NA	CTX (16), CAZ (≥32), FEP (8), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≥16), AMK (16), CIP (≤4), SXT (≥8), COL (≥8), TGC (2)	EU	CH
17	Turkey slices	*P. mirabilis*	VEB-6, TEM-1	P	NA	NA	CTX (16), CAZ (≥32), FEP (16), ATM (8), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (8), AMK (16), CIP (≤4), SXT (≥8), COL (≥8), TGC (2)	EU	CH
18	Grounded chicken	*E. coli*	CTX-M-1	F, I1, P, U	A	ST23	CTX (16), CAZ (≤0.5), FEP (≤1), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH
19	Chicken mini-steaks	-	-	-	-	-	-	CH	CH
20	Chicken slices	*E. coli*	CTX-M-1	F, I1	A	ST23	CTX (32), CAZ (≤0.5), FEP (4), ATM (4), TZP (≤4), MEM (≤0.5), ERT (≤0.125), GEN (≤0.5), AMK (≤2), CIP (≤0.125), SXT (≥8), COL (≤0.125), TGC (≤0.125)	CH	CH

CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; TZP, piperacillin-tazobactam; MEM, meropenem; ERT, ertapenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; COL, colistin; TGC, tigecycline; -, isolates possessing bla_{ESBL} or bla_{pAmpC} were not detected; NA, not applicable; GER, Germany; CH, Switzerland; AUT, Austria; EU, European Union

Conclusions:

This is the **first study analyzing the prevalence and molecular characteristics of ESC-R-*Ent* found in raw poultry meat retailed in Switzerland.**

The **prevalence of ESC-R-*Ec* (60%)** is high but consistent with recent results from surrounding countries.

ESC-R-*Ec* originating from Switzerland possessed CTX-M-1 and CMY-2, whereas meat coming from Europe carried TEM-52 and SHV-12.

In two of the three ESC-R-*Pm* we detected **VEB-6 that was encoded on an ~17 kb class I integron** co-carrying other resistance genes rendering them MDR.

Interestingly, **these two ESC-R-*Pm* strains share 94% similarity with a VEB-6 carrying ESC-R-*Pm* strain** previously detected in a French patient (100% on the class 1 integron).

This is the first study describing VEB-6 carrying ESC-R-*Pm* in a non-human setting and also the first report of a ESBL- and pAmpC-positive *P. mirabilis* strains in raw poultry meat.

Acknowledgements:

We thank Agnese Lupo, Alexandra Collaud and Catherine Neuwirth for their support. This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and by Grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office (BVET). Salome N. Seiffert is a PhD student supported by IFIK and BVET.

Chapter III

This very original study examined the contamination of pet food with ESC-R *Ent*. For this purpose 30 packages purchased at different grocery stores in Bern were tested for living bacteria as well as the presence of β-lactamase genes.
70% of the samples were contaminated with one or more β-lactamase genes. Those β-lactamase genes however are usually more related to the human reservoir (CTX-M-15, CMY-4) than to the animal reservoir. Furthermore, 27% of the samples contained *bla*$_{\text{OXA48-like}}$ carbapenemase genes.

Preliminary results were presented at the **24th European Congress of Clinical Microbiology and Infectious Diseases** (ECCMID) – Barcelona, Spain – May 10-13, **2014** (see Poster presentation at the end of this chapter).

The study was published in **Antimicrobial Agents and Chemotherapy** under the title "High Prevalence of Extended-Spectrum β-Lactamase, Plasmid-Mediated AmpC and Carbapenemase genes in Food for Pets" in **2014** (Seiffert et al., Antimicrobial Agents Chemotherapy; 58(10):6320-6323, 2014).

AAC Journals.ASM.org

High Prevalence of Extended-Spectrum β-Lactamase, Plasmid-Mediated AmpC, and Carbapenemase Genes in Pet Food

Salome N. Seiffert,[a,b,c] Alessandra Carattoli,[a,b,d] Regula Tinguely,[a] Agnese Lupo,[a] Vincent Perreten,[b] Andrea Endimiani[a]

Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Bern, Switzerland[a]; Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland[b]; Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland[c]; Istituto Superiore di Sanità, Rome, Italy[d]

We evaluated the pet food contained in 30 packages as a potential origin of extended-spectrum cephalosporin-resistant Gram-negative organisms and β-lactamase genes (*bla*). Live bacteria were not detected by selective culture. However, PCR investigations on food DNA extracts indicated that samples harbored the $bla_{CTX\text{-}M\text{-}15}$ (53.3%), $bla_{CMY\text{-}4}$ (20%), and $bla_{VEB\text{-}4\text{-}like}$ (6.7%) genes. Particularly worrisome was the presence of $bla_{OXA\text{-}48\text{-}like}$ carbapenemases (13.3%). The original pet food ingredients and/or the production processes were highly contaminated with bacteria carrying clinically relevant acquired *bla* genes.

Enterobacteriaceae possessing β-lactamase genes (*bla*) encoding extended-spectrum β-lactamase (ESBL) (e.g., CTX-Ms, VEBs), plasmid-mediated AmpC (pAmpC) (e.g., CMYs), and carbapenemase (e.g., *Klebsiella pneumoniae* carbapenemases [KPCs], OXA-48-like, NDMs) enzymes have become a major threat to public health (1–4). Pets and food-producing animals may be colonized or even infected with these drug-resistant bacteria, possibly acting as a potential reservoir for their spread to humans (5–9).

In line with analyses performed on food products for humans (e.g., retail meat, vegetables) (9–12), this study investigated pet food as a potential source of genes conferring resistance to extended-spectrum cephalosporins (ESCs). For this purpose, we analyzed the presence of ESC-resistant *Enterobacteriaceae* (ESC-R-*Ent*), *bla* genes, and plasmid-specific markers in retail pet food.

In September 2013, 30 pet food (for cats and dogs) packages containing 50 g to 1 kg of mixed wet food with different flavors were purchased from three stores in Bern, Switzerland. The advertised ingredients (content declaration), the brands, and the manufacturers, as indicated on the package labels, are listed in Table 1. For most of the packages, only a minor proportion of the component(s) constituting the overall product was declared in detail. For 73% of the food samples, meat from chicken, duck, and/or turkey was advertised on the label, but this component contributed just 4% to 18% of the final composition. Declarations of the remaining compounds (72% to 96%) were missing. Furthermore, a large proportion of the food mixtures also contained an additional compound(s), like grains, plant-derived products, and fish by-products, that were "not promoted" but listed in the declaration of the composition (data not shown).

A portion of the food content (about 10 g each) was enriched in Luria-Bertani broth plus 20 μg/ml ampicillin (Sigma-Aldrich) at 37°C overnight. From there, an aliquot of 50 μl was streaked out on MacConkey, Drigalski plus cefotaxime (1.5 μg/ml), and MacConkey plus ceftazidime (2 μg/ml) agar plates (bioMérieux). ESC-R-*Ent* or other cephalosporin-resistant bacteria were not detected by selective cultivation in any of the 30 samples.

DNA isolation was performed with the QIAamp DNA minikit (Qiagen) using about 10 g of food from each package. Each sample was examined as previously described by standard PCR and DNA sequencing for the presence of *bla* genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1\ group}$, $bla_{CTX\text{-}M\text{-}9\ group}$, bla_{VEB}, bla_{CMY}, $bla_{OXA\text{-}48\text{-}like}$, bla_{IMP}, bla_{VIM}, bla_{NDM}), insertion sequence IS*Ecp1*, and plasmid types (IncA/C, IncN, IncI1, IncF, and IncL/M and the genes *parA*, *repA*, and *traU*) (1, 13–16).

As shown in Table 1, 16 samples were positive for bla_{ESBL} genes: 14 samples harbored $bla_{CTX\text{-}M\text{-}1\ group}$, one was positive for $bla_{CTX\text{-}M\text{-}1\ group}$ and bla_{VEB}, and one had only bla_{VEB}. In seven samples, the DNA sequence of the $bla_{CTX\text{-}M\text{-}1\ group}$ amplicon led us to suspect the presence of both the $bla_{CTX\text{-}M\text{-}3}$ and the $bla_{CTX\text{-}M\text{-}15}$ genes. This was due to the observation of a double peak in the DNA sequence corresponding to amino acid position 242, discriminating between the CTX-M-3 (Asp242) and CTX-M-15 (Gly242) variants.

It was not possible to determine the complete genetic environments of the amplified genes because the pet food processing and heat treatment steps used to sterilize the food likely caused a partial fragmentation of the bacterial DNA, and it was therefore not possible to amplify targets larger than 800 to 1,000 bp. However, for many samples, a positive amplification was detected for the IS*Ecp1*-$bla_{CTX\text{-}M}$ intergenic region (Table 1), with the expected distance of 48 bp from the start codon of the $bla_{CTX\text{-}M\text{-}15}$ gene (17). This result demonstrates that $bla_{CTX\text{-}M\text{-}15}$ was an acquired, presumably plasmid-located resistance gene. Conversely, the $bla_{CTX\text{-}M\text{-}3}$ gene may be presumably derived from pet food contaminated with *Kluyvera* species progenitors, where it is intrinsic (18).

The two bla_{VEB}-positive amplicons were $bla_{VEB\text{-}4\text{-}like}$ and were very likely acquired resistance genes, since this gene has never been described in a progenitor species. We recently identified $bla_{VEB\text{-}6}$, a $bla_{VEB\text{-}4\text{-}like}$ variant, in a *Proteus mirabilis* isolate from poultry meat in Switzerland (10).

Six samples (20%) were positive for $bla_{CMY\text{-}4}$ (Table 1). Sequencing analyses indicate that $bla_{CMY\text{-}4}$ cannot be attributed to the chromosomal gene found in the ancestral species (*Citrobacter freundii*) generating this family of pAmpCs (i.e., no 100% DNA

Received 26 June 2014 Returned for modification 28 July 2014
Accepted 31 July 2014
Published ahead of print 4 August 2014
Address correspondence to Andrea Endimiani, andrea.endimiani@ifik.unibe.ch.
Copyright © 2014, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.03185-14

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

TABLE 1 Epidemiological characteristics and DNA amplicons of relevant antibiotic resistance and plasmid traits from 30 pet food samples

Sample no.	Pet type	Food component(s) (% declared on label)	Country[a]	Store	Brand[b]	Manufacturer[b]	*bla* gene(s) found						IncL/M plasmid typing[f]			IncF, repFII plasmid typing[f]
							OXA-48[c]	CTX-M-1 group[d]	CMY[e]	VEB	TEM[f]	SHV[f]	TraU	ParA	RepA	
101	Cat	Beef (4), chicken (4)	GER	A	1	I	−	IS*Ecp1*-CTX-M-15, CTX-M-3	−	VEB-4-like	+	+	−	−	+	+
102	Cat	Veal (4), poultry (4)	GER	A	1	I	−	IS*Ecp1*-CTX-M-15, CTX-M-3	CMY-4	−	+	+	−	−	+	+
103	Cat	Salmon (4), trout (4)	GER	A	1	I	−	−	−	−	−	−	+	+	+	+
104	Cat	Poultry (4), liver (4)	GER	A	1	I	−	IS*Ecp1*-CTX-M-15, CTX-M-3	−	−	+	+	−	−	+	+
106	Cat	Turkey (14), rabbit (8)	GER/NED	A	2	I, II	−	IS*Ecp1*-CTX-M-15, CTX-M-3	−	−	+	+	−	−	+	−
107	Cat	Beef (14), turkey hearts (8)	GER/NED	A	2	I, II	−	−	−	−	−	−	+	−	+	+
109	Cat	Salmon (4), chicken (4)	EU	A	3	III	−	IS*Ecp1*-CTX-M-15, CTX-M-3	−	−	+	−	+	−	+	+
1010	Cat	Beef (4)	EU	A	3	III	−	IS*Ecp1*-CTX-M-15	−	−	+	−	−	−	+	+
1011	Cat	Turkey (4), duck (4)	EU	A	3	III	−	−	CMY-4	−	+	−	+	+	+	+
1013	Dog	Beef (4)	EU	A	4	IV	−	IS*Ecp1*-CTX-M-15, CTX-M-3	−	−	+	+	+	−	+	+
1014	Dog	Lamb (4)	EU	A	4	IV	−	−	−	−	+	+	+	+	+	+
1015	Dog	Rabbit (4)	EU	A	4	IV	OXA-48	−	−	VEB-4-like	+	+	−	−	+	+
1016	Dog	Turkey (4), peas (2)/carrots (2)	LIE	A	5	V	−	−	−	−	+	+	+	+	+	+
1017	Cat	Poultry (4)	DEN	B	6	VI	−	−	−	−	−	−	+	−	+	+
1018	Cat	Chicken (8), green beans (5)	EU	B	7	VII	−	−	−	−	+	+	+	+	+	+
1020	Cat	Turkey (4)	EU	B	8	VIII	−	−	−	−	+	+	+	−	+	+
1021	Cat	Poultry (4)	EU	B	8	VIII	−	IS*Ecp1*-CTX-M-15	−	−	+	+	+	−	−	−
1023	Dog	Beef (4), chicken (4)	NED	B	9	IX	OXA-48	IS*Ecp1*-CTX-M-15	−	−	+	−	−	−	+	+
1024	Dog	Turkey (4), chicken (4)	EU	B	10	IV	−	IS*Ecp1*-CTX-M-15	CMY-4	−	+	+	+	−	+	+
1025	Dog	Beef (4), liver (4)	EU	B	10	IV	−	IS*Ecp1*-CTX-M-15, CTX-M-3	CMY-4	−	+	+	+	−	+	+
1026	Dog	Poultry (4)	FRA	B	11	X	−	IS*Ecp1*-CTX-M-15	CMY-4	−	+	+	-	−	+	+
1027	Cat	Chicken (4)	EU	C	12	VIII	−	IS*Ecp1*-CTX-M-15	−	−	+	+	+	−	+	+
1028	Cat	Poultry (4)	EU	C	12	VIII	−	IS*Ecp1*-CTX-M-15	−	−	+	+	+	−	+	+
1029	Cat	Chicken (4), turkey (4)	LIE	C	13	V	−	−	−	−	+	+	−	−	+	+
1030	Cat	Beef (4)	LIE	C	14	V	−	−	−	−	+	+	−	−	+	+
1031	Cat	Poultry (4)	LIE	C	14	V	OXA-48-like[c]	CTX-M-15	CMY-4	−	+	−	−	−	+	+
1032	Cat	Poultry (4)	EU	C	15	NA	−	CTX-M-15	−	−	+	+	+	−	+	+
1033	Dog	Beef (4)	NED	C	16	XI	−	−	−	−	+	−	−	−	+	+
1034	Dog	Chicken (14), rice (4)	GER/NED	C	17	I, II	−	−	−	−	+	−	+	−	+	+
1035	Dog	Turkey (14), rice (4)	GER/NED	C	17	I, II	OXA-48	−	−	−	+	+	−	−	+	+

[a] GER, Germany; NED, The Netherlands; FRA, France; LIE, Liechtenstein; DEN, Denmark; EU, European Union (the specific country was not declared).

[b] Brands and manufacturers are coded with 1 through 17 and I through VIII, respectively. These data are given as an indication of brands produced at the same factory (i.e., manufacturer VIII produces for brands 8 and 12) or brands receiving products from more than one manufacturer (i.e., brand 2 has manufacturers I and II). NA, not available due to unreadable labeling.

[c] OXA-48-like sequence contains the following amino acid changes compared to OXA-48 (Trp25Leu, Gln41Leu, Thr99Met, Glu168Gln, and Ser171Ala) or OXA-181 (Trp25Leu, Gln41Leu, Thr99Met, Ala104Thr, and Asp110Asn).

[d] The copresence of CTX-M-3 and CTX-M-15 is suggested by a double pick in the DNA sequence of the CTX-M-1 group amplicon, corresponding to amino acid position 242, discriminating between the CTX-M-3 and CTX-M-15 variants (Asp242 in CTX-M-3 and Gly242 in CTX-M-15 [GenBank accession no. Y10278 and AY044436, respectively]). By PCR analysis, most of the $bla_{\text{CTX-M-15}}$ genes were associated with IS*Ecp1*.

[e] By PCR analysis, no $bla_{\text{CMY-4}}$ genes were associated with an upstream IS*Ecp1* element.

[f] +, PCR-positive amplicon; −, PCR-negative amplicon. Amplicons were not sequenced.

homology) (19). Conversely, it has always been identified on plasmids and is usually found in *Enterobacteriaceae* isolates of human origin (3) and in retailed chicken meat (20, 21).

Three food samples were contaminated with bla_{OXA-48} (Table 1). In particular, DNA sequences of the amplicons revealed that they were 100% identical to the plasmid-mediated bla_{OXA-48} genes usually identified on IncL/M plasmids circulating in *Enterobacteriaceae* (13). These sequences showed three nucleotides of difference with the intrinsic $bla_{OXA-48-like}$ genes identified in *Shewanella* species progenitors. Therefore, the genes found in the food samples were likely acquired resistance genes. Conversely, in a fourth sample, a novel $bla_{OXA-48-like}$ variant was identified (see Table 1 footnotes), and this might have derived from an unknown progenitor contaminating the food. Unfortunately, because of the fragmentation of the bacterial DNA, it was not possible to determine the genetic environments (e.g., Tn*1999*-like) of the four $bla_{OXA-48-like}$ genes (13). In particular, no amplification was detected when a primer lending into the $bla_{OXA-48-like}$ gene (OXA-48RVout [5′-TCATACGTGCCTCGCCAATT-3′]) was combined with a primer specific for the genetic vicinity of its 5′ end (IS1999RVout [5′-GGATATTGGCTTCGCGCATC-3′]).

Investigation of the plasmid types showed that most of the samples were contaminated with IncL/M and IncF plasmids, while IncA/C, IncN, and IncI1 plasmids were not present. Remarkably, all the food samples were positive for one or more of the targets of the PCR-typing scheme proposed to detect the IncL/M plasmid pOXA48 (*repA*, *traU*, and *parA*; Table 1) (13, 16). All but two samples were positive for the repFII replicon, suggesting a high prevalence of IncF plasmids, which are widely diffused in *Enterobacteriaceae* and very often associated with the $bla_{CTX-M-15}$ gene (22).

Overall, our data indicate that a relevant contamination of *bla* genes was detected in the pet food, suggesting a reservoir of antibiotic resistance bacteria in the production chain preceding the final packaging. Hence, the source(s) from which the pet food acquired the resistance genes remains to be elucidated. Potential derivations may be the original food components themselves or other ingredients that were added to the mixture, as well as the various steps in the food production process. When looking at the designated ingredients, no clear correlations were detected at the level of the different *bla* genes to the respective food components. The comparison of the detected resistance genes to the respective countries of production and to the factories that produced the different brands gave no clear correlations. This is partially due to the fact that, for almost half of the packages, it was not stated from which country within the European Union the respective products were manufactured (Table 1).

It is particularly relevant that the resistance genes detected in this study (e.g., bla_{OXA-48}, $bla_{CTX-M-15}$, $bla_{VEB-4-like}$, and bla_{CMY-4}) are those typically found in human rather than in animal settings, while it would have been expected to find other gene variants such as $bla_{CTX-M-1}$ and bla_{CMY-2} (6). This observation is also supported by the fact that no plasmids demonstrated to be prevalent in food-producing animals and in food of animal origin (e.g., the IncN and IncI1 plasmids associated with $bla_{CTX-M-1}$ or the IncA/C and IncI1 plasmids associated with bla_{CMY-2}) were found in the food (6, 10, 12, 23, 24). However, we emphasize that the detection of bla_{OXA-48} is worrisome due to its epidemiological and clinical impact (25). The bla_{OXA-48} gene is usually detected in humans (3, 26), but bla_{OXA-48}-possessing *Enterobacteriaceae* isolates were found recently in pets (27).

In conclusion, our data point toward a likely environmental or human-made contamination source(s) that may have taken place during the various steps of the production process of the pet food. Since bacteria were killed during the preservation process (heat treatment step), and contaminating bacterial DNA was highly fragmented, it is unlikely that these resistance genes were transmitted among the bacterial flora in animals fed with this food. However, it is important to trace the source of the drug-resistant bacteria and to prevent the contamination of pet food. These results should raise awareness of the importance of identifying the contamination source(s) because it might be an original niche of clinically important multidrug-resistant bacteria and associated mobile genes.

ACKNOWLEDGMENTS

This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and partially by grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office (BVET). Salome N. Seiffert is a Ph.D. student supported by the IFIK and the BVET.

REFERENCES

1. **Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. J. Antimicrob. Chemother. **68:**2249–2254. http://dx.doi.org/10.1093/jac/dkt208.
2. **Pitout JD.** 2013. *Enterobacteriaceae* that produce extended-spectrum β-lactamases and AmpC β-lactamases in the community: the tip of the iceberg? Curr. Pharm. Des. **19:**257–263. http://dx.doi.org/10.2174/138161213804070348.
3. **Seiffert SN, Perreten V, Johannes S, Droz S, Bodmer T, Endimiani A.** 2014. OXA-48 carbapenemase-producing *Salmonella enterica* serovar Kentucky isolate of sequence type 198 in a patient transferred from Libya to Switzerland. Antimicrob. Agents Chemother. **58:**2446–2449. http://dx.doi.org/10.1128/AAC.02417-13.
4. **Nordmann P, Naas T, Poirel L.** 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. **17:**1791–1798. http://dx.doi.org/10.3201/eid1710.110655.
5. **Verraes C, Van Boxstael S, Van Meervenne E, Van Coillie E, Butaye P, Catry B, de Schaetzen MA, Van Huffel X, Imberechts H, Dierick K, Daube G, Saegerman C, De Block J, Dewulf J, Herman L.** 2013. Antimicrobial resistance in the food chain: a review. Int. J. Environ. Res. Public Health **10:**2643–2669. http://dx.doi.org/10.3390/ijerph10072643.
6. **Seiffert SN, Hilty M, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? Drug Resist. Updat. **16:**22–45. http://dx.doi.org/10.1016/j.drup.2012.12.001.
7. **Timofte D, Dandrieux J, Wattret A, Fick J, Williams NJ.** 2011. Detection of extended-spectrum-β-lactamase-positive *Escherichia coli* in bile isolates from two dogs with bacterial cholangiohepatitis. J. Clin. Microbiol. **49:**3411–3414. http://dx.doi.org/10.1128/JCM.01045-11.
8. **Sallem RB, Gharsa H, Slama KB, Rojo-Bezares B, Estepa V, Porres-Osante N, Jouini A, Klibi N, Saenz Y, Boudabous A, Torres C.** 2013. First detection of CTX-M-1, CMY-2, and QnrB19 resistance mechanisms in fecal *Escherichia coli* isolates from healthy pets in Tunisia. Vector Borne Zoonotic Dis. **13:**98–102. http://dx.doi.org/10.1089/vbz.2012.1047.
9. **Donati V, Feltrin F, Hendriksen RS, Svendsen CA, Cordaro G, Garcia-Fernandez A, Lorenzetti S, Lorenzetti R, Battisti A, Franco A.** 2014. Extended-spectrum-β-lactamases, AmpC beta-lactamases and plasmid mediated quinolone resistance in *Klebsiella* spp. from companion animals in Italy. PLoS One **9:**e90564. http://dx.doi.org/10.1371/journal.pone.0090564.
10. **Seiffert SN, Tinguely R, Lupo A, Neuwirth C, Perreten V, Endimiani A.** 2013. High prevalence of extended-spectrum-cephalosporin-resistant *Enterobacteriaceae* in poultry meat in Switzerland: emergence of CMY-2- and VEB-6-possessing *Proteus mirabilis*. Antimicrob. Agents Chemother. **57:**6406–6408. http://dx.doi.org/10.1128/AAC.01773-13.

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

11. **Doi Y, Paterson DL, Egea P, Pascual A, Lopez-Cerero L, Navarro MD, Adams-Haduch JM, Qureshi ZA, Sidjabat HE, Rodriguez-Bano J.** 2010. Extended-spectrum and CMY-type β-lactamase-producing *Escherichia coli* in clinical samples and retail meat from Pittsburgh, USA and Seville. Spain. Clin. Microbiol. Infect. **16:**33-38. http://dx.doi.org/10.1111/j.1469-0691.2009.03001.x.
12. **Voets GM, Fluit AC, Scharringa J, Schapendonk C, van den Munckhof T, Leverstein-van Hall MA, Stuart JC.** 2013. Identical plasmid AmpC β-lactamase genes and plasmid types in *E. coli* isolates from patients and poultry meat in the Netherlands. Int. J. Food Microbiol. **167:**359–362. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.10.001.
13. **Poirel L, Bonnin RA, Nordmann P.** 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob. Agents Chemother. **56:**559–562. http://dx.doi.org/10.1128/AAC.05289-11.
14. **Bonnin RA, Nordmann P, Carattoli A, Poirel L.** 2013. Comparative genomics of IncL/M-type plasmids: evolution by acquisition of resistance genes and insertion sequences. Antimicrob. Agents Chemother. **57:**674–676. http://dx.doi.org/10.1128/AAC.01086-12.
15. **Dallenne C, Da Costa A, Decre D, Favier C, Arlet G.** 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*. J. Antimicrob. Chemother. **65:**490–495. http://dx.doi.org/10.1093/jac/dkp498.
16. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
17. **Lartigue MF, Poirel L, Nordmann P.** 2004. Diversity of genetic environment of *bla*(CTX-M) genes. FEMS Microbiol. Lett. **234:**201–207. http://dx.doi.org/10.1111/j.1574-6968.2004.tb09534.x.
18. **Rodriguez MM, Power P, Radice M, Vay C, Famiglietti A, Galleni M, Ayala JA, Gutkind G.** 2004. Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. Antimicrob. Agents Chemother. **48:**4895–4897. http://dx.doi.org/10.1128/AAC.48.12.4895-4897.2004.
19. **Jacoby GA.** 2009. AmpC beta-lactamases. Clin. Microbiol. Rev. **22:**161–182. http://dx.doi.org/10.1128/CMR.00036-08.
20. **Zhao S, White DG, McDermott PF, Friedman S, English L, Ayers S, Meng J, Maurer JJ, Holland R, Walker RD.** 2001. Identification and expression of cephamycinase bla_{CMY} genes in *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. Antimicrob. Agents Chemother. **45:**3647–3650. http://dx.doi.org/10.1128/AAC.45.12.3647-3650.2001.
21. **Vogt D, Overesch G, Endimiani A, Collaud A, Thomann A, Perreten V.** 28 April 2014. Occurrence and genetic characteristics of third-generation cephalosporin-resistant *Escherichia coli* in Swiss retail meat. Microb. Drug Resist. http://dx.doi.org/10.1089/mdr.2013.0210.
22. **Villa L, Garcia-Fernandez A, Fortini D, Carattoli A.** 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J. Antimicrob. Chemother. **65:**2518–2529. http://dx.doi.org/10.1093/jac/dkq347.
23. **Endimiani A, Rossano A, Kunz D, Overesch G, Perreten V.** 2012. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. Diagn. Microbiol. Infect. Dis. **73:**31–38. http://dx.doi.org/10.1016/j.diagmicrobio.2012.01.004.
24. **Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ, National ESBL Surveillance Group.** 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol. Infect. **17:**873–880. http://dx.doi.org/10.1111/j.1469-0691.2011.03497.x.
25. **Guerra B, Fischer J, Helmuth R.** 2014. An emerging public health problem: acquired carbapenemase-producing microorganisms are present in food-producing animals, their environment, companion animals and wild birds. Vet. Microbiol. **171:**290–297. http://dx.doi.org/10.1016/j.vetmic.2014.02.001.
26. **Poirel L, Potron A, Nordmann P.** 2012. OXA-48-like carbapenemases: the phantom menace. J. Antimicrob. Chemother. **67:**1597–1606. http://dx.doi.org/10.1093/jac/dks121.
27. **Stolle I, Prenger-Berninghoff E, Stamm I, Scheufen S, Hassdenteufel E, Guenther S, Bethe A, Pfeifer Y, Ewers C.** 2013. Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. J. Antimicrob. Chemother. **68:**2802–2808. http://dx.doi.org/10.1093/jac/dkt259.

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

24th ECCMID Barcelona, Spain 10 – 13 May 2014
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
P1639

High prevalence of CTX-M-, CMY-, and OXA-48-like *bla* genes in food for pets

Salome N. Seiffert,[1,2,3*] Regula Tinguely,[1] Agnese Lupo,[1] Markus Hilty,[1] Vincent Perreten,[2] and Andrea Endimiani [1#]

[1] Institute for Infectious Diseases, [2]Institute of Veterinary Bacteriology, [3]Graduate School for Cellular and Biomedical Sciences; University of Bern, Switzerland; (*Email: salome.seiffert@ifik.unibe.ch; #aendimiani@gmail.com)

Introduction:

Extended-spectrum β-lactamase (**ESBL**) and plasmid-mediated AmpC (**pAmpC**) β-lactamase producing *Enterobacteriaceae* have become a major threat to public health, especially after the emergence and escalating number of community-acquired infections (Pitout JD, Curr Pharm Des., 2013). The situation has even become worse since also **carbapenemase** (e.g., KPC, OXA-48, NDM) producers, spread around the world (Nordmann P et al., Emerg Infect Dis., 2011).

The current situation in the animal population (pets and food animals) is similar, though not as dramatic as in the human context (Seiffert SN et al., Drug Resist Updat., 2013).

Moreover, a lot of studies have investigated animals as a potential reservoir as well as vector for the transmission of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (**ESC-R-*Ent***).

However, there is scarce information regarding the source(s) of their colonization or even infection.

In this study, we evaluated pet food as potential origin of ESC-R-*Ent* and/or antimicrobial resistance genes (i.e., β-lactamase genes, *bla*).

Materials and Methods:

We analyzed 30 pet food packages purchased in September 2013 at different grocery stores located in Bern (Switzerland). The main ingredients (food content declaration), country of origin, brand name and food manufacturer, as indicated on the label marking the packages, are listed in Table 1.

Ten grams of raw pet food were cultured in 20 ml of LB. After overnight incubation, 50 µl of the culture were plated onto selective chromID ESBL (bioMérieux), Drigalski plus cefotaxime, and MacConkey plus ceftazidime plates (Oxoid).

DNA isolation was directly performed on the raw pet food using the QIAamp DNA Mini kit (QIAGEN).

The DNA extract of the food samples were characterized with:

- PCR/DNA sequencing for bla_{ESBLs} (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}gr.1}$, $bla_{CTX\text{-}M\text{-}gr.9}$, and bla_{VEB}), bla_{pAmpCs} (bla_{CMY}, bla_{DHA}) and carbapenemase genes ($bla_{OXA\text{-}48\text{-}like}$, bla_{IMP}, bla_{VIM}, and bla_{NDM});
- PCRs for various Inc groups, i.e., IncA/C, IncN, IncII, IncF, and IncL/M using primers from the PBRT kit (Diatheva);
- Samples positive for the IncL/M replicon were further analyzed by amplification of the *parA*, *repA* and *traU* genes (data not shown);
- PCR for class I/II integrons;
- PCR for 16S rDNA of bacteria

Table 1: Epidemiological characteristics and DNA amplicons of relevant resistance traits from 30 pet food samples

Sample ID	Food for	Food components (% declared on label)	Country [a]	Stores	Brand	Manufactory [b]	*Bla* and class 1 integrase gene [h]								IncL/M plasmid typing [e, f, h]				IncF	16S rDNA [e, g]
							OXA-48	CTX-M-gr.1	CMY	VEB	TEM [e]	SHV [e]	DHA [e]	Int1 [e]	TraU	ParA	RepA	repFII		
101	Cat	Beef, Chicken (4% each)	GER	A	1	I	-	CTX-M-3/-15[d]	-	VEB-4-like	+	+	-	+	-	-	+	+	+	++
102	Cat	Veal, Poultry (4% each)	GER	A	1	I	+	CTX-M-3/-15[d]	CMY-4	-	+	+	-	+	-	-	+	+	+	+
103	Cat	Salmon, Trout (4% each)	GER	A	1	I	-	-	-	-	-	-	-	+	+	+	+	+	+	+++
104	Cat	Poultry, Liver (4% each)	GER	A	1	I	+	CTX-M-3/-15[d]	-	-	+	+	-	+	-	-	+	+	+	++
106	Cat	Turkey (14%), Rabbit (8%)	GER/NED	A	2	I, II	-	CTX-M-3/-15[d]	-	-	+	+	+	+	-	-	+	-	-	-
107	Cat	Beef (14%), Turkey hearts (8%)	GER/NED	A	2	I, II	-	-	-	-	-	-	+	+	+	-	+	+	+	+
109	Cat	Salmon, Chicken (each 4%)	EU	A	3	III	-	CTX-M-3/-15[d]	-	-	+	-	-	+	+	-	+	+	+	++
1010	Cat	Beef (4%)	EU	A	3	III	-	+	-	-	+	-	-	+	-	-	+	+	+	++
1011	Cat	Turkey, Duck (each 4%)	EU	A	3	III	-	-	CMY-4	-	+	-	-	+	+	+	+	+	+	++
1013	Dog	Beef (4%)	EU	A	4	IV	-	CTX-M-3/-15[d]	-	-	+	+	-	+	+	-	+	+	+	++
1014	Dog	Lamb (4%)	EU	A	4	IV	-	-	-	-	+	+	-	-	+	+	+	+	+	-
1015	Dog	Rabbit (4%)	EU	A	4	IV	OXA-48	-	-	VEB-4-like	+	+	-	+	-	-	+	+	+	++
1016	Dog	Turkey (4%), Peas/Carrots (each 2%)	LIE	A	5	V	-	-	-	-	+	+	-	+	+	+	+	+	+	+
1017	Cat	Poultry (4%)	DEN	B	6	VI	-	-	-	-	-	-	-	-	+	-	+	+	+	++
1018	Cat	Chicken (8%), green Beans (5%)	EU	B	7	VII	-	+	-	-	+	+	-	-	+	+	+	+	+	++
1020	Cat	Turkey (4%)	EU	B	8	VIII	-	-	-	-	+	+	-	+	+	-	+	+	+	+
1021	Cat	Poultry (4%)	EU	B	8	VIII	-	+	-	-	+	+	-	+	+	-	-	-	-	+
1023	Dog	Beef, Chicken (each 4%)	NED	B	9	IX	OXA-48	+	-	-	+	-	-	+	-	-	+	+	+	++
1024	Dog	Turkey, Chicken (each 4%)	EU	B	10	IV	-	CTX-M-15	CMY-4	-	+	+	+	+	-	-	+	+	+	+++
1025	Dog	Beef, Liver (each 4%)	EU	B	10	IV	-	CTX-M-3/-15[d]	CMY-4	-	+	+	-	+	+	-	+	+	+	++
1026	Dog	Poultry (4%)	FRA	B	11	X	-	CTX-M-15	+	-	+	+	-	+	-	-	+	+	+	+++
1027	Cat	Chicken (4%)	EU	C	12	VIII	-	CTX-M-15	-	-	+	+	-	+	+	-	+	+	+	+
1028	Cat	Poultry (4%)	EU	C	12	VIII	-	+	-	-	+	+	+	+	+	-	+	+	+	+
1029	Cat	Chicken, Turkey (4% each)	LIE	C	13	V	+	-	-	-	+	+	-	+	-	-	+	+	+	++
1030	Cat	Beef (4%)	LIE	C	14	V	-	-	-	-	+	+	-	+	-	-	+	+	+	++
1031	Cat	Poultry (4%)	LIE	C	14	V	OXA-48-like[c]	CTX-M-15	CMY-4	-	+	-	-	+	-	-	+	+	+	+
1032	Cat	Poultry (4%)	EU	C	15	NA	-	CTX-M-15	-	-	+	+	-	+	+	-	+	+	+	+++
1033	Dog	Beef (4%)	NED	C	16	XI	+	-	+	-	+	-	-	+	-	-	+	+	+	+++
1034	Dog	Chicken (14%), Rice (4%)	GER/NED	C	17	I, II	-	-	-	-	+	-	-	+	+	-	+	+	+	++
1035	Dog	Turkey (14%), Rice (4%)	GER/NED	C	17	I, II	OXA-48	-	-	-	+	+	-	+	-	-	+	+	+	++

Note. +, PCR positive amplicon; -, PCR negative amplicon

[a] Country of production; GER= Germany; NED= The Netherlands; FRA= France; LIE= Liechtenstein; DEN= Denmark; EU= European Union (the specific country was not declared)

[b] Declared manufacturer of a brand; some brands produce their products at the same manufactory (i.e., Manufactory VIII produces for brands #8 and #12). Brand #2 has two manufacturers (I and II). NA; not available due to unreadable labelling

[c] OXA-48-like sequence contains the following amino acid changes compared to OXA-48 (Trp25Leu, Gln41Leu, Thr99Met, Glu168Gln, and Ser171Ala) or OXA-181 (Trp25Leu, Gln41Leu, Thr99Met, Ala104Thr, and Asp110Asn). Unfortunately, the *bla* gene was not fully sequenced

[d] The presence of both CTX-M-3 and CTX-M-15 are suspected in these samples: the DNA sequence of the $bla_{CTX\text{-}M\text{-}gr.1}$ demonstrated a double pick in the DNA sequence corresponding to amino acid position 242, discriminating the CTX-M-3 and CTX-M-15 variants (Asp242 in CTX-M-3 and Gly242 in CTX-M-15; GenBank accession No.: Y10278 and AY044436, respectively)

[e] No sequencing of the amplicon was performed

[f] Primer pairs detecting the IncL/M *repA*, *parA* and *traU* genes were used as previously described by Poirel L et al., AAC, 2012.

[g] The bacterial contamination was evaluated by amplification of the 16S rDNA: i.e., "-", negative; "+", faint band; "++", clearly visible band; and "+++", strong band

[h] *Bla* and replicon genes (see material and methods section) resulted PCR negative have not been reported in the present table

Results:

The food composition showed that **only 4-14% of the component(s) constituting the overall food product was declared in detail**. The declaration of the remaining compounds (78-96%) was imprecise. On the majority of the food samples (73%, n=22), poultry meat from chicken, duck, and/or turkey was advertised on the label. Less frequently listed ingredients were: beef or veal, vegetables, fish, rabbit, liver and/or lamb.

All the pet food products were originally produced outside Switzerland.

No detectable growth after selective cultivation was observed in any of the samples. However, twenty-eight **(93%) food samples resulted PCR positive for the 16S rDNA.**

Twenty-seven **(90%) samples were PCR positive for the *intI1* gene** (Table 1), while all samples were negative for the *intI2* gene.

Most of the samples were **positive for IncL/M (100%) and IncF (93%) plasmids**, while none of the isolates carried IncA/C, IncN, and IncI1 plasmids.

In 21 out of 30 food products **(70%) one or more bla_{ESBL} and/or bla_{pAmpC} gene was detected**. Of the 18 bla_{ESBL} samples; 17 isolates harbored **$bla_{CTX\text{-}M\text{-}gr.1}$ (57%)** and two were positive for the **bla_{VEB} gene (7%)**. One isolate harbored both genes. From 12 $bla_{CTX\text{-}M\text{-}gr.1}$ positive amplicons good sequences were obtained: five revealed the presence of the **$bla_{CTX\text{-}M\text{-}15}$**, whereas seven carried both **$bla_{CTX\text{-}M\text{-}3}$** and $bla_{CTX\text{-}M\text{-}15}$ genes. The two bla_{VEB} positive amplicons were **$bla_{VEB\text{-}4\text{-}like}$** variants. Ten samples were positive for **bla_{pAmpC} (33%)**, of which seven and four of them for **bla_{CMY} (23%)** and **bla_{DHA} (13%)**, respectively. Sequences from five bla_{CMY} positive isolates revealed the presence of $bla_{CMY\text{-}4}$ gene.

Eight (27%) food samples resulted contaminated with $bla_{OXA\text{-}48\text{-}like}$. Four $bla_{OXA\text{-}48\text{-}like}$ amplicons gave a clear DNA sequences : three confirmed the presence of the **$bla_{OXA\text{-}48}$ gene**, whereas one carried a $bla_{OXA\text{-}48\text{-}like}$ variant.

Conclusions:

This is the **first study exploring the contamination of pet food with ESC-R-*Ent* as well as the prevalence of *bla* genes.**

93% of the food samples resulted PCR positive for the 16S rDNA indicating the existence of **previously living bacteria in the original products.**

In **70%** of the isolates **one or more bla_{ESBL} and/or bla_{pAmpC}** gene were detected. However these *bla*-variants (i.e., $bla_{CTX\text{-}M\text{-}15}$, $bla_{CMY\text{-}4}$, $bla_{OXA\text{-}48}$) are typically more **related to the human context** that to the animal context.

Especially worrisome is the finding that 27% of the food samples were contaminated with $bla_{OXA\text{-}48\text{-}like}$ genes.

This finding reflects an emerging treat for public health coming from acquired carbapenemase-producing bacteria which are emerging in animals.

These results should raise awareness in order to **identify the contamination source(s)** because they might be the original niches of **clinically important multidrug-resistant bacteria and associated mobile genes.**

Acknowledgements:

We thank Prof. Dr. Alessandra Carattoli for her support.

This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and by Grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office (BVET).

Salome N. Seiffert is a PhD student supported by IFIK and BVET.

Chapter IV

In this study, the whole sequence of *bla*$_{CMY}$-carrying IncK plasmids was determined from *E. coli* strains isolated from human infections, poultry and poultry meat *E.coli*. The analysis showed that all the plasmids belonged to a novel variant of incompatibility group of plasmids, the IncK2 group.

The paper will be submitted to the Journal of Antimicrobial Chemotherapy.

Novel IncK2 Plasmids Carrying *bla*$_{CMY}$ in *Escherichia coli*: Evidence of Spread among Poultry, Poultry Meat and Human Infections

Salome N. Seiffert,[1, 2, 3] Alessandra Carattoli,[2,4] Sybille Schwendener,[1] Alexandra Collaud,[1] Andrea Endimiani,[2] and Vincent Perreten[1*]

[1] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland; [2] Institute for Infectious Diseases, University of Bern, Bern, Switzerland; [3] Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland; [4] Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Short running title: Novel *bla*$_{CMY}$ plasmids in poultry and humans

Keywords: ESBL, food, antibiotic resistance, animals

*Corresponding author. Mailing address: Institute of Veterinary Bacteriology, University of Bern, Länggassstrasse 122, CH-3012 Bern, Switzerland, Phone: +41 31 631 24 30. Fax: +41 31 631 26 34. E-mail: vincent.perreten@vetsuisse.unibe.ch

Abstract

Objective: Plasmids are vehicles for transferring resistance genes between bacteria of human and animal origin. However, in-deep analyses of such shared plasmids are still scarce. This study compares whole sequences of *bla*$_{CMY}$-carrying plasmids that originated from diverse *E.coli* from humans, poultry and poultry-meat to evaluate their relationship.

Methods: Nine genetically diverse strains (three from each origin) harboring *bla*$_{CMY}$-carrying IncK-like plasmids were analyzed by PstI digestion and whole plasmid sequencing. Strains were characterized using MLST and MIC determination. Incompatibility testing was performed using conjugation assays.

Results: Plasmids from different sources showed 99% nucleotide identity. The phylogenetic analysis of the incompatibility regions showed that they were forming a new plasmid variant within the IncK and IncB/O group. This novel plasmid variant was designated as IncK2 based on conjugation assays and *inc*RNAI sequences, with respect to the IncK and IncB/O reference plasmids.

Conclusion: Comparative genetic analysis of *bla*$_{CMY}$-carrying plasmids revealed the presence of a common novel IncK plasmid variant, IncK2 in diverse *E. coli* from poultry-meat production and human infections. This finding suggests that the IncK2 plasmids originated from a common progenitor and have the capability to spread to genetically diverse *E.coli* in the different reservoirs. This discovery is highly alarming and should stimulate the rapid introduction of strict hygiene measures along the food chain to stop the spread of such plasmids in the human settings.

Introduction

E. coli represent a large group of bacteria with pathogenic and non-pathogenic strains (1). These strains colonizing or infecting humans and animals are usually specifically adapted to their hosts with the exception of some zoonotic pathogenic *E. coli* (1, 2). Additionally, a large diversity exists among strains of a same source in terms of sequence types (STs), β-lactamase-types as well as plasmid incompatibility groups (3). A few clones have been successful and became epidemic in human hospitals like ST131 (4, 5). The worldwide dissemination of such international *Enterobacteriaceae* clones along with resistance plasmids carrying β-lactamase genes is posing an increasing threat to the public health systems (5).

Poultry and poultry-meat have been revealed as a large reservoir of extended spectrum-β-lactamases (ESBL) and plasmid-mediated AmpC (pAmpC) enzymes in several countries (6-9). These resistance genes are encoded on various mobile genetic elements; however, they are detected primarily on plasmids that are self-replicating extrachromosomal elements. Such plasmids vary not only in their size and compatibility to other plasmid groups but also in their capability of transferring to various bacteria species (10).

The involvement of plasmids in the zoonotic spread of β-lactamases and carbapenemase genes between human and animal reservoirs has been already emphasized (3, 11). Additionally, the presence of similar plasmids in *E.coli* from humans, poultry and poultry-meat has been reported (12, 13). However, firm evidence of the spread of the same plasmids in *E.coli* causing human infections and in *E.coli* from the poultry-meat chain using whole plasmid sequencing (WPS) has not yet been demonstrated (14, 15).

Comparing datasets from poultry, poultry-meat, and clinical human isolates from Switzerland, we noticed that the pAmpC $bla_{\text{CMY-2}}$ and $bla_{\text{CMY-4}}$ genes were associated with plasmids belonging to the IncB/O/K groups (8, 9, 16). Other studies investigating pAmpC-producing *E.coli* have detected the same kind of plasmid in healthy humans (17, 18), poultry (19), and poultry-meat (20). This prompted us to perform an in-depth analysis of bla_{CMY}-carrying IncB/O/K plasmids to demonstrate for the first time that *E.coli* isolates found in Switzerland and in these three settings share the same plasmids, also revealing a novel IncK-group variant, IncK2.

Methods

Bacterial strains

Strains were obtained from previous studies characterizing 3rd-generation cephalosporin-resistant *E.coli* (3GC-R-*Ec*) from poultry, poultry-meat, and human infection (8, 9, 16) as well as from the Laboratory of Microbiology of the Institute of Infectious Diseases, University of Bern (Table 1).

Strains were selected because they were positive for $bla_{\text{CMY-(2/4)}}$ by PCR/DNA sequencing (16) and because they had been assigned to either IncK and/or IncB/O by PCR-based replicon typing (PBRT) of plasmids (21). Plasmids were transformed by electroporation into *E. coli* ElectroMax DH10B competent strains (Thermo Scientific), or into*E. coli* One Shot® TOP10 Electrocomp (Life Technologies) as described previously (9). The MIC of antibiotics was obtained using the microdilution Sensititre panels ESB1F and EUMVS2 (Trek Diagnostics) and interpreted following the CLSI guidelines. Sequence types (ST) were assigned following the multi-locus sequence typing (MLST) Achtman scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Restriction analysis and WPS

Plasmids were purified using the Genopure Plasmid Midi Kit (Roche Diagnostic) according to the manufacturer's procedures. Plasmids were first, digested using PstI (Bioconcept) and run on a 0.8% agarose gel electrophoresis (Figure S1).

WPS was performed using the 454-Junior Genome Sequencer procedure (Roche Diagnostic). The libraries were constructed with the plasmid DNA that was previously purified using the Genopure Plasmid Midi Kit (Roche Diagnostic). The libraries were constructed using the GS FLX Titanium Rapid Library Preparation Kit.

De novo assembly of DNA reads, gap-closure and annotation

Contigs with at least a 70-fold coverage were obtained using the GS-FLX gsAssembler software. The assembly of the contigs was first done in silico by using the 454 ReadStatus output file, generated by the gsAssembler software (Roche Diagnostics), identifying reads overlapping adjacent contigs. The assembly and gap-closure was confirmed and verified by PCR followed by Sanger DNA sequencing. The annotation was done using the Artemis Version 8 (Sanger Institute) in combination with a pairwise alignment that was performed by a BLASTN and BLASTP homology search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic analysis of IncB/O/K plasmids

For the phylogenetic analysis of the IncB/O/K plasmids, two fully sequenced reference plasmids, namely pCT belonging to IncK (GenBank acc. No. FN868832.1; unpublished) and p3521 belonging to IncB (NC_014843.1; (22) were included. These reference plasmids were chosen as they represent the genetic outgroup of the new IncK2 plasmid variant. The homology trees were calculated based on the Observed Divergence method (left panel of Figure 2), whereas the phylogenetic threes were constructed with the Jukes and Cantor model (2000 Bootstrap trials) (right panel of Figure 2) using the DNAMAN phylogenetic analysis software (Lynnon BioSoft). The following settings were applied for the Quick Alignment with a gap penalty of 7, K-tuple of 2, number of top diagonals of 4.

The trees in line A were constructed based on the backbones of the plasmids (Figure 2). This was achieved by removing all mobile genetic elements of the plasmids. For the IncK2 plasmids, the *bla*$_{CMY}$-carrying element was removed (position 42023-45695 in pTMSA970) and in addition for pTMSA970 the IS*26* (position 4801-6009 in pTMSA970). For the reference IncK and IncB plasmids, the *bla*$_{CTX\text{-}M\text{-}14}$-carrying element was removed from the IncK reference plasmid (position 68837-71981 in FN868832.1) and the 26kb large IS*26* element in the IncB reference plasmid (position 59569-86117 in NC_014843.1). The trees in line B were constructed additionally removing the shufflon. The shufflon region starts at the shufflon-specific DNA recombinase (*Rci*) and ends with the last shufflon gene in each of the plasmids including the references (position 62686- 65450 in pTMSA970).

Conjugation for incompatibility testing

Mating experiments were performed crossing the transformant strain containing plasmid p5312.29 and the one containing pTMSA970 with reference plasmids Rhh72 (IncB/O), and R387 (IncK) (provided by the Istituto Superiore di Sanità, Rome, Italy) which are used for incompatibility testing (23). Matings were performed for 24h at 37°C on solid Luria-Bertani (LB) agar plates without antibiotics. Donors and transconjugants were counted plating serial dilutions on LB plates containing 50 µg/ml of ampicillin, 50 µg/ml kanamycin, 25 µg/ml chloramphenicol, 50 µg/ml of ampicillin plus 50 µg/ml kanamycin, and 50 µg/ml of ampicillin plus 25 µg/ml chloramphenicol.

PBRT update for the IncK2 plasmid variant

A novel reverse primer (NAME: 5`-GCCACAAGCTGTCGCTTA-3`) that can be used in combination with the universal IncB/O-K forward primer (NAME: 5`-GCGGTCCGGAAAGCCAGAAAAC-3`) of the PBRT kit was designed to generate a 242bp PCR product in order to distinguish the IncK2 from the IncB/O/K group by PCR and sequence analysis. Amplification conditions were the followings: 1 cycle

of denaturation at 94°C for 10 minutes, continued by 30 cycles of denaturation at 95 °C for 1 minute, annealing at 60°C for 30 seconds and elongation at 72 °C for 1 minute, followed by 1 cycle at 72°C for 5 minutes.

Nucleotide sequence accession numbers

The complete nucleotide sequence of the seven IncK2 plasmids have been deposited into the Genebank database under accession numbers KR905385 (p5312.29), KR905389 (p4809.66), KR905387 (pTMSA992), KR905388 (pTMSA970), KR905386 (pTMSA1088), KR905384 (pDV45), and KR905390 (pDV10).

Results and Discussion

Restriction analysis and plasmid sequencing

The comparison by restriction of nine plasmids obtained from humans, poultry and poultry meat isolates illustrates high degree of relatedness among each other (Figure S1). Identical plasmid restriction profiles were observed for five plasmids including three from human (plasmids p5312.29, p4809.66, and p4901.67), one from poultry (pTMSA992), and one from poultry meat isolate (pDV45). Plasmids from four additional isolates from poultry (pTMSA1088, pTMSA970), and poultry meat strains (pDV10, pDV56) also showed highly-related restriction profiles, differing for one or two bands only.

Seven plasmids were selected for complete sequencing, as representative of the different variants detected by restriction. WPS revealed the presence of common bla_{CMY}-containing plasmids in genetically diverse *E. coli* from poultry (ST38, ST420), poultry meat (ST38, ST1564) and human infections (ST131, ST1431). Plasmids differed from each other within the shufflon region, and the highly variable region situated between *impC* and *nikA*. A major deletion of approximately 6 kb, that starts after *yfbA* and ends before *psiB*, distinguished plasmids pDV10, pTMSA1088, pTMSA970 pTMSA992 from the larger plasmids pDV45, p4809.66, and p5312.29. Plasmid pTMSA970 had also an extra insertion of IS*26*.

The highly variable region between *impC* and *nikA* also harbors, along with many genes of unknown function, the putative antirestriction protein YfeB as well as the single-stranded DNA-binding (SSB) protein. The main functions of the SSB protein are binding of ssDNA and subsequent protection from degradation by nucleases, whereas the function of the antirestriction protein YfeB is not yet defined. The direct consequences of the loss of SSB and of YfeB for the IncB/O/K-group plasmids are difficult to determine and need further investigations.

The shufflon is a biological switch that controls the C-terminal end of the PilV proteins via site-specific recombination. This mechanism was described in R64 as well as in pCT where the shufflon, despite being part of a minor component of the thin pilus, determines the recipient specificity in liquid mating (24, 25). The shufflon of R64 consists of four invertible DNA fragments embedded of seven 19bp repeat sequences. Those 19bp repeats can be subdivided into the 7bp core-site region where the DNA crossover occurs and the highly conserved 12bp right arm region (26). The shufflon of the seven plasmids is highly diverse and contains from two to eight recombination sites. Four, from R64 distinct 19-bp repeat sequences were identified. Interestingly, the 7bp core-site region is identical to the ones

identified in the IncI1/I2 plasmids. Their terminology is following the designation of the repeats in R64 (24) (i.e., repeat-e: *GTGCCAA*TCCGGTtcgTGG; repeat-f: *GTGCCAA*TCCGGTatcTGG; repeat-g: *GTGCCAA*TCCGGTGtgtGG; repeat-h: *GTGCCAA*TCCGGTttgTGG; letters in *italic* represent the core-site, small letters for the variable bases).

The three plasmids pTMSA970, pTMSA992, and p5312.29 have only two repeat-e sequences, followed by p4809.66 with four repeat sequences in the following order: e-e-e-g. The most complex shufflons with eight repeat sequences each were detected in pDV10 (g-g-e-e-e-e-h-f), pDV45 (e-e-e-e-g-g-h-f), and pTMSA1088 (e-e-h-f-e-e-g-g). Although, no apparent correlation between the numbers of recombination sites could be detected (neither with the plasmid size of the plasmid nor with their origin), the observation of such great diversity may have an impact of the mating preferences of the various plasmids and should therefore be further investigated.

Incompatibility assays

Sequence analysis of the region upstream of the *repA* including the *inc*RNAI revealed that the DNA sequences of the seven plasmids diverged from the previously reported *inc*RNAI of the IncK, IncB and IncB/O plasmids (Figure 4). This region before the *repA* is highly conserved among the specific plasmid Inc groups and is crucial for the incompatibility behavior among plasmids of the same group (23, 27). The key element for this behaviour is the *inc*RNAI that is forming a stem-and loop structure and negatively regulates the transcription of *repA*. Consequently, if two plasmids of the same or similar Inc group(s) reside in the same bacterial cell the *inc*RNAIs hybridize with each other and prohibiting translation (23). Additionally, a phylogenetic tree constructed using only the *inc*RNAI sequences which contain the stem-and loop structure strengthened the previous observations that the seven plasmids are highly similar to each other, and that they are more closely related to the IncK group that to the IncB/O groups.

The divergent *inc*RNAI sequences suggested that these plasmids could be compatible with the IncK and IncB/O reference plasmids. Therefore, incompatibility assays were performed crossing 5312.29 and TMSA970 strains with both IncB/O Rhh72 and IncK R387 reference strains, respectively. Transconjugants were obtained from all mating experiments but at different frequency. Both 5312.29 and TMSA970 showed higher frequency of conjugation when crossed with IncB/O (1×10^{-5} transconjugant/donor cell) than when crossed with IncK (1×10^{-7} transconjugant/donor cell) reference plasmid. This suggests that the plasmids under study are more closely related to IncK than to IncB/O. Based on both conjugation results and *inc*RNAI sequence analysis, the plasmids were assigned to a novel variant of the IncK group designated as IncK2.

Phylogenetic analysis of the IncK2 plasmids

A solid indication that all the IncK2 plasmids analyzed have a common ancestor is the fact that the *bla*$_{CMY}$-carrying mobile element (IS*Ecp1*-*bla*$_{CMY-(2/4)}$-*blc*-*sugE1*) is integrated at the same site in all of the seven plasmids inside the *tra* locus and between the *traT* and *TraU* (Figure 2). This identical structure and integration site have been already reported in an IncK plasmids (18). Notably, the only *bla*$_{CMY-4}$-carrying plasmid pDV10 has also developed from the same progenitor as the other plasmids and was subjected to subsequent evolution via a Trp221Arg substitution in *bla*$_{CMY-2}$, which is the single amino acid substitution which distinguishes CMY-4 from CMY-2.

The phylogenetic analysis of the IncK2 plasmids supported the hypothesis of a common ancestor, as the plasmid-backbone homology of the seven plasmids was 99% (Figure 2A). This similarity is remarkable given that the percentage of homology dropped to 84% for the two reference plasmids IncK and IncB. The new IncK2 plasmids are closely-related to the IncK and IncB references. The phylogenetic tree shows a similar topology in which the IncK2 plasmids are a very homogenous cluster with both reference plasmids at the same distance (Figure 2A). However, when deleting the shufflon region the plasmid-backbone homology among all the IncK2 plasmids increases to 100% (Figure 2B), whereas the homology stays constant at 85% regarding the two references IncK and IncB. Due to the highly probable involvement of the shufflon region in the mating preferences of the respective isolate, a process that is only indirectly linked to the evolution of the remaining plasmid sequence, the increase in homology to 100% can be taken as another firm indication for a common ancestor of all the IncK2 plasmids.

Taken together, these results lead to the assumption that the IncK2 plasmids are an overall highly homogenous cluster although they are distributed in genetically diverse *E.coli* isolated from different sources and time frames. The phylogenetic analysis points towards a common ancestor that has subsequently spread between the various reservoirs. Indeed, the most striking characteristic of the IncK2 group is the broad reservoir (including poultry, poultry-meat and humans isolates) from which they were isolated. There is strong evidence that all IncK2 plasmids originated from a common source due to: *i)* the identical integration site of the *bla*$_{CMY}$-carrying mobile element; *ii)* the overall 99% homology among all the plasmids; and *iii)* the increase of homology to 100% once the shufflon is excluded from the comparison.

Recent publications from the Netherlands, Sweden, and Japan investigating pAmpC-producing *E.coli* isolates from healthy and hospitalized humans as well as strains from poultry and poultry-meat, have reported a high prevalence of *bla*$_{CMY-2}$ in both reservoirs (14, 15, 20, 28-30). Using the PBRT kit, a substantial part of the *bla*$_{CMY-2}$-carrying plasmids was localized on either IncK plasmids or IncB/O

plasmids. It has to be noted that by using the PBRT the IncK2 plasmid-carrying strains may have had falsely identified as positive for the IncK or IncB/O plasmid groups. Indeed, all the strains of our study showed a cross-reaction with IncB/O, an issue that was already discussed in the paper from Carattoli et al. 2005 (21). Hence, it can be assumed that they may also belong to the novel IncK2 incompatibility group.

In our study we observed that five of the nine plasmids were carried by clones of the ExPEC group (i.e., ST38 in one poultry and two poultry-meat strains and ST131 in one human isolate) indicating that ExPEC may have better affinity for IncK2 plasmids. The novel IncK2 group seems to be an emerging epidemic plasmid with a preference for *E.coli* isolated from human- and poultry-related reservoirs.

To our knowledge, this is the first time that a highly conserved plasmid family is described in isolates from human infection, poultry, and poultry meat all coming from the same geographic region. However, further epidemiological studies are needed to assess if poultry and poultry meat are the main reservoir of this plasmid. Nevertheless, the presence of a common plasmid in three different settings is alarming and should stimulate rapid of adequate measures in the meat production chain in order to limit the spread of broad-host range drug-resistant plasmids.

ACKNOWLEDGMENTS

This work was supported by Grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office and internal funds of both, the Institute of Infectious Diseases (IFIK) and the Institute of Veterinary Bacteriology (IVB), University of Bern. Salome N. Seiffert is a PhD student assigned at both IFIK and IVB.

TRANSPARENCY DECLARATION

None declared

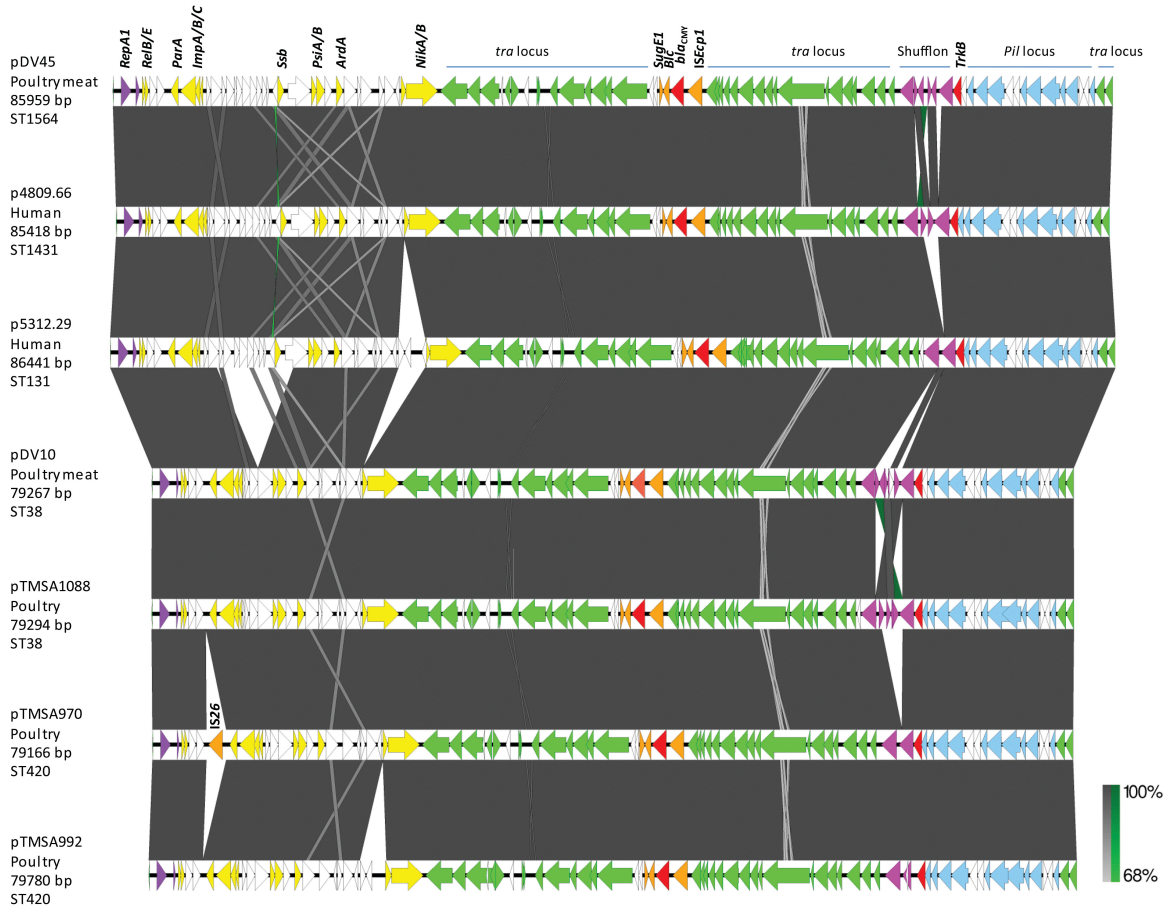


Figure 1: Structural features of seven fully sequenced IncK2 plasmids. The open reading frames are illustrated in arrows pointing towards their respective orientation (Figure created by EasyFig). The colour code is as follows: The *tra* and *trb* transfer loci are in green, antibiotic resistance genes in red, mobile elements (IS) in orange, replicase genes in purple, partitioning genes and toxin/anti-toxin and other stabilization systems in yellow, shufflon-associated genes in violett, pilus genes in pale blue, and genes with unknown function are in white. Important genes and loci are indicated above the first plasmid sequence. Plasmid sequence pTMSA970 carries an additional IS*26* element which is indicated in the figure. The blue lines above the figure indicate the different loci and the shufflon-region. The indications on the left hand side are the respective plasmid profile (see Figure S1).

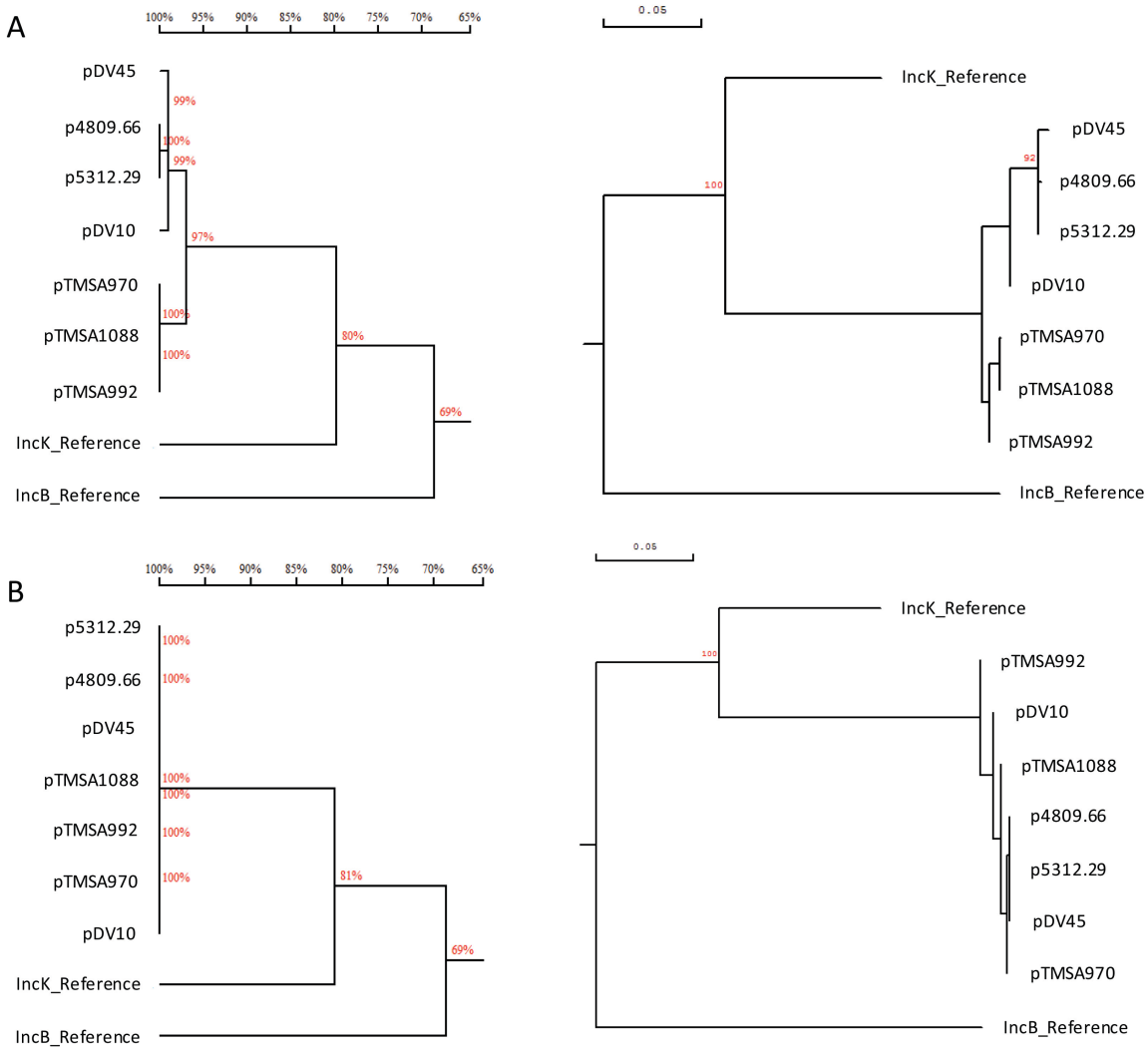
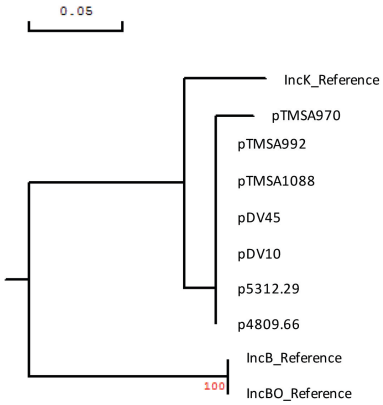


Figure 2: Phylogenetic analysis of the IncB/O/K group of plasmids. The analysis includes the plasmids of the novel IncK2 group as well as the reference plasmids pCT and p3521of the IncK (FN868832) and IncB (NC_014843) groups (Figure was created by DNAMAN). A) The two figures in the upper part are based on the analysis of the entire plasmid sequences whereas in B) the analysis is performed on the same plasmid sequences but lacking the shufflon region. On the left hand side are the homology trees that were created using the Observed Divergence method whereas the figures on the right hand side represent phylogenetic trees that were calculated based on the Jukes and Cantor method with a Bootstrap value of 2000.

*inc*RNAI phylogenetic tree
(2000 boostrap trials)



*inc*RNA nucleotide alignment

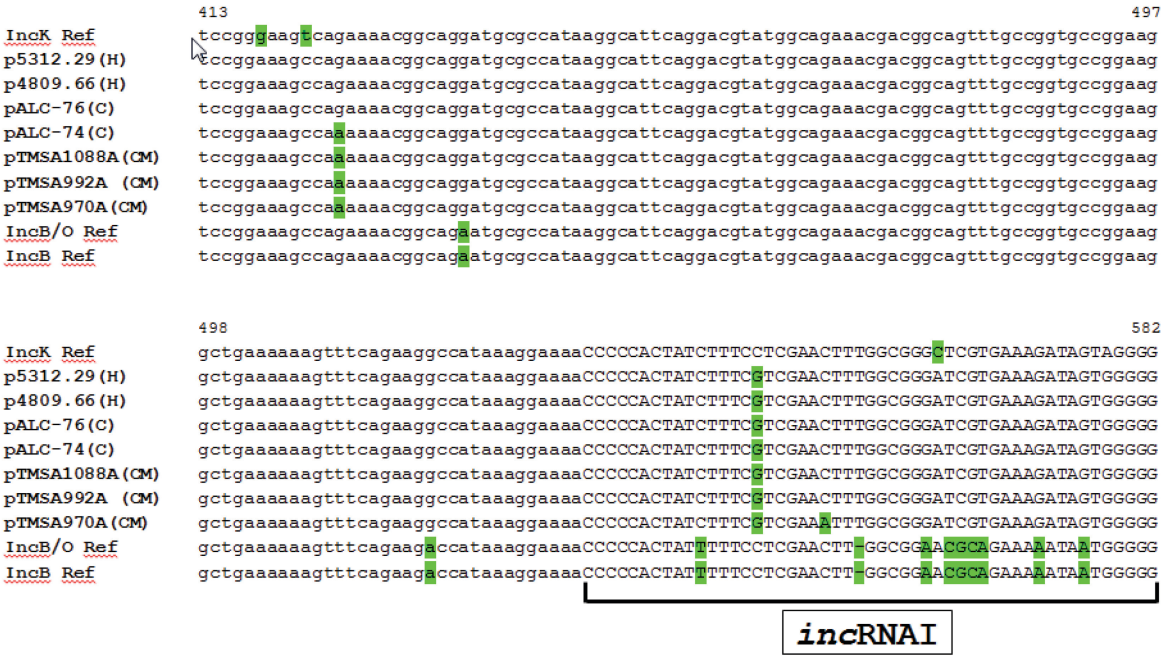


Figure 3: Phylogenetic tree and sequence alignment of the *inc*RNAI-II of the IncB/O/K-group plasmids including references. The sequences were extracted from the IncK2 plasmids as well as from the reference plasmids of IncB and IncK. The IncB/O reference sequence derived from the paper of Couturier et al, 1988 (13). The phylogenetic tree was constructed (DNAMAN) with the *inc*RNAI sequences only (in capital letters) using the Jukes and Cantor model (2000 Bootstrap trials). Abbreviations: H= human infection; C=chicken; CM=poultry meat.

Strain ID	4809.66		DV56		DV10		DV45		4901.67		5312.29		MSA1088 A		MSA992 A		MSA970 A		Perents	
Transformant/Plasmid ID		p4809.66 [a]		pDV56 [b]		pDV10 [b]		pDV45 [b]		p4901.67 [a]		p5312.29 [a]		TMSA1088 [b]		TMSA992 [b]		TMSA970 [b]		
Source	Human		Poultry meat		Poultry meat		Poultry meat		Human		Human		Poultry		Poultry		Poultry		DH10B	TOP10
Date/Origin of isolation	2011, Bern		2013, Bern		2013, Bern		2013, Bern		2011, Bern		2014, Bern		2010/11, Lucerne		2010/11, Lucerne		2010/11, Fribourg			
MLST	ST1431		ST38		ST38		ST1564		ST1431		ST131		ST38		ST420		ST420			
Plasmid-Phenotype		A		B		B		A		A		A		B		A		C		
Antibiotic MIC, mg/L (interpretation)																				
Piperacillin/tazobactam	8/4	8/4	16/4	8/4	<=4/4	<=4/4	<=4/4	<=4/4	16/4	16/4	> 64/4	16/4	<=4/4	8/4	64/4	64/4	16/4	>64/4	<=4/4	<=4/4
Cefoxitin	>64	64	>64	64	64	64	64	64	>64	>64	>64	>64	>64	64	>64	>64	>64	>64	<=4	8
Cefpodixime	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	<=0.25	1
Ceftriaxone	32	16	32	16	16	32	16	16	16	32	64	64	32	16	64	128	32	128	<=1	<=1
Cefotaxime	16	8	32	16	32	32	16	16	16	16	64	32	16	16	64	64	32	64	<=0.25	<=0.25
Cefotaxime/Clavulansäure	16/4	16/4	32/4	8/4	16/4	16/4	8/4	8/4	16/4	16/4	64/4	32/4	8/4	8/4	64/4	64/4	16/4	64/4	<=0.12/4	<=0.12/4
Ceftazidime	32	64	64	64	16	128	64	64	64	64	128	128	64	64	128	>128	32	>128	<=0.25	0.5
Ceftazidime/Clavulansäure	32/4	32/4	64/4	32/4	8/4	64/4	64/4	32/4	32/4	32/4	>128/4	64/4	32/4	32/4	64/4	>128/4	32/4	>128/4	<=0.12/4	0.5/4
Cefazolin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	<=8	<=8
Cephalothin	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	>16	<=8	<=8
Cefepime	<=1	<=1	<=1	<=1	2	2	2	<=1	<=1	<=1	2	2	<=1	<=1	<=1	2	<=1	<=1	<=1	<=1
Imipenem	<=0.5	<=0.5	2	<=0.5	<=0.5	<=0.5	1	<=0.5	4	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5
Meropenem	<=1	<=1	<=1	<=1	4	<=1	<=1	<=1	<=1	<=1	2	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1	<=1
Gentamicin	2	0.5	1	0.5	1	1	0.5	0.5	1	1	1	0.5	1	0.5	0.5	1	1	0.5	<=0.25	0.5
Ciprofloxacin	>8	<=0.008	0.5	<=0.008	0.03	<=0.008	0.5	<=0.008	>8	<=0.008	0.25	0.015	0.015	0.015	0.25	0.06	0.5	0.015	0.03	<=0.008
Chloramphenicol	8	4	8	4	8	4	4	4	8	4	4	4	8	4	4	<=2	8	4	4	4
Florfenicol	4	<=2	8	<=2	4	<=2	4	<=2	8	<=2	4	4	8	<=2	4	<=2	8	<=2	4	<=2
Nalidixinsäure	>64	<=4	>64	<=4	32	<=4	>64	<=4	>64	<=4	>64	<=4	<=4	<=4	>64	64	>64	8	32	<=4
Trimethoprim	>32	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	>32	<=0.5	>32	<=0.5	<=0.5	<=0.5	>32	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5	<=0.5
Sulfamethoxazole	>1024	16	16	16	16	16	>1024	16	>1024	16	32	16	16	32	128	32	16	32	<=8	32
Streptomycin	>128	>128	8	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	<=2	>128
Tetracyclin	4	4	4	2	2	2	64	2	4	4	<=1	2	>64	2	2	2	2	2	<=1	2
Colistin	<=2	<=2	<=2	<=2	4	<=2	4	<=2	<=2	<=2	>4	4	4	<=2	<=2	<=2	<=2	<=2	<=2	<=2
Kanamycin	<=4	<=4	<=4	<=4	<=4	<=4	>128	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4	<=4

Table 1: Phenotypic and molecular characteristics of the nine *bla*$_{CMY}$-IncK2 positive *E.coli* and their transformants

[a] Transformant obtained from DH10B cells, [b] Transformant obtained fromTOP10 cell

REFERENCES

1. **Pitout JD. 2012. Extraintestinal Pathogenic Escherichia coli: A Combination of Virulence with Antibiotic Resistance. Frontiers in microbiology 3:9.**
2. **Manges AR, Johnson JR. 2012. Food-borne origins of Escherichia coli causing extraintestinal infections. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 55:712-719.**
3. **Seiffert SN, Hilty M, Perreten V, Endimiani A. 2013. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy 16:22-45.**
4. **Chattaway MA, Jenkins C, Ciesielczuk H, Day M, DoNascimento V, Day M, Rodriguez I, van Essen-Zandbergen A, Schink AK, Wu G, Threlfall J, Woodward MJ, Coldham N, Kadlec K, Schwarz S, Dierikx C, Guerra B, Helmuth R, Mevius D, Woodford N, Wain J. 2014. Evidence of evolving extraintestinal enteroaggregative Escherichia coli ST38 clone. Emerging infectious diseases 20:1935-1937.**
5. **Mathers AJ, Peirano G, Pitout JD. 2015. The Role of Epidemic Resistance Plasmids and International High-Risk Clones in the Spread of Multidrug-Resistant Enterobacteriaceae. Clinical microbiology reviews 28:565-591.**
6. **Dierikx CM, van der Goot JA, Smith HE, Kant A, Mevius DJ. 2013. Presence of ESBL/AmpC-producing Escherichia coli in the broiler production pyramid: a descriptive study. PloS one 8:e79005.**
7. **Nilsson O, Borjesson S, Landen A, Bengtsson B. 2014. Vertical transmission of Escherichia coli carrying plasmid-mediated AmpC (pAmpC) through the broiler production pyramid. The Journal of antimicrobial chemotherapy 69:1497-1500.**
8. **Endimiani A, Rossano A, Kunz D, Overesch G, Perreten V. 2012. First countrywide survey of third-generation cephalosporin-resistant Escherichia coli from broilers, swine, and cattle in Switzerland. Diagnostic microbiology and infectious disease 73:31-38.**
9. **Vogt D, Overesch G, Endimiani A, Collaud A, Thomann A, Perreten V. 2014. Occurrence and genetic characteristics of third-generation cephalosporin-resistant Escherichia coli in Swiss retail meat. Microbial drug resistance 20:485-494.**
10. **Carattoli A. 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrobial agents and chemotherapy 53:2227-2238.**
11. **Guerra B, Fischer J, Helmuth R. 2014. An emerging public health problem: acquired carbapenemase-producing microorganisms are present in food-producing animals, their environment, companion animals and wild birds. Veterinary microbiology 171:290-297.**
12. **Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ, National Esg. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 17:873-880.**
13. **Wang J, Stephan R, Power K, Yan Q, Hachler H, Fanning S. 2014. Nucleotide sequences of 16 transmissible plasmids identified in nine multidrug-resistant Escherichia coli isolates expressing an ESBL phenotype isolated from food-producing animals and healthy humans. The Journal of antimicrobial chemotherapy 69:2658-2668.**
14. **Voets GM, Fluit AC, Scharringa J, Schapendonk C, van den Munckhof T, Leverstein-van Hall MA, Stuart JC. 2013. Identical plasmid AmpC beta-lactamase genes and plasmid types in E. coli isolates from patients and poultry meat in the Netherlands. International journal of food microbiology 167:359-362.**
15. **Borjesson S, Jernberg C, Brolund A, Edquist P, Finn M, Landen A, Olsson-Liljequist B, Tegmark Wisell K, Bengtsson B, Englund S. 2013. Characterization of plasmid-mediated AmpC-producing E. coli from Swedish broilers and association with human clinical isolates. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 19:E309-311.**
16. **Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A. 2013. Extended-spectrum cephalosporin-resistant Escherichia coli in community, specialized outpatient clinic and hospital settings in Switzerland. The Journal of antimicrobial chemotherapy 68:2249-2254.**
17. **Baudry PJ, Mataseje L, Zhanel GG, Hoban DJ, Mulvey MR. 2009. Characterization of plasmids encoding CMY-2 AmpC beta-lactamases from Escherichia coli in Canadian intensive care units. Diagnostic microbiology and infectious disease 65:379-383.**
18. **Porres-Osante N, Saenz Y, Somalo S, Torres C. 2014. Characterization of Beta-lactamases in Faecal Enterobacteriaceae Recovered from Healthy Humans in Spain: Focusing on AmpC Polymorphisms. Microbial ecology.**

19. **Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D. 2010. Increased detection of extended spectrum beta-lactamase producing Salmonella enterica and Escherichia coli isolates from poultry. Veterinary microbiology 145:273-278.**
20. **Egervarn M, Borjesson S, Byfors S, Finn M, Kaipe C, Englund S, Lindblad M. 2014. Escherichia coli with extended-spectrum beta-lactamases or transferable AmpC beta-lactamases and Salmonella on meat imported into Sweden. International journal of food microbiology 171:8-14.**
21. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. Journal of microbiological methods 63:219-228.**
22. **Papagiannitsis CC, Tzouvelekis LS, Kotsakis SD, Tzelepi E, Miriagou V. 2011. Sequence of pR3521, an IncB plasmid from Escherichia coli encoding ACC-4, SCO-1, and TEM-1 beta-lactamases. Antimicrobial agents and chemotherapy 55:376-381.**
23. **Couturier M, Bex F, Bergquist PL, Maas WK. 1988. Identification and classification of bacterial plasmids. Microbiological reviews 52:375-395.**
24. **Komano T. 1999. Shufflons: multiple inversion systems and integrons. Annual review of genetics 33:171-191.**
25. **Cottell JL, Saw HT, Webber MA, Piddock LJ. 2014. Functional genomics to identify the factors contributing to successful persistence and global spread of an antibiotic resistance plasmid. BMC microbiology 14:168.**
26. **Brouwer MS, Tagg KA, Mevius DJ, Iredell JR, Bossers A, Smith HE, Partridge SR. 2015. IncI shufflons: Assembly issues in the next-generation sequencing era. Plasmid.**
27. **Praszkier J, Wei T, Siemering K, Pittard J. 1991. Comparative analysis of the replication regions of IncB, IncK, and IncZ plasmids. Journal of bacteriology 173:2393-2397.**
28. **Huijbers PM, van Hoek AH, Graat EA, Haenen AP, Florijn A, Hengeveld PD, van Duijkeren E. 2015. Methicillin-resistant Staphylococcus aureus and extended-spectrum and AmpC beta-lactamase-producing Escherichia coli in broilers and in people living and/or working on organic broiler farms. Veterinary microbiology 176:120-125.**
29. **Shaheen BW, Nayak R, Boothe DM. 2013. Emergence of a New Delhi metallo-beta-lactamase (NDM-1)-encoding gene in clinical Escherichia coli isolates recovered from companion animals in the United States. Antimicrobial agents and chemotherapy 57:2902-2903.**
30. **de Been M, Lanza VF, de Toro M, Scharringa J, Dohmen W, Du Y, Hu J, Lei Y, Li N, Tooming-Klunderud A, Heederik DJ, Fluit AC, Bonten MJ, Willems RJ, de la Cruz F, van Schaik W. 2014. Dissemination of cephalosporin resistance genes between Escherichia coli strains from farm animals and humans by specific plasmid lineages. PLoS genetics 10:e1004776.**

Supplementary Materials for Chapter IV

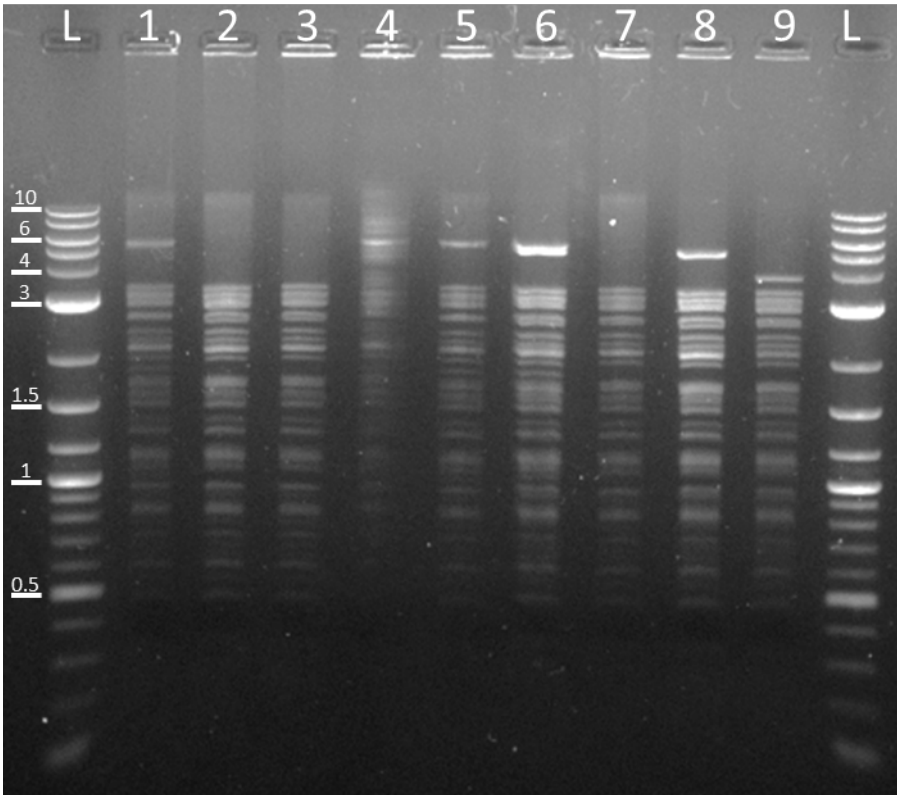


Figure S1: Figure S1: Restriction analysis of the IncK2-group plasmids using PstI. Origin is indicated in parentheses. Line 1: p4809.66 (human), line 2: pDV56 (poultry meat), line 3: pDV10 (poultry meat), line 4: pDV45 (poultry meat), line 5: p4901.67 (human), line 6: p5312.29 (human), line 7: pTMSA1088 (poultry), line 8: pTMSA992 (poultry), line 9: pTMSA970 (poultry). Line L indicates the Log2 ladder (Bioconcept) (values in kbp).

PART B

Chapter V

In this study, isolates from a patient that was transferred from Libya to Switzerland were characterized. One of the screening samples contained a MDR *Salmonella enterica* Kentucky of ST198 which was harbouring $bla_{\text{VEB-8}}$ and $bla_{\text{OXA-48}}$. This result shows that isolation of patients coming from endemic countries can prevent outbreaks.

Preliminary results were presented at the **24th European Congress of Clinical Microbiology and Infectious Diseases** (ECCMID) – Barcelona, Spain – May 10-13, **2014** (see Poster presentation at the end of this chapter).

The study was published in **Antimicrobial Agents and Chemotherapy** under the title "OXA-48 Carbapenemase Producing *Salmonella enterica* Kentucky of ST198 in a Patient Transferred from Libya to Switzerland" in **2014** (Seiffert et al., Antimicrobial Agents Chemotherapy; 58(4):2446, 2014).

AAC Journals.ASM.org

OXA-48 Carbapenemase-Producing *Salmonella enterica* Serovar Kentucky Isolate of Sequence Type 198 in a Patient Transferred from Libya to Switzerland

Salome N. Seiffert,[a,b,c] Vincent Perreten,[b] Sönke Johannes,[d] Sara Droz,[a] Thomas Bodmer,[e] Andrea Endimiani[a]

Institute for Infectious Diseases, University of Bern, Bern, Switzerland[a]; Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland[b]; Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland[c]; Rehaklinik Bellikon, Bellikon, Switzerland[d]; labormedizinisches Zentrum Dr Risch, Department of Medical Microbiology, Bern-Liebefeld, Switzerland[e]

Here, we report a case of OXA-48-producing *Salmonella enterica* serovar Kentucky of sequence type 198 (ST198) from perianal screening cultures of a patient transferred from Libya to Switzerland. The bla_{OXA-48} gene was carried by Tn*1999.2* and located on an ~60-kb IncL/M plasmid. This *Salmonella* strain also possessed the bla_{VEB-8}, *aac*(*6*)*-Ib*, *tet*(A), *sul1*, and *mphA* resistance genes and substitutions in GyrA (Ser83Phe and Asp87Asn) and ParC (Ser80Ile). This finding emphasizes that prompt screening strategies are essential to prevent the dissemination of carbapenemase producers imported from countries where they are endemic.

The rapid emergence of OXA-48 carbapenemase-producing *Enterobacteriaceae* is currently a concern, mainly because the relatively low carbapenem MICs of these bacteria make their detection difficult (1–3). In some countries, these multidrug-resistant (MDR) isolates are now more frequently detected than those producing the classic (KPC, NDM, and VIM) carbapenemases (4–6). The bla_{OXA-48} gene is usually detected in *Klebsiella pneumoniae* and *Escherichia coli*, but it can also be found in other *Enterobacteriaceae* (2). However, to date, OXA-48-producing *Salmonella enterica* has been detected only in a French patient (7).

In March 2012, a polytraumatized male with severe brain injury due to a grenade detonation (September 2011) was transferred from Libya to a rehabilitation clinic located in northeastern Switzerland. During the hospitalization, different screening samples were analyzed for the presence of MDR Gram-negative pathogens (e.g., extended-spectrum β-lactamase [ESBL] producers) by implementation of selective chromID ESBL plates (bioMérieux). Colonies were identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonik), and antimicrobial susceptibility patterns were routinely obtained using the Vitek II system (bioMérieux). This strategy is part of the hospital hygiene policy of the clinic to prevent the spread of difficult-to-treat organisms (8–10).

At arrival, *E. coli* (*Ec*-38), *Citrobacter koseri* (*Ck*-39 and *Ck*-41), and *K. pneumoniae* (*Kp*-39-1, *Kp*-39-2, and *Kp*-41) isolates with antibiotic phenotypes suspicious for ESBL production were detected in samples from urine, nasopharynx, and perianal swabs (Table 1). Based on these results, the patient was kept in isolation in a single room and strict standard hygienic procedures were implemented (8, 10).

After 2 months (May 2012), a further perianal screening culture was again positive for an ESBL-producing *K. pneumoniae* isolate (*Kp*-43) and also for an MDR *Salmonella enterica* isolate of serotype Kentucky (*Sk*-1). This isolate was resistant to ciprofloxacin and last-generation cephalosporins and was flagged by the Vitek system as a possible carbapenemase producer (MICs for imipenem and ertapenem, 1 and 2 μg/ml, respectively). The same *Salmonella* Kentucky (*Sk*-2) strain was isolated from another sample obtained from a perianal screening swab performed 17 months after the admission (August 2013) (Table 1).

In September 2013, the patient was transferred to a long-term-care facility in Tunisia. During the 18-month period of residence at the Swiss rehabilitation clinic, he never developed diarrhea, and no clinical samples indicating possible ongoing infection(s) were positive for the above-described MDR *Enterobacteriaceae*. Moreover, no other patients hospitalized at the clinic and during the same time frame developed infection or colonization with MDR *Salmonella*.

All isolates were further characterized by microdilution ESB1F and GNX2F MIC plates (Trek Diagnostics) (11); CT-103 (CheckPoints) and AMR-ve 0.5m (Alere) microarrays for detection of ESBL, plasmid-mediated AmpC (pAmpC), carbapenemase, and other resistance genes (12); PCR/DNA sequencing for *gyrA*, *parC*, *bla* genes, transposon Tn*1999*-like, and plasmid incompatibility groups (13–17); multilocus sequence typing (MLST) for *E. coli* (Environmental Research Institute [ERI] [see http://mlst.ucc.ie]), *K. pneumoniae* (Pasteur Institute [see http://www.pasteur.fr]), and *Salmonella* spp. (ERI); and analytical isoelectric focusing (aIEF) (14). Plasmids were extracted using alkaline extraction and electroporated into ElectroMAX *E. coli* DH10B (Invitrogen). Cells were selected on LB plates containing ampicillin (20 μg/ml) or cefotaxime (1 μg/ml) (Sigma) (16). Conjugation experiments were performed at both 37°C and 25°C using the rifampin-resistant *E. coli* strain JF33 and LB selective plates containing ampicillin (50 μg/ml) plus rifampin (100 μg/ml).

Ec-38 was of sequence type 131 (ST131) and produced the

Received 5 November 2013 Returned for modification 8 December 2013
Accepted 19 January 2014
Published ahead of print 27 January 2014
Address correspondence to Andrea Endimiani, aendimiani@gmail.com.
Copyright © 2014, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.02417-13

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

TABLE 1 Phenotypic and molecular characteristics of the MDR *Enterobacteriaceae* isolates detected in the screening cultures during the 18-month hospitalization of the patient

Antibiotic tested or isolate characteristic	MIC [μg/ml (interpretation[b])] or other characteristic(s) of indicated isolate from indicated sample							
	Urine (March 2012[h]): *E. coli* (*Ec*-38)	Nasopharyngeal swab (March 2012)			Perianal swab (March 2012)		Perianal swab (May 2012 [August 2013])[a]	
		C. koseri (*Ck*-39)	*K. pneumoniae* (*Kp*-39-1)	*K. pneumoniae* (*Kp*-39-2)	*C. koseri* (*Ck*-41)	*K. pneumoniae* (*Kp*-41)	*K. pneumoniae* (*Kp*-43)	*S.* Kentucky (*Sk*-1 [and *Sk*-2])
Piperacillin-tazobactam	32 (R)	64 (R)	≥128 (R)	16 (I)	32 (R)	16 (I)	32 (R)	≥128 (R)
Ticarcillin-clavulanate	128 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)
Cefoxitin	8 (NA)	8 (NA)	32 (NA)	8 (NA)	16 (NA)	8 (NA)	8 (NA)	8 (NA)
Ceftriaxone	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	64 (R)
Cefotaxime	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	64 (R)	≥128 (R)	64 (R)
Cefotaxime-clavulanate	≤0.064 (NA)	4 (NA)	8 (NA)	4 (NA)	8 (NA)	8 (NA)	4 (NA)	16 (NA)
Ceftazidime	16 (R)	32 (R)	128 (R)	64 (R)	64 (R)	64 (R)	128 (R)	≥256 (R)
Ceftazidime-clavulanate	0.25 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	≥256 (NA)
Cefepime	≥32 (R)	16 (R)	≥32 (R)	16 (R)	16 (R)	16 (R)	16 (R)	≥32 (R)
Aztreonam	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)
Imipenem	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)
Meropenem	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)
Ertapenem	≤0.125 (S)	0.5 (S)	2 (R)	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	1 (I)
Doripenem	≤0.064 (S)	≤0.064 (S)	0.25 (S)	≤0.064 (S)	≤0.064 (S)	≤0.064 (S)	≤0.064 (S)	0.25 (S)
Gentamicin	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≤0.5 (S)
Tobramycin	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)
Amikacin	≥64 (R)	8 (S)	8 (S)	8 (S)	4 (S)	≤2 (S)	≤2 (S)	8 (S)
Ciprofloxacin	≥4 (R)	≥4 (R)	≥4 (R)	≥4 (R)	≥4 (R)	2 (R)	2 (R)	≥4 (R)
Trimethoprim-sulfamethoxazole	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≤0.25 (S)
Colistin	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	≥8 (R)[c]	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)
Polymyxin B	≤0.125 (NA)	≤0.125 (NA)	0.5 (NA)	≥8 (NA)	≤0.125 (NA)	0.5 (NA)	0.5 (NA)	0.5 (NA)
Doxycycline	≤1 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≤1 (NA)
Tigecycline	≤0.125 (S)	0.5 (S)	0.5 (S)	≤0.125 (S)	0.5 (S)	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)
bla genes	CTX-M-15 TEM-1-like[d]	CTX-M-15 CMY-4 TEM-1-like	CTX-M-15 CMY-4 SHV-1-like[d]	CTX-M-15 CMY-4 SHV-1-like	CTX-M-15 CMY-4 TEM-1-like	CTX-M-15 CMY-4 SHV-1-like	CTX-M-15 CMY-4 SHV-1-like	OXA-48 VEB-8[e]
Plasmid incompatibility group(s)[f]	F, FII, Nt-1	Nt-2	Nt-1, Nt-2	F, Nt-2	Nt-2	F, Nt-2	F, Nt-2	L/M
aIEF, isoelectric points ±0.2[g]	8.9, 7.6, 5.4	9.1, 8.9, 7.6, 5.4	8.9, 7.8, 7.6, 6.8, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.0, 7.6, 7.2, 6.3
MLST	ST131	NA	ST101	ST111	NA	ST111	ST111	ST198

[a] Another perianal swab collected in August 2013 was positive for an MDR *Salmonella* Kentucky strain with the same phenotypic and molecular characteristics.
[b] According to EUCAST criteria (11); R, resistant; I, intermediate; S, susceptible; NA, not applicable or not available. MICs were obtained by implementing the ESB1F and GNX2F plates (Trek Diagnostics).
[c] The MIC for colistin was also elevated (4 μg/ml) when measured by Etest (bioMérieux).
[d] No TEM- or SHV-type ESBLs were present. For *K. pneumoniae*, SHV-1-like indicates the natural β-lactamases.
[e] *Salmonella* Kentucky was also positive for the following antibiotic resistance genes: *aac(6)-Ib*, *tet*(A), *sul1*, and *mphA*. Amino acid substitutions in GyrA (Ser83Phe and Asp87Asn) and ParC (Ser80Ile) were also found.
[f] Nt-1, nontypeable plasmid PCR positive only for replicon R; Nt-2, nontypeable plasmid PCR positive only for replicon FIB-M.
[g] pI of ~8.9, CTX-M-15; pI of ~9, CMY-4; pI of ~5.4, TEM-1-like; pI of ~7.6, SHV-1-like; pI of ~7.4, VEB-8; pI of ~7.2, OXA-48 (http://www.lahey.org/Studies/).
[h] Date of isolation.

CTX-M-15 ESBL, whereas the remaining *C. koseri* (*Ck*-39 and *Ck*-41) and *K. pneumoniae* (*Kp*-39-1, *Kp*-39-2, *Kp*-41, and *Kp*-43) isolates also coproduced the CMY-4 pAmpC. The *K. pneumoniae* isolates were of ST101 and ST111. Interestingly, *Kp*-39-2 was highly resistant to polymyxins (Table 1). Moreover, *C. koseri* and *K. pneumoniae* isolates were all PCR positive for a nontypeable plasmid of the FIB-M replicon.

Salmonella Kentucky was of ST198 and carried a plasmid of IncL/M. According to microdilution results, the isolate was resistant to ciprofloxacin and showed reduced susceptibility to ertapenem (MIC, 1 μg/ml) but not to the other carbapenems (all MICs, ≤0.25 μg/ml). This phenotype was due to amino acid substitutions in GyrA (Ser83Phe and Asp87Asn) and ParC (Ser80Ile) and to the production of OXA-48 carbapenemase. In particular, the bla_{OXA-48} gene was carried by Tn*1999.2* and located on an ~60-kb IncL/M plasmid (13, 15). The *Salmonella* isolate was also highly resistant to last-generation cephalosporins (e.g., MIC for ceftazidime, ≥256 μg/ml) because of the production of the VEB-8 ESBL. According to microarray results, genes conferring resistance to aminoglycosides, tetracyclines, sulfonamides, and macrolides [*aac(6)-Ib*, *tet*(A), *sul1*, and *mphA*, respectively] were also detected (Table 1). Only transconjugants and DH10B transformants carrying the bla_{OXA-48}-positive IncL/M plasmid were obtained. This indicates the possible chromosomal location of the bla_{VEB-8}, as usually observed for this class of genes (12).

Only two OXA-48-producing *Salmonella* Kentucky isolates of ST198 (also ciprofloxacin resistant) and one OXA-48-producing *Salmonella* Saintpaul isolate were previously reported in France. These strains were detected during 2009 to 2011 in the stools of a unique nonhospitalized female who traveled in Egypt (7). Like-

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

wise, the OXA-48-producing *Salmonella* Kentucky isolate found in Switzerland was ciprofloxacin resistant, belonged to ST198, and was responsible for prolonged intestinal colonization of the patient transferred from Libya. The bla_{OXA-48} gene was located in a typical genetic environment (Tn*1999*-like and IncL/M plasmid) (3, 15). However, the isolate also coproduced the VEB-8, an ESBL previously reported only in one OXA-48-positive *E. coli* isolate originating from Libya (1). We also noted that the other isolates found in the patient produced the CMY-4, a pAmpC usually reported in North Africa (8, 18). Overall, these results indicate that in countries with endemic spread of OXA-48 producers and which have high frequencies of *Salmonella* diseases, there are high risks for the selection of very resistant clones (e.g., the ciprofloxacin-resistant hyperepidemic *Salmonella* Kentucky of ST198) that may further spread not only among people in the community but also in the environment and among food-producing animals (19–22).

In conclusion, this is the second report of OXA-48-producing *Salmonella enterica*. The isolate copossessed other resistance genes that rendered the isolate very resistant to standard antibiotic treatments. Reference laboratories for enteric pathogens should be aware that this MDR *Salmonella* may silently disseminate in the community with the potential to cause outbreaks, and it poses a serious threat to public health. Effective strategies to screen patients at risk of colonization (e.g., those transferred or recently traveling in specific areas) must be constantly implemented by all health care institutions to prevent the diffusion of difficult-to-treat pathogens (10, 22).

ACKNOWLEDGMENTS

We thank Alexandra Collaud, Sacha Thiermann, and Christel Inauen for technical assistance.

This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and by grant 1.12.06 from the Swiss Veterinary Federal Office (BVET). S.N.S. is a Ph.D. student supported by the IFIK and the BVET.

REFERENCES

1. **Potron A, Poirel L, Rondinaud E, Nordmann P.** 2013. Intercontinental spread of OXA-48 β-lactamase-producing *Enterobacteriaceae* over a 11-year period, 2001 to 2011. Euro Surveill. **18**:pii=20549. http://www.eurosurveillance.org/ViewArticle.Aspx?ArticleId=20549.
2. **Poirel L, Potron A, Nordmann P.** 2012. OXA-48-like carbapenemases: the phantom menace. J. Antimicrob. Chemother. **67**:1597–1606. http://dx.doi.org/10.1093/jac/dks121.
3. **Lupo A, Papp-Wallace KM, Sendi P, Bonomo RA, Endimiani A.** 2013. Nonphenotypic tests to detect and characterize antibiotic resistance mechanisms in *Enterobacteriaceae*. Diagn. Microbiol. Infect. Dis. **77**:179–194. http://dx.doi.org/10.1016/j.diagmicrobio.2013.06.001.
4. **Oteo J, Saez D, Bautista V, Fernandez-Romero S, Hernandez-Molina JM, Perez-Vazquez M, Aracil B, Campos J.** 2013. Carbapenemase-producing *Enterobacteriaceae* in Spain in 2012. Antimicrob. Agents Chemother. **57**:6344–6347. http://dx.doi.org/10.1128/AAC.01513-13.
5. **Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P.** 2012. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin. Microbiol. Infect. **18**:413–431. http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x.
6. **Glasner C, Albiger B, Buist G, Tambic Andrasevic A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H, European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group.** 2013. Carbapenemase-producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. Euro Surveill. **18**:pii=20525. http://www.eurosurveillance.org/ViewArticle.Aspx?ArticleId=20525.
7. **Le Hello S, Harrois D, Bouchrif B, Sontag L, Elhani D, Guibert V, Zerouali K, Weill FX.** 2013. Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. Lancet Infect. Dis. **13**:672–679. http://dx.doi.org/10.1016/S1473-3099(13)70124-5.
8. **Levy Hara G, Gould I, Endimiani A, Pardo PR, Daikos G, Hsueh PR, Mehtar S, Petrikkos G, Casellas JM, Daciuk L, Paciel D, Novelli A, Saginur R, Pryluka D, Medina J, Savio E.** 2013. Detection, treatment, and prevention of carbapenemase-producing *Enterobacteriaceae*: recommendations from an international working group. J. Chemother. **25**:129–140. http://dx.doi.org/10.1179/1973947812Y.0000000062.
9. **Schechner V, Kotlovsky T, Kazma M, Mishali H, Schwartz D, Navon-Venezia S, Schwaber MJ, Carmeli Y.** 2013. Asymptomatic rectal carriage of bla_{KPC} producing carbapenem-resistant *Enterobacteriaceae*: who is prone to become clinically infected? Clin. Microbiol. Infect. **19**:451–456. http://dx.doi.org/10.1111/j.1469-0691.2012.03888.x.
10. **European Centre for Disease Prevention and Control.** 2011. Risk assessment on the spread of carbapenemase-producing *Enterobacteriaceae* (CPE) through patient transfer between healthcare facilities, with special emphasis on cross-border transfer. Technical report, September 2011. European Centre for Disease Prevention and Control, Solna, Sweden.
11. **EUCAST.** 2013. European Committee on Antimicrobial Susceptibility Testing clinical breakpoints. Version 3.0. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_Breakpoint_table_v_3.0.pdf.
12. **Seiffert SN, Tinguely R, Lupo A, Neuwirth C, Perreten V, Endimiani A.** 2013. High Prevalence of Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* in Poultry Meat in Switzerland: emergence of CMY-2- and VEB-6-possessing *Proteus mirabilis*. Antimicrob. Agents Chemother. **57**:6406–6408. http://dx.doi.org/10.1128/AAC.01773-13.
13. **Giani T, Conte V, Di Pilato V, Aschbacher R, Weber C, Larcher C, Rossolini GM.** 2012. *Escherichia coli* from Italy producing OXA-48 carbapenemase encoded by a novel Tn*1999* transposon derivative. Antimicrob. Agents Chemother. **56**:2211–2213. http://dx.doi.org/10.1128/AAC.00035-12.
14. **Endimiani A, Hujer AM, Perez F, Bethel CR, Hujer KM, Kroeger J, Oethinger M, Paterson DL, Adams MD, Jacobs MR, Diekema DJ, Hall GS, Jenkins SG, Rice LB, Tenover FC, Bonomo RA.** 2009. Characterization of bla_{KPC}-containing *Klebsiella pneumoniae* isolates detected in different institutions in the Eastern USA. J. Antimicrob. Chemother. **63**:427–437. http://dx.doi.org/10.1093/jac/dkn547.
15. **Poirel L, Bonnin RA, Nordmann P.** 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. Antimicrob. Agents Chemother. **56**:559–562. http://dx.doi.org/10.1128/AAC.05289-11.
16. **Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A.** 2013. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. J. Antimicrob. Chemother. **68**:2249–2254. http://dx.doi.org/10.1093/jac/dkt208.
17. **O'Regan E, Quinn T, Pages JM, McCusker M, Piddock L, Fanning S.** 2009. Multiple regulatory pathways associated with high-level ciprofloxacin and multidrug resistance in *Salmonella enterica* serovar enteritidis: involvement of RamA and other global regulators. Antimicrob. Agents Chemother. **53**:1080–1087. http://dx.doi.org/10.1128/AAC.01005-08.
18. **Dahmen S, Mansour W, Charfi K, Boujaafar N, Arlet G, Bouallegue O.** 2012. Imipenem resistance in *Klebsiella pneumoniae* is associated to the combination of plasmid-mediated CMY-4 AmpC β-lactamase and loss of an outer membrane protein. Microb. Drug Resist. **18**:479–483. http://dx.doi.org/10.1089/mdr.2011.0214.
19. **Woodford N, Wareham DW, Guerra B, Teale C.** 2014. Carbapenemase-producing *Enterobacteriaceae* and non-*Enterobacteriaceae* from animals and the environment: an emerging public health risk of our own making? J. Antimicrob. Chemother. **69**:287–291. http://dx.doi.org/10.1093/jac/dkt392.
20. **Mulvey MR, Boyd DA, Finley R, Fakharuddin K, Langner S, Allen V, Ang L, Bekal S, El Bailey S, Haldane D, Hoang L, Horsman G, Louis M, Robberts L, Wylie J.** 2013. Ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky in Canada. Emerg. Infect. Dis. **19**:999–1001. http://dx.doi.org/10.3201/eid1906.121351.
21. **Le Hello S, Hendriksen RS, Doublet B, Fisher I, Nielsen EM, Whichard**

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

JM, Bouchrif B, Fashae K, Granier SA, Jourdan-Da Silva N, Cloeckaert A, Threlfall EJ, Angulo FJ, Aarestrup FM, Wain J, Weill FX. 2011. International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. J. Infect. Dis. **204:** 675–684. http://dx.doi.org/10.1093/infdis/jir409.

22. **Le Hello S, Bekhit A, Granier SA, Barua H, Beutlich J, Zajac M, Munch S, Sintchenko V, Bouchrif B, Fashae K, Pinsard JL, Sontag L, Fabre L, Garnier M, Guibert V, Howard P, Hendriksen RS, Christensen JP, Biswas PK, Cloeckaert A, Rabsch W, Wasyl D, Doublet B, Weill FX.** 2013. The global establishment of a highly fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. Front. Microbiol. **4:**395. http://dx.doi.org/10.3389/fmicb.2013.00395.

Downloaded from http://aac.asm.org/ on May 28, 2015 by Universitaetsbibliothek Bern

24th ECCMID Barcelona, Spain 10 – 13 May 2014
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
P1110

OXA-48 and VEB-8 producing *Salmonella* Kentuky of ST198 in a patient transferred from Libya to Switzerland

Salome N. Seiffert,[1,2,3*] Vincent Perreten,[2] Sönke Johannes,[1] Sara Droz,[1] Thomas Bodmer,[4] and Andrea Endimiani[1#] (*Email: salome.seiffert@ifik.unibe.ch; #aendimiani@gmail.com)

[1] Institute for Infectious Diseases, [2]Institute of Veterinary Bacteriology, [3]Graduate School for Cellular and Biomedical Sciences; University of Bern, Switzerland; [4]Medical Microbiology, labormedizinisches zentrum Dr Risch (Bern-Liebefeld, Switzerland)

Introduction:

In the past few years the prevalence of carbapenem-resistant *Enterobacteriaceae* has reached dramatic levels.

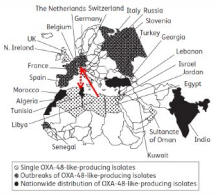
Particularly worrisome is the rapid spread of the OXA-48 carbapenemase producers (Figure 1), especially because of their relatively low carbapenems MICs that make their detection difficult (Poirel L et al., *JAC*, 2012)

These multidrug-resistant (MDR) isolates are, in some countries, getting more frequently detected than those producing the classic (KPC, NDM and VIM) carbapenemases (Canton R et al., *CMI*, 2012).

The bla_{OXA-48} gene is usually detected in *K. pneumoniae* and *E. coli* but it can also be found in other *Enterobacteriaceae* (*Ent*).

However, to date, only three OXA-48-producing *Salmonella enterica* isolates have been reported in Egypt (Le Hello S et al., Lancet Infect Dis, 2013).

Figure 1: Geographic spread of bla_{OXA-48} (from Poirel L et al., *JAC* 2012)



Our patients was transferred from Libya to Switzerland on March 2012 after severe trauma due to grenade detonation.

He was hospitalized 18 months and then transferred to a long-term-care facility in Tunisia

Materials and Methods:

On March 2012, a polytraumatized male due to a grenade detonation (September 2011) was transferred from Libya to a rehabilitation clinic located in Switzerland (Figure 1). Screening samples were taken at different time points during the 18-month stay of the patient in the Swiss clinic. The samples were plated onto selective chromID ESBL (bioMérieux) in order to investigate the presence of MDR Gram-negative bacteria.

The subsequently obtained extended-spectrum cephalosporin resistant *Ent* were further characterized with:

- Species identification using MALDI-TOF MS (Bruker Daltonik);
- Antimicrobial susceptibility pattern using Vitek II system (Card AST-N283; bioMérieux);
- Antibiotic susceptibility test (MIC) using ESBL1F and GNX2F MICs plates (Trek Diagnostics);
- Microarrays for detection of ESBL, pAmpC, and carbapenemase genes as well as other resistance genes (Check-Points and Alere);
- PCR/DNA sequencing for *bla* genes, transposon Tn*1999*-like and plasmid incompatibility group (Poirel L et al., *AAC*, 2012);
- Analytical isoelectric focusing (aIEF);
- Multilocus sequencing typing (MLST) analysis (for *E. coli* and *Salmonella* spp.; Achtman scheme, and for *K. pneumoniae*; Pasteur Scheme);
- PCRs for plasmid replicon typing (PBRT; Diathevo).

The *S.* Kentucky isolate was additionally characterized with:

- Conjugation experiments were performed at 37°C and 25°C using the rifampicin-resistant *E. coli* strains JF33 and streaked out on LB plates with ampicillin [20ug/ml] and rifampicin [100ug/ml] (Sigma);
- Plasmids were extracted using an alkaline extraction and electroporated into ElectroMAX *E. coli* DH10B (Invitrogen); cells were selected on LB plates containing ampicillin [20 µg/mL] or cefotaxime [1 µg/mL] (Sigma).

Table 1: Phenotypic and molecular characteristics of the MDR *Enterobacteriaceae* detected in the screening cultures

Sample	Urine	Nasopharyngeal swab		Perianal swab		Perianal swab [a]	
Date	March 2012	March 2012		March 2012		May 2012 (August 2013) [a]	
Species	*E. coli*	*C. koseri*	*K. pneumoniae*	*C. koseri*	*K. pneumoniae*	*K. pneumoniae*	***S.* Kentucky [e]**
Strain ID	*Ec*-38	*Ck*-39	*Kp*-39-1	*Ck*-41	*Kp*-41	*Kp*-43	***Sk*-1 (and *Sk*-2) [e]**
Antibiotic MIC, µg/mL (interpretation) [b]							
Piperacillin-tazobactam	32 (R)	64 (R)	≥128 (R)	16 (I)	32 (R)	16 (I)	32 (R)	**≥128 (R)**
Ticarcillin-clavulanate	128 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	**≥256 (R)**
Cefoxitin	8 (NA)	8 (NA)	32 (NA)	8 (NA)	16 (NA)	8 (NA)	8 (NA)	**8 (NA)**
Ceftriaxone	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	**64 (R)**
Cefotaxime	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	64 (R)	≥128 (R)	≥128 (R)	**64 (R)**
Cefotaxime-clavulanate	≤0.064 (NA)	4 (NA)	8 (NA)	4 (NA)	8 (NA)	8 (NA)	4 (NA)	**16 (NA)**
Ceftazidime	16 (R)	32 (R)	128 (R)	64 (R)	64 (R)	64 (R)	128 (R)	**≥256 (R)**
Ceftazidime-clavulanate	0.25 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	8 (NA)	**≥256 (NA)**
Cefepime	≥32 (R)	16 (R)	≥32 (R)	16 (R)	16 (R)	16 (R)	16 (R)	**≥32 (R)**
Aztreonam	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	**≥32 (R)**
Imipenem	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	**≤0.25 (S)**
Meropenem	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	**≤0.5 (S)**
Ertapenem	≤0.125 (S)	0.5 (S)	2 (R)	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	**1 (I)**
Doripenem	≤0.064 (S)	≤0.064 (S)	0.25 (S)	≤0.064 (S)	≤0.064 (S)	≤0.064 (S)	≤0.064 (S)	**0.25 (S)**
Gentamicin	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	≥32 (R)	**≤0.5 (S)**
Tobramycin	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	≥16 (R)	**≥16 (R)**
Amikacin	≥64 (R)	8 (S)	8 (S)	8 (S)	4 (S)	≤2 (S)	≤2 (S)	**8 (S)**
Ciprofloxacin	≥4 (R)	≥4 (R)	≥4 (R)	≥4 (R)	≥4 (R)	2 (R)	2 (R)	**≥4 (R)**
Trimethoprim/sulfamethoxazole	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	**≤0.25 (S)**
Colistin	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	≥8 (R) [c]	≤0.125 (S)	≤0.125 (S)	≤0.125 (S)	**≤0.125 (S)**
Polymixin B	≤0.125 (NA)	≤0.125 (NA)	0.5 (NA)	≥8 (NA)	≤0.125 (NA)	0.5 (NA)	0.5 (NA)	**0.5 (NA)**
Doxycycline	≤1 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	≥32 (NA)	**≤1 (NA)**
Tigecycline	≤0.125 (S)	0.5 (S)	0.5 (S)	≤0.125 (S)	0.5 (S)	≤0.125 (S)	≤0.125 (S)	**≤0.125 (S)**
***Bla* genes**	CTX-M-15 TEM-1-like [d]	CTX-M-15 CMY-4 TEM-1-like [d]	CTX-M-15 CMY-4 SHV-1-like [d]	CTX-M-15 CMY-4 SHV-1-like [d]	CTX-M-15 CMY-4 TEM-1-like [d]	CTX-M-15 CMY-4 SHV-1-like [d]	CTX-M-15 CMY-4 SHV-1-like [d]	**OXA-48 VEB-8 [e]**
Plasmid Inc groups [f]	F, FII, (Nt-1)	(Nt-2)	(Nt-1, Nt-2)	F, (Nt-2)	(Nt-2)	F, (Nt-2)	F, (Nt-2)	**L/M**
aIEF, isoelectric points (pIs, ±0.2) [g]	8.9, 7.6, 5.4	9.1, 8.9, 7.6, 5.4	8.9, 7.8, 7.6, 6.8, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	8.9, 7.6, 5.4	**8.0, 7.6, 7.2, 6.3**
MLST	ST131	NA	ST101	ST111	NA	ST111	ST111	**ST198**

[a] Another perianal swab collected on August 2013 resulted positive for a MDR *Salmonella* Kentucky with the same phenotypic and molecular characteristics

[b] According to the EUCAST criteria: R, resistant; I, intermediate; S, susceptible; NA, not applicable or not available.

[c] The MIC for colistin was also elevated (4 µg/mL) when measured by Etest (bioMérieux)

[d] No TEM- or SHV-type ESBL were present. For *K. pneumoniae* SHV-1-like indicates the natural β-lactamases

[e] ***Sk1 and Sk2 were also positive for the following antibiotic resistance genes: aac(6)-Ib, tet(A), sul1, and mphA. Substitutions in GyrA (Ser83Phe and Asp87Asn) and ParC (Ser80Ile) were also found***

[f] Nt-1, not typable plasmid resulting PCR positive only for replicon R; Nt-2, not typable plasmid resulting PCR positive only for replicon FIB-M

[g] pI of ~8.9, CTX-M-15; pI of ~9, CMY-4; pI of ~5.4, TEM-1-like; pI of ~7.6, SHV-1-like; pI of ~7.4, VEB-8; pI of ~7.2, OXA-48

Results:

Five samples were collected during the 18-month hospital-stay of the patient (Table 1). The three samples taken at admission (March 2012) contained *E. coli* (*Ec*-38), *C. koseri* (*Ck*-39 and *Ck*-41), and *K. pneumoniae* (*Kp*-39-1, *Kp*-39-2, and *Kp*-41) isolates, whereas the sample taken later on (May 2012) contained *K. pneumoniae* (*Kp*-43), but also an MDR *Salmonella enteritis* of serotype Kentucky (*Sk*-1). Another *Salmonella* Kentucky (*Sk*-2) was isolated in August 2013.

All the isolates (except the two *Sk*) expressed the CTX-M-15 ESBL. The *C. koseri* and *K. pneumoniae* isolates additionally co-expressed the CMY-4 pAmpC. The unique *E. coli* (*Ec*-38) was of ST131, whereas the *K. pneumoniae* isolates were of ST101 and ST111. Moreover, *C. koseri* and *K. pneumoniae* isolates were all PCR-positive for a not typable plasmid of FIB-M replicon.

The *Salmonella* Kentucky was of ST198 and produced OXA-48 as well as VEB-8. Transformation and conjugation experiments showed that bla_{OXA-48} was carried by an IncL/M plasmid, whereas bla_{VEB-8} could be located on the chromosome. Other detected resistance genes were: *aac(6)-Ib*, *tet(A)*, *sul1*, and *mphA*.

Conclusions:

This is the first report of an OXA-48-producing *S. enterica* outside Egypt as well as the first *Salmonella* co-producing OXA-48 and VEB-8.

The bla_{OXA-48} was carried by Tn*1999.2* and located on a ~60Kb IncL/M plasmid.

All the other isolates carried CMY-4, a pAmpC frequently reported in North Africa.

The co-expression of OXA-48 and VEB-8 together with other resistance genes rendered the isolate very resistant to standard antibiotic treatments.

The risk of selection and dissemination of such highly resistant bacteria is especially worrisome in countries with an endemic spread of OXA-48 producers.

Effective strategies to screen patients at risk to be colonized (e.g., those transferred or recently travelling in specific areas) must be constantly implemented by all health care institutions to prevent the diffusion of difficult to treat pathogens.

Acknowledgements:

We thank Alexandra Collaud, Sacha Thiermann, and Christel Inauen for technical assistance. This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and by Grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office (BVET). Salome N. Seiffert is a PhD student supported by IFIK and BVET.

Chapter VI

In line with the previous study a XDR *Klebsiella pneumoniae* was detected in a patient who got transferred back from Belgrade (Serbia) to Switzerland. The isolate was producing two carbapenemase genes, two *bla* genes, and carrying several other resistance genes.

The study was published in **International Journal of Antimicrobial Agents** under the title "Emergence of *Klebsiella pneumoniae* Coproducing NDM-1, OXA-48, CTX-M-15, CMY-16, qnrA, and ArmA Methyltransferase in Switzerland" in May **2014** (Seiffert et al., International Journal of Antimicrobial Agents; 44(3):260-262, 2014).

International Journal of Antimicrobial Agents 44 (2014) 260–262

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short Communication

Emergence of *Klebsiella pneumoniae* co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA and ArmA in Switzerland

Salome N. Seiffert[a,b,c], Jonas Marschall[d], Vincent Perreten[b], Alessandra Carattoli[a,b,e], Hansjakob Furrer[d], Andrea Endimiani[a,*]

[a] *Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, Postfach 61, CH-3010 Bern, Switzerland*
[b] *Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland*
[c] *Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland*
[d] *Department of Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland*
[e] *Department of Infectious, Parasitic and Immune-Mediated Diseases, Istituto Superiore di Sanità, Rome, Italy*

ARTICLE INFO

Article history:
Received 6 March 2014
Accepted 12 May 2014

Keywords:
16S rRNA
Carbapenemase
MBL
ESBL
AmpC

ABSTRACT

Extensively drug-resistant (XDR) *Klebsiella pneumoniae* isolates usually carry a single carbapenemase (e.g. KPC, NDM, OXA-48-like). Here we describe an XDR *K. pneumoniae* of sequence type 101 that was detected in the screening rectal swab of a patient transferred from the intensive care unit of a hospital located in Belgrade (Serbia) to Bern University Hospital (Switzerland). The isolate was resistant to all antibiotics with the exception of colistin [minimum inhibitory concentration] (MIC ≤ 0.125 μg/mL), tigecycline (MIC = 0.5 μg/mL) and fosfomycin (MIC = 2 μg/mL). The isolate co-possessed class B (NDM-1) and class D (OXA-48) carbapenemases, class A extended-spectrum β-lactamase (CTX-M-15), class C cephalosporinase (CMY-16), ArmA 16S rRNA methyltransferase, substitutions in GyrA and ParC, loss of OmpK35 porin, as well as other genes conferring resistance to quinolones (*qnrA*), tetracyclines [*tet*(A)], sulfonamides (*sul1*, *sul2*), trimethoprim (*dfrA12*, *dfrA14*), rifampicin (*arr-1*), chloramphenicol (*cmlA1*, *floR*) and streptomycin (*aadA1*). The patient was placed under contact isolation precautions preventing the spread of this nearly untreatable pathogen.

© 2014 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

1. Introduction

The spread of extensively drug-resistant (XDR) and pandrug-resistant Enterobacteriaceae, especially *Klebsiella pneumoniae*, imperils our last antibiotic options against Gram-negative pathogens. These isolates are usually resistant to all β-lactams because they possess one carbapenemase (e.g. class A KPC, class B NDM or class D OXA-48-like) along with class A extended-spectrum β-lactamases (ESBLs) (e.g. CTX-M) or class C plasmid-mediated AmpC enzymes (pAmpCs) (e.g. CMY) coupled with outer membrane porin loss [1,2]. However, unique and very rare *K. pneumoniae* isolates co-producing at least two classes of carbapenemases have been also reported in Colombia, several European countries, North Africa and the Middle East [3–7]. In this report, we describe the first dual carbapenemase-producing *K. pneumoniae* detected in Switzerland and its exceptional pool of antibiotic resistance genes.

2. Materials and methods

The following methodologies were implemented: microdilution ESB1F and GNX2F MIC plates (Trek Diagnostics, East Grinstead, UK) [8]; CT-103 (Check-Points, Wageningen, The Netherlands) and AMR-ve 0.8 m (Alere Technologies GmbH, Jena, Germany) microarrays for detection of β-lactamase (*bla*) and other resistance genes; PCR/DNA sequencing for *bla*, insertion sequence (IS) elements (IS*Ab125* and IS*Ecp1*), *ompK35* and *ompK36* porins, transposon Tn*1999*-like, 16S rRNA methyltransferases (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD* and *rmtF*), *gyrA*, *parC*, *qnrA*/*B*/*S*, and plasmid incompatibility group (PBRT kit; Diatheva, Fano, Italy) [9–11]. Sequencing results of chromosomal genes were compared with the deposited wild-types (GenBank nos. DQ673325 for *gyrA*, CP003785 for *parC*, AJ303057 for *ompK35* and AJ344089 for *ompK36*). Plasmids were extracted and electroporated into ElectroMAX™ *Escherichia coli* DH10B (Invitrogen, Carlsbad, CA); cells were selected on Luria–Bertani plates containing ampicillin (50 μg/mL) or cefotaxime (1 μg/mL) (Sigma, Steinheim, Germany) [9]. Multilocus sequence typing (MLST) was also performed (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

* Corresponding author. Tel.: +41 31 632 8632; fax: +41 31 632 8766.
E-mail addresses: aendimiani@gmail.com, andrea.endimiani@ifik.unibe.ch (A. Endimiani).

http://dx.doi.org/10.1016/j.ijantimicag.2014.05.008
0924-8579/© 2014 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

3. Results

3.1. Clinical case

In December 2013, a 54-year-old Swiss male was transferred from the intensive care unit of a hospital located in Belgrade (Serbia) to Bern University Hospital (Switzerland). The patient had suffered a polytrauma in a motor vehicle accident and during a 9-day hospital stay in Belgrade he underwent diagnostic work-up but no treatment of his fractures. Of note, Bern University Hospital did not receive any information on previous infections or colonisation with multidrug-resistant (MDR) organisms at the time of transfer to Switzerland.

Upon arrival at Bern University Hospital, the patient was placed under contact isolation precautions in a single room. A number of screening samples (i.e. urine, rectal and central venous catheter exit site swabs) were also taken and analysed for the presence of MDR Gram-negative pathogens (e.g. those producing ESBL and/or carbapenemase) using chromID® ESBL, MacConkey plus ceftazidime, and Drigalski plus cefotaxime selective agar plates (bioMérieux, Marcy-l'Étoile, France). This strategy is part of the hospital's infection prevention approach for patients transferred from abroad to identify colonisation with difficult-to-treat pathogens and to prevent their spread [12].

Only the stool sample revealed bacterial growth. In particular, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik, Bremen, Germany) and disc diffusion susceptibility tests, which are routinely performed in our laboratory, indicated the presence of an XDR *K. pneumoniae* isolate (AE-2247421) [13]. Infection prevention was notified and contact isolation precautions were maintained for the entire duration of the hospital stay. On Day 6, pelvic and right tibial fractures were fixated. On Day 17 the patient was discharged home without having developed an infection due to the XDR *K. pneumoniae*. As a result of the immediate implementation of contact precautions, no other patients were found to be colonised or developed infection with XDR *K. pneumoniae* isolates.

Table 1
Phenotypic and molecular characteristics of the extensively drug-resistant *Klebsiella pneumoniae* isolate detected in the screening culture (AE-2247421) and its *Escherichia coli* DH10B electrotransformants.

	K. pneumoniae AE-2247421	*E. coli* DH10B			
		Transformant #2	Transformant #7	Transformant #16	Parent
Antibiotic MIC, in μg/mL (interpretation)[a]					
Piperacillin/tazobactam	≥128 (R)	≤2	≥128	≥128	≤2
Ticarcillin/clavulanic acid	≥256 (R)	64	≥256	≥256	≤8
Cefoxitin	≥128 (NA)	≤2	≤2	≥128	≤2
Cefpodoxime	≥64 (R)	≥64	2	≥64	0.5
Ceftriaxone	≥256 (R)	≥256	≤0.5	128	≤0.5
Cefotaxime	≥128 (R)	≥128	1	≥128	≤0.125
Cefotaxime/clavulanic acid	≥128 (NA)	≤0.06	0.5	≥128	≤0.06
Ceftazidime	≥256 (R)	32	0.5	≥256	0.5
Ceftazidime/clavulanic acid	≥256 (NA)	0.5	0.5	≥256	0.5
Cefepime	≥32 (R)	16	≤0.5	16	≤0.5
Aztreonam	≥32 (R)	≥32	≤1	≥32	≤1
Imipenem	4 (R)	≤0.25	1	1	≤0.5
Meropenem	≥16 (R)	≤0.5	≤0.5	1	≤0.5
Ertapenem	≥8 (R)	≤0.125	1	2	≤0.125
Doripenem	≥4 (R)	≤0.06	0.25	0.5	≤0.06
Gentamicin	≥32 (R)	≤0.5	≤0.5	≥32	≤0.5
Tobramycin	≥16 (R)	≤0.5	≤0.5	≥16	≤0.5
Amikacin	≥64 (R)	≤2	≤2	≥64	≤2
Ciprofloxacin	≥4 (R)	≤0.125	≤0.125	≤0.125	≤0.125
Levofloxacin	≥16 (R)	≤0.5	≤0.5	≤0.5	≤0.5
Trimethoprim/sulfamethoxazole	≥8 (R)	4	≤0.25	≥8	≤0.25
Colistin	≤0.125 (S)	≤0.125	≤0.125	≤0.125	≤0.125
Polymyxin B	≤0.125 (NA)	≤0.125	≤0.125	≤0.125	≤0.125
Doxycycline	16 (NA)	≤1	≤1	4	≤1
Minocycline	8 (NA)	≤1	≤1	≤1	≤1
Tigecycline	0.5 (S)	≤0.125	≤0.125	≤0.125	≤0.125
Fosfomycin[b]	2 (S)	0.094	0.125	0.094	0.125
Resistance genes (genetic background or characteristics)	bla_{SHV-1} (NS) bla_{TEM-1} (NS) $bla_{CTX-M-15}$ (ISEcp1) bla_{CMY-16} (ISEcp1) bla_{OXA-48} (Tn1999.2) bla_{NDM-1} (ISAb125) OmpK35 (stop codon) armA (NS) GyrA (Ser83Tyr; Asp87Asn) ParC (Ser80Ile) qnrA $bla_{OXA-7-like}$, arr-1, dfrA12, dfrA14, sul1, sul2, cmlA1, floR, aadA1, tet(A), IntI1[c]	bla_{TEM-1} $bla_{CTX-M-15}$ dfrA14, IntI1 [c]	bla_{OXA-48}	bla_{CMY-16} bla_{NDM-1} armA qnrA $bla_{OXA-7-like}$, arr-1, dfr12, sul1, sul2, cmlA1, floR, aadA1, tet(A) [c]	NA
Plasmid incompatibility (Inc) group	A/C, R, L/M	R	L/M	A/C	
MLST	ST101	NA	NA	NA	

MIC: minimum inhibitory concentration; R: resistant; I: intermediate; S: susceptible; NA: not applicable; NS: not studied; MLST: multilocus sequence typing.

[a] According to European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [8]: MIC values were determined using ESB1F and GNX2F MIC plates (Trek Diagnostics, East Grinstead, UK).

[b] The fosfomycin MIC was determined by Etest (bioMérieux, Marcy-l'Étoile, France).

[c] These resistance genes were detected using the AMR-ve 0.8 m microarray (Alere Technologies GmbH, Jena, Germany).

3.2. Phenotypic and molecular analyses

As shown in Table 1, the isolate was confirmed to be an XDR *K. pneumoniae* susceptible only to colistin, tigecycline and fosfomycin [8]. AE-2247421 was of ST101 and carried at least three plasmids positive for repA/C, repL/M and repR, respectively. High-level resistance to all β-lactams was due to the simultaneous production of two different classes of carbapenemases (NDM-1 and OXA-48), CTX-M-15 ESBL and CMY-16 pAmpC as well as reduced permeability due to a disrupted OmpK35 porin (i.e. deletion of G in position 184 translating for a stop codon). AE-2247421 was also fully resistant to all aminoglycosides and quinolones owing to the production of ArmA 16S rRNA methyltransferase and amino acid substitutions in GyrA (Ser83Tyr and Asp87Asn) and ParC (Ser80Ile), respectively. Finally, microarray analysis indicated the presence of further genes such as those conferring resistance to quinolones (*qnrA*), tetracyclines [*tet*(A)], sulfonamides (*sul1*, *sul2*), trimethoprim (*dfrA12*, *dfrA14*), rifampicin (*arr-1*), chloramphenicol (*cmlA1*, *floR*) and streptomycin (*aadA1*) (Table 1).

Analysis of the genetic environment of the *bla* genes showed that: IS*Ab125* was identified upstream of bla_{NDM-1}, but ble_{MBL} encoding for bleomycin was not detected downstream [10]; bla_{OXA-48} was carried by Tn*1999.2* [2]; and both $bla_{CTX-M-15}$ and bla_{CMY-16} had upstream a complete IS*Ecp1* element [11]. These are commonly reported locations of the above *bla* genes [2,10,11]. Three phenotypically different DH10B transformants, each of which carried one plasmid, were also obtained. Based on their genotype results, we assert that: bla_{OXA-48} was located on the IncL/M plasmid; the IncA/C plasmid carried bla_{NDM-1}, bla_{CMY-16}, *qnrA* and *armA* genes; and $bla_{CTX-M-15}$ was located on the IncR plasmid (Table 1). The restriction enzyme patterns of the plasmid extracts of AE-2247421 and each single transformant were consistent with the above conclusions (Supplementary Fig. S1).

4. Discussion

AE-2247421 is a double carbapenemase-producing *K. pneumoniae* isolate possessing an extraordinary number of both chromosomal and mobile resistance genes. This *K. pneumoniae* may be a harbinger of the XDR Enterobacteriaceae that could soon infiltrate our healthcare systems, seriously compromising patient outcomes by the limited treatment options that remain. In support of this hypothesis, we note that ST101 is a rapidly emerging pan-epidemic clone with high potential for dissemination and associated with the production of important antibiotic resistance traits (e.g. ESBLs and/or carbapenemases) [2]. This *K. pneumoniae* was still susceptible to colistin, tigecycline and fosfomycin; however, one should be aware that resistant isolates are quickly selected under treatment and may eventually evolve into almost untreatable isolates [1]. Even more concerning is the fact that neither standard nor novel β-lactamase inhibitors would be able to efficiently combat isolates co-producing four classes of β-lactamases (i.e. classes A, B, C and D) such as AE-2247421. In fact, they never inhibit more than three families of enzymes (e.g. avibactam and BAL30072 do not act against classes B and A, respectively) [14]. Finally, due to the production of 16S rRNA methylases, all aminoglycosides (including those recently developed, e.g. plazomicin) become ineffective [15].

In conclusion, we show that XDR Enterobacteriaceae introduced from high-prevalence areas are arriving in Switzerland. Such isolates may combine a variety of resistance traits that make them nearly untreatable. Since only a few antibiotic options remain, we must therefore focus on rapid identification of colonised patients and implementation of isolation precautions. This may be our best chance to counter the spread of these life-threatening pathogens.

Funding: This work was supported by internal funds from the Institute of Infectious Diseases of Bern (IFIK) and by grant 1.12.06 (2012–2014) from the Swiss Veterinary Federal Office (BVET). SNS is a PhD student supported by IFIK and BVET.

Competing interests: None declared.

Ethical approval: Not required.

Acknowledgments

The authors thank Regula Tinguely and Alexandra Collaud for their technical assistance and Dr Sara Droz for providing the isolate.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2014.05.008.

References

[1] Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect 2012;18:413–31.

[2] Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 β-lactamase-producing Enterobacteriaceae over an 11-year period, 2001 to 2011. Euro Surveill 2013;18, pii: 20549.

[3] Rojas LJ, Mojica MF, Blanco VM, Correa A, Montealegre MC, De La Cadena E, et al. Emergence of *Klebsiella pneumoniae* coharboring KPC and VIM carbapenemases in Colombia. Antimicrob Agents Chemother 2013;57:1101–2.

[4] Balm MN, La MV, Krishnan P, Jureen R, Lin RT, Teo JW. Emergence of *Klebsiella pneumoniae* co-producing NDM-type and OXA-181 carbapenemases. Clin Microbiol Infect 2013;19:E421–3.

[5] Ben Nasr A, Decre D, Compain F, Genel N, Barguellil F, Arlet G. Emergence of NDM-1 in association with OXA-48 in *Klebsiella pneumoniae* from Tunisia. Antimicrob Agents Chemother 2013;57:4089–90.

[6] Samuelsen O, Naseer U, Karah N, Lindemann PC, Kanestrom A, Leegaard TM, et al. Identification of Enterobacteriaceae isolates with OXA-48 and coproduction of OXA-181 and NDM-1 in Norway. J Antimicrob Chemother 2013;68:1682–5.

[7] Dortet L, Poirel L, Al Yaqoubi F, Nordmann P. NDM-1, OXA-48 and OXA-181 carbapenemase-producing Enterobacteriaceae in Sultanate of Oman. Clin Microbiol Infect 2012;18:E144–8.

[8] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical breakpoints version 3 0. EUCAST; 2013.

[9] Seiffert SN, Perreten V, Johannes S, Droz S, Bodmer T, Endimiani A. OXA-48 carbapenemase-producing *Salmonella enterica* serovar Kentucky of sequence type 198 in a patient transferred from Libya to Switzerland. Antimicrob Agents Chemother 2014;58:2446–9.

[10] Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of bla_{NDM-1}-positive Enterobacteriaceae. Antimicrob Agents Chemother 2011;55:5403–7.

[11] Seiffert SN, Hilty M, Kronenberg A, Droz S, Perreten V, Endimiani A. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. J Antimicrob Chemother 2013;68:2249–54.

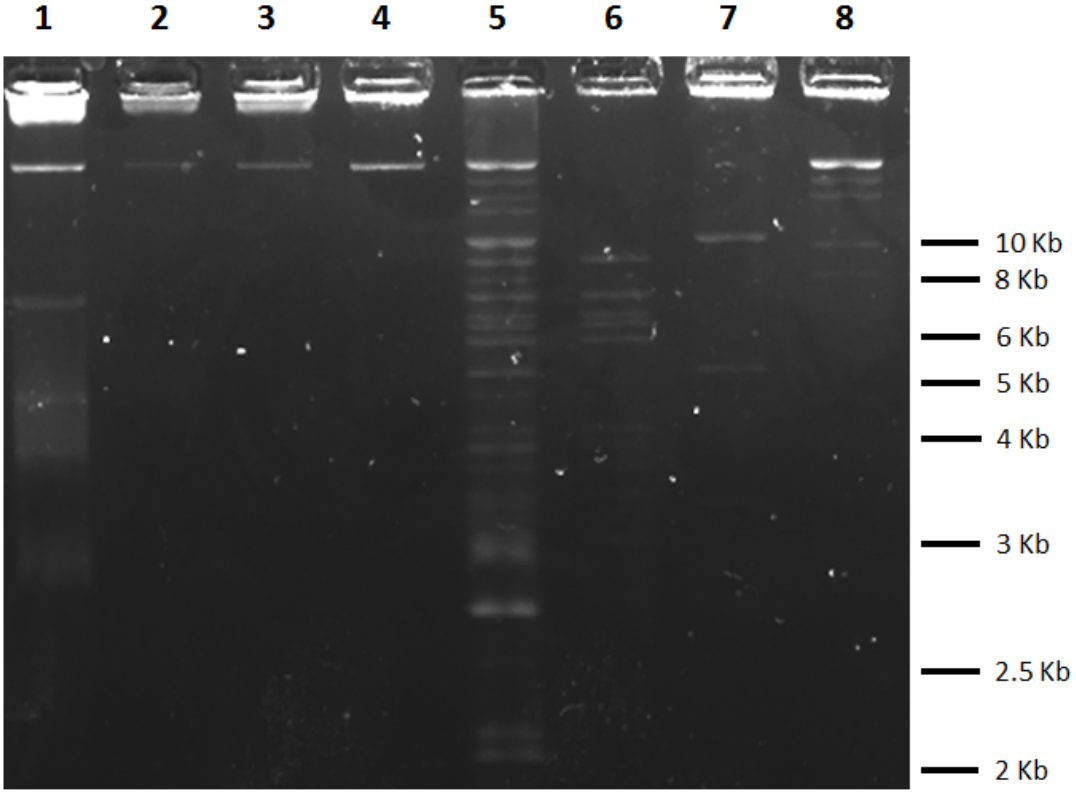
[12] European Centre for Disease Prevention and Control (ECDC). Risk assessment on the spread of carbapenemase-producing Enterobacteriaceae (CPE) through patient transfer between healthcare facilities, with special emphasis on cross-border transfer. Technical report, September 2011. ECDC; 2011.

[13] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.

[14] Watkins RR, Papp-Wallace KM, Drawz SM, Bonomo RA. Novel β-lactamase inhibitors: a therapeutic hope against the scourge of multidrug resistance. Front Microbiol 2013;4:392.

[15] Zhanel GG, Lawson CD, Zelenitsky S, Findlay B, Schweizer F, Adam H, et al. Comparison of the next-generation aminoglycoside plazomicin to gentamicin, tobramycin and amikacin. Expert Rev Anti Infect Ther 2012;10:459–73.

Supplementary Material of Chapter VI



Supplementary Fig. S1. Plasmid restriction analysis with *Pst*I of the extensively drug-resistant *Klebsiella pneumoniae* isolate detected in the screening culture (AE-2247421) and its *Escherichia coli* DH10B electrotransformants: lanes 1 and 5, AE-2247421; lanes 2 and 6, transformant #2; lanes 3 and 7, transformant #7; and lanes 4 and 8, transformant #16. Lanes 1–4, uncut plasmid extracts; lanes 5–8, plasmid extracts cut with restriction enzyme *PstI.*

Chapter VII

This study describes an interesting case of a patient that was transferred back from Belgrade (Serbia) to the University Hospital of Basel (Switzerland). The screening samples contained a MDR *Citrobacter freundii* harbouring *bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{SHV-12}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1}}$, and *armA*. Moreover, the *bla*$_{\text{NDM-1}}$ gene in a novel IncHI2 plasmid background that was subsequently on by 454-pyrosequencing.

Results were presented at the the **25$_{\text{th}}$ European Congress of Clinical Microbiology and Infectious Diseases** (ECCMID) – Copenhagen, Denmark – April 25-28, **2015**

The paper will be submitted for consideration for publication soon.

Citrobacter freundii possessing bla_{OXA-48}, bla_{SHV-12}, $bla_{CTX-M-15}$, bla_{TEM-1}, and *armA* also carries the bla_{NDM-1} gene in a novel IncHI2 plasmid background

Salome N. Seiffert,[1,2,3*] Vincent Perreten,[2] Vladimira Hinic,[4] Reno Frei,[4] Andrea Endimiani,[1] and Alessandra Carattoli [1,5#]

[1]Institute for Infectious Diseases, Faculty of Medicine, University of Bern, Bern, Switzerland; [2]Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;

[3]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland; [4]Division of Clinical Microbiology, University Hospital Basel, Basel, Switzerland; [5]Istituto Superiore di Sanità, Rome, Italy; (*Email: salome.seiffert@ifik.unibe.ch; #alessandra.carattoli@ifik.unibe.ch)

ECCMID 25-28 APRIL 2015 COPENHAGEN, DENMARK www.eccmid.org

Poster: EV0088

Introduction:

The New Delhi Metallo-β-lactamase (**NDM**) is one of the most potent and frequently detected carbapenemases in the world. Until now, the majority of the patients colonized/infected with bla_{NDM}-possessing organisms had an epidemiological link with the Indian subcontinent or Balkan countries (Seiffert SN et al, IJAA., 2014). Unlike other carbapenemase genes, bla_{NDM} has been described in an incredible number of plasmid-types which are carried by many members of *Enterobacteriaceae* as well as *Acinetobacter* spp. (Dortet L et al, BRI, 2014; Carattoli A, IJMM, 2013).

Here, we report the case of a patient who was simultaneously colonized/infected by six different MDR isolates including an XDR *Citrobacter freundii* (*Cf*) harboring bla_{NDM-1} on a novel IncHI2 plasmid.

Materials and Methods:

In August 2014, a polytraumatized patient with previous hospitalization in Serbia after a car accident was transferred to the University Hospital Basel (Switzerland). Several screening samples were taken. The samples were plated onto selective chromID ESBL (bioMérieux) in order to investigate the presence of MDR Gram-negative bacteria.

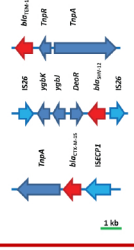
The subgroup of extended-spectrum cephalosporin-resistant *Enterobacteriaceae* (ESC-R-Ent) were further characterized with:

- Species identification using MALDI-TOF MS (Bruker Daltonik);
- Microarray CT103 for detection of ESBL, pAmpC, and carbapenemase genes (Check-Points);
- Antibiotic susceptibility testing (MIC) using ESBL1F and GNX2F MICs microdilution plates (Trek Diagnostics);
- PCR/DNA sequencing for bla_{ESBLs} (bla_{SHV}, and $bla_{CTX-M-gp\,1}$), bla_{AmpCs} (bla_{MOV}), carbapenemase genes (bla_{OXA-48}, and bla_{NDM}) as well as for *armA*, *gyrA*, *parC*, *ompK35*, *ompK36*, and transposon *Tn1999-like* environment.
- PCRs for plasmid replicon typing (PBRT; Diatheva);
- MLST analysis as well as pDLST for the IncHI2-NDM plasmid;
- Plasmid extraction was performed for the strains 804135-Nr.5 and 804135-Nr.4 using the Genopure Plasmid Midi Kit (Roche) followed by electrotransformation into DH10b *E. coli*. After O/N cultivation selection was done on plates with ampicillin [20 mg/L] and kanamycin [30 mg/L] (Sigma).

The complete nucleotide sequence of the IncHI2-NDM plasmid was obtained as follows:

- Whole sequencing of the IncHI2-NDM plasmid was performed using the 454 Junior Genome Sequencer (Roche).
- De novo assembly of DNA reads was accomplished with the GS-FLX gsAssembler software. The gap-closure was achieved using the 454 ReadStatus in combination with PCR- and cloning-based gap closure, by Sanger DNA sequencing of the amplicons.
- Sequence annotation was done using Artemis Version 8 (Sanger Institute) succeeded by pBLAST and Sequin (v 13.7).

Figure 2: Genetic environment of bla_{TEM-1}, bla_{SHV-12} and $bla_{CTX-M-15}$ on the HI2 plasmid



The β-lactamase TEM-1 was identified within a Tn*3* element.

bla_{SHV-1} is captured between two *IS26* together with the transcriptional regulator *YgbI* that controls *YgbJ* and *YgbK* which are potential oxidoreductase and tRNA synthase, respectively.

The $bla_{CTX-M-15}$ is flanked by a Tn*3* element and an *ISEcp1*, as previously described.

Table 1: Molecular characterization of the clinical samples

Species	*E. coli*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*K. pneumoniae*				*C. freundii*			
Strain/Transformant ID	804133-Nr.1	804133-Nr.2	804133-Nr.3	804133-Nr.6	804135-Nr.5	Kpn-EI.12	Kpn-EI.17	Kpn-EI.33	804135-Nr.4	Cf-EI.8	pHI2-NDM	Cf-EI.40
Sample	rectal swab	rectal swab	rectal swab	rectal swab	Urine				Urine			
Antibiotic MIC, mg/L (interpretation) [a]												
Piperacillin-tazobactam	32 (R)	≤4 (S)	≥128 (R)	8 (S)	≥128 (R)	≥128 (R)	≤4 (S)	8 (S)	≥128 (R)	≥128 (R)	≥128 (R)	≤4 (S)
Ticarcillin-clavulanate	≥256 (R)	32 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	64 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≥256 (R)	≤16 (I)
Cefoxitin	≥128 (NA)	8 (NA)	≥128 (NA)	64 (NA)	≥128 (NA)	8 (NA)	8 (NA)	≥128 (NA)	≥128 (NA)	16 (NA)	≥128 (NA)	≤4 (NA)
Cefpodoxime	≥64 (R)	≥64 (R)	≥64 (R)	≥64 (R)	≥64 (R)	1 (S)	≥64 (R)	≥64 (R)	≥64 (R)	1 (S)	≥64 (R)	2 (R)
Ceftriaxone	≥256 (R)	128 (R)	≥256 (R)	64 (R)	≥256 (R)	≤1 (S)	128 (R)	≥256 (R)	≥256 (R)	≤1 (S)	≥256 (R)	≤1 (S)
Cefotaxime	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	≥128 (R)	0.5 (S)	≥128 (R)	≥128 (R)	≥128 (R)	0.5 (S)	≥128 (R)	≤0.25 (S)
Cefotaxime-clavulanate	≥128 (NA)	≤0.12 (NA)	≥128 (NA)	64 (NA)	≥128 (NA)	0.5 (NA)	≤0.12 (NA)	32 (NA)	≥128 (NA)	1 (NA)	64 (NA)	≤0.12 (NA)
Ceftazidime	≥256 (R)	16 (R)	≥256 (R)	32 (R)	≥256 (R)	0.5 (S)	64 (R)	≥256 (R)	≥256 (R)	0.5 (S)	≥256 (R)	1 (S)
Ceftazidime-clavulanate	128 (NA)	0.25 (NA)	128 (NA)	16 (NA)	128 (NA)	0.5 (NA)	0.5 (NA)	128 (NA)	≥256 (NA)	0.5 (NA)	≥256 (NA)	0.5 (NA)
Cefepime	≥32 (R)	8 (R)	≥32 (R)	4 (R)	≥32 (R)	≤1 (S)	≥32 (R)	16 (R)	≥32 (R)	≤1 (S)	≥32 (R)	≤1 (S)
Aztreonam	>32 (R)	>32 (R)	>32 (R)	≤2 (I)	>32 (R)	≤2 (I)	>32 (R)	>32 (R)	>32 (R)	≤2 (I)	>32 (R)	≤2 (I)
Imipenem	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	2 (S)	4 (I)	≤0.5 (S)	≤0.5 (S)	≤0.5 (S)	4 (I)	2 (S)	1 (S)	≤0.5 (S)
Meropenem	≤1 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≥16 (R)	≤1 (S)	≤1 (S)	≤1 (S)	2 (S)	≤1 (S)	2 (S)	≤1 (S)
Ertapenem	≤0.25 (S)	≤0.25 (S)	12 (R)	≤0.25 (S)	≥8 (R)	1 (I)	≤0.25 (S)	≤0.25 (S)	≥8 (R)	1 (I)	2 (R)	≤0.25 (S)
Doripenem	≤0.12 (S)	≤0.12 (S)	0.5 (S)	≤0.12 (S)	≥8 (R)	0.25 (S)	≤0.12 (S)	≤0.12 (S)	2 (R)	0.5 (S)	1 (S)	≤0.12 (S)
Gentamicin	≥32 (R)	≤1 (S)	≥32 (R)	≥32 (R)	≥32 (R)	≤1 (S)	≤1 (S)	≥32 (R)	≥32 (R)	≤1 (S)	4 (I)	≥32 (R)
Tobramycin	≥16 (R)	≤1 (S)	≥16 (R)	≥16 (R)	≥16 (R)	≤1 (S)	≤1 (S)	≥16 (R)	≥16 (R)	≤1 (S)	≥16 (R)	≥16 (R)
Amikacin	≥64 (R)	≤4 (S)	≥64 (R)	≥64 (R)	≥64 (R)	≤4 (S)	≤4 (S)	≥64 (R)	≥64 (R)	≤4 (S)	≤4 (S)	≥64 (R)
Ciprofloxacin	≥0.25 (S)	≤0.25 (S)	≥4 (R)	≥4 (R)	≥4 (R)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≥4 (R)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)
Levofloxacin	≤1 (S)	≤1 (S)	≥16 (R)	≥16 (R)	≥16 (R)	≤1 (S)	≤1 (S)	≤1 (S)	≥16 (R)	≤1 (S)	≤1 (S)	≤1 (S)
Trimethoprim/sulfamethoxazole	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≥8 (R)	≤0.5	≥8 (R)	≥8 (R)	≥8 (R)	≤0.5	≥8 (R)	≤0.5
Colistin	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≥8 (R)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)
Polymyxin B	0.5 (NA)	≤0.25 (NA)	0.5 (NA)	≥8 (NA)	0.5 (NA)	≤0.25 (NA)	≤0.25 (NA)	≤0.25 (NA)	0.5 (NA)	≤0.25 (NA)	≤0.25 (NA)	0.5 (NA)
Doxycycline	≤3 (NA)	≤3 (NA)	≥32 (NA)	≥32 (NA)	16 (NA)	≤3 (NA)	≤3 (NA)	4 (NA)	16 (NA)	≤3 (NA)	≤3 (NA)	≤3 (NA)
Minocycline	≤2 (NA)	≤2 (NA)	16 (NA)	≥32 (NA)	16 (NA)	≤2 (NA)	≤2 (NA)	≤2 (NA)	8 (NA)	≤2 (NA)	≤2 (NA)	≤2 (NA)
Tigecycline	≤0.25 (S)	≤0.25 (S)	1 (S)	4 (R)	1 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)	0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤0.25 (S)
Fosfomycin [b]	0.38 (S)	0.25 (S)	8 (S)	>1000 (R)	8 (S)	0.5 (S)	2 (S)	0.38 (S)	2 (S)	2 (S)	1.5 (S)	3 (S)
Resistance genes CT103-PCR	bla_{TEM} (wt) $bla_{CTX-M-15}$ $bla_{SHV-12like}$ ArmA (PCR only)*	bla_{TEM} (wt) $bla_{CTX-M-15}$	bla_{TEM} (wt) bla_{SHV} (wt) $bla_{CTX-M-15}$ $bla_{CMY-16like}$ ArmA (PCR only)* GyrA (S83Y, D87N) ParC (S80I), Omp35 (stop codon AA 63) Omp35GY	bla_{TEM} (wt) $bla_{CMY-16like}$ ArmA (PCR only)*	bla_{TEM} (wt) bla_{SHV} (wt) bla_{OXA-48} $bla_{CTX-M-15}$ $bla_{CMY-16like}$ ArmA (PCR only)* GyrA (S83Y, D87N) ParC (S80I), Omp35 (stop codon AA 63) Omp35GY				bla_{TEM} (wt) bla_{SHV-12} $bla_{CTX-M-15}$ bla_{TEM-1} ArmA (PCR only)*		
PBRT results for plasmid:	I1, FⁱA:B⁻, A/C, R, FII	I1, FⁱA:B⁻, R, FII	I1, FⁱA:B⁻, A/C, R, FII	-	A/C, R, I, FⁱA:B⁻(Pse3)	L	R	A/C	A/C, HI2, I,	L	HI2	A/C
MLST/pDLST	ST117	ST117	ST101		ST101						pDLST: ST1	
Transformation						OXA-48 (Tn1999.2)	TEM, CTX-M-15	AmA, TEM, CMY-16-like		OXA-48 (Tn1999.2)	TEM-1, SHV-12, CTX-M-15, NDM-1	ArmA

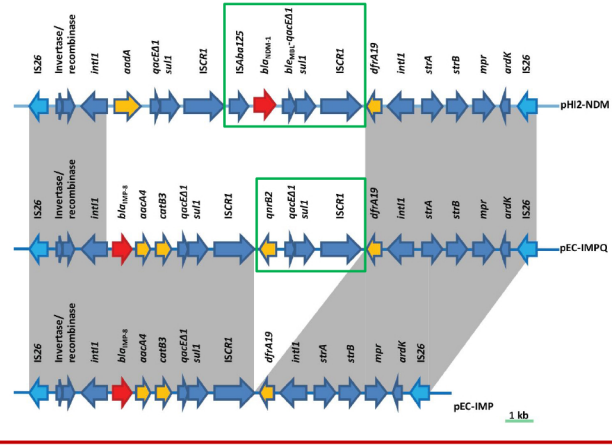
Note. [a] According to the EUCAST criteria: R, resistant; I, intermediate; S, susceptible; NA, not applicable or not available; [b] Fosfomycin MIC was obtained implementing the Etest (bioMérieux); [c] No sequencing of the amplicon was performed

Figure 1: Genetic environment of bla_{NDM-1} on the HI2 plasmid

The genetic environment of bla_{NDM-1} has structural similarity with the bla_{NMP-1} environment of anothe IncHI2 plasmid named pEC-IMPQ. Both pEC-IMPQ and pHI2-NDM plasmids show two co-linear class 1 integrons, and two copies of the *ISCR1* element. pEC-IMPQ derived from pEC-IMP by acquisition of the *qnrB2-qacEΔ1-sul1-ISCR1* module in a position comprised between *ISCR1* and the *dfrA19* gene.

In pHI2-NDM the *ISAba125*-bla_{NDM-1}-*ble*$_{MBL}$-*qacEΔ1-sul1-ISCR1* module has been identified at the same integration site comprised between *ISCR1* and the *dfrA19* gene.

Both these structures may derive from the recombination of the resisdent complex class 1 integron and a circular intermediate generated by the *ISCR1*-mediated rolling circle duplication.



Results:

Rectal swab screening yielded extended-spectrum cephalosporin-resistant *E. coli* (804133-Nr.1, 804133-Nr.2), *K. pneumoniae* (804133-Nr.3) and *P. mirabilis* (804133-Nr.6) isolates. More importantly, analysis of urine revealed the presence of another *K. pneumoniae* (804135-Nr.5) (*Kpn*-Nr.5) and of one *Cf* (804135-Nr.4) (*Cf*-Nr.4) isolate with reduced susceptibility to carbapenems (**Table 1**).

The two *E. coli* as well as both of the *K. pneumoniae* strains shared the same MLST types, ST117 and ST101, respectively. The four strains isolated from the rectal swab carried only ESBL genes (e.g.: $bla_{CTX-M-15}$, and $bla_{CMY-16-like}$), whereas the two strains isolated from urine additionally harboured carbapenemase genes (e.g.: bla_{OXA-48}, and bla_{NDM-1}).

Kpn-Nr.5 possessed three plasmids: an **IncL carrying the bla_{OXA-48} gene**; an **IncA/C with bla_{CMY-16}, bla_{TEM-1}, and 16S rRNA-methylase *armA* gene**; as well as an **IncR plasmid harbouring $bla_{CTX-M-15}$ and bla_{TEM-1}**. *Cf*-Nr.4 carried the same **IncL-OXA-48** plasmid as *Kpn*-Nr.5; an IncA/C with *armA* as well as an **IncHI2-ST1 plasmid (pHI2-NDM), containing the bla_{NDM-1}, bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-15}$** beta-lactamase genes.

The pHI2-NDM is >300kb in size. **The NDM-1 was identified in a novel genetic environment** inside a class 1 integron (Figure 1). The class 1 integron most likely acquired bla_{NDM-1} via RC and homologous recombination, within a previously described IncHI2 plasmid scaffold.

The other three β-lactamases (i.e., $bla_{CTX-M-15}$, bla_{SHV-1}, and bla_{TEM-1}) were located in frequently described genetic elements (Figure 2).

Conclusions:

This is the first description of bla_{NDM} on an IncHI2 plasmid.

These broad host-range plasmids have their optimal conjugation temperature between 22°C and 30°C, facilitating their spread among environmental species. This combination of factors could create a potent vector for the global spread of bla_{NDM}.

Additionally, this study describes bla_{NDM-1} in a novel genetic environment.

The detection of several different MDR bacterial species in the same patient is worrisome and underlines the need for effective screening strategies for patients coming from high-risk countries.

Overall, these results should raise the awareness for a bla_{NDM} spread independent from its epidemiological link with the Indian subcontinent or Balkan countries. Only tight screening strategies might prevent a global dissemination of this highly potent carbapenemase.

Acknowledgements:

This work was supported by internal funds of the Institute of Infectious Diseases of Bern (IFIK) and by Grant 1.12.06 (2012-2014) from the Swiss Veterinary Federal Office (BVET).

Salome N. Seiffert is a PhD student supported by IFIK and BVET.

CONCLUSION AND OUTLOOK

CONCLUSION

The aims of this thesis were: *i)* to investigate the potential transmission of ESC-R *Ent* between animals and human reservoirs, and *ii)* to characterize new resistance mechanisms. In order to assess the risk of ESC-R *Ent* being transmitted between the human and animal reservoirs, several studies were conducted in the different settings.

For the investigation of potential transmission pathways between the human and animal reservoirs detailed molecular characteristics from a defined region and from a similar timeframe of the respective settings were needed. As such data was scarce and not from the same location, we performed several studies in order to acquire a good overview over the spread of ESC-R *Ent* in humans and animals in the region of Bern, Switzerland.

In the first study, ESC-R *E.coli* isolated from hospitalized patients were characterized in-depth.[1] This was the first Swiss analysis that investigated the epidemiological and molecular characteristics of ESC-R isolates in human patients. Our results showed that, with an overall prevalence of 6.5%, Switzerland has still a low-prevalence unlike other neighbouring countries.[2] The molecular characterization showed that the high-risk clone ST131-carrying CTX-M-15 was also prevalent (35%).[3] Moreover, 25% of the strains presented an AmpC phenotype, caused by a plasmid-mediated AmpC (bla_{CMY}) or a hyper-expressed chromosomal AmpCs, a high prevalence which could be due to the frequent use of β-lactam/β-lactam-inhibitor combinations (e.g., amoxicillin/clavulanate) in our region. A similar rate of 28% of strains with AmpC phenotype was also detected in a study from the Netherlands.[4] Interestingly, most of the AmpC producers in our study were detected in patients referring to Specialized Outpatient Clinics (SOC)-and were mainly coming from the nephrology and urology department. This group of patients is especially at risk of spreading ESC-R *Ent* based on their long-term treatment and their frequent hospital stays.[1] A major limitation of the study was that only patients from one hospital in Switzerland were enrolled. However, the results were comparable to another study performed in Switzerland where faecal samples from healthy humans were investigated. An overall prevalence of 5.8% for ESBL producing *E.coli* was reported, with CTX-M-15 as major β-lactamase.[5] Unfortunately, no MLST analysis was done in the study of Geser et al, which did not allow comparing the *E. coli* clones between the two studies. Differences in the prevalence and/or type of resistance genes could be strongly influenced by the antibiotic usage guidelines of a certain region/country.

The second study investigated the contamination of ESC-R *Ent* in raw chicken-meat collected from different shops in the city of Bern, Switzerland.[6] The overall prevalence of 70% was in the range of other studies from Germany (43.9%) and the Netherlands (94%).[7, 8] The poultry-meat that originated from Switzerland was mainly contaminated with *E.coli* containing *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CMY-2}}$, whereas the poultry-meat imported from outside contained strains harbouring *bla*$_{\text{SHV-12}}$, *bla*$_{\text{TEM-52}}$, and *bla*$_{\text{CMY-2}}$. These results were in line with studies performed in Germany and in the Netherlands where the majority of the poultry-meat samples contained strains carrying *bla*$_{\text{CTX-M-1,}}$ *bla*$_{\text{SHV-12}}$, and *bla*$_{\text{TEM-52}}$.[7, 8] Similar data was also collected in a previously published study on broilers in Switzerland where an overall prevalence of 25% ESBL and AmpC carriage mainly due to *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CMY-2}}$ was determined.[9]

All these studies indicate that chicken-meat and broiler are contaminated/colonized with the same resistance genes. However, this epidemic spread is not driven by a specific clonal strain but rather by several different plasmid types, especially plasmids of the IncI1 group. [10, 11] The IncI1 plasmid group along with other plasmids groups have been considered as "epidemic resistance plasmids" due to their spread to various enterobacterial species as well as their zoonotic potential.[10, 11] Noteworthy in the present study was the discovery of a *Proteus mirabilis* strain that carried *bla*$_{\text{VEB-6}}$ on a ~17kb class 1 integron. Exactly the same integron was previously reported in a *P. mirabilis* strain (*Pm*VB1248) of a human patient in France.[12] Together with the fact that the *P. mirabilis* strains were indistinguishable by rep-PCR, this was the first indication of direct/indirect transmission between the human and animal reservoirs. The same human *P. mirabilis* strain (*Pm*VB1248) has been used in another recently published study where the authors investigated the genetic islands in *P. mirabilis* in dogs and humans.[13] The authors identified two *P. mirabilis* isolates from humans (one of them was the *Pm*VB1248 isolate) and one from a dog carrying nearly identical *bla*$_{\text{VEB-6}}$-carrying SGI1-V elements. As the same human *P. mirabilis* isolate (*Pm*VB1248) is closely related to strains from chicken-meat and dogs, it can be inferred that this *P. mirabilis* strain is widely dissemination in Europe.[6, 13] Limitations of the study are the relatively low sample size and that all samples were bought in the same city. However, such studies are very labour-intensive and the results are matching with data from other studies. To sum up, it is strongly suggested that poultry-meat should not be washed to prevent aeroionisation of these highly drug-resistant bacteria and that the meat should be cooked properly in order to diminish the risk of transmitting these pathogens to the human gut flora.[14]

Recent studies have detected ESBLs, AmpCs, and even carbapenemases in healthy as well as diseased companion animals.[15-17] These animals, unlike food-producing animals, represent a huge reservoir that is in very close and frequent contact to their owners.[18] Moreover, in order to treat companion animals, β-lactams are the most important drug class, which consequently leads to an enormous problem once resistances are continue to spread.[15, 18] Furthermore, several studies have described identical isolates in humans and their pets inferring that transmission from humans to animals or *vice-versa* is jet another way for the transmission of highly drug-resistant pathogens.[19, 20] However, to our knowledge there were no reports investigating alternative transmission pathways by which pets can acquire their resistant bacteria. Meanwhile, in the human reservoir many recently published studies are investigating meat, vegetables, and ready-to-eat food for the presence of highly-drug resistant bacteria.[6, 21-23]

In line with these projects, **the third study** was performed on pet food as a contamination source for companion animals.[24] For this purpose, we examined wet food (n=30) for cats and dogs for highly drug-resistant bacteria. As no living bacteria were detected, the samples were examined for the existence of acquired resistance genes using PCR/Sequencing. Overall, 70% of the samples were contaminated with *bla* genes. Surprisingly, the detected *bla* genes (i.e., $bla_{\text{CMY-2}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{OXA-48}}$) are usually more related to the human setting inferring that there was a huge human-made/environmental contamination along the production process.[25, 26] Although the risk that these highly fragmented resistance genes are taken up and integrated into a mobile genetic element is very small, our results showed that there are unknown reservoirs contaminating the pet food systematically. These reservoirs have to be traced back, as they could be the origin of highly drug-resistant bacteria.

Although not linked to the results of our study, reports on companion animals being infected/colonized with bacteria carrying $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-48}}$ are increasing.[27] One study from Germany identified $bla_{\text{OXA-48}}$ in six samples from dogs with nosocomial infections.[17] This finding is alarming as the detection of the hyperepidemic IncL plasmid carrying $bla_{\text{OXA-48}}$ is difficult and because veterinary laboratories are not commonly testing for carbapenem resistance.[18, 28] However, systematic large scale surveillance studies are needed in order to better understand the epidemiological spread of these resistance determinants in companion animals. Limitations of our study were the PCR products of less than 1000 bp due to the strong fragmentation of the DNA most likely caused by the autoclaving process during the food production process. Nevertheless, this study unveiled an unsuspected reservoir of antibiotic resistance genes.

For **the fourth study** we investigated the common characteristics among the animal and human settings. It became evident that the *bla* genes, the plasmids, and the MLST types are often varying between both settings. However, one *bla* gene, *bla*$_{CMY-2}$, was constantly detected in isolates from humans, poultry-meat, as well as livestock (including broiler samples).[1, 9, 29] Analysis of the *bla*$_{CMY}$-carrying isolates revealed that in nine of them (three from each collection) the *bla* gene was encoded on an IncK plasmid. The analysis of the whole plasmid sequences from seven of these plasmids illustrated that they all belonged to a new incompatibility group variant, the IncK2 group that was genetically distinct from the IncK, IncB/O groups. This finding gives firm evidence that the plasmid originated from common ancestor from where it has probably subsequently spread to the various settings. Tracing back the source was not possible but the close genetic relatedness infers a common source from where the plasmid has spread to the different reservoirs. To our knowledge this was the first time that a genetically identical novel plasmid type has been detected simultaneously in the human, food and animal reservoirs. Moreover, the samples were all collected within a time period of four years and originated all from a small area in central Switzerland.

The second part of the thesis was dedicated to the characterization of unknown resistance phenotypes in *Enterobacteriaceae*.

The first study described the case of a patient that was transferred from Libya to Switzerland.[26] The patient was a suffering a polytrauma due to a grenade denotation and was transferred into a rehabilitation centre in Switzerland after being hospitalized in Libya. Upon arrival several samples were taken from the patient in order to be tested for the presence of highly drug-resistant bacteria. This procedure is part of the hospital hygiene policies where patients that were previously hospitalized in a country, which is endemic for highly drug-resistant pathogens are put in isolation and samples are taken for checking if such pathogens are present. These measures are needed in order to prevent hospital outbreaks involving such highly drug-resistant pathogens. In fact one of the screening samples contained a MDR *Salmonella enterica* Kentucky harbouring *bla*$_{VEB-8}$ and *bla*$_{OXA-48}$. This was the first report of a *Salmonella enterica* Kentucky harbouring *bla*$_{OXA-48}$ outside the Balkans and the first report of Salmonella carrying *bla*$_{VEB-8}$. The first case of *bla*$_{OXA-48}$ in a *Salmonella enterica* Kentucky was isolated from a French patient with travel history to Egypt.[30] In this study they have characterized several more ciprofloxacin-resistant *Salmonella enterica* Kentucky ST198 isolates, mostly originating in Balkan countries, and detected not only the carbapenemase *bla*$_{OXA-48}$, but also *bla*$_{VIM-2}$.[30] In the past decades *Salmonella enterica* Kentucky was known for

causing outbreaks in poultry the USA, Ireland, and in Africa reflecting the potential zoonotic potential of this strain.[31, 32]

In the two following studies difficult-to-treat isolates were detected in patients that were both hospitalized in Belgrade (Serbia) and subsequently transferred back to Switzerland. The first case was a patient that carried an XDR *Klebsiella pneumoniae* co-producing two carbapenemase genes, $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$.[25] The New-Delhi Metallo-β-lactamase (bla_{NDM}) was discovered in 2008 and has meanwhile become one of the most threatened carbapenemase genes.[33, 34] The hydrolytic activity spectrum of this enzyme incorporates all of the β-lactam antibiotics leaving out only the monobactams. Moreover, bla_{NDM} has been reported along with many other resistance genes leaving out in some cases only few antibiotics for the treatment of NDM-producing organisms.[34] Of major importance is the fact that most of the bla_{NDM}-positive patients had a connection to India, the Arabian, Balkan, or North African countries.[34, 35] Noteworthy, in 2011 a study detected a variety of bacterial species carrying bla_{NDM} in seepage and in tap water samples in New-Delhi inferring a huge environmental contamination with this carbapenemase.[36] The NDM β-lactamase is a huge threat to our public health systems not only due to its hydrolytic activity but also based on its potential to sustain in the environment.[33, 36] In addition, the NDM β-lactamase is also a known for its broad bacterial host range with stable inheritance, its possibility of being transferred, and its spread into various genetic backgrounds including different plasmid groups and into resistance islands encoded on bacterial chromosomes.[36-39] The second carbapenemase, $bla_{\text{OXA-48}}$, has already been described in the previously mentioned study where pet food samples were investigated for the presence of resistance genes.[24] Like bla_{NDM}, cases of $bla_{\text{OXA-48}}$ seem to have a link to Turkey and the North African countries.[34] The epidemic spread of $bla_{\text{OXA-48}}$ is caused by a unique IncL plasmid which is depressed due to the integration of the transposon *Tn*1999 inside the *tir* gene, a gene most likely involved in the inhibition of the conjugal plasmid transfer, and is consequently highly efficient in spreading to different bacterial species.[28, 34, 40] As previously mentioned part of the $bla_{\text{OXA-48}}$ problematic is the difficulty in detecting this carbapenemase as is susceptible to oxyiminocephalosporin and shows only minor increase in MICs for the carbapenems.[41] The combination of both carbapenemase genes in one patient is alarming and indicates that constant surveillance is needed in order to prevent the spread of these enzymes inside the hospitals but also into the community and the environment.

In the second case the patient suffered a polytrauma due to a car accident and had been hospitalized in Belgrade before being transferred to Switzerland. The patient was

infected/colonized with several ESBL-carrying *Enterobacteriaceae* including an MDR double-carbapenemase producing *Citrobacter freundii*. Similarly to the preceding study the strain carried $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$. However, in this case the $bla_{\text{NDM-1}}$ gene has been identified for the first time on an IncHI2 plasmid, a plasmid group which is known for their huge size (>300kb), their broad host range, and for their conjugation temperature which is optimal below 30°C.[42, 43] This finding is worrying as a previously published paper has observed that the conjugation frequencies of NDM-positive bacteria had an increased efficacy at 30°C. [36] This temperature corresponds to the daily peak temperature reached in New Delhi inferring that such a plasmid might easily spread inside the bacterial community in the environment.[36] Although such a spread is unlikely in central Europe due to the lower temperatures, constant surveillance is needed, as NDM is known to spread to various genetic backgrounds that can also be temperature independent. In general, these cases showed that strict isolation strategies are needed in order to prevent the spread of such nearly untreatable pathogens inside the hospitals or even into the community.

Final remarks

This thesis provided strong evidences confirming active gene transfer between the human and animal reservoirs. First evidence was presented in the poultry-meat study where an identical class 1 integron has been detected in a *P. mirabilis* in a human patient as well as in two *P. mirabilis* of our poultry-meat samples. Furthermore, a recently published study has detected a $bla_{\text{VEB-6}}$-carrying *P. mirabilis* in a dog sample that was highly similar to the same human *P. mirabilis* that has been used in the poultry-meat study.[6, 13] Second evidence consisted of the discovery of the nearly 100% identical IncK2 plasmids in samples from patients, poultry-meat and broilers from in a short period of time (4 years) and all from restricted area (central Switzerland). These evidences point towards an active gene transfer between the human and animal reservoirs, suggesting that future surveillance systems should focus on both reservoirs in order to implement adequate measurements that limit the spread of multidrug-resistant genetic elements between bacteria from animals and humans as well as the transfer of bacteria between the two settings. The second part of the thesis showed that constant surveillance and strict isolation procedures are needed to prevent the spread of difficult-to-treat pathogens inside the hospital, to community, and to the environment.

In conclusion, continuous efforts should be made in all settings to either further maintain or implement strict hygienic measures in order to limit the spread of life-threatening multidrug-resistant bacteria.

References:

1. Seiffert SN, Hilty M, Kronenberg A et al. Extended-spectrum cephalosporin-resistant *Escherichia coli* in community, specialized outpatient clinic and hospital settings in Switzerland. *The Journal of antimicrobial chemotherapy* 2013; **68**: 2249-54.
2. Canton R, Novais A, Valverde A et al. Prevalence and spread of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Europe. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2008; **14 Suppl 1**: 144-53.
3. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clinical microbiology reviews* 2015; **28**: 565-91.
4. Voets GM, Platteel TN, Fluit AC et al. Population distribution of Beta-lactamase conferring resistance to third-generation cephalosporins in human clinical *Enterobacteriaceae* in the Netherlands. *PloS one* 2012; **7**: e52102.
5. Geser N, Stephan R, Korczak BM et al. Molecular identification of extended-spectrum-beta-lactamase genes from *Enterobacteriaceae* isolated from healthy human carriers in Switzerland. *Antimicrobial agents and chemotherapy* 2012; **56**: 1609-12.
6. Seiffert SN, Tinguely R, Lupo A et al. High prevalence of extended-spectrum-cephalosporin-resistant enterobacteriaceae in poultry meat in Switzerland: emergence of CMY-2- and VEB-6-possessing *Proteus mirabilis. Antimicrobial agents and chemotherapy* 2013; **57**: 6406-8.
7. Kola A, Kohler C, Pfeifer Y et al. High prevalence of extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in organic and conventional retail chicken meat, Germany. *The Journal of antimicrobial chemotherapy* 2012; **67**: 2631-4.
8. Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J et al. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2011; **17**: 873-80.
9. Endimiani A, Rossano A, Kunz D et al. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. *Diagnostic microbiology and infectious disease* 2012; **73**: 31-8.
10. Carattoli A. Plasmids in Gram negatives: molecular typing of resistance plasmids. *International journal of medical microbiology : IJMM* 2011; **301**: 654-8.
11. Seiffert SN, Hilty M, Perreten V et al. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* 2013; **16**: 22-45.
12. Siebor E, Neuwirth C. The new variant of Salmonella genomic island 1 (SGI1-V) from a *Proteus mirabilis* French clinical isolate harbours bla_{VEB-6} and qnrA1 in the multiple antibiotic resistance region. *The Journal of antimicrobial chemotherapy* 2011; **66**: 2513-20.
13. Schultz E, Haenni M, Mereghetti L et al. Survey of multidrug resistance integrative mobilizable elements SGI1 and PGI1 in *Proteus mirabilis* in humans and dogs in France, 2010-13. *The Journal of antimicrobial chemotherapy* 2015.
14. Gao L, Tan Y, Zhang X et al. Emissions of *Escherichia coli* carrying extended-spectrum beta-lactamase resistance from pig farms to the surrounding environment. *International journal of environmental research and public health* 2015; **12**: 4203-13.
15. Rubin JE, Pitout JD. Extended-spectrum beta-lactamase, carbapenemase and AmpC producing *Enterobacteriaceae* in companion animals. *Veterinary microbiology* 2014; **170**: 10-8.

16. Dierikx CM, van Duijkeren E, Schoormans AH et al. Occurrence and characteristics of extended-spectrum-beta-lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *The Journal of antimicrobial chemotherapy* 2012; **67**: 1368-74.
17. Stolle I, Prenger-Berninghoff E, Stamm I et al. Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. *The Journal of antimicrobial chemotherapy* 2013; **68**: 2802-8.
18. Abraham S, Wong HS, Turnidge J et al. Carbapenemase-producing bacteria in companion animals: a public health concern on the horizon. *The Journal of antimicrobial chemotherapy* 2014; **69**: 1155-7.
19. Johnson JR, Owens K, Gajewski A et al. *Escherichia coli* colonization patterns among human household members and pets, with attention to acute urinary tract infection. *The Journal of infectious diseases* 2008; **197**: 218-24.
20. Dolejska M, Duskova E, Rybarikova J et al. Plasmids carrying *bla*$_{\text{CTX-M-1}}$ and qnr genes in *Escherichia coli* isolates from an equine clinic and a horseback riding centre. *The Journal of antimicrobial chemotherapy* 2011; **66**: 757-64.
21. van Hoek AH, Veenman C, van Overbeek WM et al. Prevalence and characterization of ESBL- and AmpC-producing *Enterobacteriaceae* on retail vegetables. *International journal of food microbiology* 2015; **204**: 1-8.
22. Nuesch-Inderbinen M, Zurfluh K, Peterhans S et al. Assessment of the Prevalence of Extended-Spectrum beta-Lactamase-Producing *Enterobacteriaceae* in Ready-to-Eat Salads, Fresh-Cut Fruit, and Sprouts from the Swiss Market. *Journal of food protection* 2015; **78**: 1178-81.
23. Rubin JE, Ekanayake S, Fernando C. Carbapenemase-producing organism in food, 2014. *Emerging infectious diseases* 2014; **20**: 1264-5.
24. Seiffert SN, Carattoli A, Tinguely R et al. High prevalence of extended-spectrum beta-lactamase, plasmid-mediated AmpC, and carbapenemase genes in pet food. *Antimicrobial agents and chemotherapy* 2014; **58**: 6320-3.
25. Seiffert SN, Marschall J, Perreten V et al. Emergence of *Klebsiella pneumoniae* co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA and ArmA in Switzerland. *International journal of antimicrobial agents* 2014; **44**: 260-2.
26. Seiffert SN, Perreten V, Johannes S et al. OXA-48 carbapenemase-producing Salmonella enterica serovar Kentucky isolate of sequence type 198 in a patient transferred from Libya to Switzerland. *Antimicrobial agents and chemotherapy* 2014; **58**: 2446-9.
27. Schmiedel J, Falgenhauer L, Domann E et al. Multiresistant extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from humans, companion animals and horses in central Hesse, Germany. *BMC microbiology* 2014; **14**: 187.
28. Carattoli A, Seiffert SN, Schwendener S et al. Differentiation of IncL and IncM Plasmids Associated with the Spread of Clinically Relevant Antimicrobial Resistance. *PloS one* 2015; **10**: e0123063.
29. Vogt D, Overesch G, Endimiani A et al. Occurrence and genetic characteristics of third-generation cephalosporin-resistant *Escherichia coli* in Swiss retail meat. *Microbial drug resistance* 2014; **20**: 485-94.
30. Le Hello S, Harrois D, Bouchrif B et al. Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. *The Lancet Infectious diseases* 2013; **13**: 672-9.
31. Harrois D, Breurec S, Seck A et al. Prevalence and characterization of extended-spectrum beta-lactamase-producing clinical *Salmonella enterica* isolates in Dakar, Senegal, from 1999 to 2009. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2014; **20**: O109-16.

32. Wasyl D, Kern-Zdanowicz I, Domanska-Blicharz K et al. High-level fluoroquinolone resistant *Salmonella enterica* serovar Kentucky ST198 epidemic clone with IncA/C conjugative plasmid carrying *bla*(CTX-M-25) gene. *Veterinary microbiology* 2015; **175**: 85-91.
33. Yong D, Toleman MA, Giske CG et al. Characterization of a new metallo-beta-lactamase gene, *bla*(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrobial agents and chemotherapy* 2009; **53**: 5046-54.
34. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2014; **20**: 821-30.
35. Johnson AP, Woodford N. Global spread of antibiotic resistance: the example of New Delhi metallo-beta-lactamase (NDM)-mediated carbapenem resistance. *Journal of medical microbiology* 2013; **62**: 499-513.
36. Walsh TR, Weeks J, Livermore DM et al. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *The Lancet Infectious diseases* 2011; **11**: 355-62.
37. Girlich D, Dortet L, Poirel L et al. Integration of the $bla_{\text{NDM-1}}$ carbapenemase gene into Proteus genomic island 1 (PGI1-PmPEL) in a *Proteus mirabilis* clinical isolate. *The Journal of antimicrobial chemotherapy* 2015; **70**: 98-102.
38. Qin S, Qi H, Zhang Q et al. Emergence of extensively drug-resistant *Proteus mirabilis* harboring a conjugative NDM-1 plasmid and a novel Salmonella genomic island 1 variant (SGI1-Z). *Antimicrobial agents and chemotherapy* 2015.
39. Carattoli A. Plasmids and the spread of resistance. *International journal of medical microbiology : IJMM* 2013; **303**: 298-304.
40. Potron A, Poirel L, Nordmann P. Derepressed transfer properties leading to the efficient spread of the plasmid encoding carbapenemase OXA-48. *Antimicrobial agents and chemotherapy* 2014; **58**: 467-71.
41. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *The Journal of antimicrobial chemotherapy* 2012; **67**: 1597-606.
42. Page DT, Whelan KF, Colleran E. Characterization of two autoreplicative regions of the IncHI2 plasmid R478: RepHI2A and RepHI1A((R478)). *Microbiology* 2001; **147**: 1591-8.
43. Nilsen E, Haldorsen BC, Sundsfjord A et al. Large IncHI2-plasmids encode extended-spectrum beta-lactamases (ESBLs) in *Enterobacter spp*. bloodstream isolates, and support ESBL-transfer to *Escherichia coli. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2013; **19**: E516-8.

OUTLOOK

This thesis allowed having firm evidence regarding transmission between the animal and human settings. This was demonstrated by the discovery of the same plasmids present in genetically diverse *E. coli* from the poultry-meat production and human infections.

Further studies are now necessary to determine the prevalence of such plasmids in the different reservoirs and determine which one act as the source. These results should also stress the establishment of effective measures along the meat production chain to prevent the spread of antibiotic resistance plasmids in the community and in the hospitals. Further studies could investigate other reservoirs (e.g., food sources, environment, and companion animals) that might be sources of ESC-R *Ent*. Moreover, one should also try to establish the epidemiological link/transmission ways by which these highly-resistant bacteria are spreading within and/or between different settings.

Further studies should also investigate if certain promiscuous plasmids are more likely to disseminate between different *E. coli* genotype within and between the animal and human reservoir. In this regards, the molecular mechanisms responsible for causing a broad-host range and a small-host range in the different plasmid families should be examined.

CURRICULUM VITAE

Curriculum vitae



Salome Nadja Seiffert

Kornfeldstrasse 19
4125 Riehen
Switzerland

Mobile: +41 (0)79 796 49 10
Date of birth: 20. March 1988
Nationality: Swiss
salome.seiffert@gmail.com

Education and Qualifications:

June 2012 - ongoing: (End: August 2015)	PhD in Biochemistry and Molecular Biology University of Bern, Institute of Veterinary Bacteriology and Institute for Infectious Diseases, Switzerland
September 2010 - February 2012:	Masters in Infection Biology and Epidemiology (MSc), Major in Biology of Infection University of Basel, Swiss Tropical and Public Health Institute (Swiss TPH), Switzerland Grade: 5.5 (out of max. 6)
September 2007 - July 2010:	Bachelor of Science in Biology (BSc), Major in Integrative Biology University of Basel, Switzerland Grade: 5.1 (out of max. 6)

Work Experience:

PhD project: June 2012 - ongoing: (End: August 2015)

Ph.D.

„Genetic properties of extended cephalosporin-resistant ***Escherichia coli*** in humans and animals"

- Detailed genetic and phenotypic characterization of clinical enterobacterial isolates (see "Techniques" in section *Molecular Techniques*)
- Characterization and genetic manipulation of plasmids (conjugation, transformation, restriction-enzyme-digested products as well as of PCR-products)
- Whole plasmid sequencing using 454-pyrosequencing on 454 Junior Genome Sequencer (entire procedure from plasmid isolation to the generation of GeneBank annotation)
- Lab management including soliciting quotations for lab consumables, negotiation with sales representatives, ordering of lab consumables

Master project: September 2010 - February 2012:

Master`s Thesis (1 Year)

„The role of PE-PGRS genes in the antigenic diversity of ***Mycobacterium tuberculosis***"

- Genetic characterization of *Mycobacterium tuberculosis* isolates using Real-Time SNP-typing and Spoligotyping.
- Sequencing and alignment of 12 genes from the PE-PGRS family on a representative sample set of 101 *Mtb* strains.
- Analysis of the genetic diversity of the PE-PGRS genes including the detection of positive selection within the genes.

Travel Grants:

ASM Student and Post-Doctoral Fellows Travel Grant - 54th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Washington DC, September 6-9, **2014**

24th ECCMID 2014 Travel Grant - 24th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Barcelona, May 10-13, **2014**.

Referee for the following Journals:

- Journal of Antimicrobial Chemotherapy (since 2013)
- Microbial Drug Resistance (since 2014)
- Antimicrobial Agents and Chemotherapy (since 2014)

Publications:

Reviews (n=1)

1. **Salome N. Seiffert**, Markus Hilty, Vincent Perreten, and Andrea Endimiani. Extended-spectrum cephalosporins resistant Gram-negative organisms in livestock: an emerging problem for human health? ***Drug Resistance Updates***; 16(1-2):22-45, **2013**.

Articles (n=11)

1. Alessandra Carattoli, **Salome N. Seiffert**, Sybille Schwendener, Vincent Perreten, and Andrea Endimiani. Differentiation of IncL and IncM Plasmids Associated with the Spread of Clinically Relevant Antimicrobial Resistance. **PLoS One**;10(5):e0123063, **2015**.

2. Agnese Lupo, Debora Vogt, **Salome N. Seiffert**, Andrea Endimiani, and Vincent Perreten. Antibiotic Resistance and Phylogenetic Characterization of *Acinetobacter baumannii* Strains Isolated from Commercial Raw Meat in Switzerland. ***Journal of Food Protection***; 77(11):1976-1981, **2014**.

3. **Salome N. Seiffert**, Jonas Marschall, Vincent Perreten, Alessandra Carattoli, Hansjakob Furrer, Andrea Endimiani. Emergence of *Klebsiella pneumoniae* co-producing NDM-1, OXA-48,CTX-M-15, CMY-16, QnrA and ArmA in Switzerland. ***International Journal of Antimicrobial Agents***; 44(3):260-262, **2014**.

4. **Salome N. Seiffert**, Alessandra Carattoli, Regula Tinguely, Agnese Lupo, Vincent Perreten, Andrea Endimiani. High Prevalence of Extended-Spectrum Beta-Lactamase, Plasmid-Mediated AmpC, and Carbapenemase Genes in Pet Food. ***Antimicrobial Agents Chemotherapy***; 58(10):6320-6323, **2014**.

5. **Salome N. Seiffert,** Vincent Perreten, Sönke Johannes, Sara Droz, Thomas Bodmer, and Andrea Endimiani. OXA-48 carbapenemase-producing *Salmonella enterica* serovar Kentucky isolate of sequence type 198 in a patient transferred from Libya to Switzerland. ***Antimicrobial Agents Chemotherapy***; 58(4):2446, **2014**.

6. Richard Copin, Mireia Coscollá, **Salome N. Seiffert**, Graham Bothamley, Jayne Sutherland, Georgetta Mbayo, Sebastien Gagneux, Joel D. Ernst. Sequence Diversity in the ***pe_pgrs*** Genes of *Mycobacterium tuberculosis* Is Independent of Human T Cell Recognition. ***MBio***; 14:5(1):e00960-01013, **2014**.

7. **Salome N. Seiffert**, Regula Tinguely, Agnese Lupo, Catherine Neuwirth, Vincent Perreten, and Andrea Endimiani. High Prevalence of Extended-Spectrum Cephalosporin-Resistant *Enterobacteriaceae* in Poultry Meat in Switzerland: Emergence of CMY-2- and VEB-6-possessing *Proteus mirabilis*. ***Antimicrobial Agents and Chemotherapy***; 57(12):6406-6408, **2013**.

8. **Salome N. Seiffert**, Markus Hilty, Andreas Kronenberg, Sara Droz, Vincent Perreten, and Andrea Endimiani. Extended-Spectrum Cephalosporin-Resistant *Escherichia coli* in Community, Specialized Outpatient Clinic and Hospital Settings in Switzerland. ***Journal of Antimicrobial Chemotherapy***; 68(10):2249-2254, **2013**.

9. Regula Tinguely, **Salome N. Seiffert**, Hansjakob Furrer, Vincent Perreten, and Andrea Endimiani. Emergence of Extensively Drug-Resistant (XDR) *Haemophilus parainfluenzae* in Switzerland. ***Antimicrobial Agents and Chemotherapy***; 57(6):2867-2869, **2013**.

10. Markus Hilty, Parham Sendi, **Salome N. Seiffert**, Sara Droz, Vincent Perreten, Andrea M. Hujer, Robert A. Bonomo, Kathrin Mühlemann, and Andrea Endimiani. Characterization and Clinical Features of *Enterobacter cloacae* Bloodstream Infections Occurringat a Tertiary Care University Hospital in Switzerland: Is Cefepime the Adequate Therapy? ***International Journal of Antimicrobial Agents***; 41:236-249, **2013**.

11. Urs Führer, Jonas Marschall, **Salome N. Seiffert**, Parham Sendi. Repatriierung mit Problemkeim: Extensiv resistenter (XDR) *Acinetobacter baumannii*. ***Schweizerisches Medizin-Forum***; 13(40):801-803, **2013**. [German]

Abstracts/ Posters:

International Conferences (n=6):

1. **Salome N. Seiffert**, Vincent Perreten, Vladimira Hinic, Reno Frei, Andrea Endimiani, and Alessandra Carattoli. *Citrobacter* freundii possessing *bla*$_{OXA-48}$, *bla*$_{SHV-12}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1}$, and *armA* also carries the *bla*$_{NDM-1}$ gene in a novel IncHI2 plasmid background. **25th ECCMID** – Copenhagen, DK – April 25-28, **2015**

2. **Salome N. Seiffert**, Schwenderer Sybille, Vincent Perreten, Andrea Endimiani, and Alessandra Carattoli. Evolution of the IncL/M Plasmids Associated to the Spread of *bla*$_{OXA-48}$, *bla*$_{NDM-1}$ and *bla*$_{CTX-M-3}$. **54th ICAAC** – Washington, DC – September 6-9, **2014.**

3. **Salome N. Seiffert**, Regula Tinguely, Agnese Lupo, Markus Hilty, Vincent Perreten, and Andrea Endimiani. High prevalence of CTX-M-, CMY-, and OXA-48-like *bla* genes in food for pets. **24th ECCMID** – Barcelona, Spain – May 10-13, **2014**.

4. **Salome N. Seiffert**, Vincent Perreten, Sönke Johannes, Sara Droz, Thomas Bodmer, and Andrea Endimiani. OXA-48 and VEB-8 producing ***Salmonella*** Kentucky of ST198 in a patient transferred from Libya to Switzerland. **24th ECCMID** – Barcelona, Spain – May 10-13, **2014**.

5. Yuvia N. Guilarte, **Salome N. Seiffert**, Agnese Lupo, Sara Droz, Vincent Perreten, and Andrea Endimiani. ***Aerococcus urinae*** isolates detected in Switzerland: epidemiology and characteristics of an underestimated uro-pathogen. **24th ECCMID** – Barcelona, Spain – May 10-13, **2014**.

6. **Salome N. Seiffert,** Regula Tinguely, Sara Droz, Vincent Perreten, and Andrea Endimiani. High prevalence of extended-spectrum cephalosporin-resistant ***E. coli*** and emergence of VEB-6-producing ***P. mirabilis*** in poultry meat in Switzerland. **53rd ICAAC** – Denver, CO – September 9-13, **2013**.

Swiss Conferences (n=2):

1. **Salome N. Seiffert,** Mireia Coscollá Devís, Sebastien Gagneux. Role of PE-PGRS genes in antigenic diversity in *Mycobacterium tuberculosis*. **10 Years of Infection Biology Symposium – Biozentrum**, University of Basel, Switzerland – September 16th, **2011**.

2. **Salome N. Seiffert,** Mireia Coscollá Devís, Sebastien Gagneux. Role of PE-PGRS genes in antigenic diversity in *Mycobacterium tuberculosis*. **Biovalley Science Day** – Basel, Switzerland – September 20th, **2011**

Molecular Techniques and Computer Skills:

Molecular techniques:

Molecular techniques:
PCR, Electrophoresis, Sanger Sequencing, Restriction Fragment Length Polymorphism (RFLP), Rep-PCR (molecular fingerprint), Spoligotyping (***M. tuberculosis*** typing), analytical Isoelectric focusing (aIEF; for the detection of beta-lactamase genes), DNA- and Plasmid-Isolation, Cloning of restriction-enzyme-digested products as well as of PCR-products (TOPO-cloning), Electro-transformation, Conjugation, Antibiotic susceptibility tests (Disk-diffusion method, broth dilution method, and Checkerboard assay).

Advanced molecular techniques:
SNP-typing using Taqman Assay, Real-Time PCR, Agilent 2100 Bioanalyzer system (for exact Electrophoresis), whole plasmid sequencing using 454-pyrosequencing on 454 Junior Genome Sequencer (whole procedure from plasmid isolation to data analysis).

Computer skills:

Primer design for regular PCR (DNAMAN, Primer Blast), Lasergene and Staden package (Sequence assembly), ClustralW and Muscle (Sequence alignment), MEGA5.2 and DNAMAN (Sequence analysis), Bionumerics (Rep-PCR analysis), DNA SP (Analysis of genetic diversity of genes), PAML program (detection of positive selection in genes), Artemis and Sequin (Sequence annotation), MicroSope platform (Annotation and analysis of prokaryotic genomes), EndNote (reference management software package), Adobe Creative Suite CS6.

References:

Prof. Dr. med. Andrea Endimiani
Institute for Infectious Diseases
Friedbühlstrasse 51
3010 Bern
Switzerland
andrea.endimiani@ifik.unibe.ch

Prof. Dr. Vincent Perreten
Institute of Veterinary Bacteriology
Längassstrasse 122
3001 Bern
Switzerland
vincent.perreten@vetsuisse.unibe.ch
Tel.: +41 31 631 24 84

Prof. Dr. Alessandra Carattoli
Institute for Infectious Diseases
Friedbühlstrasse 51
3010 Bern
Switzerland
alessandra.carattoli@ifik.unibe.ch

Language skills:

German:	Mother tongue
English:	Fluent Listening: C1, speaking: C1, and writing: C1
French:	Reasonable knowledge Two residental language immersion courses (both 3 weeks) in Toulouse before the start of the university in 2007
Spanish:	Basic knowledge

Other experiences:

2007–2010: Internship in a graphic design office (part-time job)

Wegmüller Grafik und Design
Judith Seiffert-Wegmüller
4125 Riehen
Switzerland
Mobile: +41 (0)79 356 71 17

Main duties: Preparation of layouts for printing using Adobe Illustrator, Indesign and Photoshop

Leisure Activities:

Fly-fishing, Ski-/Snowboarding, Golf, Hiking

Declaration of Originality

Last name, first name: Seiffert, Salome Nadja

Matriculation number: 07-058-530

I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations.

All data, tables, figures and text citations which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such.

I am aware that in case of non-compliance, the Senate is entitled to withdraw the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 69, of 7 June 2011.

Place, date

Bern, 26.5.15

Signature

Salome Seiffert