Federal Department of Home Affairs FDHA
**Federal Food Safety and Veterinary Office FSVO**
Research Management

**One Health** **Molecular Epidemiology and Surveillance**

# Whole-genome-sequencing of cephalosporinase- and carbapenemase-producing Enterobacteriaceae from animals: a baseline for a One-health molecular epidemiology

*Vincent Perreten, Sonja Kittl, Gudrun Overesch*
*Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, CH-3012 Bern*

**Key words**
ESBL, cephalosporin, carbapenem, WGS, plasmid, antibiotic resistance, monitoring, One Health

**Aim of the study**
The objectives of the project were i) to perform whole genome sequence (WGS) analysis of 3rd generation cephalosporin-resistant *Escherichia coli* (3GC-R-*Ec*) from food-producing animals and of carbapenemase-producing Enterobacteriaceae (CPE) from companion animals; ii) to determine if a genetic relationship exists between human and animal strains and plasmids; iii) to implement the WGS and bioinformatics pipelines at the ZOBA for WGS-based national surveillance of antibiotic resistance.

**Material and methods**
WGS was performed using Illumina short read technology as well as ONT long read technology for selected strains to obtain complete and closed chromosome and plasmids. Assembly of the reads was obtained using Unicycler hybrid assembly. WGS data were analyzed for genetic and phylogenetic characteristics using different web-based bioinformatics tools and using SeqSphere+ software.

**Results and significance**
**Analysis of WGS of 3GC-R-*Ec* from slaughter calves and fattening pigs.** WGS and data analysis were performed with 77 3GC-R-*Ec* from pigs and calves from 2021 as a proof of concept and validation of the WGS and bioinformatic pipelines established at ZOBA. Phenotypic antimicrobial resistance results WGS showed a very high agreement (98.8%) with those obtained by the detection of corresponding underlying molecular mechanisms. 3GC-R-*Ec* were genetically diverse and resistance to 3GC was mainly associated with *bla*$_{\text{CTX-M-15}}$ in isolates from calves, *bla*$_{\text{CTX-M-1}}$ in isolates from pigs, and mutations in the ampC-promoter in isolates from both animal species. WGS provided important information on the occurrence of resistance mechanisms and phylogeny of *E. coli* from calves and pigs in Switzerland (Aebi *et al*. 2023).
**Comparative analysis of WGS of 3GC-R-Ec from poultry and humans.** A total of 352 WGS of 3GC-R-*Ec* isolated from poultry and poultry meat (2018, 2020) and 128 WGS of 3GC-R-*Ec* from humans from the same period were used for genomic comparative analysis. *E. coli* of the same STs (ST10, 69, 23, 38, 156, 361, 1431) were found in both human and poultry isolates, however they differed at the core-genome level with more than 80 loci difference. The predominant 3GC-R genes consisted of *bla*$_{\text{CMY-2}}$ (113 in poultry *vs.* 4 in humans), *bla*$_{\text{CTX-M-1}}$ (104 *vs.* 8), *bla*$_{\text{CTX-M-15}}$ (2 *vs.* 61), *bla*$_{\text{CTX-M-14}}$ (4 *vs.*10). Plasmids carrying *bla*$_{\text{CMY-2}}$ and *bla*$_{\text{CTX-M-1}}$ belonged mainly to IncI1 and IncB/O/K/Z groups. Only one strain from human had a similar IncB/O/K/Z *bla*$_{\text{CMY-2}}$-containing plasmid as the one found in poultry (Fernandez *et al*. 2023). The presence of different 3GC-R-*Ec* harboring different *bla* genes in poultry and human indicates that spillover of strains is not frequent. Presence of similar plasmids in 3GC-R-*Ec* from poultry and humans is also not common.
**Genomic comparative analysis of CPE from companion animals and humans.** A total of 157 CPE from humans (76 *E. coli*-OXA-181, 55 *E. coli*-OXA-48, 26 *Enterobacter hormaechei*-OXA-48) were obtained from NARA (2019-2021), sequenced, and compared to CPE from companion animals. The *E. coli* ST410-OXA-181 from humans clustered into two branches of the phylogenetic tree. The *E. coli* ST410-OXA-181 from animals also clustered into one of the branches but clearly diverge from the human strains, except for one strain originating from a veterinarian. These results indicate that *E. coli* ST410-OXA-181 from companion animals are different from those causing infections in humans, and that exchange may occur between animal and people

working with them (Frey, 2023). OXA-48-producing *E. hormaechei* from animals belonged to ST114, ST418 and ST78. Human *E. hormaechei*-OXA-48 belonged to diverse ST differing from the animal strains, except for two ST114. Core-gene SNP analysis confirmed the clonality of the animal ST114 and ST418 strains (0 to 10 SNPs), and close relatedness of animal and human ST114 strains (80-120 SNPs). The strains harbored the *bla*$_{OXA-48}$ gene on a 63 kb IncL plasmid and carried also multidrug-resistance plasmids. The presence of *E. hormaechei*-OXA-48 of ST114 in both human and animals indicates that this lineage can establish and cause infections in both settings (Donà *et al*, 2023). *E. coli*-OXA-48 from companion animals belonged to 10 different STs and those from human to 30 STs (Findlay *et al*. 2022). Only two *E. coli*-OXA-48 from humans belonged to the same ST (ST405, ST58) as *E. coli*-OXA-48 from animals but differed by cgMLST profile. The *bla*$_{OXA-48}$-containing plasmid of the *E. coli*-OXA-48 from animals were highly similar to those detected in *K. pneumoniae* and *E. hormaechei* (Campos-Madueno *et al*. 2022; Donà *et al*, 2023). The *E. coli*-OXA-48 from animals also contained additional multidrug-resistance plasmids. This analysis indicated a high diversity among *E. coli*-OXA-48 with different strains in animals and humans, and the spread of the plasmid-borne carbapenemase among each other, as well as among other Enterobacterales.

**Conclusions.** A continuous One-health and WGS-based surveillance will contribute to identify emerging multidrug-resistant bacteria and their reservoirs and routes of dissemination in animals, human and environment.

**Publications, posters and presentations**


Findlay J, Perreten V, Poirel L, Nordmann P. Molecular analysis of OXA-48-producing *Escherichia coli* in Switzerland from 2019 to 2020. Eur J Clin Microbiol Infect Dis. 2022 Nov;41(11):1355-1360.

Fernandez JE, Seth-Smith HMB, Nordmann P, Egli A, Endimiani A, Perreten V. Intra- and interspecies spread of a novel conjugative multidrug resistance IncC plasmid coharboring *bla*$_{OXA-181}$ and *armA* in a cystic fibrosis patient. Microbiol Spectr. 2022 Oct 26;10(5):e0312122.

Campos-Madueno EI, Moser AI, Jost G, Maffioli C, Bodmer T, Perreten V, Endimiani A. Carbapenemase-producing *Klebsiella pneumoniae* strains in Switzerland: human and non-human settings may share high-risk clones. J Glob Antimicrob Resist. 2022 Mar;28:206-215.

Aebi CB, Fernandez JE, Kittl S, Tresch ML, Perreten V, Overesch G. Characterization of third-generation cephalosporin-resistant *Escherichia coli* from slaughter calves and fattening pigs: A pilot study for monitoring antimicrobial resistance by whole genome sequencing in Switzerland. Schweiz Arch Tierheilkd. 2023 Jun;165(6):372-384.

Donà V, Nordmann P, Kittl S, Schuller S, Bouvier M, Poirel L, Endimiani A, Perreten V. Emergence of OXA-48-producing *Enterobacter hormaechei* in a Swiss companion animal clinic and their genetic relationship to clinical human isolates. J Antimicrob Chemother. 2023 Oct 31:dkad337. Epub ahead of print.

Perreten V. Animals and dissemination of clinically important antimicrobial resistance: tracking carbapenemases. Swiss Society for Microbiology Annual Congress 2022, SwissTech Convention Center, EPFL, Quartier Nord, Ecublens-Lausanne, August 30 – September 1, 2022. Invited speaker.

Perreten V. WGS-based relationship between multidrug-resistant bacteria from humans and animals: emphasis on CPE and MRSA. 9$^{th}$ Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE), Tours, France, 3 – 5 July 2023. Keynote lecture.

Perreten V. Emergence of carbapenemase-producing Enterobacterales in companion animals: a WGS-based One Health analysis. Tagung der DVG-Fachgruppe Bakteriologie und Mykologie, Berlin, Germany, 22 – 24 May 2023. Keynote lecture.

Fernandez JE, Egli A, Seth-Smith H, Endimiani A, Perreten V. Novel multi-resistant IncC plasmid carrying *bla*$_{OXA-181}$ identified in two different *E. coli* strains isolated from the same human patient. 32$^{nd}$ European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Lisbon, Portugal, 23 – 26 April 2022.

Fernandez JE, Overesch G, Perreten V. Third-generation cephalosporins resistance plasmids in *E. coli* isolated from poultry and retail meat in Switzerland. Annual congress of the Swiss Society for Microbiology 2023, Lausanne, Switzerland, 30 – 31 August 2023.

Donà V, Kittl S, Endimiani A, Perreten V. WGS-based characterization of emerging pathogenic OXA-48-producing *Enterobacter hormaechei* belonging to ST114 and ST418 in a Swiss companion animal clinic. 9$^{th}$ Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE), Tours, France, 3 – 5 July 2023.

Frey Y. Carbapenemase-producing *Escherichia coli* harboring *bla*$_{OXA-181}$ from human and animal origins in Switzerland: Phenotypic, genotypic and phylogenetic analysis. Master thesis in Veterinary Medicine, Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland, 2023.


***Project** 1.21.07*

***Project duration** January 2021 - July 2023 (extended to November 2023)*