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Prevalence and optimised detection of resistance to antibiotics vital for animal and human health (PRAHAD) (Uni Bern)

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Key words
Colistin, aminoglycosides, carbapenems, mobile elements, diagnostic tools, pig, cattle, resistance, prevalence

Aim of the study
The tasks of the IVB within the PRAHAD project were i) to evaluate the rates of resistance to colistin and carbapenems among Gram negative bacteria from the gut microflora of pigs, cattle, chicken, and from meat and ii) to elucidate the genetic environments of the mobile resistance mechanisms.

Material and methods
Caecum samples of chicken, pig, and cattle as well as chicken meat samples were obtained within the Swiss National Monitoring of Antibiotic Resistance in Food-producing Animals and Meat. Samples were screened for the presence of carbapemase-producing bacteria using selective chromogenic agar plates and for the presence of colistin-resistant Enterobacteriaceae using a newly developed selective agar plates. Bacterial isolates were identified by Maldi Tof and antibiotic resistance was confirmed by the determination of the MIC and production of carbapenemase. Antibiotic resistance genes and mobile genetic elements were identified and characterized by whole genome sequencing (WGS) using Illumina and MinIon technologies.

Results and significance
Colistin and carbapenem resistance in chicken, chicken meat, pigs and cattle:
Retrospective analysis of data from the Anresis-ARCH-VET report 2016 revealed the presence of colistin-resistant *E. coli* (MIC >4µg/ml) in four chicken meat imported in 2014 and in none of the meat from indigenous production corresponding to an overall prevalence of 1.3% (95% CI 0.4 – 3.2%). The 4 isolates contained the colistin resistance gene *mcr-1* and were also resistant to third generation cephalosporins. Further analysis of chicken meat from 2016 confirmed the presence of *mcr-1* positive *E. coli* in the imported meat with 2 of 150 samples being positive for *mcr-1* in the first half of the year.
Colistin-resistant *E. coli* with MIC>4µg/ml were found in 2 of 257 caecum samples from pigs and 5 of 257 caecum samples from calves corresponding to prevalence of 0.8% (95% CI 0.2 – 3%) in pigs and of 2% (95% CI 0.2 – 3%) in calves. Out of the 7 colistin-resistant *E. coli*, only one from pigs was found to contain the plasmid-mediated colistin resistance gene *mcr-1*.
No carbapenemase-producing Enterobacteriaceae were detected in pigs, cattle (2015, 2017), chicken and chicken meat (2014, 2016). However, the caecum samples from pigs and cattle contained carbapenemase-producing *Pseudomonas* sp. (45 of 172 samples in pigs [26% (95% CI 20 – 33%)]; 15 of 172 samples in cattle [9% (95% CI 5 – 14%)]). None were found among the 403 samples from chicken caecum. In chicken meat, 52 of 251 samples were positive with a carbapenemase-producing *Pseudomonas* sp. [20% (95% CI 16 – 26%)]. The *Pseudomonas* belonged to species which naturally produce carbapenemases like *P. fragi* and *P. otitidis.*

Characterization of mobile genetic elements:
Comparative analysis of the whole genome of the six *mcr-1* positive *E. coli* from chicken with those isolated from humans in Switzerland revealed *mcr-1* in a variety of plasmid types as well as integrated into the bacterial genome. Of note, one strain from human and one from chicken meat carried *mcr-1* on the same 33 kb IncX4

plasmids. Both plasmids were also almost identical to others previously characterized suggesting a dissemination of this plasmid type worldwide. In an *E. coli* from pig in Switzerland, the *mcr-1* gene was located on a 288-kb IncHI2 MDR conjugative plasmid (pRDB9) which contains several resistances genes conferring resistance to sulfonamides, trimethoprim, tetracyclines, penicillins and colistin. In avian pathogenic *E. coli* (APEC) from South Africa, WGS revealed a novel 62-kb plasmid IncI2 plasmid (pVT553) carrying *mcr-1* and gave the first insight into the type of *mcr-1* plasmids present in APEC in South Africa.
WGS of two enterovirulent *E. coli* from pigs (ETEC/STEC) revealed a 207-kb IncHI1/IncFIA multidrug resistance (MDR) plasmid p14ODMR conferring resistance to beta-lactams, aminoglycosides, sulfonamides, trimethoprim, and tetracycline in ETEC, as well as of a 99-kb IncI1 plasmid p15ODAR and a 112-kb IncFIB plasmid p15ODDR in an ETEC/STEC strain. Each of these plasmids contained several antibiotic resistance genes conferring resistance to beta-lactams, aminoglycosides, sulfonamides, trimethoprim, and tetracycline.
These results demonstrated that carbapenemase-producing Enterobacteriaceae were so far not detected in food-producing animals in Switzerland and that the prevalence of colistin-resistant Enterobacteriaceae is low. Nevertheless, the detection of the colistin resistance gene *mcr-1* on conjugative plasmids in *E. coli* from chicken meat and from pig feces emphasizes the need for an active surveillance using selective media to follow evolution. The co-localization of antibiotic resistance genes in the MDR conjugative plasmids of porcine *E. coli* strains may represent a risk of simultaneous selection of resistance genes, independent of which antibiotic is used as an empirical treatment.

Publications, posters and presentations

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