P29

Phenotypic and genotypic characteristics of Escherichia coli with non-susceptibility to quinolones isolated from environmental samples on pig farms

Morach M.[1], Kindle P.[1], Zurfluh K.[1], Nüesch-Inderbinen M.[1], von Ah S.[2], Sidler X.[2], Stephan R.[1], Kümmerlen D.[2]

[1]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, [2]Department of Farm Animals, Division of Swine Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Introduction

In the last decade, the growth of the pig-farming industry has led to an increase in antibiotic use, including several used in human medicine, e.g. (fluoro)quinolones. Data from several studies suggest that there is a link between the agricultural use of antibiotics and the prevalence of antibiotic-resistant bacteria in the pig farm environment, including (fluoro)quinolone resistance. This poses a threat to human and animal health. Our goal was to phenotypically and genotypically characterise 174 *E. coli* showing non-susceptibility to quinolones isolated from environmental samples from pig farms.

Material and Methods

Antimicrobial susceptibility testing (AST) was performed using the disk diffusion method. PCR and sequence analysis were performed to identify chromosomal mutations in the quinolone resistance-determining regions (QRDR) of *gyrA* and the isolates were screened for the presence of the plasmid-mediated quinolone resistance (PMQR) genes *aac-69-Ib-cr*, *qepA*, *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS*.

Strain relatedness was assessed by phylogenetic classification and multilocus sequence typing (MLST).

Results

Antimicrobial susceptibility testing by the disc diffusion method showed that 81% (n=141) of the strains were resistant and 19% (n=33) were intermediately resistant to nalidixic acid. Furthermore, 36.2% (n=63) of the isolates were also resistant to ciprofloxacin.

Additional antimicrobial resistance was most frequently observed for streptomycin (72.4% / n=126), tetracycline (60.9% /n=106), sulfamethoxazole/trimethoprim (50% /n=87), ampicillin (46.6% /n=81), kanamycin (19.5% /n=34), chloramphenicol (15.5% / n=27), and gentamicin (14.4% /n=25), respectively (Table 1). Resistance to all other tested antibiotics was detected for at least one isolate, except to nitrofurantoin.

Of the 174 isolates analysed in this study, 68.4% (n=119) were resistant to three or more classes of antibiotics and therefore categorised as MDR. The most frequent MDR combinations detected were SXT-TE-STR (n=15), AM-SXT-TE-S (n=10) and AM-SXT-STR-K (n=8) (Table 1). *E. coli* strains resistant to four and five antibiotics were the most prevalent (21.3% and 19.0%, respectively).

Of 141 isolates with a nalidixic acid resistant phenotype, 98.6% (n=139) possessed at least one nucleotide mutation in the QRDR of *gyrA*. Thereof, 49.6% (n=70) showed single amino acid substitution at codon Ser83, namely Ser83 to Leu (n=67), or Asp87 to Tyr (n=2), or Asp87 to Gly (n=1). Further, 48.9% (n=69) possessed double substitutions at Ser83 to Leu and Asp87 to Asn (n=68) or Tyr (n=1). Two isolates (isolates no. 65 and 106, respectively) tested negative for mutations in the QRDR of *gyrA* (Table 1).

A total of 38 strains possessed one or more PMQR genes, representing 21.8% of the 174 analysed strains (Table 1). Among the 19.5% (n=34) of the isolates with one PMQR gene, twenty (11.5%) possessed *qnrB*, thirteen (7.5%) *qnrS* and one isolate (0.6%) possessed *aac(6')-Ib*, respectively (Table 1). Four isolates (2.3%) possessed a combination of *qnrB* and *qnrS* genes. No isolates tested positive for *qnrA*, *qnrC*, *qnrD* or *qepA*. The occurrence of PMQR positive isolates was remarkably higher in strains exhibiting intermediate resistance to nalidixic acid (90.9% /n=30), than in nalidixic acid resistant strains (5.7% /n=8). Moreover, all *qnrB/qnrS* combinations were detected in intermediately resistant isolates (Table 1). Isolates possessing PMQR were found in 11 (22.9%) of the dust samples 16 (28.6%) of the wipe samples. and 11 (15.7%) of the slurry samples. Of the 23 farms with reported use of fluoroquinolones, 12 (52.2%) yielded environmental *E. coli* containing PMQR genes. Thereof, the majority (7 farms/58.3%) were farrowing and rearing farms, three (25%) were fattening farms and two (16.7%) were mating and gestation farms (Table 1).

By contrast, of the 32 farms without a history of fluoroquinolone use during the study period, nine (28.1%) tested positive for *E. coli* harbouring PMQR genes. Thereof, five (55.6%) were fattening farms, four (44.4%) were mating and gestation farms, and none (0%) were farrowing and rearing farms.

The majority of the isolates were assigned to phylogenetic groups A (48.3%/n=84) and group B1 (33.3% /n=58). The remaining strains were classified into group C (9.8% /n=17), E (6.9%/n=12), F (1.1%/ n=2) and D (0.6%/n=1), respectively. None of the isolates belonged to phylogenetic group B2.

Overall, a total of 50 STs were found. The most common sequence types were ST10 (n=20), ST297

(n=20), ST453 (n=10), ST88 (n=9), ST898 (n=8), ST93 (n=6), ST2197 (n=6), ST737 (n=5), and ST2509 (n=5).

Discussion and Conclusion

Quinolone non-susceptible *E. coli* are widespread in the environment of Swiss pig farms. In particular, isolates showing intermediate resistance to nalidixic acid frequently possess transmissible PMQR genes. This is worrisome, since the presence of *qnr* genes may increase the ability of bacteria to acquire point mutations in the gyrase and topoisomerase IV genes, resulting in high level resistance to (fluoro)quinolones. Furthermore, plasmids harbouring *qnr* genes may contribute to the horizontal spread of antibiotic resistance in livestock and in the environment. In pig farms which are part of sow pool systems, inter-farm measures that aim to reduce the risk of spreading resistant bacteria and resistance genes from one stage of production to the next need to be assessed and promoted. Our data further show that farm environments contain commensal MDR *E. coli* as well as *E. coli* with zoonotic potential. In particular, we demonstrate for the first time the presence of EPEC O80:H2 in an environmental sample from a pig farm.