Genomic basis of emerging enrofloxacin and ceftiofur resistance in *Escherichia coli* isolated from swine clinical samples collected across USA

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Key points:

- Nearly one-third of clinical *E. coli* isolates collected from swine samples were ceftiofur or enrofloxacin resistant
- Genetic analysis revealed presence of rarely reported genes in antimicrobial resistant isolates
- Most of the isolates were multi-drug resistant on both routine lab tests and genetic analysis

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Fluoroquinolones (i.e. Enrofloxacin, Ciprofloxacin, Norfloxacin among others) and cephalosporins (i.e Ceftiofur) are critically important antimicrobial families for both human and veterinary medicine. In a previous study, we analyzed the antimicrobial resistance in *Escherichia coli* isolates recovered from swine clinical samples from across USA during 2006-2016 at the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL), and found a 47% annual increase in the prevalence of enrofloxacin resistance (from 1.5 in 2006 to 32% in 2016) while no trend was observed for the resistance to ceftiofur (that ranged between 32-39%). A follow-up study was conducted to evaluate the genetic basis of resistance against enrofloxacin and ceftiofur in *E. coli* isolates using whole genome sequencing (WGS).

One hundred and fifty-three swine clinical *E. coli* isolates (106 ceftiofur-resistant and 106 enrofloxacin–resistant, with 59 resistant to both antimicrobials) collected in 2014-15 from 14 states across USA were selected and genes causing ceftiofur and enrofloxacin resistance were identified using WGS.

Twenty-one (21/106) enrofloxacin-resistant isolates from 6 states harbored diverse plasmid mediated quinolone resistance (PMQR) genes (*qnr*B19, *qnr*B2, *qnr*S1, *qnr*S2 and *qnr*S15). The presence of PMQR genes alone was associated with clinical levels of resistance. The most prevalent genes associated with ceftiofur resistance were $bla_{\text{CMY-2}}$ (89/106, 84%). Moreover, 24 ceftiofur-resistant isolates harbored various $bla_{\text{CTX-M}}$ and bla_{SHV} genes. Moreover, bacteria carrying $bla_{\text{CTX-M}}$ and *qnr genes* also contained genes coding for resistance mechanisms against other antimicrobial classes and were commonly resistant against ampicillin, tetracyclines, gentamycin, trimethoprim and sulfonamides.

These genes ($bla_{\text{CTX-M}}$, *qnr*) have been rarely reported from farm animals in USA and have been implicated as important genetic mechanisms behind extended spectrum cephalosporin and fluoroquinolone resistance in human and animal populations in several countries. These genes are present on plasmids, making their dissemination across bacterial populations faster by horizontal transfer. The presence of multiple antimicrobial resistance genes on the same plasmids also makes mitigation of this problem more difficult because of the possibility that using one antimicrobial class will exert positive selection pressure for resistance against other antimicrobial classes.

The results of this study show the evolution of novel resistance mechanisms against critical antimicrobials that have been used in swine production in the last decade. These results emphasize the need for continued antimicrobial stewardship programs, so that we can continue the judicious use of critical antimicrobials in swine production for animal health concerns as well as for producing safer pork for consumers.