Genetic determinants of extended spectrum cephalosporin and fluoroquinolone resistance in *Escherichia coli* isolated from diseased pigs in the U.S.A

Shivdeep Singh Hayer[1], Seunghyun Lim[1,2], Samuel Hong[1,3], Ehud Elnekave[1], Timothy Johnson[4], Albert Rovira[1,5], Fabio Vannucci[1,5], Andres Perez,[1] Julio Alvarez*[1,6,7,]

[1]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA

[2]Bioinformatics and Computational Biology Program, University of Minnesota-Rochester, Rochester, Minnesota, USA

[3]Department of Microbiology and Immunology, Rega Institute, KU Leuven, Leuven, Belgium

[4]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA

[5]Veterinary Diagnostic Laboratory, College of Veterinary Medicine, University of Minnesota, Saint Paul, Minnesota, USA

[6]VISAVET Health Surveillance Center, Universidad Complutense Madrid, Spain

[7]Department of Animal Health, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain

* Corresponding Author

Email- jalvarez@visavet.ucm.es

Running title: ESBL, PMQR and pAmpC genes in E. coli from diseased pigs in the U.S.A

Abstract

Fluoroquinolones and cephalosporins are critically important antimicrobial classes for both human and veterinary medicine. We have previously found a drastic increase in enrofloxacin resistance in clinical *Escherichia coli* isolates collected from diseased pigs from the U.S.A over ten years (2006-2016). However, the genetic determinants responsible for this increase are yet to be determined. The aim of the present study was to identify and characterize the genetic basis of resistance against fluoroquinolones (enrofloxacin) and extended-spectrum cephalosporins (ceftiofur) in swine *E. coli* isolates using whole genome sequencing (WGS). Based on Illumina short read WGS, $bla_{\text{CMY-2}}$ and chromosomal mutations in quinolone resistance determining regions of the genes *gyrA*, *gyrB*, *parA* and *parC* were the major genetic determinants mediating ceftiofur and enrofloxacin resistance, respectively. However, genes encoding extended spectrum Beta-Lactamases (ESBLs) ($bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{SHV-12}}$) and plasmid mediated quinolone resistance (PMQR) genes (*qnrB77*, *qnrB2*, *qnrS1*, *qnrS2* and *aac-(6)-lb'-cr*) were also present in more than 20% of ceftiofur and enrofloxacin resistant isolates, respectively. Additionally, colistin resistance gene (*mcr-9*) were present in several isolates. Some plasmids carrying ESBL and PMQR genes were assembled by using both short (Illumina) and long reads (PacBio). Most of these plasmids were similar (> 90% nucleotide identity and similar genetic contexts around ESBL genes) to previously described plasmids isolated from humans and animals globally. Comparative studies are needed to further elucidate the transmission of these mobile genetic determinants (pAmpC, ESBL, PMQR genes) between humans, swine and environment.

Importance

Food animal production has been identified as a potential contributor to the spread of antimicrobial resistance in both human and animal populations. Understanding the genetic mechanisms conferring resistance is critical to design informed control and preventive measures, particularly when involving critically important antimicrobial classes such as extended-spectrum cephalosporins and fluoroquinolones. Here, we studied the genetic traits associated with resistance in *E. coli* from diseased pigs in the U.S. We found extended spectrum beta-lactamase genes ($bla_{\text{CTX-M}}$-$bla_{\text{SHV-12}}$) in cephalosporin (ceftiofur) resistant isolates at higher levels than previously reported, and identified several combinations of both chromosomal mutations and plasmid-borne genes mediating fluoroquinolone (enrofloxacin) resistance. We also assembled the plasmid sequences carrying some of these genes, demonstrating their similarity with others previously found worldwide, which suggests that these plasmids might be part of a complex, global reservoir of antimicrobial resistance. We also detected for the first time *mcr-9* genes in U.S farm animal isolates.

***Keywords*:** plasmids, ESBLs, swine, USA, PMQR, WGS, cephalosporin, fluoroquinolone, antimicrobial resistance

Introduction

Antimicrobial resistance has emerged as an issue of grave concern in both human and veterinary medicine. Food animals are considered potential reservoirs of antimicrobial resistant and zoonotic pathogens such as *Escherichia coli*, although the extent of spread of resistant bacteria via food chain is still under debate (1). Critically important antimicrobials for human medicine such as cephalosporins and fluoroquinolones are still used in many parts of the world to treat diseased food animals, including swine in the U.S.A (2–4). Furthermore, certain genetic determinants responsible for resistance to antimicrobials approved for use in animals (such as ceftiofur and enrofloxacin) and those used in human medicine (such as cefoxitin and ciprofloxacin) are the same (5, 6). It is therefore important to monitor the circulation of genes responsible for resistance to such critically important antimicrobials in bacteria present in humans and animals to develop better source attribution models and targeted interventions in both humans and veterinary medicine (7).

Resistance to extended-spectrum cephalosporins is mediated by extended spectrum beta-lactamases (ESBLs) (commonly encoded by the *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{CTX-M}}$ genes) and plasmidic AmpC (pAmpC, commonly encoded by the *bla*$_{\text{CMY}}$ genes) (8). These genes may be inserted on bacterial chromosomes but are usually present on plasmids with the potential to disseminate horizontally to other bacterial strains (9). *bla*$_{\text{CTX-M}}$ genes are reported as the most prevalent ESBL encoders worldwide in humans and animals (10). However, *bla*$_{\text{CMY-2}}$ genes were primarily responsible for extended-spectrum cephalosporin resistance in bacteria of food animal origin in North America, while other ESBL-encoding genes were not reported until the late 2000's (11). Nevertheless, recent reports have also suggested the emergence of ESBL genes in bacteria of food animal origin in USA over the last decade (12).

Resistance to fluoroquinolones is mainly mediated by multiple chromosomal mutations in certain genes (*gyrA, gyrB, parE* and *parC*). Additionally, plasmid mediated quinolone resistance genes (such as *qnr*) and upregulation of efflux pumps confer variable levels of resistance to this antimicrobial family (13). *qnr* genes encoded in plasmids were also found in *Salmonella* isolates collected from retail pork, cecal samples from healthy pigs and clinical samples from diseased pigs in the same period, suggesting a likely role in the increase in phenotypic resistance (14–16). An increase in fluoroquinolone resistance was recently reported in *Salmonella enterica* isolates from diseased pigs in Minnesota between 2006 to 2015 (2). A similar increase in phenotypic resistance to a fluoroquinolone (enrofloxacin) was also reported for the same timeframe in swine *E. coli* clinical isolates (17), though the genetic determinants mediating this increase has not been determined yet.

Although increasing information on the prevalence of phenotypic resistance in bacteria (including *E. coli*) of animal origin is generated by national AMR monitoring programs such as NARMS (18), there is limited information on the genetic backbone mediating these resistance phenotypes. This may be of particular importance in the case of critically important antimicrobials such as fluoroquinolones, cephalosporins or carbapenems. The objective of this study was to characterize the genetic basis of fluoroquinolone and extended spectrum cephalosporin resistance in phenotypically resistant *E. coli* isolates collected from diseased pigs in the U.S.A between 2014-15 using both short read (Illumina) and long read (PacBio) whole genome sequencing (WGS).

Materials and methods

A total of 211 *E. coli* isolates recovered from diseased pigs at the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL) between 2014-2015 were included in this study. These isolates were selected on the basis of results of broth microdilution tests routinely performed at the laboratory following Clinical and Laboratory Standards Institute guidelines and were classified as ceftiofur non-wild type (minimum inhibitory concentrations (MIC) ≥ 2 μg/ml) and enrofloxacin non-wild type (MIC ≥ 0.25 μg/ml) (19). For ease of interpretation, "non-wild type" and "wild type" isolates are referred to as "resistant" and "susceptible", respectively. Out of these 211 isolates, 110 were enrofloxacin resistant and 106 were ceftiofur resistant, with 41 isolates being resistant to both ceftiofur and enrofloxacin. Forty-six isolates susceptible to both antimicrobials were added to assess the presence of resistance genes and chromosomal mutations in susceptible isolates. Only one isolate per farm was selected in order to avoid duplicity of potentially identical clones circulating in the same farm.

Isolates were first subjected to short read sequencing using Illumina HiSeq 2500 (2 x 125bp). The raw reads were uploaded to and assembled using the pipeline provided at Enterobase webserver. Draft genomes were uploaded to the Center for Genomic Epidemiology (CGE) webserver to identify multilocus sequence type (MLST version 2.0.4) (20), acquired resistance genes (Resfinder version 3.2) (21), plasmid sequence type (pMLST version 0.1.0) (22) and plasmid replicon types (Plasmid Finder version 2.0.1) (22). Chromosomal mutations in quinolone resistance determining regions (QRDRs) were identified by downloading sequences of *gyrA*, *gyrB*, *parC* and *parE* from reference *E. coli* K-12 substr. MG1655 genome (Genbank accession number- NZ_AJGD00000000.1) and performing nucleotide BLAST against the draft genomes locally (version 2.9.0, E-value threshold-10). Draft genomes were annotated using PROKKA (version 1.13) (23).

For the phylogenetic analysis, raw reads were first mapped to a reference genome (*E. coli* str. K-12 substr. MG1655, accession- NZ_AJGD00000000.1) and full gene alignments were assembled using snippy (default values, version 4.4.5) (24). From these full gene alignments, Gubbins (default values, version 0.1.0) was used to detect and remove loci present in recombinant regions and extract single nucleotide polymorphisms (25). Maximum likelihood trees were then built using a general time-reversible with gamma substitution model through RaxML (version 8.0) (26). Support for nodes on trees was assessed using 1000 bootstrap replicates and phylogenetic tree was made using iTOL (version 4.0) (27).

Additionally, long read sequencing was performed on a subset of isolates carrying ESBL genes ($bla_{\text{SHV-12}}$, $bla_{\text{CTX-M}}$) in the analysis above using Pacific Biosciences (PacBio) RSII technology (SMRT Cell 1M v3) (28). Long reads were first corrected for errors using LoRDEC (version 0.9) (28). Unicycler (version 0.4.7) (29) was used to obtain *de-novo* hybrid assemblies of these isolates using both long and short reads, and assemblies were visualized using Bandage (version 0.8.1) (30). Complete plasmid genomes (here on referred to as "assembled plasmids") were uploaded to ISSaga webserver (31) for identification of insertion sequences and to the CGE webserver to perform analyses as mentioned above. The assembled plasmids were also blasted against a database of reference plasmids available at the PLSDB webserver (32) to identify closely related plasmids also carrying antimicrobial resistance genes of interest (ESBL, PMQR). Plasmids sequences with a query coverage of >80% and nucleotide identity >90% were downloaded and the top five closely related plasmids genomes to each of the ones found here were visually compared using BRIG (version 0.95) (33).

Results

Genetic determinants conferring extended spectrum cephalosporin and fluoroquinolone resistance

Out of 106 ceftiofur-resistant isolates, 89 (84%) carried bla_{CMY-2} genes (figure 1). These genes were not present in the remaining 105 non-resistant isolates. Isolates carrying this gene belonged to 24 different ST types, with ST12 (n=21) and ST101 (n=10) being the dominant ST types (figure 1). Twenty of the 21 ST12 isolates varied only by 9-32 single nucleotide polymorphisms (SNPs) while ST10 and ST101 isolates varied by 7-4185 and 8-1367 SNPs, respectively. Twenty-four isolates belonging to 13 different ST types carried bla_{SHV-12} (5 isolates) or bla_{CTX-M} genes (19 isolates), of which 22 were ceftiofur resistant (figure 1). The two ceftiofur susceptible isolates carried the bla_{SHV-12} gene.

Multiple fluoroquinolone-resistant associated genes and mutations were detected in 106 of the 110 enrofloxacin-resistant *E. coli* isolates (table 1), while only four out of the 101 susceptible isolates presented any of them (specifically, single mutations in the *gyrA* gene (S83L or D87Y)). Isolates resistant to enrofloxacin belonged to 30 different ST types. The dominant ST types were ST100 (n=37) and ST744 (n=17) (table 1, figure 1). Thirty-six of these ST100 isolates varied by less than 20 SNP and these isolates were collected from 6 different states in USA. In contrast, ST744 isolates varied by 8-606 SNPs.

Six different types of PMQR genes were identified in a total of 24 isolates spread across 7 states (figure 1). These 24 isolates belonged to 16 different ST types (table 1, figure 1). Enrofloxacin MIC values for isolates with a single PMQR gene, two PMQRs genes and one PMQR gene plus a chromosomal mutation (*gyrA*- S83L, D87G or *parE*- D476A) ranged between 0.5-1.0 μg/ml, with the exception of two isolates that carried only *qnrB19* but had enrofloxacin MIC values of 2 μg/ml.

Description of assembled plasmids carrying PMQRs and ESBLs genes

We assembled complete *E. coli* chromosomes and plasmids using both long and short reads from 10 isolates (seven isolates carrying *bla*$_{\text{CTX-M}}$ genes, two carrying *bla*$_{\text{SHV-12}}$ genes and one carrying a *bla*$_{\text{CTX-M}}$ and a *qnrB77* gene) (table 2). In seven of the isolates, *bla*$_{\text{CTX-M}}$ genes were present on IncFII (*bla*$_{\text{CTX-M-14, 15, 27}}$) and IncHI2 (*bla*$_{\text{CTX-M-55}}$) plasmids with sizes ranging between 69 and 240 kbp. *bla*$_{\text{CTX-M}}$ genes were present in regions flanked by IS*26*, IS*Ecp1*, IS*5*, IS*6* and Tn*3* family transposases, which were often truncated (table 2, figures 2-4). In one isolate, *bla*$_{\text{CTX-M-15}}$ was present on the *E. coli* chromosome, flanked by transposases similar to those surrounding *bla*$_{\text{CTX-M-15}}$ in the IncFII plasmids. Plasmids with *bla*$_{\text{CTX-M-14}}$ or *bla*$_{\text{CTX-M-27}}$ carried only *bla*$_{\text{CTX-M}}$ or one other AMR gene (*erm(B)*, a macrolide resistant gene); whereas the plasmids carrying *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTX-M-55}}$ also bore genes which can confer resistance to aminoglycosides, penicillins, macrolides or trimethoprim (table 2, figures 2-4). Additionally, some of these *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTX-M-55}}$ plasmids also carried genes that can cause resistance to sulphonamides, phenicols or tetracyclines (table 2, figures 2 and 4). Concerningly, two of the *bla*$_{\text{CTX-M-15}}$ carrying IncFII plasmids also harbored *aac(6')-Ib-cr* gene which can confer resistance to both aminoglycosides and fluoroquinolones (table 2, figure 4).

The two plasmids carrying *bla*$_{\text{SHV-12}}$ genes assembled were of large IncHI2 type plasmids (approx. 287-300kbp), and carried genes for resistance to aminoglycosides, sulphonamides, trimethoprim, tetracyclines, penicillins, phenicols (only p39) and macrolides (table 2, figure 5). *bla*$_{\text{SHV-12}}$ genes were present in a region flanked by intact IS*6* family transposases. One of these plasmids also carried genes for resistance to fluoroquinolones (*qnrB2, aac(6')-Ib-cr*) and both of these plasmids also carried a colistin resistance gene (*mcr-9*) (table 2, figure 5).

In addition to these ESBL-encoding plasmids, we also assembled a 59 kbp IncN plasmid carrying a *qnrB77* gene (table 2, figure 6). This plasmid was present in a ST4981 isolate which also carried an ESBL-encoding gene ($bla_{\text{CTX-M-15}}$) chromosomally. This plasmid also carried resistance genes to trimethoprim and aminoglycosides. The *qnrB77* gene was flanked by a complete and a truncated transposase of IS*91* family of transposases (table 2, figure 6).

Some of these plasmids (p1, p23, p33 and p65) also carried genes (*qacΔE*) that determine resistance to quarternary ammonium compounds. Genes related to heavy metal resistance such as mercury (*merCDEPTR*), arsenic (*arsHB*), copper (*pcoES*) and tellurium (*terABCDWX*) resistance were also present on plasmids carrying $bla_{\text{SHV-12}}$. Plasmid carrying $bla_{\text{CTX-M-55}}$ genes also carried tellurium resistance genes (*terABCDWX).* Additionally, all the plasmids assembled in this study carried mobility genes (*tra* set of genes) and genes that can aid in plasmid maintenance and stability. All the IncFII and IncHI2plasmids carried genes coding for at least one toxin-antitoxin system, e.g., the IncFII plasmids carried pemI-pemK genes and all the IncHI2 carried higA-higB genes. Similarly, the *qnrB77* carrying IncN plasmid also carried mobility genes (*tra*) and genes encoding for proteins that aid in plasmid stability (stbB-stbC genes), antirestriction systems (ardA-ardB genes) and mutagenesis (mucA-mucB genes).

The comparison of these assembled plasmids with the PLDSB database resulted in the identification of several previously described plasmids with a high similarity (>80% coverage, and >98% nucleotide identity). To summarize, most of the plasmids carrying ESBL encoding genes assembled in this study were similar to plasmids harbored on various *Enterobactericaceae* and collected from various sources (animals, humans, environment) across different continents and shared the same molecular context around the genes of interest (*qnr, bla* genes) (figures 2-5).

In contrast, we were not able to identify similar plasmids to the *bla*$_{\text{CTX-M-15}}$ carrying IncFII (pMLST- F48:A1:B49) and the *qnrB77* carrying IncN plasmids found in this study.

Resistance determinants to other critical antimicrobials

No carbapenem resistance genes were identified in our collection, but the *mcr-9* gene was present in 7 isolates belonging to 6 different ST types. These isolates carried both the *mcr-9* gene and either a pAmpC, an ESBL or a PMQR gene (table 3). Descriptions of these isolates are presented briefly in table 3. *mcr-9* was also present in two of the ESBL plasmids assembled in this study (table 2).

Discussion

Whole genome sequencing (WGS) of enrofloxacin and ceftiofur resistant *E. coli* revealed multiple determinants conferring resistance to these critical antimicrobials, which were present on a wide spectrum of ST types recovered from the major swine producing states in the U.S.A. The use of both long and short read WGS technologies identified the genetic context of these resistance determinants for several isolates suggesting determinants by which resistance may be spreading such as plasmids carrying *bla*$_{\text{CMY-2}}$, which previously established in *Salmonella* and *E. coli* populations circulating in food animals in the U.S.A (11). We also assembled plasmids not previously described in isolates from swine or other food animals or retail meat in USA.

Nearly 84% of the ceftiofur resistant *E. coli* isolates carried a *bla*$_{\text{CMY-2}}$ gene, which is consistent with findings in ceftiofur-resistant *Salmonella* isolates from diseased pigs collected during the same study period (15). However, 24 *E. coli* isolates in this study (including 2 isolates non-resistant to ceftiofur) carried *bla*$_{\text{CTX-M}}$ or *bla*$_{\text{SHV-12}}$ genes, indicating a much higher prevalence (18%) of *bla*$_{\text{CTX-M}}$ in our isolates compared to ceftiofur-resistant *Salmonella* of swine

origin (15). Still, our data suggest a more limited distribution of *bla*$_{\text{CTX-M}}$ genes compared with reports in extended spectrum cephalosporin resistant *E. coli* isolates retrieved from swine in other upper income countries in Europe and Asia such as Belgium and Hong Kong (34, 35). ESBL-encoding genes are the predominant genes responsible for extended spectrum cephalosporin resistance globally in food animals (10). However, until the late 2000's these genes were not found in food animal isolates collected in North America (36). In a study on *E. coli* isolates collected from diseased pigs at the UMN-VDL in 2008, all ceftiofur resistant isolates carried *bla*$_{\text{CMY-2}}$ genes (37); whereas *bla*$_{\text{CTX-M}}$ carrying *E. coli* in finishing pigs in USA were first identified in 2011 (38). Since then, more recent studies have also reported the sporadic occurrence of *bla*$_{\text{CTX-M}}$ genes in *Enterobacteriaceae* isolates of swine origin (including pork) in the U.S.A (39, 40). Our study reinforces the results that prevalence of ESBLs might have increased in *E. coli* collected from pigs during late 2000s-early 2010s.

Similar to ESBLs, presence of PMQR genes (*qnr*, *aac(6')-Ib-cr*) in food animal isolates in the U.S.A had not been reported until recently (14, 15, 41, 42). There has also been an increase in PMQR genes in clinical *Salmonella* isolates from humans in the U.S.A; and animal sources have been postulated to contribute to this surge (42). In this study, presence of PMQR genes without additional QRDR mutations was sufficient to yield MIC values to the intermediate-susceptibility levels (0.25-1 μg/ml) but not above (with the exception of 2 *qnrB19* carrying isolates). This is consistent with previous reports suggesting PMQR genes like *qnrB* and *qnrS* confer only lower level resistance to quinolones by inhibiting binding of quinolones to DNA gyrase (43). However, these PMQRs are known to supplement resistance caused by other determinants such as altered target enzymes (DNA gyrase), efflux pump activities and deficiencies in outer membrane porin channels (44). The presence of PMQRs in zoonotic

bacteria and their clinical impact on both human and animal health should be therefore continuously monitored.

ESBLs have been associated with pandemic ST131 *E. coli* in humans (45). However, in this study only one ST131 isolate was identified, and it was considered susceptible to both antimicrobial classes under study. The main enrofloxacin resistant swine-specific ST type identified in this study was ST100, which is associated with porcine enterotoxigenic infections (46). Enrofloxacin was approved to treat swine enteric infections in the U.S.A in 2012 (2) and the association of ST100 with enrofloxacin resistance might be of concern for swine health. Some of the major cephalosporin and /or fluoroquinolone resistant ST types (ST744, ST10, ST23, ST88, ST90, ST410, ST58) identified in our panel (figure 1) have been associated with carriage of $bla_{\text{CTX-M}}$ in multiple animal species, have been implicated in human infections and are considered "zoonotic ST types" (47–49). The presence of these resistant ST types in swine may suggest a potential health risk to other animal species and humans. However, further comparative studies and detailed outbreak investigations are needed to substantiate the spread of these resistant bacteria from diseased pigs to food products and humans.

To the best of our knowledge, this is the first study to describe completely assembled plasmids carrying $bla_{\text{CTX-M-14, -15, -27, -55}}$, $bla_{\text{SHV-12}}$ and *qnrB77* in *E. coli* isolates of swine origin in the U.S.A. However, the close identities between some plasmids in this study and those already described in humans and animals globally indicate that the presence of ESBL genes in this isolate collection could be part of the pandemic expansion of ESBLs (10). $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ are considered the predominant ESBL genes in humans globally (10) and have been also identified in food animals including pigs worldwide (50–53). The plasmids carrying $bla_{\text{CTX-M-15}}$ identified in our study were highly similar (98% coverage, >99% nucleotide identity) to

other plasmids found in human *E. coli* isolates collected in the U.S.A between 2009-10 (54), (Genbank accession number-CP009232) which were also described to have the same plasmid backbone as other ESBL gene carrying plasmids reported worldwide (54). $bla_{\text{CTX-M-14}}$ carrying plasmids identical to those found here have been previously reported in human isolates in Hong Kong and characterized as an epidemic plasmid type (pHK01) (55) which has spread globally to other Asian (China, Vietnam, South Korea) and European countries (Finland) (unpublished; Genbank accession numbers- NC_013727.1, KU932024.1, KU987452.1, NC_013542.1, NZ_CP018973.1). Families of insertion sequences (IS*26*, IS*Ecp9*, IS*6*) that were part of the above-mentioned genetic contexts have also been demonstrated to be involved in transposing ESBL-encoding genes across plasmids and bacterial chromosomes (56).

It has been widely believed that the presence of plasmids in the absence of selective pressure imposes a metabolic fitness cost to the bacterial host (57). However, the fitness cost imposed due to plasmid carriage depends on the plasmid-bacterial host combination (58–60). There are several plasmid characteristics that facilitate plasmid stability in bacterial hosts: for example, IncF plasmids similar to those assembled here have a narrow host range and carry factors such as toxin-antitoxin systems which help in maintaining their stability in bacterial hosts in the absence of antimicrobial pressure (61). Similarly, IncHI2 plasmids similar to those assembled here carry genes which confer resistance to heavy metals, mutagenesis induction system etc. which can also contribute to their stability (62). Endemic plasmids identical to those found in our study such as pHK01-like plasmids have been demonstrated to be conjugative *in-vitro* (63). Hence, it can be postulated that these plasmids might aid in the establishment of ESBLs as dominant determinants behind extended spectrum cephalosporin resistance in swine in

316 the U.S.A as occurred globally. We are planning to conduct *in-vitro* conjugation and fitness
317 experiments to test these hypotheses.

318 To the best of our knowledge, this is the first report of the presence of *mcr-9* genes in
319 bacteria isolates from food animals in the U.S.A. *mcr-9* gene was recently described for the first
320 time in a *S.* Typhimurium isolate collected from a human patient in Washington State, the U.S.A,
321 and was able to confer colistin resistance to *E. coli* isolates cloned with this gene (64). Colistin
322 has never been used in swine in the U.S.A and therefore the presence of *mcr-9* gene in swine
323 could be an indicator of the complex transmission dynamics of resistant determinants across
324 different ecosystems and/or co-selection of resistant determinants due to use of other unrelated
325 antimicrobials.

326 Several considerations must be accounted for when interpreting these results. An
327 association between antimicrobial use and presence of these resistance genes cannot be
328 established due to the lack of information on use of antimicrobials. Also, the public health
329 implications of our findings could be limited by the removal of diseased pigs, such as the ones
330 from which these resistant and potentially zoonotic ST types were retrieved, from the food chain.

331 ***Conclusions***
332
333 We have identified and characterized a wide range of genetic determinants of resistance to some
334 critically important antimicrobial classes in swine clinical *E. coli* isolates, some of which had
335 never been described in isolates of animal origin in the U.S.A. Future studies will focus on
336 assembling finished genomes of isolates carrying *mcr-9* genes as well as conducting conjugation
337 and fitness experiments on selected isolates to predict the success of these plasmids and bacterial
338 hosts.

339 ***Data availability***

Short reads generated during this project have been submitted at NCBI Genbank under Bioprojects PRJNA605257, PRJNA605064 and PRJNA604903. Complete plasmid sequences have been submitted at Genbank under accession numbers MT077880, MT077881, MT077882, MT077883, MT077884, MT077885, MT077886, MT077887, MT077888 and MT077889.

Funding

This work was supported by the USDA National Institute of Food and Agriculture (Animal Health Formula Fund project MIN-62-091), Swine Disease Eradication Center fund and the Rapid Agricultural Response Fund (RARF) at the University of Minnesota.

Tables

Table 1. Pattern of genetic determinants of enrofloxacin resistance in *E. coli* clinical isolates of swine origin

Footnote:

'±' symbolizes that this genetic determinant might or might not be present in isolates with that particular MIC value.

[*]- Other determinants were *parC* (A56T or E84G), *parE* (S458A or L416F) and single PMQR (*aac(6')-Ib-cr, qnrB77 or qnrB19*)

Table 2. Characteristics of plasmids assembled in this study

Footnote: Colors in the farthest right column represent genes that can confer resistance to different antimicrobial families: **dark blue**- aminoglycosides, **purple**- penicillins, **light blue**- fluoroquinolones, **dark green**- macrolides, **pink**- trimethoprim (*dfrA-type*) and sulphonamide (*sul1*, *sul2*), **light green**-phenicols, **red**- tetracyclines, **orange**- colistin.

Table 3. Characteristics of isolates carrying *mcr-9* genes

Footnote: Colors in the farthest right column represent genes that can confer resistance to different antimicrobial families: **dark blue**- aminoglycosides, **purple**- penicillins, **light blue**-fluoroquinolones, **dark green**- macrolides, **pink**- trimethoprim (*dfrA-type*) and sulphonamide (*sul1*, *sul2*), **light green**-phenicols, **red**- tetracyclines, **black**- extended spectrum cephalosporin.

Figures

Figure 1. Maximum-likelihood tree constructed using the core-gene alignment of *Escherichia coli* isolates collected from diseased pigs at UMN-VDL between 2014-15.

Footnote: Ceftiofur and enrofloxacin MIC values (in μg/ml), sequence types (ST) and geographical location of isolation are presented in text columns. Ceftiofur and enrofloxacin MIC values are labelled in red and blue to denote resistant and non-resistant isolates, respectively. Heat map shows presence of chromosomal mutations in quinolone resistance determining regions (QRDRs), plasmid mediated quinolone resistance genes (PMQRs), extended spectrum beta-lactamase encoding genes (ESBL) and plasmidic AmpC genes ($bla_{\text{CMY-2}}$)

Figure 2. Circular maps representing comparisons of $bla_{\text{CTX-M-14}}$ (p77) and $bla_{\text{CTX-M-55}}$ (p65) carrying plasmids available at Genbank and plasmids assembled in this study.

Footnote: The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmids (p77 and p65). The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes

and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

Figure 3. Circular maps representing comparisons of *bla*$_{\text{CTX-M-27}}$ (p37 and p62) carrying plasmids available at Genbank and plasmids assembled in this study.

Footnote: The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmid (p37). The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

Figure 4. Circular maps representing comparisons of *bla*$_{\text{CTX-M-15}}$ (p1, p2 and p4) carrying plasmids available at Genbank and plasmids assembled in this study.

Footnote: The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmids (p1). There were no plasmids similar to p4. The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

Figure 5. Circular maps representing comparisons of *bla*$_{\text{SHV-12}}$ (p33 and p39) carrying plasmids available at Genbank and plasmids assembled in this study.

Footnote: The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmids (p33 and p39). The legend

on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

Figure 6. Circular maps representing region carrying antimicrobial resistance genes in *qnrB77* carrying plasmid (p23) assembled in this study.

Footnote: AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

References

1. Aarestrup FM. 2015. The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. Philos Trans R Soc B Biol Sci 370:20140085.

2. Hong S, Rovira A, Davies P, Ahlstrom C, Muellner P, Rendahl A, Olsen K, Bender JB, Wells S, Perez A, Alvarez J. 2016. Serotypes and Antimicrobial Resistance in Salmonella enterica Recovered from Clinical Samples from Cattle and Swine in Minnesota, 2006 to 2015. PLOS ONE 11:e0168016.

3. Carmo LP, Nielsen LR, Alban L, Müntener CR, Schüpbach-Regula G, Magouras I. 2017. Comparison of Antimicrobial Consumption Patterns in the Swiss and Danish Cattle and

Swine Production (2007–2013). Front Vet Sci 4.

4. Food and Drug Administration. 2017. Summary Report On Antimicrobials Sold or Distributed for Use in Food-Producing Animals.

5. Neuert S, Nair S, Day MR, Doumith M, Ashton PM, Mellor KC, Jenkins C, Hopkins KL, Woodford N, de Pinna E, Godbole G, Dallman TJ. 2018. Prediction of Phenotypic Antimicrobial Resistance Profiles From Whole Genome Sequences of Non-typhoidal Salmonella enterica. Front Microbiol 9.

6. Edirmanasinghe R, Finley R, Parmley EJ, Avery BP, Carson C, Bekal S, Golding G, Mulvey MR. 2017. A Whole-Genome Sequencing Approach To Study Cefoxitin-Resistant Salmonella enterica Serovar Heidelberg Isolates from Various Sources. Antimicrob Agents Chemother 61:e01919-16, /aac/61/4/e01919-16.atom.

7. Magouras I, Carmo LP, Stärk KDC, Schüpbach-Regula G. 2017. Antimicrobial Usage and -Resistance in Livestock: Where Should We Focus? Front Vet Sci 4.

8. Lalak A, Wasyl D, Zajac M, Skarzynska M, Hoszowski A, Samcik I, Wozniakowski G, Szulowski K. 2016. Mechanisms of cephalosporin resistance in indicator Escherichia coli isolated from food animals. Vet Microbiol 194:69–73.

9. Rodríguez I, Thomas K, Van Essen A, Schink A-K, Day M, Chattaway M, Wu G, Mevius D, Helmuth R, Guerra B. 2014. Chromosomal location of blaCTX-M genes in clinical isolates of Escherichia coli from Germany, The Netherlands and the UK. Int J Antimicrob Agents 43:553–557.

461 10. Bevan ER, Jones AM, Hawkey PM. 2017. Global epidemiology of CTX-M β-lactamases:
462 Temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145–2155.

463 11. Frye JG, Jackson CR. 2013. Genetic mechanisms of antimicrobial resistance identified in
464 Salmonella enterica, Escherichia coli, and Enteroccocus spp. isolated from U.S. food
465 animals. Front Microbiol 4.

466 12. Wittum TE, Mollenkopf DF, Erdman MM. 2012. Detection of Salmonella enterica Isolates
467 Producing CTX-M Cephalosporinase in U.S. Livestock Populations. Appl Environ
468 Microbiol 78:7487–7491.

469 13. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ,
470 Piddock LJV. 2016. Understanding the mechanisms and drivers of antimicrobial resistance.
471 The Lancet 387:176–187.

472 14. Tyson GH, Tate HP, Zhao S, Li C, Dessai U, Simmons M, McDermott PF. 2017.
473 Identification of Plasmid-Mediated Quinolone Resistance in Salmonella Isolated from
474 Swine Ceca and Retail Pork Chops in the United States. Antimicrob Agents Chemother
475 61:e01318-17, /aac/61/10/e01318-17.atom.

476 15. Elnekave E, Hong SL, Lim S, Hayer SS, Boxrud D, Taylor AJ, Lappi V, Noyes N, Johnson
477 TJ, Rovira A, Davies P, Perez A, Alvarez J. 2019. Circulation of Plasmids Harboring
478 Resistance Genes to Quinolones and/or Extended-Spectrum Cephalosporins in Multiple
479 Salmonella enterica Serotypes from Swine in the United States. Antimicrob Agents
480 Chemother 63:e02602-18.

481 16. Elnekave E, Hong S, Mather AE, Boxrud D, Taylor AJ, Lappi V, Johnson TJ, Vannucci F,

Davies P, Hedberg C, Perez A, Alvarez J. 2018. Salmonella enterica Serotype 4,[5],12:i:- in Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade. Clin Infect Dis 66:877–885.

17. Hayer SS. 2019. Monitoring of phenotypic and genotypic changes in antimicrobial resistance in clinical swine bacterial isolates circulating in the U.S. Ph.D Dissertation, University of Minnesota, Minneapolis, USA.

18. Food and Drug Administration. 2019. NARMS now.

19. EUCAST. 2019. MIC and zone distributions and ECOFFs.

20. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Ponten T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus Sequence Typing of Total-Genome-Sequenced Bacteria. J Clin Microbiol 50:1355–1361.

21. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644.

22. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. *In Silico* Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. Antimicrob Agents Chemother 58:3895–3903.

23. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069.

502 24. Seemann T. snippy: fast bacterial variant calling from NGS reads.

503 25. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris
504 SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole
505 genome sequences using Gubbins. Nucleic Acids Res 43:e15.

506 26. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis
507 of large phylogenies. Bioinformatics 30:1312–1313.

508 27. Letunic I, Bork P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new
509 developments. Nucleic Acids Res 47:W256–W259.

510 28. Salmela L, Rivals E. 2014. LoRDEC: accurate and efficient long read error correction.
511 Bioinformatics 30:3506–3514.

512 29. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome
513 assemblies from short and long sequencing reads. PLOS Comput Biol 13:e1005595.

514 30. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de
515 novo genome assemblies. Bioinformatics 31:3350–3352.

516 31. Varani AM, Siguier P, Gourbeyre E, Charneau V, Chandler M. 2011. ISsaga is an ensemble
517 of web-based methods for high throughput identification and semi-automatic annotation of
518 insertion sequences in prokaryotic genomes. Genome Biol 12:R30.

519 32. Galata V, Fehlmann T, Backes C, Keller A. 2019. PLSDB: a resource of complete bacterial
520 plasmids. Nucleic Acids Res 47:D195–D202.

521 33. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator

(BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402.

34. Ho PL, Chow KH, Lai EL, Lo WU, Yeung MK, Chan J, Chan PY, Yuen KY. 2011. Extensive dissemination of CTX-M-producing Escherichia coli with multidrug resistance to "critically important" antibiotics among food animals in Hong Kong, 2008-10. J Antimicrob Chemother 66:765–768.

35. Van Damme I, Garcia-Graells C, Biasino W, Gowda T, Botteldoorn N, de Zutter L. 2017. High abundance and diversity of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli in faeces and tonsils of pigs at slaughter. Vet Microbiol 208:190–194.

36. Roach S, Wallinga D. 2013. Commentary on genetic mechanisms of antimicrobial resistance in bacteria from U.S. food animals: ESBLs are here. Front Microbiol 4.

37. Johnson TJ, Shepard SM, Rivet B, Danzeisen JL, Carattoli A. 2011. Comparative genomics and phylogeny of the IncI1 plasmids: A common plasmid type among porcine enterotoxigenic Escherichia coli. Plasmid 66:144–151.

38. Mollenkopf DF, Mirecki JM, Daniels JB, Funk JA, Henry SC, Hansen GE, Davies PR, Donovan TS, Wittum TE. 2013. Escherichia coli and klebsiella pneumoniae producing CTX-M cephalosporinase from swine finishing barns and their association with antimicrobial use. Appl Environ Microbiol 79:1052–1054.

39. Tadesse DA, Zhao SH, Tong E, Ayers S, Singh A, Bartholomew MJ, McDermott PF. 2012. Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002. Emerg Infect Dis 18:741–749.

40. Jiang F, Wu Z, Zheng Y, Frana TS, Sahin O, Zhang Q, Li G. 2019. Genotypes and Antimicrobial Susceptibility Profiles of Hemolytic Escherichia coli from Diarrheic Piglets. Foodborne Pathog Dis 16:94–103.

41. Awosile B, McClure J, Sanchez J, Rodriguez-Lecompte JC, Keefe G, Heider LC. 2018. Salmonella enterica and extended-spectrum cephalosporin-resistant Escherichia coli recovered from Holstein dairy calves from 8 farms in New Brunswick, Canada. J Dairy Sci 101:3271–3284.

42. Cummings KJ, Rodriguez-Rivera LD, Norman KN, Ohta N, Scott HM. 2017. Identification of a Plasmid-Mediated Quinolone Resistance Gene in Salmonella Isolates from Texas Dairy Farm Environmental Samples. Zoonoses Public Health 64:305–307.

43. Tran JH, Jacoby GA, Hooper DC. 2005. Interaction of the Plasmid-Encoded Quinolone Resistance Protein Qnr with Escherichia coli DNA Gyrase. Antimicrob Agents Chemother 49:118–125.

44. Martínez-Martínez L, Pascual A, García I, Tran J, Jacoby GA. 2003. Interaction of plasmid and host quinolone resistance. J Antimicrob Chemother 51:1037–1039.

45. Pitout JDD, DeVinney R. 2017. Escherichia coli ST131: a multidrug-resistant clone primed for global domination. F1000Research 6.

46. Abraham S, Jordan D, Wong HS, Johnson JR, Toleman MA, Wakeham DL, Gordon DM, Turnidge JD, Mollinger JL, Gibson JS, Trott DJ. 2015. First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant Escherichia coli in Australian food-producing animals. J Glob Antimicrob Resist 3:273–277.

563 47. Maluta RP, Logue CM, Casas MRT, Meng T, Guastalli EAL, Rojas TCG, Montelli AC,
564 Sadatsune T, Ramos M de C, Nolan LK, Silveira WD da. 2014. Overlapped Sequence
565 Types (STs) and Serogroups of Avian Pathogenic (APEC) and Human Extra-Intestinal
566 Pathogenic (ExPEC) Escherichia coli Isolated in Brazil. PLOS ONE 9:e105016.

567 48. Day MJ, Rodríguez I, van Essen-Zandbergen A, Dierikx C, Kadlec K, Schink A-K, Wu G,
568 Chattaway MA, DoNascimento V, Wain J, Helmuth R, Guerra B, Schwarz S, Threlfall J,
569 Woodward MJ, Coldham N, Mevius D, Woodford N. 2016. Diversity of STs, plasmids and
570 ESBL genes among Escherichia coli from humans, animals and food in Germany, the
571 Netherlands and the UK. J Antimicrob Chemother 71:1178–1182.

572 49. Hasman H, Hammerum AM, Hansen F, Hendriksen RS, Olesen B, Agersø Y, Zankari E,
573 Leekitcharoenphon P, Stegger M, Kaas RS, Cavaco LM, Hansen DS, Aarestrup FM, Skov
574 RL. 2015. Detection of mcr-1 encoding plasmid-mediated colistin-resistant escherichia coli
575 isolates from human bloodstream infection and imported chicken meat, denmark 2015.
576 Eurosurveillance 20:1–5.

577 50. Liu BT, Yang QE, Li L, Sun J, Liao XP, Fang LX, Yang SS, Deng H, Liu YH. 2013.
578 Dissemination and Characterization of Plasmids Carrying oqxAB-blaCTX-M Genes in
579 Escherichia coli Isolates from Food-Producing Animals. PLOS ONE 8:e73947.

580 51. Norizuki C, Kawamura K, Wachino JI, Suzuki M, Nagano N, Kondo T, Arakawa Y. 2018.
581 Detection of Escherichia coli Producing CTX-M-1-Group Extended-Spectrum beta-
582 Lactamases from Pigs in Aichi Prefecture, Japan, between 2015 and 2016. Jpn J Infect Dis
583 71:33–38.

584 52. Horton RA, Randall LP, Snary EL, Cockrem H, Lotz S, Wearing H, Duncan D, Rabie A,
585 McLaren I, Watson E, La Ragione RM, Coldham NG. 2011. Fecal Carriage and Shedding
586 Density of CTX-M Extended-Spectrum β-Lactamase-Producing Escherichia coli in Cattle,
587 Chickens, and Pigs: Implications for Environmental Contamination and Food Production.
588 Appl Environ Microbiol 77:3715–3719.

589 53. Tian GB, Wang HN, Zhang AY, Zhang Y, Fan WQ, Xu CW, Zeng B, Guan ZB, Zou LK.
590 2012. Detection of clinically important β-lactamases in commensal Escherichia coli of
591 human and swine origin in western China. J Med Microbiol 61:233–238.

592 54. Li JJ, Spychala CN, Hu F, Sheng JF, Doi Y. 2015. Complete Nucleotide Sequences of
593 blaCTX-M-Harboring IncF Plasmids from Community-Associated Escherichia coli Strains
594 in the United States. Antimicrob Agents Chemother 59:3002–3007.

595 55. Ho PL, Lo WU, Wong RCW, Yeung MK, Chow KH, Que TL, Tong AHY, Bao JYJ, Lok S,
596 Wong SSY. 2011. Complete sequencing of the FII plasmid pHK01, encoding CTX-M-14,
597 and molecular analysis of its variants among Escherichia coli from Hong Kong. J
598 Antimicrob Chemother 66:752–756.

599 56. Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile Genetic Elements Associated
600 with Antimicrobial Resistance. Clin Microbiol Rev 31:e00088-17.

601 57. San Millan A, MacLean RC. 2017. Fitness Costs of Plasmids: a Limit to Plasmid
602 Transmission. Microbiol Spectr 5.

603 58. Johnson TJ, Singer RS, Isaacson RE, Danzeisen JL, Lang K, Kobluk K, Rivet B, Borewicz
604 K, Frye JG, Englen M, Anderson J, Davies PR. 2015. In Vivo Transmission of an IncA/C

Plasmid in Escherichia coli Depends on Tetracycline Concentration, and Acquisition of the Plasmid Results in a Variable Cost of Fitness. Appl Environ Microbiol 81:3561–3570.

59. Buckner MMC, Saw HTH, Osagie RN, McNally A, Ricci V, Wand ME, Woodford N, Ivens A, Webber MA, Piddock LJV. 2018. Clinically Relevant Plasmid-Host Interactions Indicate that Transcriptional and Not Genomic Modifications Ameliorate Fitness Costs of *Klebsiella pneumoniae* Carbapenemase-Carrying Plasmids. mBio 9:e02303-17, /mbio/9/2/mBio.02303-17.atom.

60. Schaufler K, Semmler T, Pickard DJ, de Toro M, de la Cruz F, Wieler LH, Ewers C, Guenther S. 2016. Carriage of Extended-Spectrum Beta-Lactamase-Plasmids Does Not Reduce Fitness but Enhances Virulence in Some Strains of Pandemic E. coli Lineages. Front Microbiol 7.

61. Carattoli A. 2013. Plasmids and the spread of resistance. Int J Med Microbiol 303:298–304.

62. García-Fernández A, Carattoli A. 2010. Plasmid double locus sequence typing for IncHI2 plasmids, a subtyping scheme for the characterization of IncHI2 plasmids carrying extended-spectrum β-lactamase and quinolone resistance genes. J Antimicrob Chemother 65:1155–1161.

63. Ho PL, Lo WU, Yeung MK, Li Z, Chan J, Chow KH, Yam WC, Tong AHY, Bao JYJ, Lin CH, Lok S, Chiu SS. 2012. Dissemination of pHK01-like incompatibility group IncFII plasmids encoding CTX-M-14 in Escherichia coli from human and animal sources. Vet Microbiol 158:172–179.

64. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. 2019.

626 Identification of Novel Mobilized Colistin Resistance Gene mcr-9 in a Multidrug-Resistant,
627 Colistin-Susceptible Salmonella enterica Serotype Typhimurium Isolate. mBio 10.

628
629
630
631
632
633
634
635
636
637
638
639
640
641
642

643

Table 1. Pattern of genetic determinants of enrofloxacin resistance in *E. coli* clinical isolates of swine origin

MIC value (in μg/ml)	Pattern of genetic determinants (n=number of isolates)	ST types (n=number of isolates)
>2	*gyrA*(S83L) + *gyrA*(D87Y or D87N or D87G) + *parC*(S80I or S80R) ± other genetic determinants* (n=49)	744 (n=11), 100 (n=10), 224 (n=4), 410 (n=3), 10 (n=2), 457 (n=2), 617 (n=2), 4981 (n=2), 88 (n=1), 93 (n=1), 167 (n=1), 977 (n=1), 1585 (n=1), 2161 (n=1), 3901 (n=1)
2	*gyrA*(S83L) + *parC*(S80I or S80R) (n=23)	100 (n=21), 58 (n=1), 90 (n=1)
	qnrB19 (n=2)	361 (n=1), 2496 (n=1)
	No genetic determinants (n=1)	5926 (n=1)
1	*gyrA*(S83L) + *parC*(S80I or S80R) (n=7)	100 (n=6), 69 (n=1)
	gyrA(S83L) only (n=1)	6234 (n=1)
	gyrA(D87G) + *qnrB2* (n=1)	10 (n=1)
	qnrB19 + *qnrS2* (n=1)	101 (n=1)
	aac(6')-Ib-cr + *qnrB2* (n=1)	540 (n=1)
	Single PMQR (*qnrB19*, *qnrS1*, *qnrS2*, *qnrB2* or *qnrB77*) (n=6)	10 (n=3), 641 (n=1), 847 (n=1), 5759 (n=1)

	No genetic determinants (n=1)	10 (n=1)
0.25-0.5	*gyrA*(S83L) ± *aac(6')-Ib-cr* (n=6)	6234 (n=2), 10 (n=1), 58 (n=1), 101 (n=1), 410 (n=1)
	Single PMQR (*qnrB19*, *qnrS2*, *qnrB2*) (n=5)	10 (n=3), 93 (n=1), 1112 (n=1)
	gyrA (D87G or D87Y) (n=3)	10 (n=1), 88 (n=1), 641 (n=1)
	aac(6')-Ib-cr + *qnrB2* (n=1)	641 (n=1)
	No genetic determinants (n=2)	641 (n=1), 3057 (n=1)
≤ 0.125	*gyrA*(S83L) (n=3)	10 (n=1), 847 (n=1), Unknown ST (n=1)
	gyrA(D87Y) (n=1)	90 (n=1)

'±' symbolizes that this genetic determinant might or might not be present in isolates with that particular MIC value.

*- Other determinants were *parC*(A56T or E84G), *parE*(S458A or L416F) and single PMQR (*aac(6')-Ib-cr, qnrB77 or qnrB19*)

Table 2. Characteristics of plasmids assembled in this study

Plasmid (Genbank Accession number)	Gene of interest	Size of plasmid	Replicon type (pMLST)	ST type	Other AMR genes present in the plasmid sequence
p77 (MT077889)	*bla*$_{\text{CTX-M-14}}$	77 kbp	IncF (F2:A8:B56)	10	-
p37 (MT077885)	*bla*$_{\text{CTX-M-27}}$	69 kbp	IncF (F21*:A-:B-)	744	***erm(B)***
p62 (MT077887)	*bla*$_{\text{CTX-M-27}}$	69 kbp	IncF (F21*:A-:B-)	10	-
p1 (MT077880)	*bla*$_{\text{CTX-M-15}}$	170 kbp	IncF (F31:A4:B1)	617	***aadA5***, ***aac(3)-IIa***, ***aac(6')-Ib-cr***, ***bla***$_{\text{OXA-1}}$, ***mph(A)***, ***sul1***, ***dfrA17***, ***catB3***, ***tet(B)***
p2 (MT077881)	*bla*$_{\text{CTX-M-15}}$	170 kbp	IncF (F31:A4:B1)	58	***aac(6')-Ib-cr***, ***bla***$_{\text{OXA-1}}$, ***mph(A)***, ***dfrA17***, ***catB3***, ***tet(B)***
p4 (MT077882)	*bla*$_{\text{CTX-M-15}}$	115 kbp	IncF (F48:A1:B49)	744	***aac(3)-IIa***, ***bla***$_{\text{TEM-1b}}$, ***mph(A)***, ***dfrA17***
p65 (MT077888)	*bla*$_{\text{CTX-M-55}}$	240 kbp	IncHI2 (ST-2)	165	***aac(3)-IId***, ***aadA2***, ***aph(3")-Ib***, ***aph(3')-Ia***, ***aph(6)-Id***, ***bla***$_{\text{TEM-1b}}$, ***mph(A)***, ***sul1***, ***dfrA12***, ***tet(M)***
p33 (MT077884)	*bla*$_{\text{SHV-12}}$	302 kbp	IncHI2 (ST-1)	641	***aac(6')-Ib3***, ***aac(6')-IIc***, ***aph(6')-Id***, ***aph(3')-Ib***, ***aadA2***, ***aac(6')-Ib-cr***, ***bla***$_{\text{TEM-1b}}$, ***qnrB2***,

					ere(A)*, *sul1*, *sul2*, *dfrA19*, *tet(D)*, *mcr-9
p39 (MT077886)	*bla*$_{SHV-12}$	289 kbp	IncHI2 (ST-1)	1112	***aph(3'')-Ib*, *aph(6')-Id*, *aph(3')-Ia*, *aac(6')-IIc*, *bla*$_{TEM-1b}$, *ere(A)*, *sul1*, *catA2*, *tet(D)*, *mcr-9***
p23 (MT077883)	*qnrB77*	60 kbp	IncN (unknown)	4981	***aac(3)-VIa*, *aadA1*, *dfrA15***

Colors in the farthest right column represent genes that can confer resistance to different antimicrobial families: **dark blue**-aminoglycosides, **purple**- penicillins, **light blue**- fluoroquinolones, **dark green**- macrolides, **pink**- trimethoprim (*dfrA-type*) and sulphonamide (*sul1*, *sul2*), **light green**-phenicols, **red-** tetracyclines, **orange**- colistin.

Table 3. Characteristics of isolates carrying *mcr-9* genes

Isolate (Biosample accession)	ST type	Other AMR genes in the isolate
8 (SAMN14052773)	540	***aac(6')-IIc*, *aadA2b*, *aac(6')-Ib3*, *aph(3")-Ib*, *aph(6)-Id*, *aac(6')Ib-cr*, *bla*$_{\text{TEM-1b}}$, *qnrB2*, *ere(A)*, *mdf(A)*, *sul1*, *sul2*, *sul3*, *dfrA12*, *dfrA19*, *tet(A)*, *tet(M)***
33 (SAMN14069745)	641	***aac(6')-Ib3*, *aac(6')-IIc*, *aph(6')-Id*, *aph(3")-Ib*, *aadA2*, , *aac(6')-Ib-cr*, *bla*$_{\text{TEM-1b}}$, *qnrB2*, *ere(A)*, *mdf(A)*, *sul1*, *sul2*, *dfrA19*, *tet(D)*, *tet(B)*, *bla*$_{\text{SHV-12}}$**
39 (SAMN14069776)	1112	***aph(3'')-Ib*, *aph(6')-Id*, *aph(3')-Ia*, *aac(6')-IIc*, *aadA2*, *bla*$_{\text{TEM-1b}}$, *qnrB2*, *ere(A)*, *mdf(A)*, *sul1*, *dfrA19*, *catA2*, *tet(B)*, *tet(D)*, *bla*$_{\text{SHV-12}}$**
81 (SAMN14070155)	90	***aac(3)-VIa*, *aadA1*, *aadA2*, *aadA5*, *aph(3")-Ib*, *aph(3')-Ia*, *aph(6)-Id*, *armA*, *bla*$_{\text{TEM-1b}}$, *mph(E)*, *msr(E)*, *mdf(A)*, *sul2*, *dfrA1*, *floR*, *tet(A)*, *tet(B)*, *bla*$_{\text{CMY-2}}$**
243 (SAMN14088537)	101	***aac(3)-VIa*, *aadA1*, *aph(3")-Ib*, *aph(6)-Id*, *mdf(A)*, *sul1*, *sul2*, *floR*, *tet(A)*, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{CTX-M-55}}$**
279 (SAMN14089329)	10	***aac(6')-IIc*, *aadA2b*, *aph(3")-Ib*, *aph(3')-Ia*, *aph(6)-Id*, *bla*$_{\text{TEM-1b}}$, *qnrB2*, *ere(A)*, *mdf(A)*, *dfrA19*, *sul1*, *sul2*, *tet(B)*, *tet(D)*, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{SHV-12}}$**
283	10	***aac(6')-IIc* , *aadA2b* , *aph(3')-Ib*, *aph(3')-Ia*, *aph(6)-Id*, *bla*$_{\text{TEM-1b}}$, *qnrB2*,**

(SAMN14089333)		***ere(A)*, *mdf(A)*, *dfrA19*, *sul1*, *sul2*, *tet(B)*, *tet(D)*, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{SHV-12}}$**

Colors in the farthest right column represent genes that can confer resistance to different antimicrobial families: **dark blue**-aminoglycosides, **purple**- penicillins, **light blue**- fluoroquinolones, **dark green**- macrolides, **pink**- trimethoprim (*dfrA-type*) and sulphonamide (*sul1*, *sul2*), **light green**-phenicols, **red-** tetracyclines, **black**- extended spectrum cephalosporin.

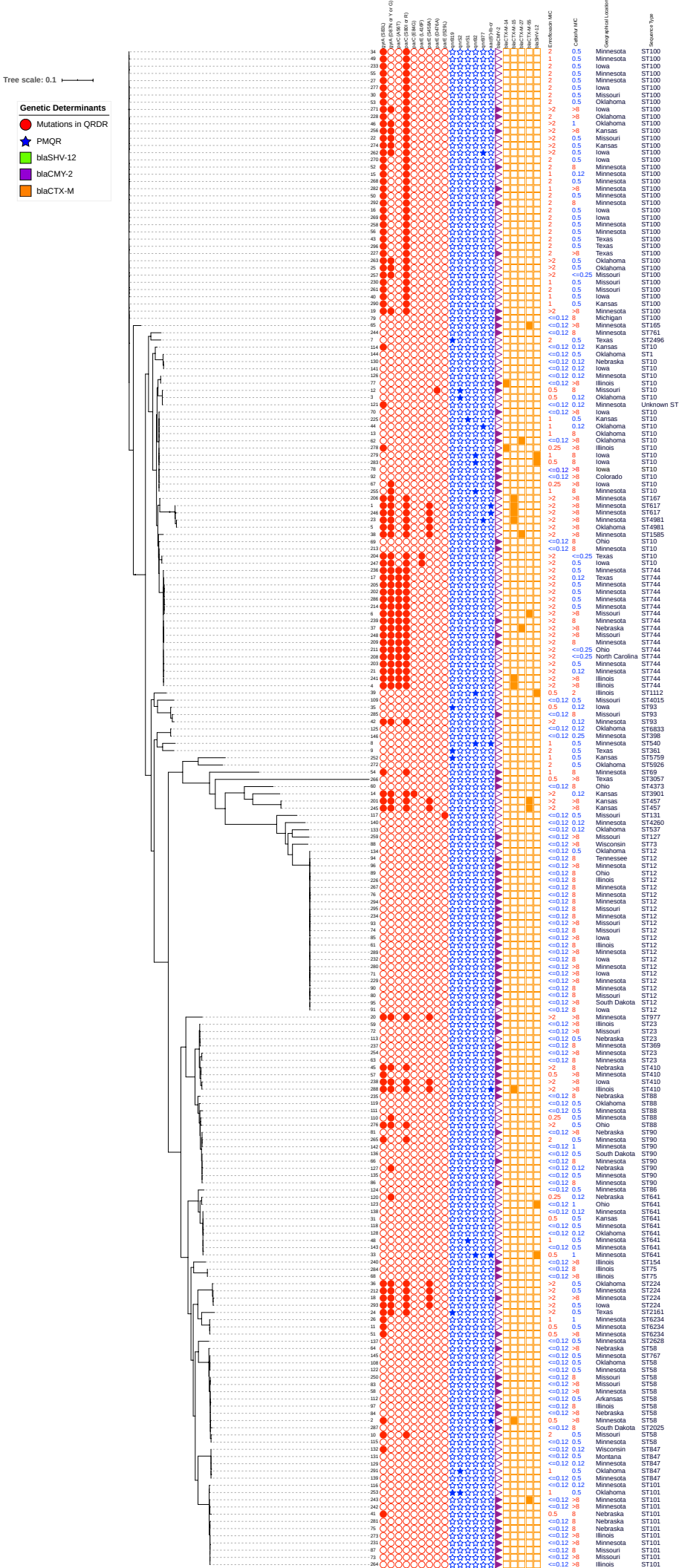
Tree scale: 0.1
Genetic Determinants
Mutations in QRDR
PMQR
blaSHV-12
blaCMY-2
blaCTX-M
gyrA (S83L)
parC (S80I or T or G)
parC (A56T)
parC (S80I or R)
parC (E84G)
parE (S458A)
parE (D474A)
parE (I529L)
qnrS1
qnrS2
qnrB2
qnrB19
oqxB9+B-nr
blaCMY-2
blaCTX-M-14
blaCTX-M-65
blaCTX-M-27
blaCTX-M-15
blaSHV-12
In Enrofloxacin MIC
Ceftiofur MIC
Geographical Location
Sequence Type

Figure 1. Maximum-likelihood tree constructed using the core-gene alignment of *Escherichia coli* isolates collected from diseased pigs at UMN-VDL between 2014-15.

Footnote: Ceftiofur and enrofloxacin MIC values (in μg/ml), sequence types (ST) and geographical location of isolation are presented in text columns. Ceftiofur and enrofloxacin MIC values are labelled in red and blue to denote resistant and non-resistant isolates, respectively. Heat map shows presence of chromosomal mutations in quinolone resistance determining regions (QRDRs), plasmid mediated quinolone resistance genes (PMQRs), extended spectrum beta-lactamase encoding genes (ESBL) and plasmidic AmpC genes (*bla*$_{\text{CMY-2}}$)

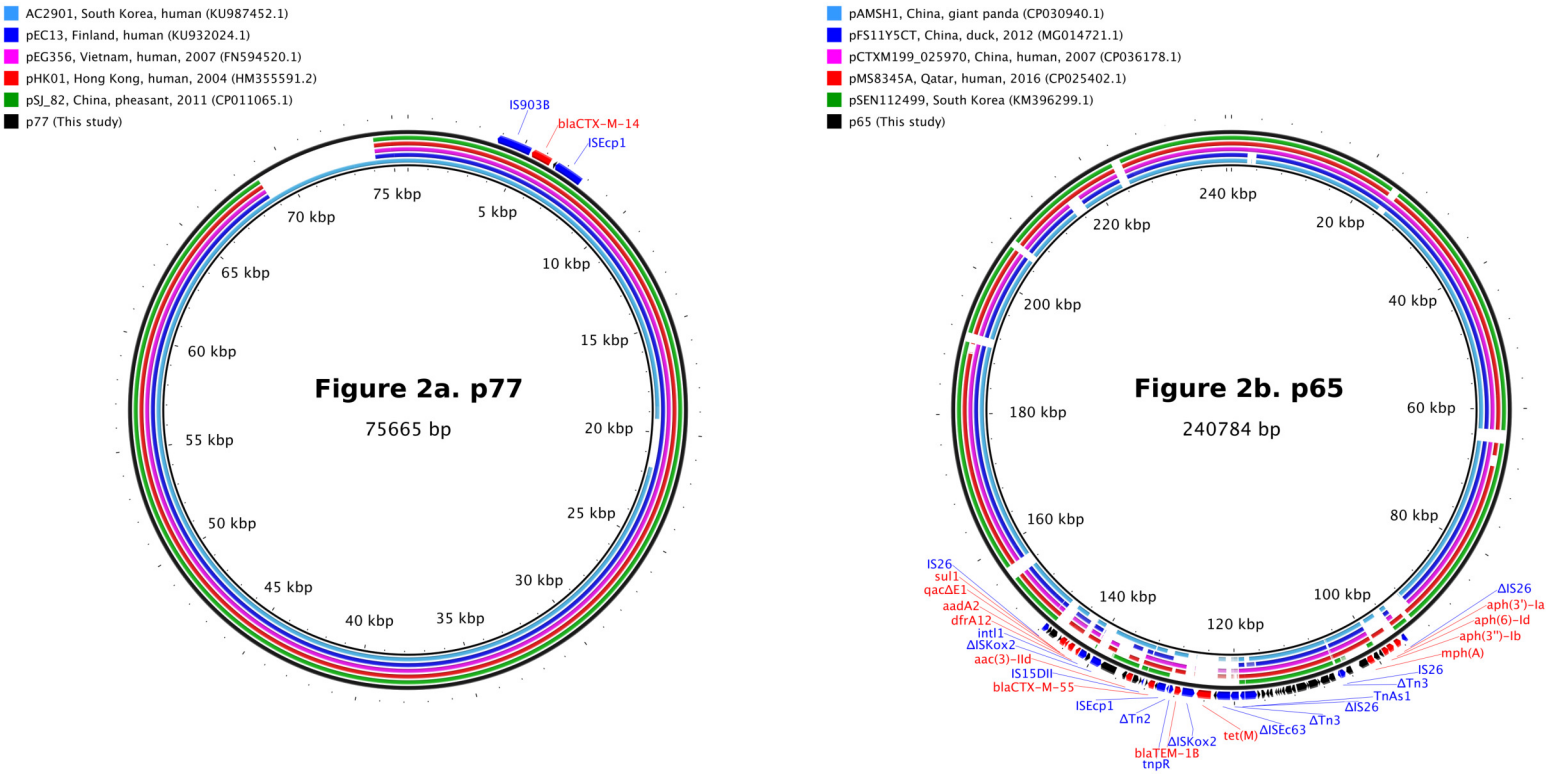

Figure 2. Circular maps representing comparisons of *bla*$_{\text{CTX-M-14}}$ (p77) and *bla*$_{\text{CTX-M-55}}$ (p65) carrying plasmids available at Genbank and plasmids assembled in this study.

The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmids (p77 and p65). The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

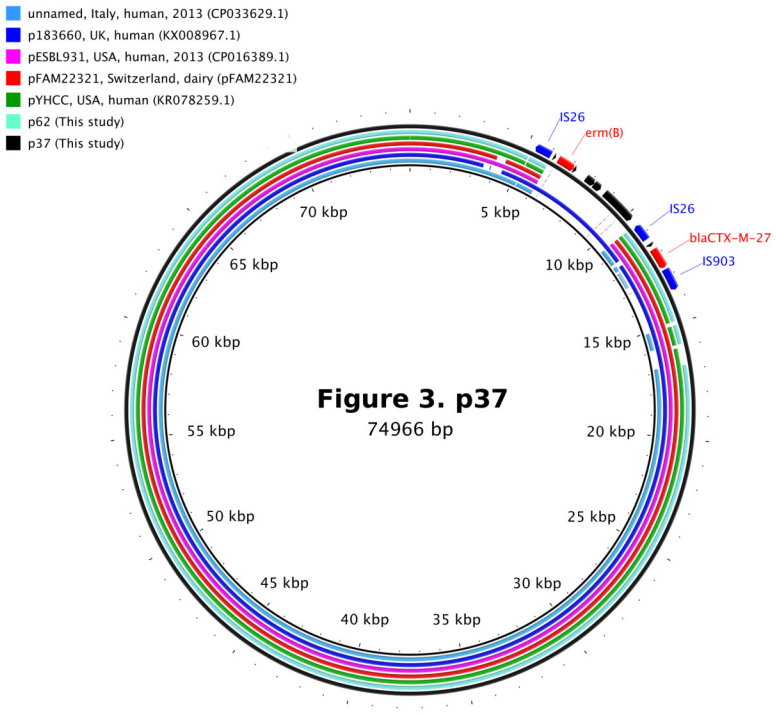

Figure 3. Circular maps representing comparisons of *bla*$_{\text{CTX-M-27}}$ (p37 and p62) carrying plasmids available at Genbank and plasmids assembled in this study.

The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmid (p37). The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

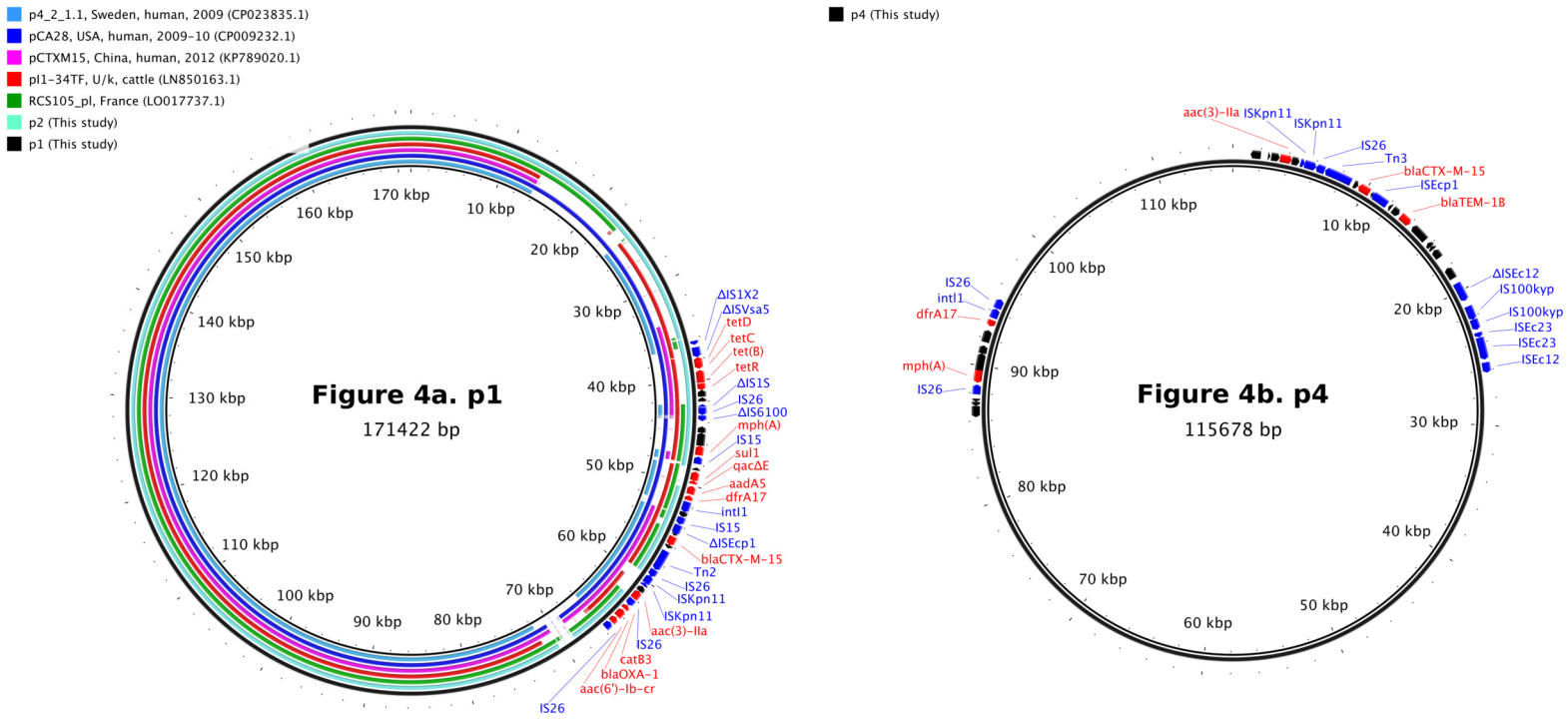

Figure 4. Circular maps representing comparisons of *bla*$_{\text{CTX-M-15}}$ (p1, p2 and p4) carrying plasmids available at Genbank and plasmids assembled in this study.

The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmids (p1). There were no plasmids similar to p4. The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR

genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

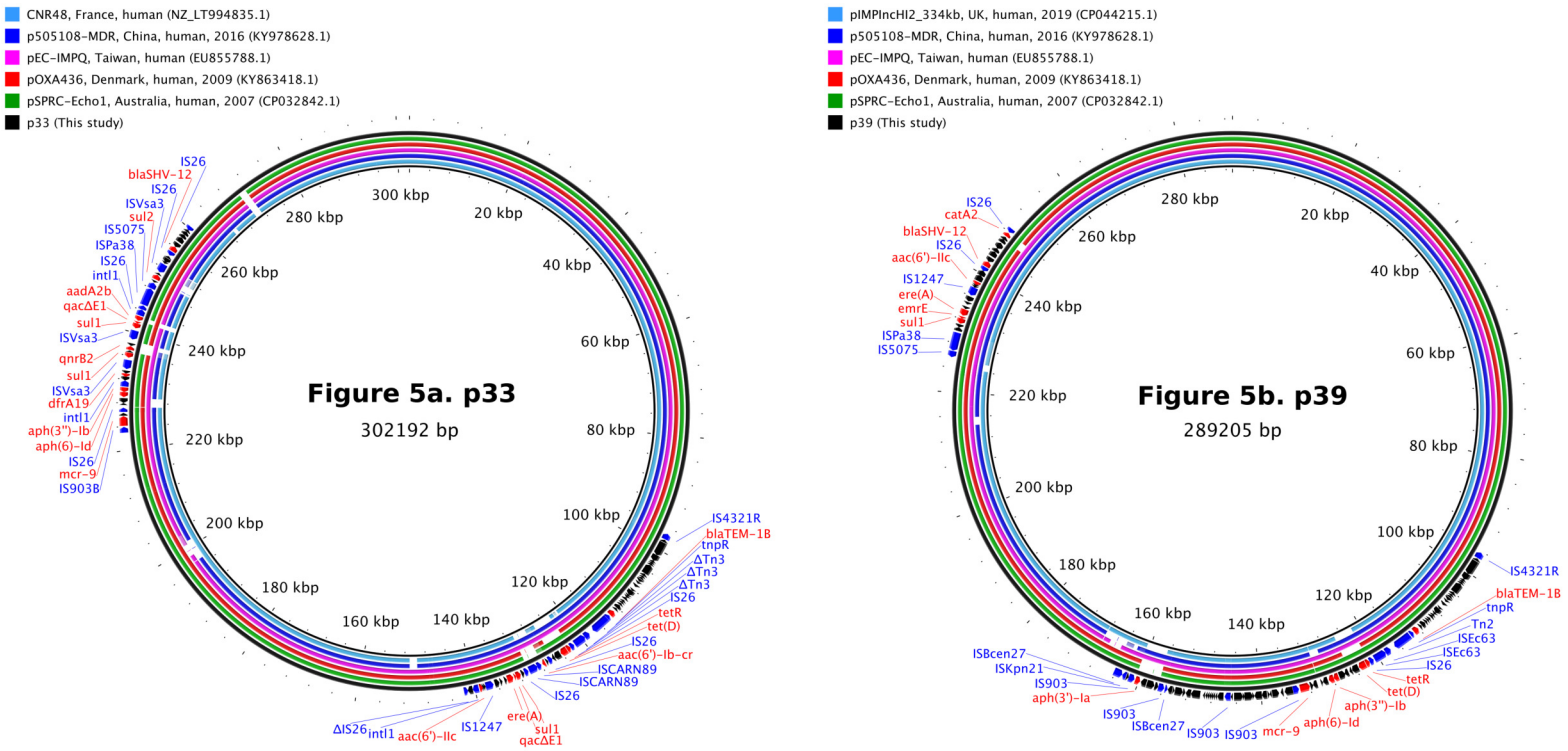

Figure 5. Circular maps representing comparisons of *bla*$_{\text{SHV-12}}$ (p33 and p39) carrying plasmids available at Genbank and plasmids assembled in this study.

The innermost rings (not colored black) represent the top plasmids with high nucleotide identity and coverage with respect to reference plasmids (p33 and p39). The legend on upper-left presents plasmid name, country, animal species/human and year of isolation, where available. Area of the plasmid carrying AMR genes is presented in outermost ring. AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.

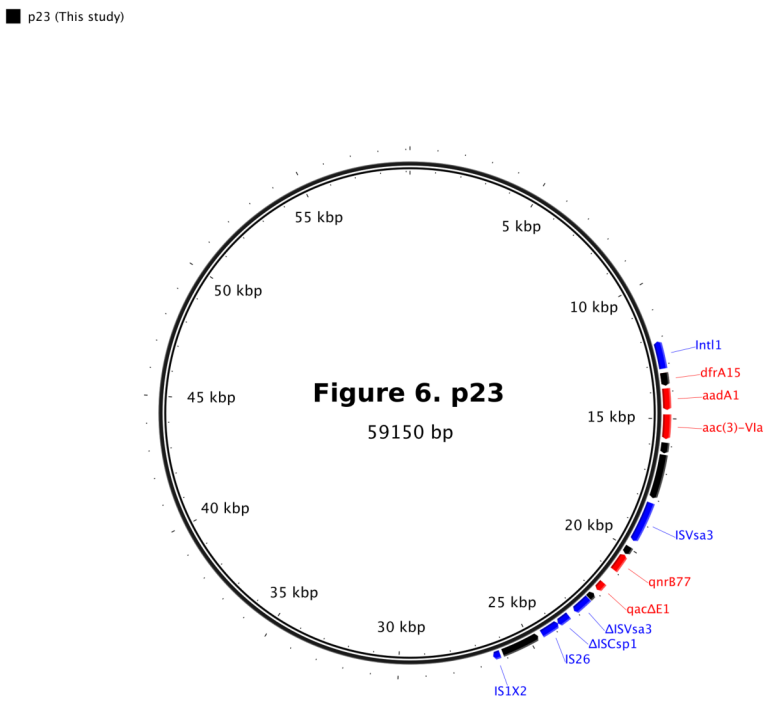

Figure 6. Circular maps representing region carrying antimicrobial resistance genes in *qnrB77* carrying plasmid (p23) assembled in this study.

AMR genes and genes associated with mobile elements are colored and labelled in red and blue, respectively. Truncated genes are represented with Δ as prefix.