

- 1 Carbapenemase-producing *Enterobacteriaceae* recovered from the environment of a swine farrow-to-
2 finish operation in the United States
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13 Running title: CRE recovered from a US swine production environment
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22 **Abstract**

23 Carbapenem-resistant *Enterobacteriaceae* (CRE) present an urgent threat to public health. While
24 carbapenem antimicrobials are restricted in food-producing animals, other β -lactams, such as ceftiofur,
25 are used in livestock. This use may provide selection pressure favoring the amplification of carbapenem
26 resistance but this relationship has not been established. Previously unreported from US livestock,
27 plasmid-mediated CREs have been reported from livestock in Europe and Asia.
28 Environmental and fecal samples were collected from a 1,500 sow, US farrow-to-finish operation during
29 4 visits over a 5 month period, 2015. Samples were screened using selective media for the presence of
30 CRE, with resulting carbapenemase-producing isolates further characterized.
31 Of 30 environmental samples collected from a nursery room on our initial visit, 2 (7%) samples yielded 3
32 isolates: 2 ST 218 *Escherichia coli* and 1 *Proteus mirabilis*, carrying the metallo- β -lactamase gene *bla*_{IMP-27}
33 on IncQ1 plasmids. We recovered 15 IMP-27-bearing isolates of multiple *Enterobacteriaceae* species
34 from 11 of 24 (46%) environmental samples from 2 farrowing rooms collected on our third visit. These
35 isolates each also carried *bla*_{IMP-27} on IncQ1 plasmids. No CRE isolates were recovered from fecal swabs
36 or samples in this study.
37 As is common in US swine production, piglets on this farm receive ceftiofur at birth, with males receiving
38 a second dose at castration (\approx day 6). This selection pressure may favor the dissemination of *bla*_{IMP-27}-
39 bearing *Enterobacteriaceae* in this farrowing barn. The absence of this selection pressure in the nursery
40 and finisher barns likely resulted in the loss of the ecological niche needed for maintenance of this
41 carbapenem resistance gene.

42

43

44 Introduction

45 The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) has been described as heralding the
46 end of the antibiotic era (1) with their global expansion presenting an urgent threat to public health (2).
47 These potential pathogens can harbor highly-mobile genes that confer resistance to the most critically
48 important, live-saving antimicrobial drugs. The plasmid-mediated class A (KPC), class B (NDM, IMP, VIM),
49 and class D (OXA-48, OXA-181) carbapenemase genes have disseminated beyond the realm of hospitals,
50 nursing homes, and other human healthcare settings, to now cause critical community-acquired
51 infections (3). Often by acquiring mobile resistance elements through horizontal gene transfer, CRE
52 infections are especially threatening because they approach pan-resistance, frequently delaying and
53 greatly reducing successful therapeutic treatment options for invasive infections. These bacteria,
54 harboring mobile carbapenemase genes are now identified with some regularity from both hospital- and
55 community-acquired human infections (4) and have been recovered from healthcare environments (5),
56 waste and surface water flows, soil, and companion animals (6, 7).

57

58 While they are considered “Last Line of Defense” drugs in human medicine, carbapenem antimicrobials
59 are not approved for use in food animal veterinary medicine. However, other β -lactams are commonly
60 used in almost all food animal species worldwide including ceftiofur and cefquinome extended-
61 spectrum cephalosporin drugs. While the exact relationship between extended-spectrum cephalosporin
62 use and carbapenem resistance has not yet been established, use of these drugs is likely to provide
63 significant selection pressure favoring organisms expressing carbapenem resistance because they will
64 also be resistant to all extended-spectrum cephalosporins. While most people today do not have direct
65 livestock exposure, enteric flora from livestock commonly contaminate fresh retail meat products that
66 are distributed over wide geographic areas (8, 9). Thus, if CRE are present in food animal populations, a

67 large number of consumers may be exposed through the food chain, resulting in a critically important
68 emerging food safety issue.
69
70 While bacteria harboring plasmid-borne carbapenemase genes have never been recovered from
71 livestock in the US, CRE have been reported in multiple bacterial species recovered from livestock in
72 Europe and Asia. In France, *Acinetobacter* spp. cultured from dairy cattle rectal swabs harbored *bla*_{OXA-23}
73 (10). *Salmonella* spp. and *Escherichia coli* isolates from two German swine farms and a poultry farm
74 were found to carry *bla*_{VIM-1} (11, 12). Lung samples from diseased pigs in China were reported to have *E.*
75 *coli*, *A. baumannii*, and *A. calcoeticus* isolates producing *bla*_{NDM-1}-mediated carbapenem resistance (13).
76 *Pseudomonas aeruginosa* producing *bla*_{VIM-2} and *A. baumannii* with *bla*_{OXA-23} and *bla*_{OXA-58} have been
77 reported in cattle, swine, and poultry in Lebanon (14). This report documents the dissemination of CRE
78 in the environment of a single swine farrow-to-finish operation in the US, including its observed
79 relationship with ceftiofur use on the farm.

80

81 **Materials and methods**

82 Sampling was conducted at a single swine farrow-to-finish operation in the US that followed typical US
83 production practices. Sterile gauze, electrostatic clothes, fecal swabs and fecal samples were collected
84 and transported at ambient temperature from the farrowing, nursery, and finishing barns during four
85 visits, July, August, October, and November of 2015. On the initial and second visit, environmental and
86 fecal samples were collected from floors and upright swine-contact surfaces in the farrowing and
87 nursery barns using sterile gauze sponges. Electrostatic clothes were used to collect environmental
88 samples in the human contact areas of the barns such as door knobs and break rooms. On the third visit,
89 50 rectal swabs were collected from piglets and 24 environmental electrostatic clothes were collected
90 from surfaces in both the farrowing and nursery barns. On the fourth visit, 72 fresh fecal samples and

91 36 electrostatic cloth samples were collected from harvest-ready pigs and the environment of a single
92 finishing barn in the same production flow.
93
94 In the laboratory, sterile gauze and electrostatic cloth samples were added to buffered peptone water
95 (BPW) in volumes of 36 ml and 90 ml, respectively. After incubation, 1 ml of each was inoculated to
96 nutrient broth modified with 2 µg/ml cefotaxime. After overnight incubation, samples were streaked to
97 MacConkey agar modified with 1 µg/ml meropenem (initial visit) or 0.5 µg/ml meropenem and 70 µg/ml
98 zinc sulfate heptahydrate (2nd, 3rd, and 4th visits) to identify isolates with the CRE phenotype. Rectal
99 swabs were added to 9 ml MacConkey broth supplemented with 2 µg/ml cefotaxime. Fecal samples
100 were reduced to 4 g and homogenized with MacConkey/cefotaxime broth. Rectal swab and homogenate
101 fecal samples were streaked to MacConkey agar containing 0.5 µg/ml meropenem and 70 µg/ml zinc
102 sulfate heptahydrate to identify isolates with a CRE phenotype. All samples were incubated overnight at
103 37°C.
104
105 For resulting isolates with reduced susceptibility to meropenem, bacterial speciation was accomplished
106 using biochemical assays – Indole, Methyl Red- Voges-Proskauer, Simmon’s citrate, motility – with
107 ambiguous species identified using MALDI-TOF mass spectrometry. Isolates were tested for
108 carbapenemase production using Carba NP (15), with Carba NP positive isolates assessed for genotype
109 using previously reported PCR assays and Sanger sequencing of PCR products to identify possible
110 carbapenemase genes including *bla*_{KPC}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM}, and *bla*_{OXA} (16-19). Specific *bla*_{IMP-27} forward
111 (5’ CGCAGGTGAGACTTTGCCTA) and reverse (3’ GCTTAACAAAGCAACGCCA) primers were designed
112 with NCBI Primer-BLAST (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) from sequence results of
113 the product from PCR reactions using IMP-1 primers (16). The plasmid content and plasmid size carried
114 by each isolate was visualized by electrophoresis using a standard plasmid profiling procedure (20).

115 Plasmid incompatibility groups were codified according to a plasmid PCR-based replicon typing
116 procedure (PBRT) detecting 18 replicon types based on incompatibility group loci (21-23). Susceptibility
117 profiles were generated using a semiautomated broth microdilution system (NARMS CMV3AGNF and
118 ESBL ESB1F panels, (Thermo Fisher Scientific, Oakwood Village, OH) following Clinical and Laboratory
119 Standards Institute (CLSI) guidelines (24).

120

121 Plasmid content of a subset of the isolates was more fully characterized using whole genome
122 sequencing (WGS, Illumina MiSeq; San Diego, CA). Plasmids were assembled from WGS data using
123 plasmidSPAdes (25) following trimming for adapters and quality (26) with visualization of plasmid de
124 Bruijn graphs in Bandage (27). Preliminary annotation of plasmids was performed for antimicrobial
125 resistance genes using the Comprehensive Antibiotic Resistance Database (CARD, (28)), and for other
126 functional elements using NCBI Conserved Domain Database (CDD, (29)) and European Bioinformatics
127 Institute Database (EMBL-EBI, (30)), and insertion sequences using the ISfinder database (31). Plasmid
128 incompatibility PCR results were confirmed with sequence data using PlasmidFinder 1.3 (32).

129

130 Results

131 This operation farrows approximately 1500 sows in one farrowing barn with 11 rooms containing 16 to
132 24 individual-sow farrowing crates in each room. All piglets in the farrowing barn routinely receive a
133 prophylactic ceftiofur (ceftiofur crystalline free acid, Excede™, Zoetis, Florham Park NJ) treatment at 0-1
134 d of age, and males receive a second prophylactic ceftiofur treatment when they are castrated at 5-7 d
135 of age. Sows in the farrowing barn receive therapeutic ceftiofur (ceftiofur hydrochloride, Excenel™,
136 Zoetis) as needed for treatment of metritis and other bacterial infections. Piglets are weaned at 21 days
137 of age into 1 of 2 enclosed nursery barns located at a single site. The nursery barns have 12 rooms each
138 with 8 pens per room and approximately 25 piglets are housed in each pen until 10 weeks of age. From

139 the nursery, pigs are moved to finishing barns where they are housed until approximately 6 months of
140 age when they are sold for harvest. In this production system, piglets do not normally receive ceftiofur
141 in the nursery or finishing barns. In addition to these typical swine production and marketing practices,
142 this operation also markets some individual piglets at approximately 10 weeks of age for youth 4-H and
143 FFA livestock projects as well as some older animals sold as breeding stock. This operation has been
144 managed as a closed herd since the 1960's.

145

146 As part of another project, our initial sampling at the farm included 30 environmental gauze samples of
147 animal contact surfaces, with 15 collected from both the farrowing room crates and nursery barn pens,
148 and 10 human-contact electrostatic cloth samples. These samples yielded 3 isolates from 2 animal-
149 environment samples (7%) expressing the CRE phenotype from the upright pen surface and floor gauze
150 sponge samples collected in room A of the nursery barn (Table 1). One gauze sponge sample taken from
151 the floor of a nursery pen harbored two carbapenemase producing isolates, an *E. coli* and a *Proteus*
152 *mirabilis*, both of which carried the metallo- β -lactamase gene, *bla*_{IMP-27} on an IncQ1 plasmid. The third
153 isolate was also an IMP-27-bearing *E. coli* recovered from a nursery pen gate using a gauze sponge. Both
154 *E. coli* isolates, MLST type 218 (MLST 1.8, (33)), were resistant to multiple antimicrobial classes and
155 carried multiple incompatibility group plasmids. In addition to *bla*_{IMP-27}, these *E. coli* both carried the
156 AmpC *bla*_{CMY-2}. The *P. mirabilis* carried only a single IncQ1 plasmid, similar to the *E. coli* isolates,
157 suggesting that the plasmid may have been transferred *in vitro* among the organisms during our
158 selective enrichment.

159

160 To gain a better understanding of the prevalence of this rare genotype, we sampled the same nursery
161 barn again, including the same pens of piglets, approximately one month later in August of 2015. We
162 collected 15 sterile gauze sponges from floor and upright surfaces and 4 electrostatic clothes from

163 human-contact surfaces in the 2 nursery rooms previously sampled on our initial visit. Additionally, we
164 collected a total of 54 fecal samples, with 4 to 5 convenience samples collected from random pens in
165 each of the 12 rooms. To optimize our recovery of these metallo- β -lactamase-bearing
166 *Enterbacteriaceae*, we reduced the meropenem concentration from 1 $\mu\text{g}/\text{ml}$ to 0.5 $\mu\text{g}/\text{ml}$ and included
167 70 $\mu\text{g}/\text{ml}$ zinc sulfate heptahydrate to our MacConkey agar media. However, these samples did not
168 produce isolates expressing the CRE phenotype.
169
170 We did not follow the same cohort of piglets as previously sampled for visit three, but rather re-sampled
171 the nursery and farrowing barns, focusing on the most recently weaned pens of nursery piglets and
172 crates of piglets in the farrowing barn which had received ceftiofur selection pressure within the past 7
173 to 10 days. At this visit in October of 2015, 12 environmental samples were collected using electrostatic
174 clothes from floor and upright surfaces in 2 farrowing rooms and 2 nursery rooms, the same nursery
175 rooms sampled in July and August. A convenience sample of 100 rectal swabs were also collected from
176 25 piglets in each of the 4 rooms. We recovered 15 IMP-27-bearing isolates of multiple bacterial species
177 from both farrowing room environments with multiple morphologies recovered from samples in both
178 rooms (Table 1). In 1 farrowing room (room A), 5 environmental samples (42%) produced isolates
179 harboring *bla*_{IMP-27}, and 7 samples (58%) were positive from the second farrowing room (room B). With
180 the exception of the exhaust fan vent covers, all carbapenemase-positive isolates were from pig-contact
181 surfaces – farrowing crate bars, side panels, floor mats, and sow feeders (Table 1). We did not recover
182 isolates expressing the CRE phenotype from any environmental samples in the nursery barn or piglet
183 rectal swabs collected in either barn. No isolates were recovered from the human-contact door knobs or
184 feed scoop handles.
185

186 The following month, we collected 72 fresh fecal samples from market-ready finishing pigs from the
187 same pig flow, along with 36 samples of the finishing barn environment using electrostatic cloths.
188 Sampled pigs were housed in a three-room finishing barn in close proximity to the nursery barn. In each
189 room, 2 fresh fecal samples were collected from each of 12 pens with care taken to avoid sampling the
190 same animal more than once. Environmental samples included pen gates, feeders, alley and pen floors,
191 window ledges, and door knobs. No isolates with reduced susceptibility to carbapenems were recovered
192 from these 108 samples.

193

194 Of the 18 IMP-27-bearing isolates from environmental samples collected on our initial and third farm
195 visit, all carried an IncQ1 plasmid of approximately 10 Kb as confirmed by plasmid profiling and replicon
196 typing. To confirm the location of IMP-27 on the IncQ1 plasmid, plasmid DNA was extracted (QIAfilter
197 plasmid midi kit, Qiagen, Hilden, Germany) from the *P. mirabilis* isolate 13-19B, which carried only the
198 IncQ1, and transformed (Electroporator 2510, Eppendorf, Hamburg, Germany) to an electrocompetant
199 *E. coli* strain (MegaX DH10B, Invitrogen, Carlsbad, CA). Confirmation of the IMP-27 gene in the resulting
200 transformants was accomplished using conventional PCR with IMP-27 specific primers. Individual
201 replicon type PCR reactions revealed carriage of additional self-transmissible helper plasmid replicons
202 including: IncP, IncF, IncI, IncX, and IncW, by 13 of these isolates (Table 1) (21). While the presence of
203 the IncQ1 plasmids in multiple bacterial host backgrounds strongly suggests that they are mobilizable,
204 conjugation experiments using the *E. coli* ST 218 (Isolate 13-19A) or ST 101 (Isolate S23) donors and an *E.*
205 *coli* K-12 MG 1655 recipient *in vitro* using broth or filter mating methodologies were unsuccessful (34,
206 35). No helper plasmids were detected in the remaining 5 isolates, suggesting an inability of those
207 isolates to successfully mobilize the IncQ1 plasmid.

208

209 Each isolate expressed reduced susceptibility or resistance to meropenem while minimum inhibitory
210 concentrations (MIC) for imipenem ranged from ≤ 0.5 to $4 \mu\text{g/ml}$. Most isolates showed reduced
211 susceptibility to 1st, 2nd, and 3rd generation cephalosporins, sulfonamides, and tetracyclines, but were
212 susceptible to aminoglycosides and fluoroquinolones. Resistance to cefepime and ceftazidime was
213 inconsistent (Table 2). WGS identified additional antimicrobial resistance genes located on the IncQ1
214 plasmid including *sul2*, *sat-1*, and *aph(3')*-Ia. All functional alleles located on the IncQ1 plasmid are
215 presented in Figure 1.

216

217 Discussion

218 Carbapenem-resistant *Enterobacteriaceae* harboring plasmid-borne carbapenemase genes have not
219 previously been reported in US livestock populations. Although not detected in sampled piglets,
220 environmental samples from the swine farrowing and nursery barns at this farm yielded multiple
221 bacterial species expressing carbapenem resistance, each isolate carried the metallo β -lactamase gene
222 *bla*_{IMP-27} located on an IncQ1 plasmid. Unlike *bla*_{KPC}, which has become endemic in human healthcare in
223 some parts of the US (36), IMP variants have been infrequently reported in North America. Originally
224 identified in 1988 in a *Pseudomonas aeruginosa* isolate collected in Japan and in *Enterobacteriaceae*
225 collected in a Japanese hospital 5 years later, IMP variants are now the most prevalent transmissible
226 carbapenemase genes in Japan and found in multiple species of gram-negative bacteria internationally
227 (37). In the US, the first occurrence of the IMP gene was reported in a *P. aeruginosa* isolate recovered
228 from a tracheal aspirate of a trauma patient in the southwestern US in 2006 (38). The first detection of
229 IMP-producing *Enterobacteriaceae* strains was reported in *Klebsiella pneumoniae* isolates collected from
230 urine samples of three infants in the pediatric intensive care unit of a single health care facility (39).
231 These closely related isolates each carried an IMP-4 gene harbored on a common transferrable plasmid
232 of approximately 100 Kb. While the early detection of metallo β -lactamases in the US was often

233 associated with a history of international travel, these pediatric patients had no travel history and, in
234 fact, one patient had never been outside the hospital setting (39).
235
236 The IMP-27 gene is rare even in the realm of the metallo β -lactamases in North America. *bla*_{IMP-27} has
237 only been reported twice previously from human cases. The first, reported in 2011, described the
238 recovery of a *Proteus mirabilis* harboring *bla*_{IMP-27} which was cultured from a patient in Iowa (40). The
239 second report, from 2012 in Toronto, described the recovery of another *P. mirabilis* harboring *bla*_{IMP-27}
240 from a urine culture of a patient with no history of international travel (41). *bla*_{IMP-27} differs from the first
241 reported IMP-1 by 50 amino acid substitutions (42) and from its closest relative, IMP-8 by 31 amino acid
242 substitutions (40).
243
244 We detected isolates carrying *bla*_{IMP-27} in multiple bacterial species. The dissemination of this resistance
245 determinant across a broad host range can likely be attributed to the highly mobilizable nature of the
246 IncQ1 plasmid harboring this gene. IncQ plasmids have the broadest host range of any known replicating
247 elements in bacteria and have been found in gram-negative, gram-positive, and cyanobacteria (43).
248 These small (5.1 – 14.0 kb) plasmids replicate host-independently, allowing for IncQ to be found in high
249 copy-numbers (44). While IncQ plasmids are not self-transmissible, they can be mobilized at high
250 frequency by a variety of type IV transporters provided by larger, self-transmissible, co-resident helper
251 plasmids from incompatibility groups including: IncP, IncF, IncI, IncM, IncX, IncN, and IncW (45). IncQ's
252 combination of high copy-number, broad host range, and ease of mobilization make this plasmid
253 extremely promiscuous, being found in a vast variety of environments (43). Our inability to conjugate
254 the IncQ plasmid to a recipient strain may have been hampered by our use of the IncQ-bearing strain
255 acting as both donor and helper plasmid. Triparental mating with donor, recipient, and helper strains
256 may help overcome any plasmid mobilization barriers.

257

258 While carbapenem antimicrobial drugs are not approved for use in food animals, other β -lactam
259 antimicrobials are formulated, labeled, and frequently applied in a variety of food animal species
260 worldwide including both ceftiofur and cefquinome extended-spectrum cephalosporin drugs. While the
261 exact relationship between cephalosporin use and carbapenem resistance has not yet been established,
262 use of these drugs may provide significant selection pressure favoring organisms expressing carbapenem
263 resistance because they will also be resistant to all extended-spectrum cephalosporins. However,
264 selection pressure favoring carbapenem-resistant strains provided by extended-spectrum cephalosporin
265 use has not been established. In the swine production system we sampled, all piglets receive ceftiofur at
266 0 to 1 day after birth, with males receiving a second dose of ceftiofur at castration (day 5 to 7). Our
267 observation that environmental recovery of isolates with *bla*_{IMP-27} was highest in the farrowing barn
268 where ceftiofur was frequently used, but much lower in the nursery and finishing barns where ceftiofur
269 is only used for the treatment of sick individual animals, is consistent with the hypothesis that ceftiofur
270 use in livestock can result in the expansion of bacterial strains harboring mobile carbapenemase genes.

271

272 While we initially detected 3 *bla*_{IMP-27}-bearing *Enterobacteriaceae* from the nursery barn environment
273 and later readily recovered this genotype from the farrowing barn environment, we did not recover
274 IMP-27 from pig fecal swabs or fecal samples collected on visits 2, 3, and 4. The fecal samples or swabs
275 collected at visits 2 and 3 were taken in both the farrowing and nursery from piglets ranging in age from
276 8 to 16 days in farrowing and 4 to 10 weeks in the nursery. Given our frequency of recovery of isolates
277 harboring *bla*_{IMP-27} in the farrowing barn environment, we expected to recover similar isolates from fecal
278 swabs of piglets in the same barn recently treated with ceftiofur. Our inability to detect those isolates
279 suggests that the small mass of feces that can be collected from a piglet may not be a sensitive sampling
280 method to detect a rare bacterial genotype in the fecal flora, even with selective enrichment. However,

281 we have since recovered fecal isolates from sows and piglets in the farrowing barn harboring *bla*_{IMP-27} as
282 part of a second study (data not shown).

283

284 We sampled harvest-ready pigs in a single finisher barn on visit 4 and were not able to recover isolates
285 harboring *bla*_{IMP-27}. This result suggests that enteric bacteria harboring *bla*_{IMP-27} are unlikely to have
286 entered the food supply through contamination of fresh pork products. The absence of ceftiofur use in
287 the nursery and finisher barns likely removed antimicrobial selection pressure on the enteric flora of the
288 pigs, resulting in the loss of the ecological niche allowing the maintenance of *bla*_{IMP-27}-bearing
289 *Enterobacteriaceae* in the farrowing barn.

290

291 Carbapenem-resistant and carbapenemase-producing bacteria have previously been reported from
292 feces of dairy cattle in the US (46). They reported *Enterobacteriaceae*, *Aeromonas* spp., and
293 *Pseudomonas* spp. with chromosomal elements conferring carbapenem resistance or reduced
294 susceptibility. Chromosomally-mediated resistance is vertically transmitted to daughter cells, and these
295 bacteria can be clinically relevant if they produce invasive infections requiring antimicrobial therapy.
296 Bacterial carbapenemase genes located on mobile plasmids, reported here, pose a far greater health
297 threat because they may be transmitted horizontally among commensal bacterial and potential
298 pathogens (47). The implication of our finding is that there is a real risk that CRE may disseminate in
299 food animal populations and eventually contaminate fresh retail meat products. Food-borne
300 transmission may then produce a reservoir of mobile carbapenemase genes in the enteric flora of
301 consumers.

302

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445 **Table and Figure Legends**

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447 Table 1. Conjugative plasmid content of 18 environmental isolates harboring *bla*_{IMP-27} on an IncQ1
448 plasmid recovered from the nursery and farrowing barns of a single swine production system

449

450 Table 2. Minimum inhibitory concentration of 24 antimicrobials^a for 18 *bla*_{IMP}-harboring environmental
451 isolates recovered from the nursery and farrowing barns of a single swine production system^b

452

453 Figure 1. Map of functional genes and truncated open reading frames (*) on an IncQ1 plasmid present in
454 multiple bacterial species isolated from the environment of a piglet nursery barn at a US swine
455 operation depicting replication (rep), mobilization (mob), integration, and antibiotic resistance genes

456

Table 1. Conjugative plasmid content of 18 environmental isolates harboring *bla*_{IMP-27} on an IncQ1 plasmid recovered from the nursery and farrowing barns of a single swine production system

Isolate	Recovery Date	Location	Barn	Sample type	Species	Conjugative plasmid content
13-19A	7/25/2015	Floor	Nursery Rm A	Gauze sponge	<i>Escherichia coli</i> ^a	IncX, Inc1, IncF
13-19B	7/25/2016	Floor	Nursery Rm A	Gauze sponge	<i>Proteus mirabilis</i>	
13-28A	7/25/2017	Pen gate	Nursery Rm A	Gauze sponge	<i>Escherichia coli</i> ^a	IncX, Inc1, IncF
S4 - A	10/2/2015	Crate floor mats	Farrowing Rm A	Electrostatic cloth	<i>Morganella morganii</i>	
S4 - B	10/2/2015	Crate floor mats	Farrowing Rm A	Electrostatic cloth	<i>Providencia rettgeri</i>	
S5 - A	10/2/2015	Sow feeders	Farrowing Rm A	Electrostatic cloth	<i>Proteus vulgaris</i>	IncP
S8 - A	10/2/2015	Crate bars	Farrowing Rm A	Electrostatic cloth	<i>Enterobacter cancerogenus</i>	IncP
S8 - B	10/2/2015	Crate bars	Farrowing Rm A	Electrostatic cloth	<i>Citrobacter braakii</i>	IncP, IncW
S11	10/2/2015	Exhaust vent cover	Farrowing Rm A	Electrostatic cloth	<i>Enterobacter cloacae</i>	IncP
S13 - A	10/2/2015	Crate dividers	Farrowing Rm B	Electrostatic cloth	<i>Citrobacter sp.</i>	IncP, Inc1
S13 - B	10/2/2015	Crate dividers	Farrowing Rm B	Electrostatic cloth	<i>Enterobacter cancerogenus</i>	IncP
S14	10/2/2015	Crate dividers	Farrowing Rm B	Electrostatic cloth	<i>Citrobacter farmeri</i>	IncP
S15 - A	10/2/2015	Crate floor mats	Farrowing Rm B	Electrostatic cloth	<i>Citrobacter koseri</i>	IncP
S15 - B	10/2/2015	Crate floor mats	Farrowing Rm B	Electrostatic cloth	<i>Morganella morganii</i>	
S17	10/2/2015	Sow feeders	Farrowing Rm B	Electrostatic cloth	<i>Citrobacter farmeri</i>	IncP
S18	10/2/2015	Sow feeders	Farrowing Rm B	Electrostatic cloth	<i>Klebsiella oxytoca</i>	
S19	10/2/2015	Crate bars	Farrowing Rm B	Electrostatic cloth	<i>Citrobacter koseri</i>	IncP
S23	10/2/2015	Exhaust vent cover	Farrowing Rm B	Electrostatic cloth	<i>Escherichia coli</i> ^a	IncX, Inc1, IncF, IncW

^a *Escherichia coli* isolates from the nursery barn floor and pen gate are sequence type 218 while the *E. coli* from the farrowing room exhaust fan is sequence type 101.

Table 2. Minimum inhibitory concentration of 24 antimicrobials^a for 18 *bla*_{IMP}-harboring environmental isolates recovered from the nursery and farrowing barns of a single swine production system^b

Isolate	AMC	AMP	AZM	CFZ	FEP	CTX	FOX	CPD	CAZ	CTF	CRO	CHL	CIP	GEN	IPM	MEM	NAL	TZP	STR	SFZ	TXC	TZC	TET	SXT
13-19A	>32	>32	8	>16	16	64	>64	>32	128	>8	>64	8	≤1	>16	≤0.5	4	4	8	>64	>256	64	128	≤4	0.25
13-19B	16	>32	>16	>16	>16	>64	>64	>32	8	>8	>64	4	≤1	≤4	4	8	4	≤4	8	>256	64	8	32	>4
13-28A	>32	>32	8	>16	16	64	>64	>32	128	>8	>64	8	≤1	>16	≤0.5	4	4	8	>64	>256	64	128	≤4	0.25
S4 - A	>32	>32	>16	>16	>16	>64	>64	>32	8	>8	>64	16	≤1	≤4	4	4	4	≤4	>64	>256	>64	16	>32	0.5
S4 - B	4	≤8	>16	>16	2	16	>64	16	8	>8	16	16	≤1	4	4	8	2	≤4	8	>256	16	4	32	1
S5 - A	8	>32	>16	>16	>16	>64	>64	>32	32	>8	>64	4	≤1	≤4	4	8	4	≤4	8	>256	>64	32	>32	>4
S8 - A	8	8	8	>16	>16	>64	>64	>32	128	>8	>64	16	≤1	≤4	≤0.5	8	4	≤4	≤2	>256	64	64	>32	0.5
S8 - B	>32	>32	8	>16	16	>64	>64	>32	64	>8	>64	8	≤1	≤4	1	8	4	≤4	>64	>256	>64	32	>32	≤0.12
S11	>32	>32	>16	>16	8	64	>64	>32	32	>8	>64	8	≤1	≤4	≤0.5	4	4	≤4	64	>256	64	32	>32	>4
S13 - A	>32	>32	8	>16	>16	>64	>64	>32	>128	>8	>64	8	≤1	≤4	≤0.5	8	4	64	≤2	>256	>64	>128	>32	0.5
S13 - B	8	16	8	>16	>16	>64	>64	>32	128	>8	>64	16	≤1	≤4	≤0.5	8	4	≤4	≤2	>256	>64	128	>32	0.5
S14	8	8	8	>16	>16	>64	>64	>32	64	>8	>64	16	≤1	≤4	≤0.5	8	4	≤4	≤2	>256	64	64	>32	0.25
S15 - A	16	8	8	>16	16	>64	>64	>32	64	>8	>64	16	≤1	≤4	≤0.5	8	4	≤4	≤2	>256	64	64	>32	0.5
S15 - B	>32	>32	>16	>16	>16	>64	>64	>32	16	>8	>64	32	≤1	≤4	4	4	4	≤4	16	>256	>64	16	>32	0.5
S17	8	16	8	>16	>16	>64	>64	>32	64	>8	>64	16	≤1	≤4	1	8	4	≤4	≤2	>256	64	64	>32	0.5
S18	8	32	8	>16	4	32	>64	>32	32	>8	64	4	≤1	≤4	≤0.5	2	1	≤4	>64	>256	32	32	>32	≤0.12
S19	16	16	8	>16	>16	>64	>64	>32	64	>8	>64	16	≤1	≤4	≤0.5	8	4	≤4	≤2	>256	64	64	>32	0.5
S23	>32	>32	2	>16	>16	>64	>64	>32	128	>8	>64	4	≤1	≤4	≤0.5	8	2	≤4	8	>256	>64	64	>32	≤0.12

^a Antimicrobials tested and resistant MIC cut-off value (R): AMC (Amoxicillin/clavulanic acid 2:1 ratio) R: ≥ 32, AMP (Ampicillin) R: ≥ 32, AZM (Azithromycin) R: >16, CFZ (Cefazolin) R: ≥ 8, FEP (Cefepime) R: ≥ 32, CTX (Cefotaxime) R: ≥ 4, FOX (Cefoxitin) R: ≥ 32, CPD (Cefpodoxime) R: ≥ 8, CAZ (Ceftazidime) R: ≥ 16, CTF (Ceftiofur) R: ≥ 8, CRO (Ceftriaxone) R: ≥ 4, CHL (Chloramphenicol) R: ≥ 32, CIP (Ciprofloxacin) R: ≥ 4, GEN (Gentamicin) R: ≥ 16, IPM (Imipenem) R: ≥ 4, MEM (Meropenem) R: ≥ 4, NAL (Nalidixic acid) R: ≥ 32, TZP (Piperacillin/tazobactam) R: ≥ 128/4, STR (Streptomycin) R: ≥ 64, SFZ (Sulfisoxazole) R: >256, TXC (Cefotaxime-Clavulanic Acid), TZC (Ceftazidime-Clavulanic Acid), TET (Tetracycline) R: ≥ 16, SXT (Trimethoprim/sulfamethoxazole) R: ≥ 4/76

^b Resistance indicated in bold text

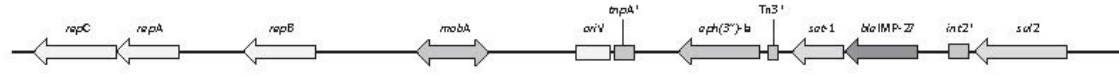


Figure 1. Map of functional genes and truncated open reading frames (*) on an IncQ1 plasmid present in multiple bacterial species isolated from the environment of a piglet nursery barn at a US swine operation depicting replication (rep), mobilization (mob), integration, and antibiotic resistance genes.