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Carbapenemase-Producing *Enterobacteriaceae* Recovered from the Environment of a Swine Farrow-to-Finish Operation in the United States

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

KEYWORDS carbapenemase-producing *Enterobacteriaceae*, IMP-27, livestock, plasmid-mediated resistance

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The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) has been described as heralding the end of the antibiotic era (1), with their global expansion presenting an urgent threat to public health (2). These potential pathogens can harbor highly mobile genes that confer resistance to the most critically important, live-saving antimicrobial drugs. The plasmid-mediated class A (KPC), class B (NDM, IMP, VIM), and class D (OXA-48, OXA-181) carbapenemase genes have disseminated beyond the realm of hospitals, nursing homes, and other human health care settings to now cause critical community-acquired infections (3). Often by acquiring mobile resistance elements through horizontal gene transfer, CRE infections are especially threatening because they approach pan-resistance, frequently delaying and greatly reducing successful therapeutic treatment options for invasive infections. These bacteria harboring mobile

carbapenemase genes are now identified with some regularity from both hospital- and community-acquired human infections (4) and have been recovered from health care environments (5), waste and surface water flows, soil, and companion animals (6, 7).

While they are considered the "last line of defense" drugs in human medicine, carbapenem antimicrobials are not approved for use in food animal veterinary medicine. However, other β-lactams are commonly used in almost all food animal species worldwide, including ceftiofur and cefquinome extended-spectrum cephalosporin drugs. While the exact relationship between extended-spectrum cephalosporin use and carbapenem resistance has not yet been established, use of these drugs likely provides significant selection pressure favoring organisms expressing carbapenem resistance, because they will also be resistant to all extended-spectrum cephalosporins. While most people today do not have direct livestock exposure, enteric flora from livestock commonly contaminate fresh retail meat products that are distributed over wide geographic areas (8, 9). Thus, if CRE are present in food animal populations, a large number of consumers may be exposed through the food chain, resulting in a critically important emerging food safety issue.

While bacteria harboring plasmid-borne carbapenemase genes have never been recovered from livestock in the United States, CRE have been reported in multiple bacterial species recovered from livestock in Europe and Asia. In France, *Acinetobacter* spp. cultured from dairy cattle rectal swabs harbored $bla_{\text{OXA-23}}$ (10). *Salmonella* spp. and *Escherichia coli* isolates from two German swine farms and a poultry farm were found to carry $bla_{\text{VIM-1}}$ (11, 12). Lung samples from diseased pigs in China were reported to have *E. coli*, *Acinetobacter baumannii*, and *Acinetobacter calcoaeticus* isolates producing $bla_{\text{NDM-1}}$-mediated carbapenem resistance (13). *Pseudomonas aeruginosa* producing $bla_{\text{VIM-2}}$ and *A. baumannii* with $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$ have been reported in cattle, swine, and poultry in Lebanon (14). This report documents the dissemination of CRE in the environment of a single swine farrow-to-finish operation in the United States, including its observed relationship with ceftiofur use on the farm.

RESULTS

The operation in our study farrows included approximately 1,500 sows in one farrowing barn, with 11 rooms containing 16 to 24 individual-sow farrowing crates in each room. All piglets in the farrowing barn routinely receive prophylactic ceftiofur (ceftiofur crystalline free acid; Excede; Zoetis, Florham Park, NJ) treatment at 0 to 1 day of age, and males receive a second prophylactic ceftiofur treatment when they are castrated at 5 to 7 days of age. Sows in the farrowing barn receive therapeutic ceftiofur (ceftiofur hydrochloride; Excenel; Zoetis) as needed for treatment of metritis and other bacterial infections. Piglets are weaned at 21 days of age into 1 of 2 enclosed nursery barns located at a single site. The nursery barns have 12 rooms each with 8 pens per room, and approximately 25 piglets are housed in each pen until they are 10 weeks of age. From the nursery, pigs are moved to finishing barns, where they are housed until approximately 6 months of age, at which time they are sold for harvest. In this production system, piglets do not normally receive ceftiofur in the nursery or finishing barns. In addition to these typical swine production and marketing practices, this operation also markets some individual piglets at approximately 10 weeks of age for youth 4-H and FFA livestock projects, and some older animals are sold as breeding stock. This operation has been managed as a closed herd since the 1960s.

As part of another project, our initial sampling at the farm included 30 environmental gauze samples of animal contact surfaces, with 15 collected from both the farrowing room crates and nursery barn pens and 10 human contact electrostatic cloth samples. These samples yielded 3 isolates from 2 animal environment samples (7%) expressing the CRE phenotype from the upright pen surface and floor gauze sponge samples collected in room A of the nursery barn (Table 1). One gauze sponge sample taken from the floor of a nursery pen harbored two carbapenemase-producing isolates, an *E. coli* and a *Proteus mirabilis* isolate, both of which carried the metallo-β-lactamase gene $bla_{\text{IMP-27}}$ on an IncQ1 plasmid. The third isolate was also an IMP-27-bearing *E. coli*

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 room A	Gauze sponge	*Escherichia coli*[a]	IncX, IncI1, IncF
13-19B	7/25/2016	Floor	Nursery room A	Gauze sponge	*Proteus mirabilis*	
13-28A	7/25/2017	Pen gate	Nursery room A	Gauze sponge	*Escherichia coli*[a]	IncX, IncI1, IncF
S4-A	10/2/2015	Crate floor mats	Farrowing room A	Electrostatic cloth	*Morganella morganii*	
S4-B	10/2/2015	Crate floor mats	Farrowing room A	Electrostatic cloth	*Providencia rettgeri*	
S5-A	10/2/2015	Sow feeders	Farrowing room A	Electrostatic cloth	*Proteus vulgaris*	IncP
S8-A	10/2/2015	Crate bars	Farrowing room A	Electrostatic cloth	*Enterobacter cancerogenus*	IncP
S8-B	10/2/2015	Crate bars	Farrowing room A	Electrostatic cloth	*Citrobacter braakii*	IncP, IncW
S11	10/2/2015	Exhaust vent cover	Farrowing Rm A	Electrostatic cloth	*Enterobacter cloacae*	IncP
S13-A	10/2/2015	Crate dividers	Farrowing room B	Electrostatic cloth	*Citrobacter* sp.	IncP, IncI1
S13-B	10/2/2015	Crate dividers	Farrowing room B	Electrostatic cloth	*Enterobacter cancerogenus*	IncP
S14	10/2/2015	Crate dividers	Farrowing room B	Electrostatic cloth	*Citrobacter farmeri*	IncP
S15-A	10/2/2015	Crate floor mats	Farrowing room B	Electrostatic cloth	*Citrobacter koseri*	IncP
S15-B	10/2/2015	Crate floor mats	Farrowing room B	Electrostatic cloth	*Morganella morganii*	
S17	10/2/2015	Sow feeders	Farrowing room B	Electrostatic cloth	*Citrobacter farmeri*	IncP
S18	10/2/2015	Sow feeders	Farrowing room B	Electrostatic cloth	*Klebsiella oxytoca*	
S19	10/2/2015	Crate bars	Farrowing room B	Electrostatic cloth	*Citrobacter koseri*	IncP
S23	10/2/2015	Exhaust vent cover	Farrowing room B	Electrostatic cloth	*Escherichia coli*[a]	IncX, IncI1, IncF, IncW

[a]*Escherichia coli* isolates from the nursery barn floor and pen gate were sequence type 218, while the *E. coli* isolate from the farrowing room exhaust fan was sequence type 101.

isolate recovered using a gauze sponge from a nursery pen gate. Both *E. coli* isolates were multilocus sequence type 218 (MLST 218) (MLST 1.8 [15]) and were resistant to multiple antimicrobial classes; they carried multiple incompatibility group plasmids. In addition to bla_{IMP-27}, these *E. coli* isolates both carried AmpC bla_{CMY-2}. The *P. mirabilis* isolate carried only a single IncQ1 plasmid, similar to the *E. coli* isolates, suggesting that the plasmid may have been transferred *in vitro* among the organisms during our selective enrichment.

To gain a better understanding of the prevalence of this rare genotype, we sampled the same nursery barn again, including the same pens of piglets, approximately 1 month later in August 2015. We collected 15 sterile gauze sponges from floor and upright surfaces and 4 electrostatic clothes from human contact surfaces in the 2 nursery rooms previously sampled on our initial visit. Additionally, we collected a total of 54 fecal samples, with 4 to 5 convenience samples collected from random pens in each of the 12 rooms. To optimize our recovery of metallo-β-lactamase-bearing *Enterobacteriaceae*, we reduced the meropenem concentration from 1 μg/ml to 0.5 μg/ml and included 70 μg/ml zinc sulfate heptahydrate to our MacConkey agar medium. However, these samples did not produce isolates expressing the CRE phenotype.

We did not follow the same cohort of piglets as previously sampled for visit three, but rather we resampled the nursery and farrowing barns, focusing on the most recently weaned pens of nursery piglets and crates of piglets in the farrowing barn which had received ceftiofur selection pressure within the past 7 to 10 days. At this visit in October 2015, 12 environmental samples were collected using electrostatic cloths on floor and upright surfaces in 2 farrowing rooms and 2 nursery rooms, the same nursery rooms sampled in July and August. A convenience sample of 100 rectal swabs was also collected from 25 piglets in each of the 4 rooms. We recovered 15 IMP-27-bearing isolates of multiple bacterial species from both farrowing room environments with multiple morphologies recovered from samples in both rooms (Table 1). In 1 farrowing room (room A), 5 environmental samples (42%) produced isolates harboring bla_{IMP-27}, and 7 samples (58%) were positive from the second farrowing room (room B). With the exception of the exhaust fan vent covers, all carbapenemase-positive isolates were from pig contact surfaces: farrowing crate bars, side panels, floor mats, and sow feeders (Table 1). We did not recover isolates expressing the CRE phenotype from any environmental samples in the nursery barn or from piglet rectal swabs collected in either barn. No isolates were recovered from the human contact doorknobs or feed scoop handles.

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

Of the 18 IMP-27-bearing isolates from environmental samples collected on our initial and third farm visits, all carried an IncQ1 plasmid of approximately 10 kb, as confirmed by plasmid profiling and replicon typing. To confirm the location of IMP-27 on the IncQ1 plasmid, plasmid DNA was extracted (QIAfilter plasmid midi kit; Qiagen, Hilden, Germany) from the *P. mirabilis* isolate 13-19B, which carried only IncQ1, and transformed (Electroporator 2510; Eppendorf, Hamburg, Germany) to an electrocompetent *E. coli* strain (MegaX DH10B; Invitrogen, Carlsbad, CA). Confirmation of the IMP-27 gene in the resulting transformants was accomplished using conventional PCR with IMP-27-specific primers. Individual replicon-type PCRs revealed carriage of additional self-transmissible helper plasmid replicons, including IncP, IncF, IncI, IncX, and IncW, by 13 of these isolates (Table 1) (16). While the presence of the IncQ1 plasmids in multiple bacterial host backgrounds strongly suggests that they are mobilizable, conjugation experiments using the *E. coli* ST 218 (isolate 13-19A) or ST 101 (isolate S23) donors and an *E. coli* K-12 MG1655 recipient *in vitro* using broth or filter mating methodologies were unsuccessful (17, 18). No helper plasmids were detected in the remaining 5 isolates, suggesting an inability of those isolates to successfully mobilize the IncQ1 plasmid.

Each isolate expressed reduced susceptibility or resistance to meropenem, while MICs for imipenem ranged from $\leq$0.5 to 4 μg/ml. Most isolates showed reduced susceptibility to first-, second-, and third-generation cephalosporins, sulfonamides, and tetracyclines but were susceptible to aminoglycosides and fluoroquinolones. Resistance to cefepime and ceftazidime was inconsistent (Table 2). Whole-genome sequencing (WGS) identified additional antimicrobial resistance genes located on the IncQ1 plasmid, including *sul-2*, *sat-1*, and *aph*(3′)-*Ia*. All functional alleles located on the IncQ1 plasmid (GenBank accession no. KY126032) are presented in Fig. 1.

DISCUSSION

Carbapenem-resistant *Enterobacteriaceae* harboring plasmid-borne carbapenemase genes have not previously been reported in U.S. livestock populations. Although not detected in sampled piglets, environmental samples from the swine farrowing and nursery barns at this farm yielded multiple bacterial species expressing carbapenem resistance, each isolate carried the metallo-β-lactamase gene $bla_{\text{IMP-27}}$ located on an IncQ1 plasmid. Unlike bla_{KPC}, which has become endemic in human health care settings in some parts of the United States (19), IMP variants have been infrequently reported in North America. Originally identified in 1988 in a *Pseudomonas aeruginosa* isolate collected in Japan and in *Enterobacteriaceae* collected in a Japanese hospital 5 years later, IMP variants are now the most prevalent transmissible carbapenemase genes in Japan and are found in multiple species of Gram-negative bacteria internationally (20). In the United States, the first occurrence of the IMP gene was reported in a *P. aeruginosa* isolate recovered from a tracheal aspirate of a trauma patient in the southwestern United States in 2006 (21). The first detection of IMP-producing *Enterobacteriaceae* strains was reported in *Klebsiella pneumoniae* isolates collected from urine samples of three infants in the pediatric intensive care unit of a single health care facility (22). These closely related isolates each carried an IMP-4 gene harbored on a common transferrable plasmid of approximately 100 kb. While the early detection of metallo-β-lactamases in the United States is often associated with a history of international travel, these pediatric patients had no travel history and, in fact, one patient had never been outside the hospital setting (22).

TABLE 2 MICs of 24 antimicrobials for 18 *bla*$_{IMP}$-harboring environmental isolates recovered from nursery and farrowing barns of a single swine production system[a]

Isolate	MIC (μg/ml)																						
	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	≤	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 resistance MIC cutoff values (R, in micrograms per milliliter) when determined: 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. Isolate-antibiotic combinations that demonstrated resistance are shown in boldface.

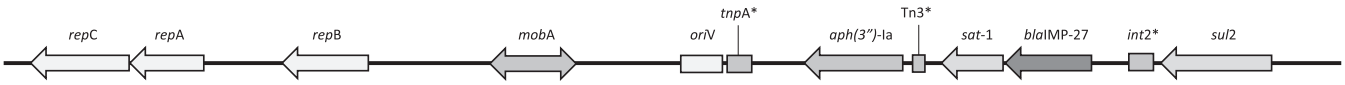

FIG 1 Map of functional genes and truncated open reading frames (*) on an IncQ1 plasmid (GenBank accession no. KY126032) present in multiple bacterial species isolated from the environment of a piglet nursery barn at a U.S. swine operation. The replication (rep), mobilization (mob), integration, and antibiotic resistance genes are depicted.

The IMP-27 gene is rare even in the realm of the metallo-β-lactamases in North America. $bla_{\text{IMP-27}}$ has only been reported from human cases three times previously. The first, reported in 2011, described the recovery of a *Proteus mirabilis* isolate harboring $bla_{\text{IMP-27}}$ which was cultured from a patient in Iowa (23). The second report, from 2012 in Toronto, described the recovery of another *P. mirabilis* isolate harboring $bla_{\text{IMP-27}}$ from a urine culture of a patient with no history of international travel (24). A third *P. mirabilis* isolate harboring $bla_{\text{IMP-27}}$ was recovered from a patient in the U.S. upper plains region in 2015 (48). $bla_{\text{IMP-27}}$ differs from the first reported IMP-1 by 50 amino acid substitutions (25) and from its closest relative, IMP-8, by 31 amino acid substitutions (23).

We detected isolates carrying $bla_{\text{IMP-27}}$ in multiple bacterial species. The dissemination of this resistance determinant across a broad host range can likely be attributed to the highly mobilizable nature of the IncQ1 plasmid harboring this gene. IncQ plasmids have the broadest host range of any known replicating elements in bacteria and have been found in Gram-negative and Gram-positive bacteria and cyanobacteria (26). These small (5.1 to 14.0 kb) plasmids replicate independently of their host, allowing for IncQ to be found in high copy numbers (27). While IncQ plasmids are not self-transmissible, they can be mobilized at high frequency by a variety of type IV transporters provided by larger, self-transmissible, coresident helper plasmids from incompatibility groups, including IncP, IncF, IncI, IncM, IncX, IncN, and IncW (28). IncQ's combination of high copy number, broad host range, and ease of mobilization makes this plasmid extremely promiscuous, and it is found in a vast variety of environments (26). Our inability to conjugate the IncQ plasmid to a recipient strain may have been hampered by our use of the IncQ-bearing strain acting as both donor and helper plasmid. Triparental mating with donor, recipient, and helper strains may help overcome any plasmid mobilization barriers.

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

While we initially detected 3 $bla_{\text{IMP-27}}$-bearing *Enterobacteriaceae* from the nursery barn environment and later readily recovered this genotype from the farrowing barn environment, we did not recover IMP-27 from pig fecal swabs or fecal samples collected on visits 2, 3, and 4. The fecal samples or swabs collected at visits 2 and 3 were taken in both the farrowing area and nursery from piglets ranging in age from 8 to 16 days in the farrowing area and 4 to 10 weeks in the nursery. Given our frequency of recovery of isolates harboring $bla_{\text{IMP-27}}$ in the farrowing barn environment, we expected to

recover similar isolates from fecal swabs of piglets in the same barn and recently treated with ceftiofur. Our inability to detect those isolates suggests that the small mass of feces that can be collected from a piglet may not be a sensitive sampling method to detect a rare bacterial genotype in the fecal flora, even with selective enrichment. However, as part of a second study, we have since recovered fecal isolates from sows and piglets in the farrowing barn that harbor bla_{IMP-27} (data not shown).

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

Carbapenem-resistant and carbapenemase-producing bacteria have previously been reported from feces of dairy cattle in the United States (29). The bacteria reported included *Enterobacteriaceae*, *Aeromonas* spp., and *Pseudomonas* spp. with chromosomal elements conferring carbapenem resistance or reduced susceptibility. Chromosomally mediated resistance is vertically transmitted to daughter cells, and these bacteria can be clinically relevant if they produce invasive infections requiring antimicrobial therapy. Bacterial carbapenemase genes located on mobile plasmids, as reported here, pose a far greater health threat because they may be transmitted horizontally among commensal bacterial and potential pathogens (30). The implication of our finding is that there is a real risk that CRE may disseminate in food animal populations and eventually contaminate fresh retail meat products. Foodborne transmission may then produce a reservoir of mobile carbapenemase genes in the enteric flora of consumers.

MATERIALS AND METHODS

Sampling was conducted at a single swine farrow-to-finish operation in the United States that followed typical U.S. production practices. Sterile gauze, electrostatic cloths, fecal swabs, and fecal samples were collected and transported at ambient temperature from the farrowing, nursery, and finishing barns during four visits, in July, August, October, and November 2015. On the initial and second visits, environmental and fecal samples were collected from floors and upright swine contact surfaces in the farrowing and nursery barns by using sterile gauze sponges. Electrostatic cloths were used to collect environmental samples in the human contact areas of the barns, such as door knobs and break rooms. On the third visit, 50 rectal swabs were collected from piglets, and 24 environmental electrostatic cloths were collected from surfaces in both the farrowing and nursery barns. On the fourth visit, 72 fresh fecal samples and 36 electrostatic cloth samples were collected from harvest-ready pigs and the environment of a single finishing barn in the same production flow.

In the laboratory, sterile gauze and electrostatic cloth samples were added to buffered peptone water (BPW) in volumes of 36 ml and 90 ml, respectively. After incubation, 1 ml of each mixture was inoculated to nutrient broth modified with 2 μg/ml cefotaxime. After overnight incubation, samples were streaked to MacConkey agar modified with 1 μg/ml meropenem (initial visit) or 0.5 μg/ml meropenem and 70 μg/ml zinc sulfate heptahydrate (second, third, and fourth visits) to identify isolates with the CRE phenotype. Rectal swabs were added to 9 ml MacConkey broth supplemented with 2 μg/ml cefotaxime. Fecal samples were reduced to 4 g and homogenized with MacConkey/cefotaxime broth. Rectal swab and homogenate fecal samples were streaked to MacConkey agar containing 0.5 μg/ml meropenem and 70 μg/ml zinc sulfate heptahydrate to identify isolates with a CRE phenotype. All samples were incubated overnight at 37°C.

For resulting isolates with reduced susceptibility to meropenem, bacterial species determination was accomplished using biochemical assays, including indole, methyl red, Voges-Proskauer, Simmon's citrate, and motility assay, with ambiguous species identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry. Isolates were tested for carbapenemase production using Carba NP (31), with Carba NP-positive isolates assessed for genotype using previously reported PCR assays and Sanger sequencing of PCR products to identify possible carbapenemase genes, including bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, and bla_{OXA} (32–35). Specific bla_{IMP-27} forward (5′-CGCAGGTGAGACTTTGCCTA) and reverse (3′-GCTTAACAAAGCAACCGCCA) primers were designed via NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) from sequence results of the products from PCRs using IMP-1 primers (32). The plasmid content and plasmid size carried by each isolate were visualized by electrophoresis using a standard plasmid profiling procedure (36). Plasmid incompatibility groups were codified according to a plasmid PCR-based replicon typing procedure (PBRT) that detected 18 replicon types based on incompatibility group loci (16, 37, 38). Susceptibility profiles were generated using a semiautomated broth

microdilution system (Narms CMV3AGNF and ESβL ESB1F panels; Thermo Fisher Scientific, Oakwood Village, OH) following Clinical and Laboratory Standards Institute (CLSI) guidelines (39).

Plasmid content of a subset of the isolates was more fully characterized using WGS (MiSeq; Illumina, San Diego, CA). Plasmids were assembled from WGS data using plasmidSPAdes (40) following trimming for adapters and quality (41) with visualization of plasmid via de Bruijn graphs generated in the Bandage program (42). Preliminary annotation of plasmids was performed for antimicrobial resistance genes using the Comprehensive Antibiotic Resistance Database (CARD [43]) and for other functional elements using the NCBI Conserved Domain Database (CDD [44]) and European Bioinformatics Institute Database (EMBL-EBI [45]); insertion sequences were identified by using the ISfinder database (46). Plasmid incompatibility PCR results were confirmed with sequence data by using PlasmidFinder 1.3 (47).

Accession number(s). Sequence data for the IncQ1 plasmid has been deposited in GenBank under accession no. KY126032.

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REFERENCES

1. Nordmann P, Poirel L, Toleman MA, Walsh TR. 2011. Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother 66:689–692. https://doi.org/10.1093/jac/dkq520.
2. CDC. 2013. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control, Atlanta, GA. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.
3. Nordmann P, Couard JP, Sansot D, Poirel L. 2012. Emergence of an autochthonous and community-acquired NDM-1-producing *Klebsiella pneumoniae* in Europe. Clin Infect Dis 54:150–151. https://doi.org/10.1093/cid/cir720.
4. Nordmann P, Cornaglia G. 2012. Carbapenemase-producing *Enterobacteriaceae*: a call for action! Clin Microbiol Infect 18:411–412. https://doi.org/10.1111/j.1469-0691.2012.03795.x.
5. Rock C, Thom KA, Masnick M, Johnson JK, Harris AD, Morgan DJ. 2014. Frequency of *Klebsiella pneumoniae* carbapenemase (KPC)-producing and non-KPC-producing *Klebsiella* species contamination of healthcare workers and the environment. Infect Control Hosp Epidemiol 35:426–429. https://doi.org/10.1086/675598.
6. Woodford N, Wareham DW, Guerra B, Teale C. 2014. Carbapenemase-producing *Enterobacteriaceae* and non-*Enterobacteriaceae* from animals and the environment: an emerging public health risk of our own making? J Antimicrob Chemother 69:287–291. https://doi.org/10.1093/jac/dkt392.
7. Wang B, Sun D. 2015. Detection of NDM-1 carbapenemase-producing *Acinetobacter calcoaceticus* and *Acinetobacter junii* in environmental samples from livestock farms. J Antimicrob Chemother 70:611–613. https://doi.org/10.1093/jac/dku405.
8. Mollenkopf DF, Cenera JK, Bryant EM, King CA, Kashoma I, Kumar A, Funk JA, Rajashekara G, Wittum TE. 2014. Organic or antibiotic-free labeling does not impact the recovery of enteric pathogens and antimicrobial-resistant *Escherichia coli* from fresh retail chicken. Foodborne Pathog Dis 11:920–929. https://doi.org/10.1089/fpd.2014.1808.
9. Mollenkopf DF, Kleinhenz KE, Funk JA, Gebreyes WA, Wittum TE. 2011. *Salmonella enterica* and *Escherichia coli* harboring bla_{CMY} in retail beef and pork products. Foodborne Pathog Dis 8:333–336. https://doi.org/10.1089/fpd.2010.0701.
10. Poirel L, Bercot B, Millemann Y, Bonnin RA, Pannaux G, Nordmann P. 2012. Carbapenemase-producing *Acinetobacter* spp. in cattle, France. Emerg Infect Dis 18:523–525. https://doi.org/10.3201/eid1803.111330.
11. Fischer J, Rodríguez I, Schmoger S, Friese A, Roesler U, Helmuth R, Guerra B. 2012. Escherichia coli producing VIM-1 carbapenemase isolated on a pig farm. J Antimicrob Chemother 67:1793–1795. https://doi.org/10.1093/jac/dks108.
12. Fischer J, Rodriguez I, Schmoger S, Friese A, Roesler U, Helmuth R, Guerra B. 2013. *Salmonella enterica* subsp. *enterica* producing VIM-1 carbapenemase isolated from livestock farms. J Antimicrob Chemother 68:478–480. https://doi.org/10.1093/jac/dks393.
13. Zhang WJ, Lu Z, Schwarz S, Zhang RM, Wang XM, Si W, Yu S, Chen L, Liu S. 2013. Complete sequence of the bla_{NDM-1}-carrying plasmid pNDM-AB from *Acinetobacter baumannii* of food animal origin. J Antimicrob Chemother 68:1681–1682. https://doi.org/10.1093/jac/dkt066.
14. Al Bayssari C, Dabboussi F, Hamze M, Rolain J. 2015. Emergence of carbapenemase-producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in livestock animals in Lebanon. J Antimicrob Chemother 70:950–951. https://doi.org/10.1093/jac/dku469.
15. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11.
16. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018.
17. Gebreyes WA, Thakur S. 2005. Multidrug-resistant *Salmonella enterica* serovar Muenchen from pigs and humans and potential interserovar transfer of antimicrobial resistance. Antimicrob Agents Chemother 49:503–511. https://doi.org/10.1128/AAC.49.2.503-511.2005.
18. Zhang H, Shi L, Li L, Guo S, Zhang X, Yamasaki S, Miyoshi S, Shinoda S. 2004. Identification and characterization of class 1 integron resistance gene cassettes among *Salmonella* strains isolated from healthy humans in China. Microbiol Immunol 48:639–645. https://doi.org/10.1111/j.1348-0421.2004.tb03473.x.
19. Mathers AJ, Stoesser N, Sheppard AE, Pankhurst L, Giess A, Yeh AJ, Didelot X, Turner SD, Sebra R, Kasarskis A, Peto T, Crook D, Sifri CD. 2015. *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* at a single institution: insights into endemicity from whole-genome sequencing. Antimicrob Agents Chemother 59:1656–1663. https://doi.org/10.1128/AAC.04292-14.
20. Notake S, Matsuda M, Tamai K, Yanagisawa H, Hiramatsu K, Kikuchi K. 2013. Detection of IMP metallo-beta-lactamase in carbapenem-nonsusceptible Enterobacteriaceae and non-glucose-fermenting Gram-negative rods by immunochromatography assay. J Clin Microbiol 51:1762–1768. https://doi.org/10.1128/JCM.00234-13.
21. Hanson ND, Hossain A, Buck L, Moland ES, Thomson KS. 2006. First occurrence of a *Pseudomonas aeruginosa* isolate in the United States producing an IMP metallo-beta-lactamase, IMP-18. Antimicrob Agents Chemother 50:2272–2273. https://doi.org/10.1128/AAC.01440-05.
22. Limbago BM, Rasheed JK, Anderson KF, Zhu W, Kitchel B, Watz N, Munro S, Gans H, Banaei N, Kallen AJ. 2011. IMP-producing carbapenem-resistant *Klebsiella pneumoniae* in the United States. J Clin Microbiol 49:4239–4245. https://doi.org/10.1128/JCM.05297-11.
23. Moulds NM, Thomson KS, Hanson ND. 2011. IMP-27, a novel metallo-β-lactamase (MBL) associated with a class II integron identified in an isolate of *Proteus mirabilis*, abstr C1-1212. Proc 51st Annu Intersci Conf Antimicrob Agents Chemother. American Society for Microbiology, Washington, DC.
24. Tijet N, Lo S, Siebert H, MacNeill M, Rawte P, Farrell DJ, Low DE, Patel SN, Melano RG. 2012. Detection of IMP-27 metallo-β-lactamase in *Proteus mirabilis*, abstr C2-090. Proc 52nd Annu Intersci Conf Antimicrob Agents Chemother. American Society for Microbiology, Washington, DC.
25. Widmann M, Pleiss J, Oelschlaeger P. 2012. Systematic analysis of metallo-

beta-lactamases using an automated database. Antimicrob Agents Chemother 56:3481–3491. https://doi.org/10.1128/AAC.00255-12.
26. Rawlings DE, Tietze E. 2001. Comparative biology of IncQ and IncQ-like plasmids. Microbiol Mol Biol Rev 65:481–496. https://doi.org/10.1128/MMBR.65.4.481-496.2001.
27. Meyer R. 2009. Replication and conjugative mobilization of broad host-range IncQ plasmids. Plasmid 62:57–70. https://doi.org/10.1016/j.plasmid.2009.05.001.
28. Loftie-Eaton W, Rawlings DE. 2012. Diversity, biology and evolution of IncQ-family plasmids. Plasmid 67:15–34. https://doi.org/10.1016/j.plasmid.2011.10.001.
29. Webb HE, Bugarel M, den Bakker HC, Nightingale KK, Granier SA, Scott HM, Loneragan GH. 2016. Carbapenem-resistant bacteria recovered from faeces of dairy cattle in the high plains region of the USA. PLoS One 11:e0147363. https://doi.org/10.1371/journal.pone.0147363.
30. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. 2001. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother 45:2716–2722. https://doi.org/10.1128/AAC.45.10.2716-2722.2001.
31. Nordmann P, Poirel L, Dortet L. 2012. Rapid detection of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 18:1503–1507. https://doi.org/10.3201/eid1809.120355.
32. Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka S, Shimokata K, Kato N, Ohta M. 1996. PCR detection of metallo-beta-lactamase gene (bla_{IMP}) in gram-negative rods resistant to broad-spectrum beta-lactams. J Clin Microbiol 34:2909–2913.
33. Smith Moland E, Hanson ND, Herrera VL, Black JA, Lockhart TJ, Hossain A, Johnson JA, Goering RV, Thomson KS. 2003. Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. J Antimicrob Chemother 51:711–714. https://doi.org/10.1093/jac/dkg124.
34. Gröbner S, Linke D, Schütz W, Fladerer C, Madlung J, Autenrieth IB, Witte W, Pfeifer Y. 2009. Emergence of carbapenem-non-susceptible extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates at the University Hospital of Tübingen, Germany. J Med Microbiol 58:912–922. https://doi.org/10.1099/jmm.0.005850-0.
35. Peirano G, Ahmed-Bentley J, Woodford N, Pitout JD. 2011. New Delhi metallo-beta-lactamase from traveler returning to Canada. Emerg Infect Dis 17:242–244. https://doi.org/10.3201/eid1702.101313.
36. Kado CI, Liu ST. 1981. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 145:1365–1373.
37. Carattoli A, Miriagou V, Bertini A, Loli A, Colinon C, Villa L, Whichard JM, Rossolini GM. 2006. Replicon typing of plasmids encoding resistance to newer beta-lactams. Emerg Infect Dis 12:1145–1148. https://doi.org/10.3201/eid1207.051555.
38. Johnson TJ, Wannemuehler YM, Johnson SJ, White DG, Doetkott C, Nolan LK. 2007. Plasmid replicon typing of commensal and pathogenic types of *Escherichia coli*. Appl Environ Microbiol 73:1976–1983. https://doi.org/10.1128/AEM.02171-06.
39. CLSI. 2014. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 9th ed. Document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
40. Antipov D, Hartwick N, Shen M, Raiko M, Lapidus A, Pevzner P. 2016. plasmidSPAdes: assembling plasmids from whole genome sequencing data. Bioinformatics https://doi.org/10.1093/bioinformatics/btw493.
41. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
42. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of *de novo* genome assemblies. Bioinformatics 31:3350–3352. https://doi.org/10.1093/bioinformatics/btv383.
43. McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock LJ, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD. 2013. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 57:3348–3357. https://doi.org/10.1128/AAC.00419-13.
44. Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, Geer RC, He J, Gwadz M, Hurwitz DI, Lanczycki CJ, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Bryant SH. 2015. CDD: NCBI's conserved domain database. Nucleic Acids Res 43:D222–D226. https://doi.org/10.1093/nar/gku1221.
45. McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, Cowley AP, Lopez R. 2013. Analysis tool web services from the EMBL-EBI. Nucleic Acids Res 41:W597–W600. https://doi.org/10.1093/nar/gkt376.
46. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32–D36. https://doi.org/10.1093/nar/gkj014.
47. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller 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. https://doi.org/10.1128/AAC.02412-14.
48. Dixon N, Fowler RC, Yoshizumi A, Horiyama T, Ishii Y, Harrison L, Geyer CN, Moland ES, Thomson K, Hanson ND. 2016. IMP-27, a unique metallo-β-lactamase identified in geographically distinct isolates of *Proteus mirabilis*. Antimicrob Agents Chemother 60:6418–6421. https://doi.org/10.1128/AAC.02945-15.