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EPIDEMIOLOGY AND SURVEILLANCE

IncX3 Epidemic Plasmid Carrying bla_{NDM-5} in *Escherichia coli* from Swine in Multiple Geographic Areas in China

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ABSTRACT Six imported pigs originating from Guangdong, Henan, and Hunan provinces in China during October 2015 to February 2017 were cultured and found to be positive for meropenem-resistant *Escherichia coli*. The samples yielded 9 *E. coli* isolates of diverse sequence types carrying bla_{NDM-5} on IncX3 (8 isolates from 5 farms) or IncFII (1 isolate from 1 farm) plasmids. The *mcr-1* gene was coharbored by 4 isolates. The IncX3 plasmids (~46 kb) carrying bla_{NDM-5} were identical or nearly identical to each other.

KEYWORDS carbapenems, antimicrobial resistance epidemiology, molecular epidemiology, *Enterobacteriaceae*

The presence of carbapenemase-producing *Enterobacteriaceae* (CPE) in livestock animals is of concern because this may facilitate expansion of the gene pool from which pathogenic bacteria can pick up resistance genes, and consumers may subsequently be exposed through the food chain (1, 2). For this reason, there is a need to enhance the monitoring of carbapenem resistance in the food supply (1). In Hong Kong, 80% of the food animals are imported from mainland China and involve farm suppliers from multiple provinces in the country (3).

From September 2008 to February 2017, rectal swabs were obtained from randomly selected fresh pig carcasses at a centralized slaughterhouse in Hong Kong by trained veterinary staff, as part of an ongoing surveillance (3). Each swab was collected from a single animal and was inoculated into nutrient broth with 10 mg/liter vancomycin and 0.5 mg/liter meropenem (4), followed by subculture on a MacConkey agar plate supplemented with 2 mg/liter meropenem. Five to ten colonies from each selective plate were picked. Matrix-assisted laser desorption ionization–time of flight mass spectrometry was used for bacterial identification. The agar dilution (for colistin) and disc diffusion (for other antibiotics) methods were used to determine antimicrobial susceptibility (5, 6). Isolates from the same animal were considered to be unique if the resistance profiles for meropenem and colistin were different.

In total, 856 pigs were cultured over 263 sampling dates (see Table S1 in the supplemental material). Six pigs originating from six different farms were cultured and found to be positive for meropenem-resistant *Escherichia coli* (Table 1). According to the susceptibility patterns, a total of nine isolates were considered to be unique and were investigated further. All isolates had positive CarbaNP test results and were resistant to ertapenem, imipenem, and meropenem. The presence of carbapenemase genes and *mcr-1* was investigated by PCR and sequencing (7–9). The bla_{NDM-5} gene was identified in all nine isolates, of which four were resistant to colistin and coharbored *mcr-1*. The isolates were further investigated by multilocus sequence typing (MLST) and replicon typing (7, 9, 10). Plasmids carrying bla_{NDM-5} were X3 ($n = 8$, ~45 kb) or F36 ($n = 1$, ~100 kb). Of the four *mcr-1* genes identified, three were harbored on plasmids of different replicon types (X4, FIB, and Y). In conjugation experiments, no cotransfer of

Received 7 November 2017 **Returned for modification** 11 December 2017 **Accepted** 20 December 2017

Accepted manuscript posted online 8 January 2018

Citation Ho P-L, Wang Y, Liu MC-J, Lai EL-Y, Law PY-T, Cao H, Chow K-H. 2018. IncX3 epidemic plasmid carrying bla_{NDM-5} in *Escherichia coli* from swine in multiple geographic areas in China. Antimicrob Agents Chemother 62:e02295-17. https://doi.org/10.1128/AAC.02295-17.

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TABLE 1 Sources and characteristics for nine NDM-positive *E. coli* isolates

Strain	Specimen	Source[b]	Date collected	MLST	bla_{NDM-5}	*mcr-1*	Replicon type of plasmid harboring:		Resistance pattern[d]
							bla_{NDM}	*mcr-1*	
P744A	Pig 1	Henan (A1)	October 2015	ST10	+	−	X3	None	Chl, Nit
P744T[a]	Pig 1	Henan (A1)	October 2015	ST1602	+	+	X3	X4	Chl, Cip, Nit
P748[a]	Pig 2	Hunan (B)	January 2016	ST167	+	−	F36	None	Gen, Chl, Cip, Nit
P768	Pig 3	Henan (A2)	May 2016	ST117	+	−	X3	None	Gen, Chl, Cip
P768-11[a]	Pig 3	Henan (A2)	May 2016	ST871	+	+	X3	FIB	Cip
P785[a]	Pig 4	Guangdong (C1)	June 2016	ST7512	+	+	X3	Chromosomal[c]	Gen, Chl, Cip, Nit
P788A	Pig 5	Guangdong (C2)	June 2016	ST1286	+	−	X3	None	Nit
P788A-32[a]	Pig 5	Guangdong (C2)	June 2016	ST7510	+	+	X3	Y	Gen, Chl, Nit
P855[a]	Pig 6	Guangdong (C3)	February 2017	ST7511	+	−	X3	None	Gen, Chl, Cip, Nit

[a]The six isolates were investigated further by genome sequencing.
[b]Province (farm) origin of the pig.
[c]Chromosomal location of *mcr-1* in the isolate was confirmed by genome sequencing.
[d]Resistance patterns for amikacin (Ak), chloramphenicol (Chl), ciprofloxacin (Cip), fosfomycin (Fos), gentamicin (Gen), and nitrofurantoin (Nit).

carbapenem and colistin coresistance was seen, but the plasmids carrying bla_{NDM} or *mcr-1* could be transferred separately at frequencies of 10^{-4} to 10^{-5} and 10^{-1} to 10^{-6} transconjugants per donor cell, respectively (9, 10).

Six isolates (one from each animal) were sequenced by an Illumina MiSeq platform at >150-fold coverage (Table 1). The plasmids were assembled *de novo* using a CLC Genomics Workbench (Qiagen, Redwood City, CA), and gaps were closed by additional PCR and Sanger sequencing (7, 9, 10). ISfinder (https://www-is.biotoul.fr/about.php) was used to identify and annotate insertion sequences. In strain P748, bla_{NDM-5} was found in a contig (~32 kb) with 100% coverage and 98% identity to p28078-NDM (GenBank accession no. MF156713).

Complete sequences of the five ~46-kb IncX3 plasmids were obtained (see Table S2 in the supplemental material). They were found to have plasmid scaffolds typical of IncX3 plasmids (Fig. 1a). The genetic load regions in the five plasmids were compared with two reference IncX3 plasmids (pNDM-HN380 and pIncX-SHV) (Fig. 1b). In the bla_{NDM}-carrying plasmids, an IS*L3* with 8-bp flanking direct repeats (ATATGCAT) was found downstream of the resolvase gene. The *umuD* gene was split into two fragments (*umuD*Δ1 and *umuD*Δ2) at the same position as in pIncX-SHV, resulting in a pair of 3-bp direct repeats (TGT). In pNDM-HN380, bla_{NDM} was inserted as an IS*26*-IS*Aba125* transposon-like structure (Fig. 1b). Subsequently, the upstream IS*Aba125* was disrupted by IS*5* (10). In four plasmids with links to the Guangdong and Henan provinces, the sequences inserted between the two *umuD* fragments were 100% identical (10,117 bp in length). This inserted sequence differed from that in pNDM-HN380 by a deletion of 7,874 bp (Fig. 1b). The remaining plasmid with a link to Henan had an additional deletion (616 bp) at the junction between the IS*5* and IS*Aba125*Δ1 remnants. In the five NDM-5 plasmids, IS*5* was inserted at the same position, leading to the flanking 4-bp direct repeats (CTAA). In pNDM-HN380, IS*5* was inserted at a different position in the opposite orientation.

To explore the geographic distribution of potentially related bla_{NDM}-carrying IncX3 plasmids, the complete sequence of pP768-NDM-5 (chosen as a representative) was used to query the GenBank database. Twenty-two plasmids related to pP768-NDM-5 were identified (Fig. 1c), including 14 plasmids from China, 4 from Myanmar, and 1 each from Canada, India, Kuwait, and Oman (see Table S3 in the supplemental material). The plasmids did not carry resistance genes other than bla_{NDM}. Multiple NDM variants were carried by the plasmids. These include NDM-1 and variants that differed by one to three amino acids, including NDM-4 (M154L), NDM-5 (V88L, M154L), NDM-7 (D130N, M154L), and NDM-17 (V88L, M154L, E170K).

We identified the occurrence of similar IncX3 plasmids carrying bla_{NDM-5} in pigs originating from multiple farms across three different Chinese provinces. The involvement of IncX3 plasmids (represented by pNDM-HN380) in the dissemination of NDM in

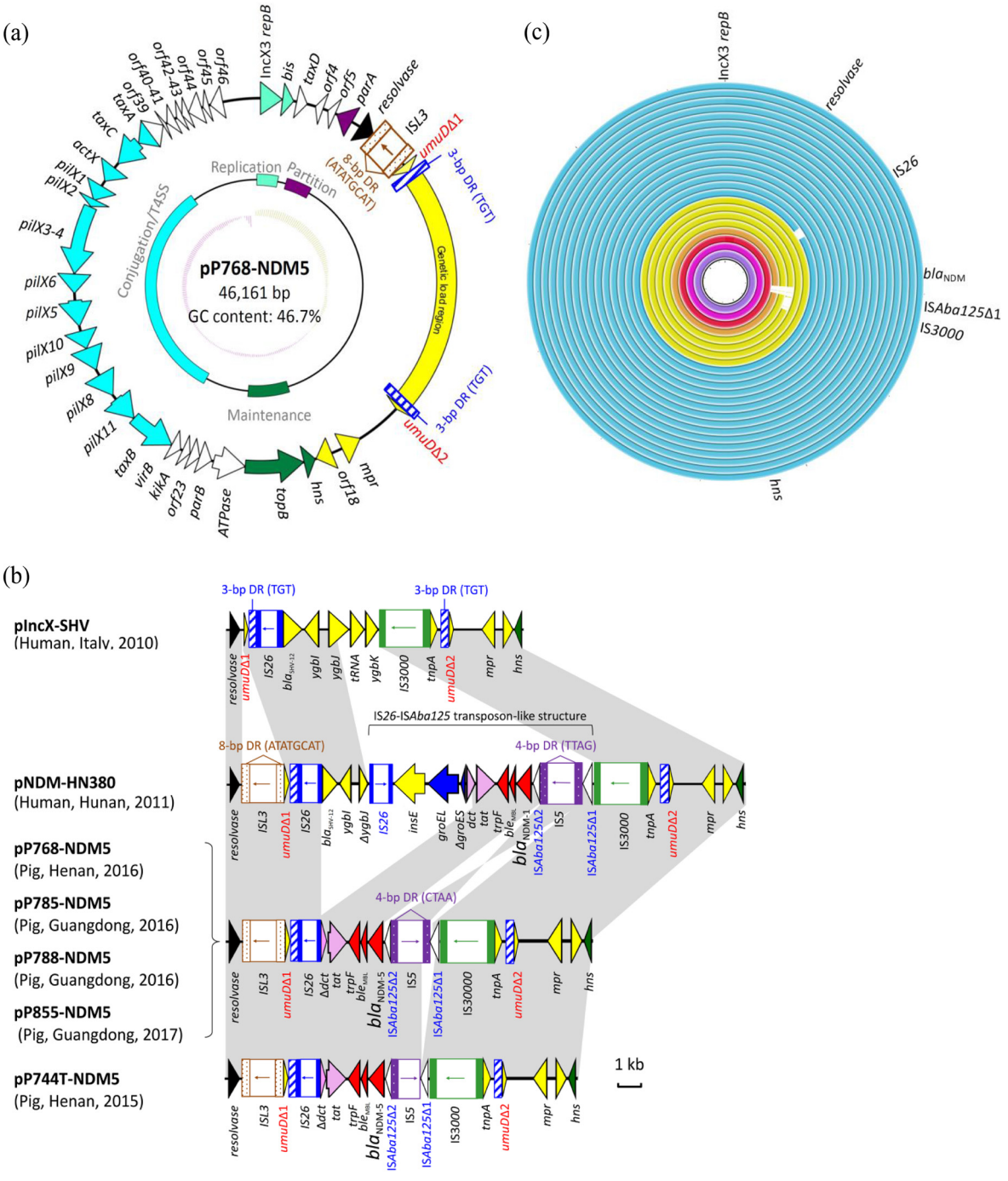

FIG 1 Comparisons of IncX plasmids in this study. (a) Circular map of plasmid pP768-NDM-5. This plasmid was used to illustrate the backbone shared by all the analyzed plasmids and the location of the genetic load region. (b) Comparison of the genetic load region in 5 plasmids harboring bla_{NDM-5} with 2 reference plasmids (pIncX-SHV and pNDM-HN380). (c) Alignment of pP768-NDM-5 with 22 plasmids identified in GenBank (last accessed 27 October 2017). The circular maps were generated with the BLAST Ring Image Generator, and each plasmid was colored by the geographic origin (China, blue; Myanmar, yellow; Oman, orange; India, red; Canada, pink; and Kuwait, purple) in the following order (outer to inner circles): pP768-NDM-5, pCREC-A6-NDM, pSCE516-2, pNDM-5_IncX3, pEc1929, pECNDM101, pAD-19R, pNDM-5_WCHEC0215, pK518_NDM-5, pK516_NDM-5, NUHL24835, pNDM-QD28, pNDM-QD29, pEC50-NDM-7, pZHDC40, pJEG027, pM216_X3, pM213_X3 and pM110_X3, pOM26-1, pNDM-MGR194 and pKpN01-NDM7, and pKW53T-NDM (see full details of plasmids in Table S3).

multiple geographic areas in China was initially reported by our group in 2012 (7). Subsequently, sporadic reports of pNDM-HN380-like plasmids carrying NDM variants have been reported in India, the Arabian Peninsula, Europe, and Australia (11–14). Recently, a Chinese national survey and several provincial studies revealed that IncX3

plasmids harboring different bla_{NDM} variants were frequently found among clinical isolates of different MLSTs and species, suggesting that they represent an important vector responsible for the wide dissemination of NDM in China (15–17). IncX3 plasmids have a narrow host range and have been found mainly in *Enterobacteriaceae* (18). Our finding from analysis of complete plasmid sequences indicates that the five IncX3 plasmids originating from pigs are related to pNDM-HN380 and plasmids originating from many other geographic areas, thus confirming that this mobile NDM vector is widespread in the ecosystem.

As carbapenems have never been licensed for use in food animals in China, the NDM-producing pig isolates detected in the present study may have been introduced to the farms via human activity or contaminated feeds. It is worrisome that some of the NDM-producing isolates in the present study were found to coharbor *mcr-1* in another plasmid or the chromosome. Nonetheless, our isolates that coharbored *mcr-1* were recovered before the ban of colistin in animal feeds was implemented in China in November 2016.

In conclusion, this study identified an epidemic IncX3 plasmid carrying bla_{NDM-5} disseminated among *E. coli* originating from pigs with epidemiological links to geographically segregated areas in China.

Accession number(s). The complete sequences of the five IncX3 plasmids were deposited in the GenBank database under accession numbers MF547511 (pP744-NDM-5), MF547510 (pP768-NDM-5), MF547509 (pP785-NDM-5), MF547507 (pP788A-NDM-5), and MF547508 (pP855-NDM-5).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02295-17.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We thank the staff at the Food and Environmental Hygiene Department (FEHD) of the Hong Kong Special Administrative Region (HKSAR) for assistance with specimen collection.

This work was supported by grants from the Health and Medical Research Fund of the Food and Health Bureau of the HKSAR and the Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Disease for the HKSAR Department of Health.

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