Extended-spectrum beta-lactamase-producing *Escherichia coli* harbouring *mcr-1* gene isolated from pigs in South Africa

To the Editor: Extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) produce enzymes that confer resistance to penicillins, cephalosporins and monobactams.[1] ESBL-PE represent a global concern in humans, in animals and in the environment and have therefore been recognised as pathogens of critical priority.[2] Of additional concern is the emergence of the plasmid-borne colistin resistance (*mcr-1*) genes that encode for resistance to polymyxins, the antibiotics of last resort for treatment of serious difficult-to-treat infections.[3] They have both been detected in developed and developing countries, and food animals have been recognised as their principal reservoir.[4]

Nasal and rectal swabs were collected from 432 pigs during a multicentre study carried out from March to October 2016 in five abattoirs in Cameroon (n=3) and South Africa (SA) (n=2). Samples were screened on a selective medium and putative ESBL-PE were tested for their antimicrobial susceptibility using the Vitek 2 System and Vitek 2 Gram Negative Susceptibility card (AST-N255) (bioMérieux, France). The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guideline,[5] with the exception of colistin, amoxicillin plus clavulanic acid, piperacillin plus tazobactam and amikacin, which were based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.[6] Closely related isolates underwent whole-genome sequencing (WGS) analysis on an Illumina MiSeq platform (Illumina Inc., USA) with 100 × coverage. ResFinder,[7] VirulenceFinder[8] and PlasmidFinder[9] were used to identify resistance genes, virulence factors and plasmids, respectively. The multi-locus sequence type was also determined from WGS data.

One of the ESBL-producing *Escherichia coli* isolated, PN256E8 (GenBank accession no. QJRZ00000000), showed resistance to several beta-lactam and non-beta-lactam antibiotics, including colistin. This resistance phenotype was corroborated by the identification of the $bla_{\text{TEM-1B}}$ and $bla_{\text{CTX-M-55}}$ genes through WGS, which also evidenced the colistin (*mcr-1*)-resistant gene (Table 1). The isolate PN256E8 was ascribed to the sequence type (ST) 446 and phylogenetic group A. It further harboured two virulence factors and two replicons along with three plasmid incompatibility groups, IncHI2 (ST-3-like), IncHI2A (ST-3-like) and IncN (ST-1).

To the best of our knowledge, this is the first report on the presence of an *mcr-1* encoding colistin resistance in ESBL-*E. coli* ST446 isolated from a pig abattoir in SA. The dissemination of *mcr-1*-producing *E. coli* strains in food animals and humans has been reported worldwide. In SA, a 79% prevalence of *mcr-1* in colistin-resistant cultures of *E. coli* was reported in a nationwide surveillance programme of poultry settings. The *mcr-1* gene was detected among clinical *E. coli* isolates of hospitalised patients (n=3) and outpatients (n=6) not previously exposed to colistin.[10] Furthermore, in the Western Cape region, 83% of clinical colistin-resistant Enterobacteriaceae harboured the *mcr-1* gene.[3] The concomitant presence of ESBL and *mcr-1* genes in *E. coli* ST 446 is an important food safety and public health concern, as these resistances could be transferred from commensal to pathogenic bacteria prevailing in the food chain and subsequently disseminate to communities and/or healthcare settings. Increased surveillance of ESBL producers for colistin resistance is essential to monitor their acquisition and spread. Efforts to ensure rational antibiotic use in agriculture are vital to preserve antibiotics for future generations.

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Author contributions. LLF co-conceptualised the study, undertook sample collection and microbiological laboratory and data analyses, prepared tables, interpreted results, contributed to bioinformatics analysis, and drafted the manuscript. RCF undertook sample collection and microbiological laboratory analyses, contributed to bioinformatics analysis and vetted the results. MA undertook bioinformatics analyses. AI performed whole-genome sequencing analysis. SYE co-conceptualised the study and undertook critical revision of the manuscript. All authors read and approved the final manuscript.

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Table 1. Antimicrobial susceptibility results of selected beta-lactam and non-beta-lactam antibiotics

Isolate name	Beta-lactam antibiotics								Non-beta-lactam antibiotics						Antibiotic resistance genes	Virulence genes	Plasmids
	AMP	AMC	TZP	CXM	CTX	CAZ	FEP	ETP	MEM	IMP	GEN	AN	CIP	TGC	FT	CS	TMP/SXT			
PN256E8	R	S	S	R	R	R	R	S	S	S	R	R	S	S	S	R	R	$bla_{\text{TEM-1B}}$, $bla_{\text{CTX-M-55}}$, *aacA4*, *aadA5*, *aac(6′)Ib-cr*, *oqxB*, *fosA*, *tet*(A), *floR*, *drfA7*, *drfA17*, *sul2*, *mcr-1*	*astA*, *gad*	Col(MG828) RepA, IncHI2 (ST-3-like), IncHI2A (ST-3-like), IncN (ST-1)

AMP = ampicillin; AMC = amoxicillin plus clavulanic acid; TZP = piperacillin plus tazobactam; CXM = cefuroxime; CTX = cefotaxime; CAZ = ceftazidime; FEP = cefepime; ETP = ertapenem; MEM = meropenem; IMP = imipenem; GEN = gentamicin; AN = amikacin; CIP = ciprofloxacin; TGC = tigecycline; FT = nitrofurantoin; CS = colistin; TMP/SXT = trimethoprim plus sulfamethoxazole; R = resistant; S = susceptible; *astA* = EAST-1 heat stable toxin; *gad* = glutamate decarboxylase.

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