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Short Communication

First report of an *Escherichia coli* strain from swine carrying an OXA-181 carbapenemase and the colistin resistance determinant MCR-1

Sandra Pulss [a], Torsten Semmler [b], Ellen Prenger-Berninghoff [a], Rolf Bauerfeind [a], Christa Ewers [a,*]

[a] Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Frankfurter Str. 85–89, 35398 Giessen, Germany
[b] Robert Koch Institute, Berlin, Germany

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ABSTRACT

Plasmid-mediated resistance to carbapenems and colistin in Enterobacteriaceae represents an emerging public health threat. Although animals have been identified as a relevant source of multidrug-resistant (MDR) bacteria, there are only a few reports on the presence of carbapenemases in animal isolates. In this study, 7850 faecal *Escherichia coli* isolates obtained from 2160 pigs were screened for carbapenem non-susceptibility using Mueller–Hinton agar supplemented with meropenem. Eleven isolates showed growth on meropenem-containing agar but only two proved positive by PCR for a carbapenemase gene, namely $bla_{\text{OXA-48-like}}$. The two isolates were obtained from different pigs housed at the same farm in Italy and were not genetically related by multilocus sequence typing (MLST), comprising ST359 and ST641. Whole-genome sequencing revealed the presence of $bla_{\text{OXA-181}}$ in both isolates; in addition, the colistin resistance gene *mcr-1* and aminoglycoside resistance gene *armA* were found in one isolate. The $bla_{\text{OXA-181}}$ resistance gene was located on a 51.5-kb non-conjugative plasmid of replicon type IncX3 and the *mcr-1* gene on a 33.3-kb transferable IncX4 plasmid. The high nucleotide similarity (>99%) of plasmids pEcIHIT31346-OXA-181 and pEcIHIT31346-MCR-1 to published plasmids from various human and animal sources suggests that specific antibiotic resistance plasmids are circulating among *E. coli* strains worldwide and across vertebrate species barriers. Although carbapenems are not licensed for use in livestock and the overall prevalence of carbapenemases in porcine *E. coli* appears to be low, the current findings indicate that even pigs can host MDR strains with accumulated plasmid-mediated resistance against several last-line antibiotics.

1. Introduction

The emergence and global spread of acquired carbapenemases together with the recent finding of plasmid-mediated colistin resistance (*mcr-1* gene) in Enterobacteriaceae constitutes a global threat to public health [1,2]. Since 2012, a growing number of studies have reported the emergence of carbapenemases, including VIM-1, NDM-1 and OXA-48, in Enterobacteriaceae from livestock and companion animals [3–5]. None the less, carbapenemase-producing Enterobacteriaceae (CPE) from animal sources are still rare, particularly compared with findings in humans [1]. In contrast, the plasmid-encoded colistin resistance gene *mcr-1*, which was first described in *Escherichia coli* from livestock, food and humans in China, so far appears to be more abundant among livestock animals and food than in human patients [2,6,7]. However, there is great concern over single reports about co-production of carbapenemases and MCR-1 in Enterobacteriaceae from humans, as this significantly reduces therapeutic options for the treatment of infections with multidrug-resistant (MDR) bacteria [6,8].

This study aimed to assess the prevalence of carbapenemases in *E. coli* isolates from pigs. Isolates carrying a carbapenemase gene were further tested for other resistance features.

2. Materials and methods

2.1. Bacterial strains, antimicrobial susceptibility testing and search for carbapenemase genes

From May 2015 to August 2016, a total of 2253 porcine faecal samples, predominantly from Germany ($n = 2069$) and also from The Netherlands ($n = 105$), Belgium ($n = 30$), Denmark ($n = 25$), Italy ($n = 19$) and Great Britain ($n = 5$), were sent to the diagnostic laboratory of the Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen (Giessen, Germany) for molecular typing of virulence-associated genes that are predictive for *E. coli* pathovars

* Corresponding author. Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, Frankfurter Str. 85–89, 35398 Giessen, Germany.
E-mail address: christa.ewers@vetmed.uni-giessen.de (C. Ewers).

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implicated in swine diarrhoea and oedema disease. According to their morphology and haemolytic phenotype, up to six colonies per sample ($n = 7850$ isolates) were screened for carbapenem non-susceptibility using Mueller–Hinton agar supplemented with 0.5 mg/L meropenem (Sigma-Aldrich, Seelze, Germany). Strains that grew on this medium were tested for the carbapenemase genes $bla_{\text{KPC-like}}$, $bla_{\text{NDM-like}}$, $bla_{\text{VIM-like}}$ and $bla_{\text{OXA-48-like}}$ by PCR [9]. Isolates carrying one of these genes were investigated for antimicrobial susceptibility using the AST cards GN38 and GN248 of the VITEK®2 system (bioMérieux, Nürtingen, Germany). Minimum inhibitory concentrations (MICs) were interpreted according to breakpoints set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) [10,11].

2.2. Whole-genome sequencing (WGS) and sequence analysis

DNA for WGS was prepared using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Sequencing was performed on a MiSeq sequencer (MiSeq Reagent Kit v.3; Illumina Inc., San Diego, CA) and resulted in 300-bp paired-end reads and an average coverage of 100×. Genome sequences were assembled with SPAdes v.3.7.1 (http://cab.spbu.ru/software/spades/) and were annotated using the Rapid Annotation using Subsystem Technology (RAST) server (http://rast.nmpdr.org/). Services provided at the Center of Genomic and Epidemiology (https://cge.cbs.dtu.dk/services/) were used to identify resistance genes (ResFinder), plasmid incompatibility groups (PlasmidFinder), virulence-associated genes (MyDbFinder), genoserotype (SerotypeFinder) and multilocus sequence types (MLST 1.8) according to the scheme hosted at Warwick University (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). Plasmid sequences were obtained from WGS data using Geneious 8.1.3 (Biomatters Ltd., Auckland, New Zealand), and contig arrangements were verified by PCR using the primers depicted in Supplementary Table S1. The genetic environment of *mcr-1* and $bla_{\text{OXA-181}}$ genes was identified using ISfinder (https://www-is.biotoul.fr/).

2.3. Genomic location of resistance genes and conjugation assays

To determine the genomic location of $bla_{\text{OXA-181}}$ *and mcr-1*, plasmid DNA and I-*Ceu*I-digested whole-cell DNA were separated by agarose or pulsed-field gel electrophoresis (PFGE), respectively, and were analysed by Southern blot hybridisation. DNA probes were prepared using the PCR Dig Probe Synthesis Kit (Boehringer Mannheim GmbH, Mannheim, Germany) and consisted of a 1486-bp PCR fragment specific for 16S rRNA genes (primers SK16R/SK16F) [12] as well as internal PCR fragments specific for *mcr-1* (309 bp; primers CLR-F/CLR-R) and $bla_{\text{OXA-48-like}}$ genes (743 bp; primers OXA-48A/B), respectively [7,9]. Detection of hybridised DNA molecules was facilitated with the DIG Luminescent Detection Kit (Boehringer Mannheim GmbH).

Conjugation was performed using *E. coli* J53 as recipient. Transconjugants were selected on Mueller–Hinton agar plates supplemented with 100 mg/L sodium azide (Merck Chemicals GmbH, Darmstadt, Germany) and either 0.5 mg/L meropenem (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) or 2 mg/L colistin sulfate (Carl Roth GmbH, Karlsruhe, Germany). Transconjugants were tested for $bla_{\text{OXA-181}}$ and *mcr-1* by PCR and by performing susceptibility tests and plasmid preparations of wild-type and transconjugant isolates as described above.

3. Results

3.1. Carbapenem non-susceptibility, resistance genes and antimicrobial susceptibility

Screening of the 7850 porcine *E. coli* isolates revealed 11 non-duplicate isolates able to grow on the meropenem screening agar. These isolates originated from nine farms in Germany and from one farm (two isolates) in Italy. The latter two isolates (IHIT31345 and IHIT31346) tested positive for $bla_{\text{OXA-48-like}}$ by PCR and for $bla_{\text{OXA-181}}$ by WGS. They showed imipenem MICs of 1–2 mg/L (Table 1), whereas the nine carbapenemase gene-negative isolates had MICs of ≤0.25 mg/L. Both $bla_{\text{OXA-181}}$-carrying isolates were MDR but differed from each other in their resistance determinants and profiles (Table 1). IHIT31346 harboured the AmpC β-lactamase gene $bla_{\text{CMY-2}}$ and *mcr-1* in addition to $bla_{\text{OXA-181}}$, with each gene on its own plasmid. In addition, this isolate harboured the 16S rRNA methyltransferase gene *armA*, which so far has only been reported once from a porcine *E. coli* in Spain in 2005 [13] and confers resistance to amikacin, gentamicin, tobramycin and kanamycin. The *armA* gene was located on an 8742-bp contig and was flanked by transposon gene sequences

Table 1
Characteristics of $bla_{\text{OXA-181}}$-carrying *Escherichia coli* isolates from swine faeces.

Strain	IHIT31345	IHIT31346
Date of isolation	April 2016	April 2016
ST/STC (phylogroup)	359/101 (B1)	641/86 (A)
Genoserotype	Ont:H21	O9:H25
Carbapenemase gene	$bla_{\text{OXA-181}}$ (51.5-kb IncX3 plasmid)	$bla_{\text{OXA-181}}$ (51.5-kb IncX3 plasmid)
Colistin resistance gene	None	*mcr-1* (33.3-kb IncX4 plasmid)
ESBL/AmpC genes	None	$bla_{\text{CMY-2}}$ (IncI1 plasmid)
Plasmid incompatibility groups	IncX3, IncFIB, ColKP3	IncX3, IncX4, IncI1, IncR, IncFII, IncFIB, ColKP3
Resistance genes [a]	$bla_{\text{TEM-1B}}$, *aph(3′)-Ia*, *aadA1*, *aadA2*, *aac(3)-IId*, *lnu*(F), *qnrS1*, *floR*, *cmlA1*, *sul2*, *sul3*, *tet*(A), *tet*(M), *dfrA12*	$bla_{\text{TEM-1B}}$, *aph(4)-Ia*, *aph(3′)-Ia*, *aadA1*, *aadA2*, *aadA5*, *aac(3)-IVa*, *armA*, *qnrS1*, *floR*, *cmlA1*, *sul1*, *sul3*, *tet*(A), *tet*(M), *dfrA12*, *dfrA17*
GyrA/ParC mutations	GyrA, S83L, D87N; ParC, S80I	None
Antimicrobial resistance (MIC in mg/L) [b]	AMP (≥32), AMC (≥32), PIP (≥128), GEN (≥16), TOB (≥16), CIP (≥4), ENR (≥4), MAR (≥4), TET (≥16), CHL (≥64), SXT (≥32)	AMP (≥32), AMC (≥32), PIP (≥128), TZP (≥128), CTX (8), CAZ (≥16), CPD (≥8), GEN (≥16), AMK (≥64), TET (≥16), CHL (≥64), PMB (8), COL (8), SXT (≥32)
COL/PMB MIC (mg/L) [b]	≤0.5/≤0.25	8/8
IPM/MEM MIC (mg/L) [b]	1/0.5	2/0.5
Virulence-associated genes	*csgA*, *sitA*, *malX*, *traT*, *fimH*, *astA*	*csgA*, *ompT*, *traT*, *malX*, *fimH*, *astA*, *aidA*

ST, sequence type; STC, ST complex; ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; PIP, piperacillin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; ENR, enrofloxacin; MAR, marbofloxacin; TET, tetracycline; CHL, chloramphenicol; SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam; CTX, cefotaxime; CAZ, ceftazidime; CPD, cefpodoxime; AMK, amikacin; PMB, polymyxin B; COL, colistin; IPM, imipenem; MEM, meropenem.

[a] Other than ESBL/AmpC, carbapenemase and *mcr-1* genes.

[b] MICs for COL and PMB were interpreted according to breakpoints set for Enterobacteriaceae by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [10] and for *Acinetobacter* spp. by the Clinical and Laboratory Standards Institute (CLSI) [11], respectively. MICs for the other substances were interpreted according to CLSI breakpoints [11].