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Short Communication

Characterisation of multidrug-resistant Shiga toxin-producing *Escherichia coli* cultured from pigs in China: co-occurrence of extended-spectrum β-lactamase- and *mcr-1*-encoding genes on plasmids

Li Bai [a], Daniel Hurley [b], Juan Li [c], Qiong Meng [d], Juan Wang [e], Séamus Fanning [a,b,*,1], Yanwen Xiong [c,**]

[a] *Key Laboratory of Food Safety Risk Assessment, Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing, China*
[b] *UCD–Centre for Food Safety, School of Public Health, Physiotherapy and Sports Science, University College Dublin, Belfield, Dublin 4, Ireland*
[c] *Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China*
[d] *Zhejiang Provincial Center for Disease Control and Prevention, Zhejiang, China*
[e] *College of Veterinary Medicine, Northwest A&F University, No. 22 Xinong Road, Yangling, Shaanxi 712100, China*

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ABSTRACT

Identification of Enterobacteriaceae harbouring the plasmid-mediated transferable colistin resistance gene *mcr-1* presents a new challenge to public health. The aim of this study was to characterise multidrug-resistant Shiga toxin-producing *Escherichia coli* (STEC) harbouring the *mcr-1* gene on plasmids cultured from pigs in China. Using CHROMagar™ ECC plates combined with *stx* gene detection by PCR, 93 STEC were recovered from 326 faecal, 351 small intestine content and 326 colon content samples taken from healthy pigs in 2011 and 2012 in China. This study, in which ten colistin-resistant isolates with minimum inhibitory concentrations (MICs) of 8–12 mg/L were identified and found to be positive by PCR for the *mcr-1* gene, is a follow-up to an earlier investigation. Plasmid profiling by S1-nuclease digestion followed by pulsed-field gel electrophoresis (PFGE) identified several high-molecular-weight plasmids and these were typed by PCR-based replicon typing (PBRT). Two of the ten isolates, namely STEC-CQ09 (O116:H11/CC23/ST88) and CQ10 (O2:H32/ST3628), were selected for further study as described in this report.

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1. Introduction

Plasmids carrying antimicrobial resistance-encoding genes can arise from multiple sources and be disseminated by horizontal gene transfer. This feature limits the available chemotherapeutic treatment options, leading to an ever-increasing reliance on compounds such as tigecycline and colistin. The increase in carbapenemase-producing Enterobacteriaceae has resulted in a greater reliance on the use of polymyxin B and polymyxin E (colistin), with the inevitable risk of emerging resistance among bacteria [1]. Colistin belongs to the family of polymyxins, which are cationic polypeptides, that target the lipid A component of the lipopolysaccharide (LPS) structure. These compounds have a broad spectrum of activity against Gram-negative bacteria [1]. Colistin is indicated for use in food-producing animals and very low rates of resistance have been reported. Where resistance does arise, associated mechanisms, until recently, have been mediated by chromosomally located genes (e.g. *pmrAB*, *phoPQ* and its negative regulator *mgrB*) [2]. A transferrable colistin resistance-encoding gene, *mcr-1*, has been detected in food, animals and humans in different species across the word [1,3–6]. Considering the importance of colistin in the treatment in human infections and its use in food-producing animals in China and elsewhere, identification of the *mcr-1* gene in food-producing animals presents a new challenge to veterinary public health.

Shiga toxin-producing *Escherichia coli* (STEC) is recognised as an important human pathogen causing diarrhoea, haemorrhagic colitis

* Corresponding author. Key Laboratory of Food Safety Risk Assessment, Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing, China. Fax: +353-1 716 2869.
E-mail address: sfanning@ucd.ie (S. Fanning).
** Corresponding author. Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China. Fax: +86 10 5890 0748.
E-mail address: xiongyanwen@icdc.cn (Y. Xiong).
1 Present address: UCD–Centre for Food Safety, University College Dublin, Room S1.04, Belfield, Dublin D04 N2E5, Ireland.

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and haemolytic–uraemic syndrome [7]. Swine play an important role as a carrier of this pathogen [8]. In this study, 93 STEC isolates were retrospectively assessed for the presence of the *mcr-1* gene by PCR [1]. The MCR-1 resistance mechanism appears to have spread to extended-spectrum β-lactamase (ESBL)-producing STEC cultured from pigs.

2. Materials and methods

2.1. Bacterial study collection

In an earlier study, 93 STEC were collected from 326 faecal, 351 small intestine content and 326 colon content samples taken from healthy pigs between May 2011 and August 2012 at sites located in Beijing, Chongqing and Guizhou Province in China. One gram of each sample was enriched in 5 mL of modified tryptone soya broth supplemented with novobiocin (10 mg/L) (Oxoid Ltd., Basingstoke, UK) and was incubated at 37 °C for 18–24 h. Isolation of STEC microorganisms was carried out using CHROMagar™ ECC plates (CHROMagar, White City, OR) combined with the *stx* PCR detection method [8].

2.2. Detection of the mcr-1 gene by standard PCR

Primers for the *mcr-1* gene as described previously were used to screen the study isolates [1].

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for all *mcr-1*-positive isolates by agar disk diffusion (Oxoid Ltd.; Thermo Fisher Scientific Inc.). The minimum inhibitory concentration (MIC) of colistin (Sigma, St Louis, MO) was determined by broth dilution according the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org; accessed 22 March 2016). *E. coli* ATCC 25922 was included as a quality control strain. Results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) [9] and EUCAST.

2.4. Plasmid profiling

Plasmid DNA profiles of ten colistin-resistant STEC isolates and their corresponding transconjugants were obtained using the S1-nuclease pulsed-field gel electrophoresis (PFGE) molecular subtyping method [10].

2.5. PCR-based replicon typing (PBRT)

Eighteen pairs of primers were used to determine the plasmid incompatibility (Inc) types by PBRT using purified genomic DNA as template [11].

2.6. Conjugation-based mating experiments and verification

Two of the *mcr-1*-positive STEC isolates (STEC-CQ09 and -CQ10) were selected for conjugation experiments and were analysed individually for their ability to transfer the colistin resistance gene to the plasmid-free recipient *E. coli* J53 (sodium azide-resistant). Conjugation experiments were carried out using a broth mating protocol [10]. Antimicrobial susceptibility tests were again performed to confirm plasmid transfer, followed by S1-nuclease PFGE, PBRT and PCR as described previously.

2.7. Whole-genome sequencing (WGS) and phylogenetic analysis

Genomic DNA was sequenced using an Illumina HiSeq 2500 platform (Illumina, San Diego, CA) generating 90-bp paired-end reads from a library with an average insert size of 500 bp and a total amount of quality-filtered raw sequence of >550 Mbp per isolate. Reads were assembled de novo using SOAPdenovo v.2.04 [12]. The core genome-based phylogeny was determined by Harvest Suite [13] and a maximum composite likelihood phylogenetic tree was generated by MEGA v.6 software (http://www.megasoftware.net/) using *E. coli* MG1655 as reference. Accession numbers were assigned as follows: **FLOA01000000** (STEC-CQ09); and **FLOF01000000** (STEC-CQ10).

3. Results and discussion

Among the previous isolate collection of 93 STEC, 10 colistin-resistant isolates were identified and were denoted as STEC-CQ01, -CQ02, -CQ03, -CQ04, -CQ05, -CQ06, -CQ07, -CQ08, -CQ09, -CQ10. The *mcr-1* gene was identified by PCR in all ten of these isolates. The colistin-resistant isolates were additionally resistant to several compounds in different antimicrobial classes including aminoglycosides, aminopenicillins, cephalosporins, fluoroquinolones, phenicols, tetracyclines, trimethoprim and sulphonamides (Table 1). S1-nuclease-based PFGE profiling experiments identified small and large plasmids ranging in size from 40 kbp to 250 kbp in the ten colistin-resistant isolates, of which STEC-CQ01, -CQ02, -CQ03, -CQ04, -CQ05, -CQ06, -CQ07, -CQ08 and -CQ10 had an indistinguishable

Table 1
Characteristics of colistin-resistant Shiga toxin-producing *Escherichia coli* (STEC) isolated from pig faecal samples and their phylogenetic group, sequence type (ST), plasmid content and incompatibility (Inc) group(s) along with their antimicrobial resistance profiles and minimum inhibitory concentration (MIC) to colistin.

STEC isolate	Phylogroup	ST	Plasmid(s) (kbp)	PBRT based on WGS	ESBL type	Antimicrobial resistance profile	Colistin MIC (mg/L)	Additional resistance determinants identified based on WGS
CQ09 (O116:H11)	A	ST88	241, 224, 206, 186, 90, 68, 44	IncI2, IncHI2, IncHI2A, IncA/C, IncFIB, IncFIC, IncX1, IncFII	CTX-M-55	AMC, AMP, ATM, CHL, CIP, CRO, COL, CTX, CXM, NIT, FEP, KAN, KF, NAL, NOR, PIP, STR, SAM, SXT, TET	12	*aadA1*, *aadA2*, *aph(3′)-Ia*, *strAB*, *sul1*, *sul2*, *sul3*, *tet(A)*, *dfrA12*, *ermB*, *mphA*, *cmlA1*, *floR*
CQ10 (O2:H32)[a]	A	ST3628	248, 124, 94, 66	IncFIC, IncHI2, IncHI2A, IncN, IncFIB, IncX4	CTX-M-65	AMP, ATM, CHL, CRO, COL, CTX, CXM, NIT, KAN, KF, NAL, PIP, STR, SXT, TET	8	*aadA1*, *aadA2*, *aph(3′)-Ia*, *strAB*, *tet(A)*, *dfrA12*, *dfrA14*, *cmlA1*, *floR*, *qnrS1*, *oqxA*

PBRT, PCR-based replicon typing; WGS, whole-genome sequencing; ESBL, extended-spectrum β-lactamase; AMC, amoxicillin/clavulanic acid; AMP, ampicillin; ATM, aztreonam; CHL, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; COL, colistin; CTX, cefotaxime; CXM, cefuroxime; NIT, nitrofurantoin; FEP, cefepime; KAN, kanamycin; KF, cefalotin; NAL, nalidixic acid; NOR, norfloxacin; PIP, piperacillin; STR, streptomycin; SAM, ampicillin/sulbactam; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

[a] STEC-CQ01, -CQ02, -CQ03, -CQ04, -CQ05, -CQ06, -CQ07 and -CQ08 had the same phylogroup, ST, plasmids, ESBL type, antimicrobial resistance profile and colistin MIC as STEC-CQ10.

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