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Occurrence and characterization of *mcr-1*-harbouring *Escherichia coli* isolated from pigs in Great Britain from 2013 to 2015

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Objectives: To determine the occurrence of *mcr-1*-harbouring *Escherichia coli* in archived pig material originating in Great Britain (GB) from 2013 to 2015 and characterize *mcr-1* plasmids.

Methods: Enrichment and selective culture of 387 archived porcine caecal contents and recovery from archive of 1109 *E. coli* isolates to identify colistin-resistant bacteria by testing for the presence of *mcr-1* by PCR and RT–PCR. *mcr-1*-harbouring *E. coli* were characterized by WGS and compared with other available *mcr-1* WGS.

Results: Using selective isolation following enrichment, the occurrence of *mcr-1 E. coli* in caeca from healthy pigs at slaughter from unique farms in GB was 0.6% (95% CI 0%–1.5%) in 2015. *mcr-1 E. coli* were also detected in isolates from two porcine veterinary diagnostic submissions in 2015. All isolates prior to 2015 were negative. WGS analysis of the four *mcr-1*-positive *E. coli* indicated no other antimicrobial resistance (AMR) genes were linked to *mcr-1*-plasmid-bearing contigs, despite all harbouring multiple AMR genes. The sequence similarity between *mcr-1*-plasmid-bearing contigs identified and those found in GB, Chinese and South African human isolates and Danish, French and Estonian livestock-associated isolates was 90%–99%.

Conclusions: *mcr-1*-harbouring plasmids were diverse, implying transposable elements are involved in *mcr-1* transmission in GB. The low number of *mcr-1*-positive *E. coli* isolates identified suggested *mcr-1* is currently uncommon in *E. coli* from pigs within GB. The high sequence similarity between *mcr-1* plasmid draft genomes identified in pig *E. coli* and plasmids found in human and livestock-associated isolates globally requires further investigation to understand the full implications.

Introduction

Colistin is becoming increasingly prescribed to combat MDR bacteria in humans and has been licensed for use in treatment of livestock in the UK since 2004. Occurrence of a plasmid-borne colistin resistance gene, *mcr-1*, was first reported by Liu *et al.*[1] in November 2015. Since then, *mcr-1*-harbouring plasmids have been reported in livestock, food and humans across the globe, with new reports of identification in countries occurring each month.[2] Despite the recent identification of *mcr-1*, it has subsequently been identified in archived Chinese chicken *Escherichia coli* isolates from the 1980s; there has also been an increase in reporting of *mcr-1* from such isolates from the past 5 years.[3] The Animal and Plant Health Agency (APHA) previously reported the characterization of colistin-resistant *Salmonella* and *E. coli* isolated from pigs with diarrhoeal disease on a farm in Great Britain (GB). In this study we examined porcine bacterial isolates, frozen pig caecal samples and WGS data of *E. coli* isolated from pigs, archived from 2013 to 2015, for the presence of *mcr-1*.

Materials and methods

Archived porcine samples and culture

Details of the samples are given in Table 1 and include their origin, number of pig herds and numbers of *E. coli*, including colistin-resistant *E. coli*, examined.

For enrichment of colistin-resistant bacteria from caecal samples, 0.5 g of caecal contents was added to 4.5 mL of buffered peptone water and incubated for 18 h at 37 °C. Plating of enriched contents was on MacConkey agar with/without 2 mg/L colistin.[4]

PCR screening for mcr-1

A sweep of ~10–20 colistin-resistant lactose-fermenting colonies were picked and PCR was performed on colony lysates as described previously.

Table 1. Dates, sources and numbers of samples tested for prevalence of *mcr-1* in GB

Date and project type	Sample origin and location	Number of pig herds represented in the sample		Number of archived *E. coli* isolates	Number of colistin-resistant isolates	Number of *mcr*-1-positive herds	Herd prevalence (95% CI)
		healthy	diseased/veterinary diagnostic				
2013 surveillance	abattoir, UK	115	–	190[a]	–	0	0% (–)
2014–15 research project	abattoir, England	57	–	556[b]	63	0[g]	0% (–)
2015 surveillance	abattoir, GB	313[c]	–	200[d]		0[e]	0% (–)
				NA[c]	204[f]	2[g]	0.6% (0%–1.5%)
2015/16 veterinary diagnostic submissions	veterinary, England and Wales	–	105	163[h]	4	2[g]	1.9% (0.0%–4.5%)

NA, not applicable.
[a]ESBL-producing *E. coli* recovered from porcine caecal samples from a UK-wide surveillance project[20] for which WGS was available.
[b]*E. coli* cultured from randomly selected porcine caecal samples collected from England.
[c]387 randomly selected porcine caecal samples originating from 313 different pig herds in GB as part of an EU-wide surveillance programme.
[d]*E. coli* recovered from MacConkey plates without colistin from these porcine caecal samples.
[e]Isolates tested by PCR for *mcr-1*.
[f]*E. coli* recovered on selective MacConkey plates containing 2 mg/L colistin following overnight enrichment.
[g]Isolates tested by RT–PCR for *mcr-1*.
[h]*E. coli* recovered from clinical submissions from diseased animals in England and Wales.

For RT–PCR, we used the forward primer 5′ → 3′ CCGATCATGCCAATCTACTC and reverse primer 5′ → 3′ CAGGCTTGGTTGCTTGTA in colony lysates under standard RT–PCR conditions.

WGS and analysis of mcr-1-positive isolates

Isolates positive for *mcr-1* were sequenced using an Illumina MiSeq 2 × 150 bp run. Sequences were assembled using SPAdes 3.7.0[5] and annotated using Prokka 1.11.[6] Contigs with *mcr-1* were identified using BLASTN and SeqFinder was used to establish the presence of antimicrobial resistance (AMR) and virulence genes (Table 2). PlasmidFinder was used to determine plasmid compatibility type[7] and BRIG[8] was used for plasmid comparisons. WGS data were deposited in the sequence read archive (SRA; Table 2) and used for comparison against all publicly available *mcr-1* sequences in the SRA and NCBI (Table S1, available as Supplementary data at *JAC* Online).

Results

Laboratory investigations

All *E. coli* recovered from abattoir samples (2013–15) were tested for the presence of *mcr-1* by PCR,[1] RT–PCR or WGS. The 200 *E. coli* recovered from MacConkey plates from 2015 were negative by PCR, as were the WGS available for the 190 ESBL *E. coli* from 2013, scanned for the presence of *mcr-1* using SeqFinder4 (Table 1). However, two *mcr-1*-positive isolates were identified following selective culturing and RT–PCR of caecal samples taken from pigs at slaughter in 2015 and originating from two separate anonymized farms (Table 1). Of the 556 *E. coli* isolates from 2014–15, 63 *E. coli* showing a colistin-resistant phenotype were tested by RT–PCR, and all 63 were *mcr-1*-negative. Testing of 163 clinical *E. coli* identified 4 colistin-resistant isolates, which were *mcr-1* positive, originating from two pig farms; 3 were from one farm and 1 (E4), already reported,[4] was from a second farm. The unadjusted herd prevalence estimates for *mcr-1* in *E. coli* considering these different sample and isolate collections were between 0% and 1.9% (Table 1).

WGS analysis of mcr-1-positive isolates

WGS was performed on five *mcr-1*-positive isolates (excluding E4), three of which were clones from the same farm. Three isolates, one representative of each farm, were analysed further. Two of these harboured non-synonymous SNPs in other genes associated with colistin resistance, as well as *mcr-1* (Table 2). All isolates harboured multiple AMR genes, suggesting MDR, and multiple plasmid Inc types, suggesting multiple plasmids were present, which was confirmed by plasmid profiling (Figure S1). None of the *mcr-1*-containing plasmids harboured other AMR genes and they varied in size from 32.7 to 91.2 kb. Plasmids of the size predicted from WGS analyses were identified in all isolates by plasmid profiling (Figure S1). A variety of virulence genes were detected, with the *E. coli* O149:H10 clinical isolate harbouring a heat-stable toxin gene (*stb*), as well as *eaeH* and *eltA-B*, which have been linked with disease in pigs caused by enterotoxigenic *E. coli*.[9,10] One isolate carried *mcr-1* on a pO111-like plasmid, one isolate on an IncI2 plasmid and another on an IncX4 plasmid; the IS*ApI1* element was only detected in the pO111-like plasmid (Figure S2).

Comparison of mcr-1 plasmid sequences

The three *mcr-1* plasmids from *E. coli* in pigs shared varying degrees of similarity to pHNSHP45,[1] with the highest being 90% in isolate PO169, which had the same plasmid incompatibility group (pPO169; Table 2 and Figure S2). Plasmid pPO169 also shared 99% identity with pS3,[4] but further comparison indicated pP169 had lost ∼3 kb of its plasmid genome compared with pS3. pPO169 also had 96% identity with a GB human isolate[11] and ≥97% identity with isolates from China[12] and Malaysia (Table S1 and Figure S3).

Table 2. AMR and virulence genes present in four *mcr-1*-positive isolates from WGS

Isolate and origin	Inc-types present	Estimated size of *mcr-1*-containing plasmid (kb)[a]	Similarity to pHNSHP45 (%)	Maximum similarity to publicly available *mcr-1* plasmids (%)	Colistin resistance genes	Other AMR genes	Virulence genes
• Clinical isolate[c] • O149:H10 • 2015 veterinary submission	Inc1, **IncX4**, IncFII(pCoo), IncFIB(A-P001918), IncFIC(FII), IncY	32.7	28	99	*phoP*[b], *phoQ*[b], *pmrA*[b], *etk*[b], *mcr-1*	*aadA1b*, *ant3-Ia*, *dfrA1*, *folP*[b], *sul2*	*aec15-19*, *aec22-27*, *aec29-32*, *astA9*, *cah*, *eaeH*, *ecpA-E*, *ecpR*, *ehaB*, *eltA-B*, *espL4*, *espR1*, *faeC-E*, *faeG-J*, *fimF-G*, *hlyA-E*, *ibeB-C*, *ItcA*, *stb1*, *shf*
• PO155[c] • –:H56 • 2015 surveillance study	Incl1, Col8282, **pO111**, IncX1	91.2	19	90	*mcr-1*	*aac3-IVa*, *aadA2*, *ant3-Ia*, *aph3-Ib*, *aph4-Ia*, *aph6-Id*, *blaTEM-1*, *cml*, *dfrA12*, *inuF*, *sul2*, *tetA*	*aec19*, *aec32*, *astA*, *ecpA-B*, *ecpD-E*, *ecpR*, *espL1*, *espL4*, *espX5*, *fimB-C*, *fimF-G*, *fimI*, *hlyE*, *iss*, *iucA*, *sitA-C*
• PO169[c] • –:H2 • 2015 surveillance study	IncX1, **IncI2**, IncFII(pCoo), IncB/O/K/Z	59.2	90	99	*acrR*[b], *phoP*[b], *mcr-1*	*blaTEM-1*, *gyrA*[b], *qnrS1*, *tetA*	*aec31-32*, *ecpA-E*, *ecpR*, *espR1*, *fimA-C*, *fimE-I*, *hlyE*, *ibeC*, *iss*, *mchF*, *tia*

Bold formatting indicates the Inc type of the *mcr-1*-harbouring plasmid.
[a]Estimated from size of *mcr-1*-containing contigs present in WGS and plasmid profiling, as described previously.
[b]Indicates the presence of non-synonymous SNPs on chromosomal genes that may result in AMR.
[c]WGS accession number PRJEB13576.

The IncX4 plasmid present in *E. coli* O149:H10 also had ≥96% identity with *mcr-1*-plasmid-bearing contigs from human isolates from GB,[11] the USA, China,[12] Brazil,[13] Italy[14] and South Africa[15] in addition to Danish[16] and French[17] meat products, Estonian pig slurry and a Swiss environmental isolate (Figure S4).[18] pPO155 showed 99% identity with pE4;[4] however, pE4 had lost ∼12.7 kb, which probably resulted from recombination events and demonstrates elasticity of the plasmid genome (Figure S5). The second most similar was a meat isolate from Denmark, which showed 90% identity.[16]

The area around *mcr-1* is conserved in the IncX4 and IncI2 plasmids, but neither harboured the preceding IS element seen in pHNSHP45[1] (Figures S2–S4), and the *mcr-1* gene may be stably integrated. Furthermore, the IncI2 *mcr-1* plasmid was highly similar to ones found in *E. coli*, *Salmonella enterica* and *Kluyvera ascorbata*, with sizes ranging from ∼57 to 65 kb (Figure S3). However, the pO111-type plasmids, pPO155 and pE4,[4] both contained the IS*ApI1* element (Figure S5), so the *mcr-1* gene may be unstable and mobilized from these plasmids.

Discussion

The presence of the plasmid-mediated *mcr-1* gene was reported in humans in England and Wales and from a pig farm in GB,[4,11] shortly following its first description.[1] This study describes further investigation of a large number of archived samples held at APHA to investigate the occurrence of *mcr-1* in *E. coli*. *mcr-1 E. coli* was detected on two pig farms in GB through anonymized surveillance of 387 caecal samples collected in 2015 from pigs at slaughter originating from 313 different herds; thus 0.6% of those pig herds sampled were considered to be positive on the basis of this sample set. *E. coli* positive for *mcr-1* were also detected on 2/105 (1.9%) pig farms from which archived *E. coli* isolates were available from veterinary diagnostic investigations performed in 2015/16. The surveillance of pigs at slaughter was anonymized and therefore it is unknown whether the two herds detected were epidemiologically related to those detected through examination of isolates from veterinary diagnostic submissions. The transferable colistin resistance gene *mcr-1* was, however, detected in only a small number of pig herd samples in GB. Although only single or limited numbers of caecal samples were screened from individual herds, detailed studies of two positive pig herds (L. Randall, unpublished results) indicated that a high proportion of animals carried *E. coli* with *mcr-1*, albeit as a minor component of the intestinal microbiota, so the estimate of herd prevalence is likely to be realistic, and colistin is not used on the majority of British pig farms.

WGS was used to characterize *mcr-1* plasmids from three positive isolates from separate farms. High sequence similarity was found between the three *mcr-1*-harbouring plasmids in *E. coli* from GB pigs and other *mcr-1* plasmids from within the UK and internationally, including in different bacterial species, and from both livestock and humans.[11,12,15–17,19] The IncX4 *mcr-1* plasmid from this study was highly similar to that found in *E. coli*, *Klebsiella pneumoniae* and *Salmonella* Typhimurium, isolated from humans, meat products and pig slurry between 2012 and 2015 globally, indicating the dissemination of a stable plasmid.[11–18] The variable presence of the IS element preceding the *mcr-1* gene possibly indicates that the *mcr-1* gene is transmitting in different ways, either via the IS element or through plasmid dissemination. With release of more WGS data from isolates carrying *mcr-1*, further comparisons between plasmids can be completed, which will facilitate the understanding of *mcr-1* plasmid transfer and global dissemination in enteric bacteria from humans, livestock and the food chain.

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Transparency declarations

None to declare.

Disclaimer

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health or Public Health England.

Supplementary data

Supplementary data, including Table S1 and Figures S1 to S5, are available at *JAC* Online (http://jac.oxfordjournals.org/).

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