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Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015

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The plasmid-mediated colistin resistance gene, *mcr-1*, was detected in an *Escherichia coli* isolate from a Danish patient with bloodstream infection and in five *E. coli* isolates from imported chicken meat. One isolate from chicken meat belonged to the epidemic spreading sequence type ST131. In addition to IncI2*, an incX4 replicon was found to be linked to *mcr-1*. This report follows a recent detection of *mcr-1* in E. coli from animals, food and humans in China.

Very recently, in November 2015, *Liu et al.* reported the finding of a transferable plasmid-mediated colistin resistance gene, *mcr-1*, detected in *Escherichia coli* isolates from animals, food and patients in China. Moreover, they found *mcr-1* in *Klebsiella pneumoniae* isolates from patients [1]. Horizontal gene transfer represents a paradigm shift in colistin resistance, which until then only was found to be mediated by chromosomal mutations and thus spread by vertical transmission.

National surveillance of antimicrobial resistance in food animals, food and humans in Denmark using whole genome sequencing

Since 2012, the national surveillance of antimicrobial resistance in food animals, food and humans in Denmark (www.DANMAP.org) has used whole-genome sequence (WGS) analysis for detection of resistance genes and multilocus sequence typing (MLST) using the open-access bioinformatic web-tools ResFinder and MLST, respectively from www.genomicepidemiology.org for characterisation of extended spectrum beta-lactamase (ESBL)- and AmpC-producing *E. coli* isolates [2-4]. The *mcr-1* sequence from China was added on 24 November 2015 to the ResFinder database as soon as it was available from The National Center for Biotechnology Information (NCBI).

Investigation of presence of *mcr-1* in *E. coli* isolates from food animals, food and human bloodstream infections

The updated version of ResFinder was used to analyse the WGS data from ESBL- and AmpC-producing *E. coli* isolates from food animals and food for the years 2012 to 2014, as well as ESBL- and AmpC-producing *E. coli* isolates from human bloodstream infections, and carbapenemase-producing organisms (CPOs) from humans, from January 2014 to beginning of November 2015 (Table 1), for the presence of *mcr-1*. Furthermore, fluoroquinolone resistance determinants were investigated by searching manually for mutations in the GyrA, ParC and ParE. [5].

The *mcr-1* gene was detected in one *E. coli* isolate from a human bloodstream infection from 2015 and in five *E. coli* isolates obtained from chicken meat of European origin imported to Denmark from 2012, 2013 and 2014 (Table 2). None of the CPOs were positive for *mcr-1* (Table 1).

The patient infected with the *mcr-1*-positive *E. coli* was an elderly man with prostate cancer and repeated urinary tract infections with ESBL-producing *E. coli* resulting in four positive urine samples over five month prior to the bloodstream infection, all resistant to third generation cephalosporins, gentamicin, sulfamethoxazole, trimethoprim and ciprofloxacin (these isolates were not

Table 1

Numbers of extended spectrum beta-lactamase and AmpC-producing *E. coli* isolates obtained and analysed by WGS from chicken meat, humans and carbapenemase-producing isolates from humans tested for *mcr-1* using ResFinder, Denmark, November 2015 (n=914)

Isolate origin	No. of isolates analysed by WGS	No. of sequences positive for *mcr-1*
ESBL- and AmpC-producing *E. coli* isolates from Danish chicken meat (2012–2014)	125	0
ESBL- and AmpC-producing *E. coli* isolates from imported chicken meat (2012–2014)	255	5
ESBL- and AmpC-producing *E. coli* isolates from human bloodstream infections (January 2014– beginning of November 2015)	417	1
Carbapenemase-producing isolates from humans (January 2014–beginning of November 2015)	117	0

ESBL: extended spectrum beta-lactamase; No: number; WGS: whole-genome sequence.

Table 2

Genotypic characterisation of *mcr-1*-positive *E. coli* isolates, Denmark, November 2015 (n=6)

Isolate name	Origin	Year of detection	MLST	Resistance genes detected by ResFinder besides *mcr-1*	Detection of chromosomal mutations encoding resistance to quinolones
0412016126	Chicken meat	2012	ST359	*aadA1*, *aadA5*, *aph(3')-Ic*, bla_{CMY-2}, bla_{TEM-1B}, *dfrA1*,*strA*, *strB*, *sul1*, *sul2*, *tet*(B)	GyrA (S83L, D87N) ParC (E62K)
0412044854	Chicken meat	2012	ST48	*aadA1*, bla_{CMY-2}, bla_{TEM-1B}, *dfrA1*, *mph(B)*, *strA*, *strB*, *sul1*, *sul2*, *tet*(A)	GyrA (S83L)
0412049521	Chicken meat	2012	ST131	*aadA1*, bla_{SHV-12}, bla_{CMY-2}, *strA*, *strB*, *sul1*, *dfrA1*, *tet*(A)	ND
0413040864	Chicken meat	2013	ST1112	*aadA1*, *aadA2*, bla_{SHV-12}, *cmlA1*, *sul3*, *tet*(A)	ND
14042624	Chicken meat	2014	ST2063	*aadA1*, *aadA2*, bla_{SHV-12}, *cmlA1*, *sul3*	ND
ESBL20150072	Human, bloodstream infection	2015	ST744	*aadA5*, bla_{CMY-2}, $bla_{CTX-M-55}$, bla_{TEM-1B}, *catA1*, *dfrA17*, *floR*, *fosA*, *mph*(A), *rmtB*, *strA*, *strB*, *sul1*, *sul2*, *tet*(A)	GyrA (S83L, D87N) ParC (E62K)

MLST: multilocus sequence typing; ND: not detected.

investigated further). He had been treated empirically with piperacillin/tazobactam and subsequently meropenem after susceptibility testing of the bloodstream isolate, but not with colistin according to the available patient data.

Besides *mcr-1*, the human isolate from the Danish patient contained 15 different resistance genes including $bla_{CTX-M-55}$ and bla_{CMY-2} conferring resistance to extended-spectrum beta-lactam antibiotics as well as two GyrA mutations (S83L, D87N) and a ParC mutation (E62K) leading to high-level fluoroquinolone resistance (Table 2). The human *mcr-1* positive *E. coli* isolate belonged to ST744, a rare sequence type in both humans and animals in Denmark. The patient had not been travelling abroad recently and the origin of the isolate is unknown.

The bla_{CMY-2} gene was detected in three of the five *mcr-1*-positive chicken meat isolates. In addition, three of the chicken meat *E. coli* isolates carried bla_{SHV-12} conferring resistance to extended-spectrum beta-lactam antibiotics excluding cephamycins.

One of the *mcr-1* positive *E. coli* isolates from chicken meat belonged to ST131. The other chicken meat isolates belonged to sequence types not frequently found in Denmark (Table 2). The human MCR-1-producing *E. coli* isolate was only susceptible to piperacillin/tazobactam, carbapenems and tigecycline according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [6], whereas the chicken meat isolates were less resistant (Table 3).

WGS analysis using the web-tool PlasmidFinder [9] identified an IncI2 replicon present in the human isolate as well as in three of the chicken meat isolates, but this replicon was not detected in the remaining two chicken meat isolates. De novo assembly using CLCbio Genomics Workbench (v8.5.1; Qiagen, Aarhus,

Table 3

Antimicrobial susceptibility profiles of the five MCR-1-producing *E. coli* isolates from chicken meat and the MCR-1-producing *E. coli* isolate from human bloodstream infection, Denmark November 2015

Origin	Human		Chicken meat									
Isolate name	ESBL20150072		14042624		0413040864		0412049521		0412016126		0412044854	
Antimicrobial agent	MIC (mg/L)	S/R	MIC (mg/L)	S/R	MIC (mg/L)	S/R	MIC (mg/L)	S/R	MIC (mg/L)	S/R	MIC (mg/L)	S/R
Polymyxins												
Colistin	>4	R	>4	R	>4	R	>4	R	>4	R	>4	R
Polymyxin B[a]	4	R	>4	R	4	R	>4	R	>4	R	4	R
Beta-lactam/beta-lactam inhibitor combinations												
Ticarcillin/clavulanic acid	128/2	R	>128/2	R	≤16/2	S	64/2	R	64/2	R	≤16/2	S
Piperacillin/tazobactam	≤8/4	S	≤8/4	S	≤8/4	S	≤8/4	S	≤8/4	S	≤8/4	S
Cephalosporins												
Cefotaxime	>32	R	8	R	8	R	8	R	8	R	4	R
Ceftazidime	>16	R	>16	R	8	R	>16	R	16	R	8	R
Cefepime	>2	R	≤2	S	≤2	S	≤2	S	≤2	S	≤2	S
Monobactams												
Aztreonam	>16	R	>16	R	>16	R	>16	R	8	R	8	R
Carbapenems												
Ertapenem	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Meropenem	≤1	S	≤1	S	≤1	S	≤1	S	≤1	S	≤1	S
Imipenem	≤1	S	≤1	S	≤1	S	≤1	S	≤1	S	≤1	S
Doripenem	≤0.125	S	≤0.125	S	≤0.125	S	≤0.125	S	≤0.125	S	≤0.125	S
Aminoglycosides												
Gentamicin	>8	R	≤1	S	2	S	≤1	S	≤1	S	≤1	S
Tobramycin	>8	R	≤1	S	2	S	≤1	S	2	S	≤1	S
Amikacin	>4	R	≤4	S	≤4	S	≤4	S	≤4	S	≤4	S
Fluoroquinolones												
Ciprofloxacin	>2	R	≤0.25	S	≤0.25	S	≤0.25	S	>2	R	≤0.25	S
Levofloxacin	8	R	≤1	S	≤1	S	≤1	S	>8	R	≤1	S
Tetracyclines												
Doxycycline[b]	8	I	≤2	S	16	R	8	I	16	R	8	I
Minocycline[b]	4	S	≤2	S	4	S	≤2	S	16	R	≤2	S
Glycylcyclines												
Tigecycline	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Folate pathway inhibitors												
Trimethoprim/ sulfamethoxazole	>4/76	R	≤0.5/9.5	S	≤0.5/9.5	S	>4/76	R	>4/76	R	>4/76	R

R/S according to the European Committee on Antimicrobial Susceptibility (EUCAST) clinical breakpoints [6].
I: intermediate; MIC: Minimal Inhibitory Concentration; R: resistant: S: sensitive.
[a] Breakpoint according to Société Francaise de Microbiologie [7]
[b] R/I/S according to The Clinical and Laboratory Standards Institute (CLSI) guidelines (M100-S25) [8].

Denmark) of the genomic data produced a direct link between the *mcr-1* gene and an IncX4 replicon in one of the two isolates not containing IncI2 replicons. An identical IncX4 replicon was detected in four of the chicken meat isolates including both isolates lacking an IncI2 replicon (but not in the human isolate).

Discussion and conclusion

Here we describe a MCR-1 producing *E. coli* isolate from a human infection coproducing both an ESBL (CTX-M-55) and an AmpC (CMY-2) cephalosporinase as well as five MCR-1 producing *E. coli* from chicken meat coproducing either and ESBL (SHV-12) or an AmpC (CMY-2) cephalosporinase, or both. Human and animal CTX-M-55-producing isolates are commonly reported from Asia [10,11], but are relatively rarely seen in Denmark. CTX-M-55-producing *E. coli* isolates were detected in only 3% of the *E. coli* from bloodstream infections in 2014 [4]. CMY-2-producing *E. coli* isolates have commonly been detected from chicken meat both in Denmark and other countries [2-4,12,13], but bla_{CMY-2} has been relatively rare in the Danish human bloodstream *E. coli* isolates. Similarly, only two of the 245 human bloodstream *E. coli* isolates from 2014 were

SHV-12-producing [4]. Based on antibiogram data it seems plausible that the bloodstream infection is related to the repeated urinary tract infections, but this will need to be confirmed by additional WGS analysis. At this point in time, the origin of the human isolate is unresolved.

MLST analysis did not show any close clonal relationship between any of the six isolates. However, one of the chicken meat isolates belonged to ST131. This sequence type is commonly associated with human *E. coli* urinary tract and blood infection isolates worldwide, but are rare in animal *E. coli* isolates [4,14,15]. The fact that a ST131 MCR-1-producing *E. coli* isolate was found is of special concern, since ST131 isolates have spread epidemically during the last decade [14,15] and the ability of *mcr-1* to be acquired by this sequence type has been demonstrated here.

The *mcr-1* gene was initially reported to be located on an IncI2 plasmid without other known resistance markers [1]. Here only four of the isolates were found to contain an IncI2 replicon, suggesting that the *mcr-1* gene was either located on the chromosome or on a plasmid type belonging to another group. In support of the latter is the fact that *de novo* assembly of the genomic data from one of the isolates produced a continuous DNA fragment containing both an IncX4 and the *mcr-1* gene, strongly suggesting that the *mcr-1* gene is not restricted to the IncI2 plasmid group, but additional studies are needed to clarify this further.

In conclusion, this study is to our knowledge, the first proof of colistin-resistant *mcr-1* positive *E. coli* outside China. The human isolate was only susceptible to very few antimicrobial classes such as carbapenems. Should an isolate like this acquire carbapenem resistance, it would leave very few, if any, suitable treatment options. Finally, our findings underline the importance of continuous microbiological surveillance programs and not the least the benefit of employing comprehensive WGS-based surveillance of antimicrobial resistance, as it allows for rapid re-analysis of large datasets *in silico* and thus make early detection and risk assessment possible when new resistance genes emerge.

*Authors' correction

Upon request of the authors, Christina Aaby Svendsen's name was corrected in the Acknowledgement section on 14 December 2015. In addition, the sentence "In addition to IncN2, an incX4 replicon was found to be linked to mcr-1." was corrected to read "In addition to IncI2, an incX4 replicon was found to be linked to mcr-1." on 16 December 2015 upon request of the authors.

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Conflict of interest

None declared.

Authors' contributions

HH and AMH collected the data and drafted the manuscript, HH, MS, PL, EZ and RK did the molecular analysis, FMA, FH, YA, RSH, LC, DSH, BO produced phenotypic data and participated in the coordination and concept of the manuscript, RLS coordinated and edited the manuscript.

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