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The first human report of mobile colistin resistance gene, *mcr-1*, in Finland

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Colistin resistance mediated by mobile *mcr-1* gene has raised concern during the last years. After steep increase in *mcr-1* reports, other *mcr*-gene variants (*mcr-2* to *mcr-5*) have been revealed as well. In 2016, a clinical study was conducted on asymptomatic stool carriage of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* among Finnish adults. All suspected ESBL producing bacterial isolates were first tested by phenotypic ESBL-confirmation methods, and then further analyzed with whole genome sequencing to identify the resistance genes. We found one study subject carrying a colistin resistant *E. coli* with a transferrable *mcr-1* gene. This multi-drug resistant isolate, although initially suspected to be an ESBL producer, did not carry any ESBL genes, but was proven to carry several other resistance genes by using whole genome sequencing. Sequence type was ST93. The *mcr-1* gene was connected to IncX4 plasmid which suggests that the colistin resistance gene locates in the respective plasmid. Here, we report the finding of a *mcr-1* harboring human *E. coli* isolate from Finland. Clinical antimicrobial resistance (AMR) rates are low in Finland, and mobile colistin resistance has not been reported previously. This highlights the importance of AMR surveillance also in populations with low levels of resistance.

Key words: antimicrobial resistance; carriage; *Escherichia coli*; colistin; mcr-1.

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The global spread of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBL) and carbapenemases (CPE) has limited the options of suitable antibiotics for the treatment of infections caused by these gram-negative bacteria. A new chapter in the era of emerging antimicrobial resistance started in 2015 when Liu et al. reported the first finding of transferable plasmid-encoded colistin resistance in *Enterobacteriaceae* isolated from humans and animals (1). Colistin is considered as the last resort of treatment against multi-drug resistant bacteria. Since the first report from China, several countries from five continents, have detected the *mcr-1* gene in *Enterobacteriaceae* isolated from humans, animals or the environment (2, 3). Sporadic findings of *mcr-1* carrying isolates have previously been reported from three other Nordic countries, namely Sweden, Norway and Denmark (4–6). After the first wave of *mcr-1* reports, several countries including Denmark from the Nordic countries, have identified isolates carrying new gene variants of *mcr* gene (currently *mcr-1* to *mcr-5*) (7, 8). In Finland, the overall antimicrobial resistance level has remained relatively low, and use of colistin is reserved for only very rare clinical indications. Now we report the first *Escherichia coli* isolate from Finland with colistin resistance mediated by *mcr-1* gene.

MATERIALS AND METHODS

During 2016, we conducted a prospective, clinical study to investigate asymptomatic fecal ESBL carriage in

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Southwest Finland as part of the Northern Dimension Antibiotic Resistance Study (NoDARS, http://www.ndphs.org/?database,view,project,1468). Fecal samples were collected from 176 healthy, anonymous volunteers, and the samples were screened for ESBL producing *E. coli* and *K. pneumoniae* from ESBL specific chromogenic media (unpublished data). Antimicrobial susceptibility testing (AST) was performed for all isolates according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (version 6.0, http://www.eucast.org/clinical_breakpoints/). Minimal inhibitory concentration for colistin was preliminarily determined with a gradient strip test (Liofilchem, Roseto degli Abruzzi, Italy) and confirmed by broth microdilution (Thermo Scientific™ Sensititre™ System, Thermo Scientific, East Grinsted, England). Strains with reduced susceptibility to third generation cephalosporins were tested for ESBL production with the combination disk test according to EUCAST recommendations.

To analyze the molecular mechanisms of ESBL, all phenotypically confirmed *E. coli* and *K. pneumoniae* ESBL isolates were analyzed using paired-ended (2 × 150 bp) whole-genome sequencing (WGS) with Illumina MiSeq platform. ResFinder (database version 2.1) (9) and *in silico* PCR, using previously reported primers (10), were used to identify the resistance genes and plasmid replicons from the WGS data. The reads of the *E. coli* isolate carrying *mcr-1* gene have been deposited in NCBI SRA database under the accession numbers SRR6656065.

RESULTS

During our clinical screening study (Ny et al. unpublished data), we decided to extend our analysis by whole genome sequencing all phenotypically confirmed ESBL isolates (*E. coli* and *K. pneumoniae*). The analysis revealed that one of the *E. coli* isolates carried the *mcr-1* gene. The WGS analysis also revealed that the only acquired β-lactam resistance genes found from this isolate were $bla_{TEM\text{-}1b}$ and $bla_{CMY\text{-}2}$.

Mcr-1 gene and the IncX4 replicon were connected in the de Bruijn graph of the assembly which was visualized with Bandage (11). This strongly suggests that *mcr-1* is located in an IncX4 plasmid. The close proximity region of *mcr-1* gene is illustrated in Fig. 1.

The strain belonged to sequence type (ST) 93. Colistin resistance was confirmed both by the gradient strip test and microdilution methods. All detected plasmid replicons, antimicrobial resistance genes and the AST panel of this isolate are listed in Table 1.

The *mcr-1* carrying *E. coli* was isolated from a fecal sample of a 26 year old healthy male. The exclusion criteria for the study included antibiotic treatment 3 months prior to sampling. Previous usage of antibiotics was not recorded. A background questionnaire revealed that he had travelled to Central Europe and South-America within 6 months before the sampling.

DISCUSSION

During the last years there has been a vast expansion of reports on findings of colistin resistant organisms in humans without prior colistin therapy or clonal transmission (12). Many of these arise from retrospective surveys that have screened past clinical or environmental sample collections for the *mcr-1* gene. Our finding was isolated from a healthy volunteer in 2016. Stool carriage of *mcr-1* gene has been reported from healthy individuals in Europe at least from the Netherlands, Switzerland and Sweden (4, 13, 14). Other reports are from China, Laos and Thailand (12). Of the Nordic countries, Norway and Denmark have both also reported of finding of *Enterobactericeae* isolates carrying *mcr-1* from clinical sample collections (5, 6). A recent publication from Norway, a Nordic country with very low usage of colistin, detected two ESBL producing *E. coli* isolates carrying *mcr-1* gene from environmental samples collected in 2010 (15).

Although the phenotypic characterization of the isolate reported here suggested it to be an ESBL and AmpC producer, it did not carry any classical

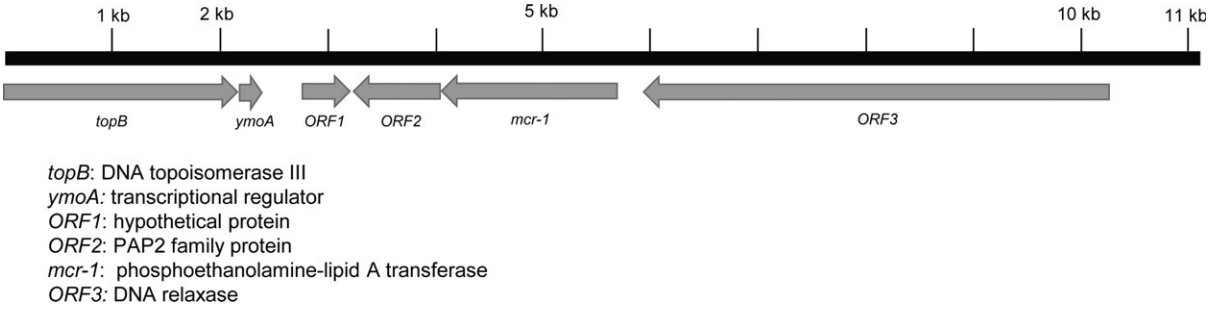

Fig. 1. Structure of the *mcr-1* gene region (SRA accession number SRR6656065).

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Table 1. Characteristics of the *Escherichia coli* isolate carrying *mcr-1* gene. Antimicrobial susceptibility testing was performed with disk diffusion method, in addition, MIC testing for fosfomycin with Etest and for colistin with Etest and Sensititre

Antimicrobial class	Antimicrobial agent	Zone diameter (mm) or MIC (μg/ml)	S/R[1]	Resistance genes		Combination disk test	Zone diameter (mm)	Plasmids
Penicillin	Ampicillin	6	R	*blaTEM*-1B, *blaCMY*-2	Beta-lactam resistance	Cefotaxime/ Cefotaxime + clavulanic acid	19/24	ColRNAI
	Amoxicillin clavulanic acid	11	R			Ceftazidime/ Ceftazidime + clavulanic acid	16/14	ColRNAI
2nd generation cephalosporin	Cefoxitin	10	R			Cefotaxim/ Cefotaxim + cloxacillin	23/32	IncFII
3rd generation cephalosporin	Cefotaxime	13	R			Ceftazidim/ Ceftazidim + cloxacillin	21/31	IncX4
	Ceftazidime	14	R					I1
Carbapenem	Meropenem	33	S					FIB
Fluoroquinolone	Ciprofloxacin	24	S	*qnrB19*	Quinolone resistance			
Trimethoprim	Trimethoprim	6	R	*dfrA8*	Trimethoprim resistance			
	Trimethoprim-sulfamethoxazole	6	R					
Aminoglycoside	Gentamicin	21	S					
Polymyxin	Colistin	3[2]/4[3]	R	*mcr-1*	Colistin resistance			
Other	Nitrofurantoin	22	S					
	Temocillin	27	–					
	Fosfomycin	0.38	S					
	Mecillinam	22	S					
				strA, strB	Kanamycin, neomycin, paromycin, streptomycin resistance			
				sul2	Sulfonamide resistance			
				floR	Florfenicol/ chloramphenicol resistance			

[1]Interpretation according to EUCAST clinical breakpoints. S = sensitive, R = resistant, – = no currently available breakpoint.
[2]As determined by Etest (μg/ml).
[3]As determined by Sensititre (μg/ml).

ESBL genes based on WGS analysis. However, the isolate can be considered as multi-drug resistant based on criteria by Magiorakos et al. (16). Instead of ESBL genes, the isolate carried a bla_{CMY-2} gene which encodes for a plasmid-mediated AmpC β-lactamase. This specific bla_{CMY-2} gene has especially been reported from animal isolates, mainly from poultry (17). ST-93 is found both from human and animal sources (https://enterobase.warwick.ac.uk/). The plasmid composition and location of the *mcr-1* gene in IncX4 resembles an isolate that has been reported from a pig in Austria (18). Recent studies from Sweden (19) and Norway (20) have identified close genetic relatedness of *E. coli* isolates carrying the bla_{CMY-2} gene both from human samples and retail poultry meat, which suggests a possible common source. The source of the Finnish *mcr-1 E. coli* isolate, however, remains unknown.

Antimicrobial resistance is routinely monitored from clinical bacterial isolates in Finland. Based on our latest nation-wide antimicrobial report (Finres 2016; http://urn.fi/URN:ISBN:978-952-302-958-3), antimicrobial susceptibility of *E. coli* isolates from bacteremic cases is still relatively good. Resistance to third generation cephalosporins is 5–6% in *E. coli* blood isolates. However, the rates have

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shown an increase during the last few years. National Institute for Health and Welfare has retrospectively screened all CPE isolates (n = 145) found from Finland between 2008–2015 for the *mcr-1* gene. No such isolates have been found (unpublished data). Our current *mcr-1* finding is not from a clinical case but from a healthy volunteer. AST for colistin is routinely often limited to the most resistant strains, *i.e.* those with reduced susceptibility to carbapenems. In the lack of an easy screening test, the presence of transferrable colistin resistance may remain undetected in strains fully susceptible to carbapenems.

We detected the presence of *mcr-1* only in WGS, which may prove a reliable means to detect transferrable colistin resistance. Our report clearly highlights the presence of transferrable colistin resistance among human non-ESBL producing *E.coli* isolates also in countries with proven history of low rates of antimicrobial resistance or colistin use.

ETHICS

The study was conducted in accordance with the Declaration of Helsinki and national and institutional standards. Written informed consent was obtained from all study participants. Study design was approved by Ethics Committee, Hospital District of Southwest Finland (ETMK 157/2015).

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TRANSPARENCY DECLARATIONS

None to declare.

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