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The first report of mobile colistin resistance gene (*mcr-1*) carrying *Escherichia coli* in Turkey

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Sir,

Colistin has recently regained great attention as a last resort antimicrobial drug playing a vital role to treat certain bacterial infections in humans [1]. A plasmid-mediated colistin resistance gene, *mcr,* was first reported in 2015 in China [2] which added more on the view of resistance mechanisms. Follow-up studies confirmed the high abundance of *mcr* positive *Enterobactriaceae* isolates, particularly *Escherichia coli*, from different locations of the world within various environments including humans, animals and foods of animal origin [1, 3-5]. In our study, we have characterized colistin resistant (ColR) *mcr*-carriying *E. coli* strains isolated from chicken meat samples for the first time in Turkey.

Four ColR *E. coli* strains (designated ID= A1, A5, A7 and A9) were isolated from different chicken meat samples (n=80), which were purchased from butchers and retail markets in two provinces (Hatay and Adana). All isolates were identified using MALDI-TOF (Bruker

Daltonics, Billerica, MA). Pulsed-field gel electrophoresis typing of ColR *E. coli* strains was performed by XbaI restriction. Antimicrobial susceptibility testings of ColR *E. coli* isolates were determined by Vitek 2 system and broth microdilution method was applied to determine the MIC of colistin. Total bacterial DNA extraction was done using commercial kits (Qiagen, Hilden, Germany). PCR was run to determine the presence of *mcr-1* gene as previously described [3]. Whole-genome sequencing (WGS) of the strains was performed using NextSeq500 sequencer (Illumina). Sequencing reads were assembled using SPAdes genome assembler and resulting contigs were annotated using Blast tool. Multilocus sequence types of strains, plasmid replicon types and resistance gene contents of plasmids were characterized *in silico* (http://www.genomicepidemiology.org/).

In the current study, a total of four *E. coli* strains (5%) displaying colistin MICs of >8 µg/ml were isolated from 80 chicken meat samples and all isolates were confirmed to harbor the *mcr-1* gene by PCR. To the best of our knowledge, this is the first report of *mcr-1* positive *E. coli* in Turkey. Among the four ColR *E. coli* isolates, PFGE revealed 3 different pulsotypes (Figure 1). One strain from each pulsotypes (A1, A5 and A9) as well as the plasmid of these strains was subjected to sequencing [Accession No (AN): QBRX00000000, QBRV00000000 and QBRW00000000]. The *mcr* genes were found in all three strains and they showed 100% nucleotide similarity with previously annotated *mcr-1* gene [2]. Plasmid sequencing of three strains (A1, A5 and A9) resulted in long contigs of multiple plasmids in each strain. For A9, 31203 bp plasmid sequence (Plasmid replicon type IncX4) was found to be almost completely covering plasmid pICBEC171Smcr harboring *mcr*-1 gene, which was previously isolated in Brazil (AN: CP021418.1). For A1 and A5, *mcr-1* carrying contigs were short assemblies that could not be scaffolded to large regions of neither plasmids nor chromosomes.

The analysis of WGS data revealed that *E. coli* strains A1 and A5 belonged to the same MLST type (ST-3941), although these strains were clustered separately by PFGE method, whereas the strain A9 was identified as ST-1049. Recently Corbella et al. [4] reported the *mcr-1* positive *E. coli* strain of type ST-3941 from human bloodstream infection in Italy. As suggested previously, this epidemiological relation also highlights the significance of likely transmission of ColR *E. coli* to humans via contaminated animal products. Interestingly, an mcr-producer belonging to the ST-1049 recovered from chicken was previously identified in Switzerland [5]. These findings might arise the hypothesis of possible dissemination of ColR *E. coli* from breeder flocks in Europe since the poultry industry in Turkey does not export any products to the European Union countries. In fact, the epidemiological situation for ColR *E. coli* requires further comprehensive investigation to predict potential spreading pathways.

Antimicrobial resistance patterns of these isolates were given in Figure 1. Of these four isolates, three strains (A1, A7 and A5) exhibited resistance against piperacillin, tetracycline, levofloxacin and ciprofloxacin and the strain A5 was also resistant to trimethoprim-sulphamethoxazole. The strain A9 was found to be resistant to piperacillin, ceftazidime, cefepime and aztreonam.

In addition to *mcr-1*, screening for other acquired resistance determinants revealed the presence of ten additional antibiotic resistance genes including $bla_{\text{TEM-1b}}$, *aadA5*, *aph(3")-Ib*, *abh(6)-Id*, *mphA*, *catA1*, *sul1*, *sul2*, *dfrA17* and *tetA* in strain A5, whereas A1 had six additional genes including $bla_{\text{TEM-1c}}$, *aph(3")-Ib*, *abh(6)-Id*, *floR*, *sul2* and *tetB*. On the other hand, the strain A9 only had $bla_{\text{CTX-M-15}}$ gene additionally.

In conclusion, we report the presence of *mcr-1* carrying strains for the first time in Turkey. Due to potential exposure to these bacteria via contaminated foods, colistin resistance could be potentially transferred to human microbiome and clinical pathogens in Turkey as well. Therefore, the environmental isolates needs to be studied carefully to monitor presence of mobile colistin resistance genes and to control the dissemination of *mcr* positive strains.

Declarations

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Competing Interests: None

Ethical Approval: Not required

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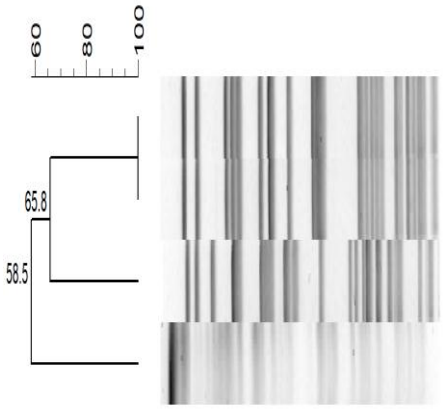

Strains (MLST type)	Antibiotic Resistance Profile	Resistance Genes
A1 (ST-3941)	PRL, TE, LEV, CIP, CT	*bla*$_{TEM-1c}$, *aph(3")-Ib*, *abh(6)-Id*, *floR*, *sul2*, *tetB*, *mcr-1*
A7 (ND)	PRL, TE, LEV, CIP, CT	ND
A9 (ST-1049)	PRL, FEP, ATM, CAZ, CT	*bla*$_{CTX-M-15}$, *mcr-1*
A5 (ST-3941)	PRL, TE, SXT, LEV, CIP, CT	*bla*$_{TEM-1b}$, *aadA5*, *aph(3")-Ib*, *abh(6)-Id*, *mphA*, *catA1*, *sul1*, *sul2*, *dfrA17*, *tetA*, *mcr-1*

Figure 1:PFGE profile and genetic characterization of mcr-1 positive *E. coli* isolates from chicken meat samples. ND: not determined; PRL: Piperacillin, TE: Tetracycline, SXT: Trimethoprim/Sulphamethoxazole, LEV: Levofloxacin, FEP: Cefepime, ATM: Aztreonam, CAZ: Ceftazidime, CIP: Ciprofloxacin, CT: Colistin.