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Plasmid-Borne Mobile Colistin Resistance (MCR-1) In Healthy Humans and Poultry

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ABSTRACT

Background and aim: The emergence of antimicrobial-resistant bacteria has become a menace to global public health and socio-economic development. Colistin is used as the last resort antibiotic in refractory infections. However, resistant isolates are reported frequently. The aim of this study was to find mcr genes in healthy people poultry farms in Sanandaj, west of Iran.

Methods and materials: In this study, faecal samples were collected from two groups of humans and poultry. Colistin-containing screening media as well as colistin-EDTA-containing medium to screen plasmid-mediated colistin resistance. PCR for prevalent mcr genes was performed. Antimicrobial susceptibility testing was done for MCR-harboring isolates.

Results: In this study, no colistin-resistant bacteria were isolated from poultry samples (0%) while two human E. coli isolates showed resistance to colistin (0.59%). Of which, one isolate was inhibited by EDTA and harboured the MCR-1 variant. The MIC for colistin was 16 mg/L.

Conclusion: The results indicated the low prevalence of this gene in healthy individuals and the poultry industry. To the best of our knowledge, this is the first report of MCR in healthy individuals from Iran.

1. Introduction

The emergence of bacteria resistant to antimicrobial agents has become a serious threat to global public health, healthy food and socio-economic development. Amongst antibiotic-resistant bacteria, carbapenems, and colistin have strongly challenged the antimicrobial treatment of life-threatening infections (Elbediwi et al., 2019; Sun et al., 2020) . Colistin as a polycationic antimicrobial peptide in polymyxins was introduced to the market in 1949 and used as the last resort treatment for refractory hospital-acquired infections caused by multidrug-resistant (MDR) gram-negative bacteria such as carbapenemase producing *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae* (Borowiak et al., 2017; Brennan-Krohn, Pironti, & Kirby, 2018; Elbediwi et al., 2019). The two polymyxins currently in use are polymyxin B and polymyxin E (colistin), which differ in only one amino acid but have different biological activity (Liu et al., 2016).

Prior to 2015, colistin resistance was thought to be due to mutations in chromosomal regulatory genes including *phoPQ*, *pmrAB*, and *mgrB*, and the discovery of plasmid-borne mobile colistin resistance (*mcr*) genes had a pronounced effect on antibiotic treatment regimens (Liu et al., 2016). The *mcr* genes have been identified in a wide range of plasmid types such as lncl2, lncHl2, lncX4, lncP, lncF, and lncY. Bacteria carrying these genes have been isolated from pigs, poultry, cattle, and food products from these animals, as well as from environmental sources such as hospital wastewater, rivers, and seas (Elbediwi et al., 2019). Many isolates carrying mcr-1 also carry other different resistance genes, such as carbapenemases (Sonnevend et al., 2016). The mobile colistin resistance gene, *mcr-1*, has attracted worldwide attention because of its polymyxins degradation, one of the antibiotics to treat severe clinical infections caused by multidrug-resistant gram-negative bacteria (Yin et al., 2017).

Colistin resistance in *Enterobacteriaceae* may be due to chromosomal mechanisms or the acquisition of motile plasmids carrying colistin resistance (*mcr*) genes. Since the first mcr gene, *mcr*-1, was reported in 2015 in *Escherichia coli* strain SHP45, isolated from farmed pigs in China, several other *mcr* genes, including *mcr*-2, *mcr*-3, mcr-4, *mcr* -5, *mcr*-6, *mcr*-7, *mcr*-8, *mcr*-9, in *Enterobacteriaceae* as well as *mcr*-1, and *mcr*-4 in Acinetobacter, and *mcr*-9 recently reported in Salmonella enterica *Typhimurium* serotype (Carroll et al., 2019; Franklin et al., 2020; Liu et al., 2016; Wang et al., 2020). All Mcr proteins are phosphoethanol amine transferases that catalyze the binding of phosphoethanol amine to lipid A lipopolysaccharide, and by reducing the negative charge on the outer layer of the LPS of gram-negative bacteria as well as structural changes in lipid A lead to colistin resistance. Interestingly, these enzymes are inhibited by ethylene diamine tetra-acetic acid (EDTA), which is a way to characterize them in clinic (Borowiak et al., 2017; Kieffer, Aires-de-Sousa, Nordmann, & Poirel, 2017).

The aim of this study was to identify *mcr* genes in healthy people who applied for a health card as well as poultry farms in Sanandaj, western part of Iran.

1. Methods and Materials

2.1. Sampling method, culture media and screening method

In this cross-sectional study, during 2020, fecal samples were collected from humans and poultry. By referring to the Central Health Laboratory and Central Poultry Slaughterhouse in Sanandaj city located in the western region of Iran, 339 human feces and 400 poultry rectal swabs were collected.

Culture media used included i) Nutrition Broth (Himedia, India) as enrichment media, ii) MacConkey (Himedia, India) containing 3.75 mg/L colistin (Sigmaaldrich, Germany) as the first screening medium, iii) cation adjusted Müller Hinton Broth (CAMHB) (Himedia, India) containing variable doses of colistin as the minimum inhibitory concentration (MIC) determination media, and, iv) CAMHB containing and 4 mg/L colistin and 1 mM EDTA, as phenotypic identification of *mcr* genes (Bell et al., 2019).

2.2Antimicrobial susceptibility testing

To determine the MIC of colistin, CAMHB with different concentrations of colistin (0.12-64 mg/L) was used by broth microbroth-dilution method (CLSI, 2012). Isolated colonies from MacConkey were subjected to define colistin resistance MIC. Briefly, a freshly bacterial suspension equivalent to 0.5 McFarland was prepared and with the final concentration of 5×10^5 CFU/mL were inoculated into the CAMHB-colistin medium. The last clear well was considered as the MIC of the colistin. To evaluate the resistance to other antibiotics, Vitek2 instrument (BioMérieux, Marcy l'Etoile, France) was used. *Escherichia coli* strain ATCC

25922 was used as a negative control. The results were interpreted according to CLSI 2020 (CLSI, 2020).

2.3. Phenotypic detection of *mcr* genes

Since Mcr enzymes are inhibited by chelating agents, EDTA is used for their phenotypic detection (Bell et al., 2019). In this method, CAMHB containing 4 mg/L colistin and 1 mM EDTA are used. From the bacterial isolates grown in the screening stage, 0.5 McFarland suspension was prepared and inoculated the tubes with a final concentration of 5×10^5 CFU/mL. Lack of bacterial growth in this medium indicates resistance to plasmid-carrying colistin.

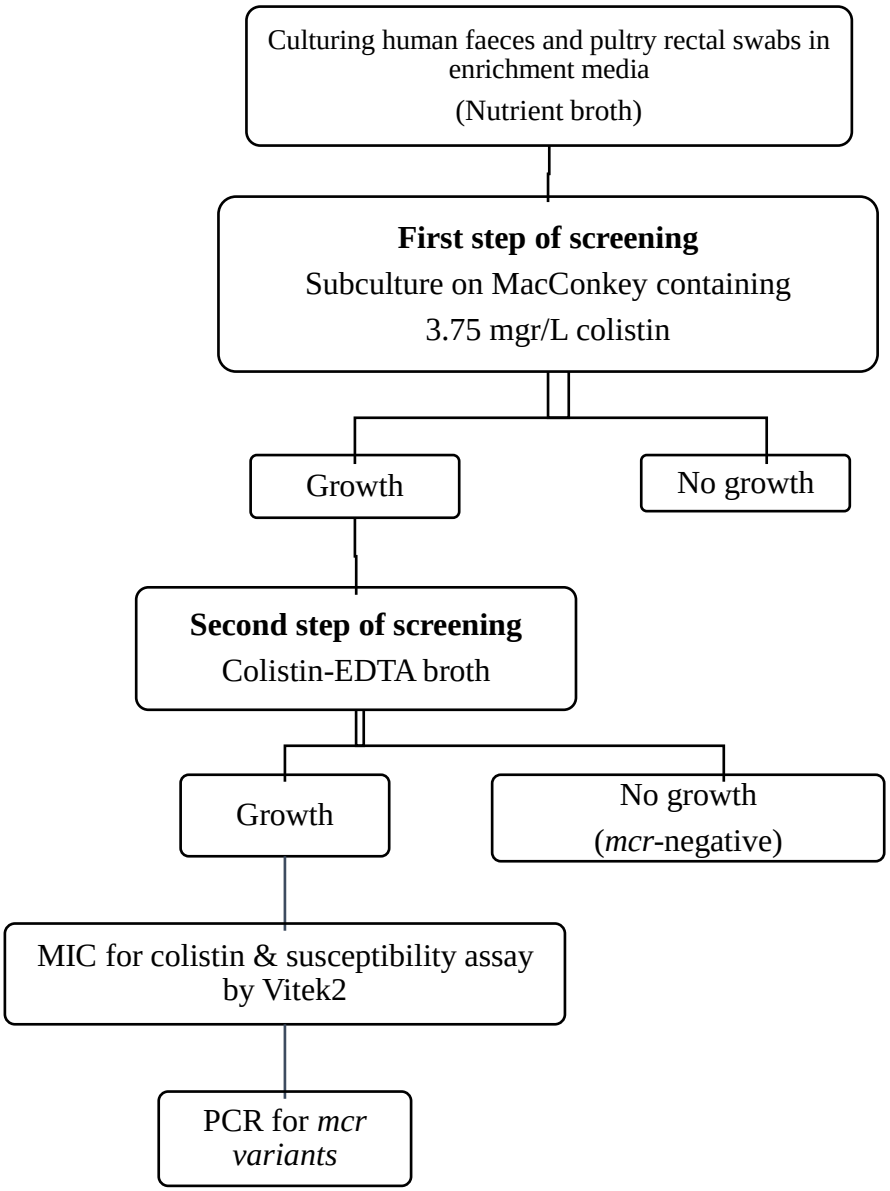

Figure 1: Flowchart of detecting mcr containing isolates

2.4. Genotypic detection of *mcr* variants and sequencing

DNA was extracted from isolated colonies in the screening stage by boiling. Mastermix (Parstous, Iran) was used and PCR test was performed in the final volume of 20 µL with *mcr* primers (Table 1) (Jousset et al., 2019). PCR products were run on 1% gel and finally photographed. PCR product was sequenced by PCR primers and results were embedded in national center for biotechnology, nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find the exact match.

Table 1: Primers used in this study

Primer name	Sequence (5' → 3')	Target	Size (bp)
Multi mcr-1-/2-Fw	TATCGCTATGTGCTAAAGCCTG	*mcr*-1	1139
Multi mcr-1-Rv	CGTCTGCAGCCACTGG		

Multi mcr-1-/2-Fw	TATCGCTATGTGCTAAAGCCTG	*mcr*-2	816
Multi mcr-2-Rv	AAAATACTGCGTGGCAGGTAGC		
Multi mcr-3-Fw	CAATCGTTAGTTACACAATGATGAAG	*mcr*-3	676
Multi mcr-3-Rv	AACACATCTAGCAGGCCCTC		
Multi mcr-4-Fw	ATCCTGCTGAAGCATTGATG	*mcr*-4	405
Multi mcr-4-Rv	GCGCGCAGTTTCACC		
Multi mcr-5-Fw	GGTTGAGCGGCTATGAAC	*mcr*-5	207
Multi mcr-5-Rv	GAATGTTGACGTCACTACGG		

3 Results

3.1. Phenotypic detection of *mcr* harboring bacteria

In this study, contrary to expectations, excluding Proteus species that are intrinsically colistin-resistant, no colistin-resistant bacteria were isolated from poultry samples (0%). In human samples, two samples showed resistance to colistin (0.59%), both of which were isolated from *E. coli*. Of which, one isolate of human colistin-resistant sample was inhibited by EDTA. The person carrying the bacterium was a baker and had not the history of working in livestock, poultry industry or even a hospital stays in the past three years.

3.2. Genotypic detection of *mcr* genes

PCR results showed that one *E. coli* from human sample had *mcr*-1 variant. The rest of the *mcr* genes were not detected. The relevant band was sequenced to ensure the specificity of PCR. The sequence results were completely consistent with the *mcr*-1 variant.

3.3. Antimicrobial resistance pattern of *E. coli* encoding *mcr*-1

E. coli carrying *mcr*-1 gene was sensitive to all other antibiotics except colistin and ampicillin. The MIC for colistin and ampicillin were 16 mg/L and 64 mg/L, respectively.

4 Discussion

The aim of this study was to identify *mcr* in healthy individuals as well as farmed poultry from Sanandaj, west of Iran. The results indicated the low prevalence of this gene in healthy individuals and poultry industry. The only human specimen that contained bacteria carrying the *mcr-1* gene. Because colistin is used extensively in livestock and poultry (Rhouma, Beaudry, Thériault, & Letellier, 2016), it was expected to be more prevalent in poultry. To the best of our knowledge, this is the first report of *mcr* in healthy individuals from Iran.

In Iran, several studies have been conducted on the prevalence of these genes. One study was performed in southwest of Iran, which was conducted in 2019 (Moosavian & Emam, 2019). In this study, eight enterobacterial containing *mcr*-1 gene were identified from 712 clinical samples. Of which, six isolates were *E. coli* and two isolates were *K. pneumoniae*. From livestock and sewage was also *mcr*-1 detected in *E. coli* (Nikkhahi et al., 2021). In contrast to our study, all of previous projects detected *mcr* in animals rather than healthy humans.

In the Middle East and neighboring countries of Iran, the first case of this gene was reported in the Arabian Peninsula, all of which in *E. coli*. There were two isolates from Bahrain, one from Saudi Arabia and one from the United Arab Emirates (Sonnevend et al., 2016). In another study conducted in Oman in 2018 (Mohsin et al., 2017) an *E. coli* isolate was identified among colistin-resistant bacteria, a sample of which was from 2016 and was isolated from a patient blood culture. On the other hand, in Turkey, in 2018, the *mcr*-1 gene in *E. coli* was identified (Kurekci, Aydin, Nalbantoglu, & Gundogdu, 2018). In 2019, a case of *E. coli* carrying *mcr*-1 was also detected in Syrian refugee living in Lebanon (Sulaiman & Kassem, 2019).

E. coli seems to have a special potential to receive the plasmid harboring *mcr* genes. This plasmid was mostly reported in the IncH group (Elbediwi et al., 2019). The type of plasmid was not investigated in the *E. coli* isolated in our study. On the other hand, the sequence type (ST) of this bacterium was not characterized. An important difference between our strains and other studies was that most strains isolated in different parts of the world were resistant to a variety of antibiotics, while our isolates were sensitive to most antibiotics. Examination of the plasmid carrying this gene could provide a new perspective on how this gene was distributed in western Iran. Further studies are needed in this regard.

5 Conclusion

The results of this study indicated the low prevalence of *mcr* genes in healthy individuals and poultry industry. To the best of our knowledge, this is the first report of *mcr* in healthy individuals from Iran.

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