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ORIGINAL RESEARCH PAPER

Detection of colistin resistance among multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* clinical isolates in Turkey

NILGÜN KANSAK[1*], SEBAHAT AKSARAY[2], MÜGE ASLAN[1], RIZA ADALETI[1] and NEVRIYE GÖNÜLLÜ[3]

[1] Medical Microbiology Laboratory, Haydarpaşa Numune Training and Research Hospital, University of Health Sciences, Istanbul, Turkey

[2] Department of Medical Microbiology, University of Health Sciences-Hamidiye Medical Faculty, Istanbul, Turkey

[3] Department of Medical Microbiology, Istanbul University-Cerrahpasa School of Medicine, Istanbul, Turkey

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ABSTRACT

In this study investigation of plasmid-mediated *mcr 1-5* resistance genes was performed among multidrug-resistant (MDR) colistin sensitive and resistant *Klebsiella pneumoniae* and *Escherichia coli* strains isolated in our laboratory. We aimed to evaluate automated system (Vitek-2), broth microdilution (BMD) reference method and chromogenic media performance. Totally 94 MDR *K. pneumoniae* and six *E. coli* isolates were included in the study. CHROMID® Colistin R agar (COLR) (bioMerieux, France) was used to determine the colistin resistance by chromogenic method. Standard PCR amplification was performed using specific primers to screen the plasmid-mediated *mcr 1-5* genes. Sixty-one isolates were resistant to colistin and 39 were susceptible with reference BMD. The essential and categorical agreement of Vitek-2 was determined as 100 and 99%. The sensitivity of COLR medium was 100%, the specificity was 97.5%. In our study *mcr-1* was detected in eight isolates, while other *mcr* genes were not detected. Due to the high sensitivity and specificity of the COLR medium, it can be used in routine diagnostics for the detection of colistin resistance. In our study we detected 8% prevalence of *mcr-1* among MDR strains however, two *mcr-1* positive isolates were found sensitive to colistin by BMD.

KEYWORDS

colistin, broth microdilution, resistance, COLR, *mcr 1-5*

INTRODUCTION

Colistin is a polycationic antibiotic isolated from *Paenibacillus polymyxa subsp. colistinus*, effective against Gram-negative bacteria [1]. Use of colistin was abandoned since the 1970s due to its nephrotoxic side effect. The dramatic increase of multidrug-resistant (MDR) Gram-negative bacterial infections, especially the emergence of carbapenem resistance, revived the use of colistin as a last option antibiotic.

Colistin resistance can be intrinsic or acquired. The most common cause of resistance development is mutation in genes encoding the regulatory system responsible for lipid A synthesis. As a result of mutation, negative charge of outer membrane and colistin binding are reduced. Mutations in the *PmrA-PmrB* or *PhoP-PhoQ* regulatory system are chromosomally encoded resistance mechanisms [2].

Plasmid-mediated colistin resistance encoded by *mcr-1* gene, was identified in an *Echerichia coli* isolate in China, in 2015. Despite there is no clinical use of colistin until 2017

*Corresponding author.
Tel.: +90 5057980003; fax: +90 2163360565.
E-mail: nilkansak@gmail.com

in China, identification of *mcr-1* and after a while the identification of variants of the *mcr-1* and the other *mcr 2-8* genes from human, animal and environment isolates, has showed that colistin resistance is a global public health problem. Because plasmid-mediated colistin resistance can spread horizontally, colistin sensitivity should be determined quickly and reliably [3].

Polymyxin susceptibility tests are technically problematic for many reasons. Disk diffusion and gradient strip test methods have poor performance due to poor agar diffusion of large and cationic colistin molecule and these methods yield inconsistent results [4]. The binding of polymyxins to plastic surfaces (pipette tip, titration plate, etc.) is another technical reason that makes it difficult to detect colistin sensitivity [3]. Although broth microdilution (BMD) method is the recommended technique, it is limited in routine laboratory practice due to the difficulty and the time it takes [5, 6]. There are several commercially available selective culture media [CHROMagar COL-APSE, Super Polymyxin media, CHROMID® Colistin R Agar (COLR)] to identify and rapidly detect the polymyxin resistant bacteria from both culture and stool samples. In our study, it was aimed to examine the usability of the culture medium in routine laboratory studies for screening and qualitative colistin resistance detection by comparing the COLR medium and BMD results.

In this study, investigation of plasmid-mediated *mcr 1-5* resistance genes was performed in MDR colistin sensitive/resistant strains. It was also aimed to evaluate the performance of commercial chromogenic media produced for ease of use in routine detection of colistin resistance together with the automated system (Vitek-2) and reference BMD results.

MATERIAL AND METHODS

Ninety-four multidrug resistant *Klebsiella pneumoniae* and six *E. coli* strains isolated from various clinical specimens (37% blood, 35% urine, 9% wound, 7% tracheal aspirate, 12% other) between 2017 and 2019 were included in our study. The identification and antibiotic susceptibility tests of the strains were performed using MALDI-TOF MS (bioMérieux, France) and Vitek-2 (bioMérieux, France) system.

BMD reference method

Reference BMD test was performed using polystyrene microplate and colistin sulfate salt (Sigma-Aldrich C4461, USA) according to ISO-standard (20776-1) recommendations. A stock solution was made by dissolving 25.6 mg of colistin in 10 mL sterile distilled water (dH_2O) and from this a 1/10 diluted solution containing 256 mg/L colistin was prepared from the stock solution for the use. Minimum inhibitory concentration (MIC) was studied in the 0.125–128 mg/L dilution range. A suspension suitable for 0.5 McFarland turbidity (1.5–2 $\times$ 10^8 colony forming units; cfu/mL) was prepared in sterile saline water by using bacterial colonies from fresh culture by direct colony suspension method. The bacterial suspension was diluted using CAMHB (Mueller-Hinton II Broth Cation-Adjusted Becton-Dickinson, 212322, France) and distributed to a final bacterial concentration of 5 $\times$ 10^5 in the wells. BMD plates were incubated at 35–37 °C for 18–24 h. After incubation, the results were evaluated independently of each other in a double eye control. Quality control was performed with colistin susceptible ATCC 25922 *E. coli* and colistin resistant NCTC 13846 (*mcr-1* positive) *E. coli* strains. Strains with MIC values ≤2 mg/L were considered susceptible and strains with MIC >2 mg/L were considered resistant in accordance with the EUCAST version 10.0 recommendations [5]. The essential agreement (EA) (MIC results within ±1 dilution) and categorical agreement (CA) (number of overlapping (S,R) results) between Vitek-2 and reference method; the rates of major errors (ME) (susceptible strains with the reference method that are detected as false resistant) and very major errors (VME) (resistant strains with the reference method that are detected as false susceptible) were calculated. Results were evaluated according to ISO criteria (EA and CA 90%; ME and VME <3%) [6].

CHROMID® Colistin R Agar

For the CHROMID® Colistin R Agar (COLR) (bioMerieux, France) qualitative resistance detection procedure, 0.5 McFarland (1.5–2 $\times$ 10^8 CFU/mL) bacterial suspension was prepared with sterile saline from pure colonies grown in selective/non-chromogenic medium in line with the company recommendations. Ten µL of sample from the 10^6 CFU/mL bacterial suspension prepared by diluting 1/100, was inoculated on CHROMID® Colistin R Agar. After 18–24 h of incubation, culture medium were evaluated for bacterial growth. *E. coli* was seen as pink-burgundy and *K. pneumoniae* as blue-green colonies on the COLR medium (Fig. 1). For the medium quality control, NCTC 13846 *E. coli* strain (*mcr-1* positive) and colistin susceptible ATCC 25922 *E. coli* strain were used in accordance with the manufacturer's recommendation.

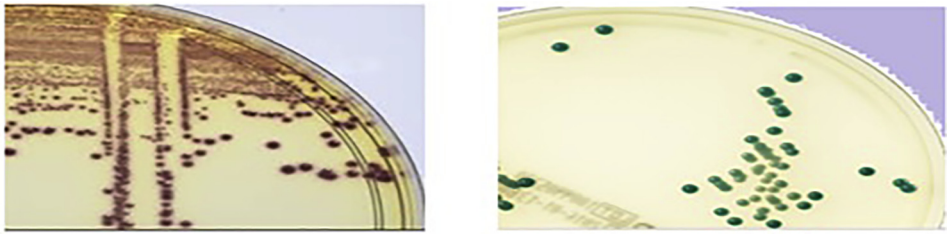

Fig. 1. Colistin resistant *E. coli* and *K. pneumoniae* in COLR medium (left to right)

Table 1. Primers and PCR conditions

Target Gene	Primers	PCR conditions	Reference
mcr-1 (40–359)	Forward (5′-CGG TCA GTC CGT TTG TTC-3′) Reverse (5′-CTT GGT CGG TCT GTA GGG-3′)	Initial denaturation: 95 °C 3′; 30×; 95 °C 45″, 56 °C 1′, 72 °C 1′; final elongation 72 °C 7′	Bardet and Rolain JM [4]; Liu et al. [13]
mcr-2 (401–1,115)	Full forward (5′-ATG ACA TCA CAT CAC TCT TGG-3′) Full reverse (5′-TTA CTG GAT AAA TGC CGC GC- 3′)	Initial denaturation: 95 °C 3′; 30×; 95 °C 45″, 52 °C 1′, 72 °C 1′; final elongation 72 °C 7′	Bardet and Rolain JM [4]; Liassine et al. [20]
mcr-3 (17–945)	Forward (5′-AAA TAA AAA TTG TTC CGC TTA TG-3′) Reverse (5′-AAT GGA GAT CCC CGT TTT T- 3′)	Initial denaturation: 95 °C 3′; 30×; 95 °C 45″, 52 °C 1′, 72 °C 1′; final elongation 72 °C 7′	Bardet and Rolain JM [4];
mcr-4 (38–1,153)	Forward 5′-AAT TGT CGT GGG AAA AGC CGC-3′ Reverse 5′-CTG CTG ACT GGG CTA TTA CCG TCA T-3	Initial denaturation: 95 °C 3′; 30×; 95 °C 45″, 60 °C 1′, 72 °C 1′; final elongation 72 °C 7′	Zhang et al. [25]
mcr-5 (1–1,644)	Forward 5′-GTG AAA CAG GTG ATC GTG ACT TAC CG-3′ Reverse 5′-CGT GCT TTA CAC CGA TCA TGT GCT -3′	Initial denaturation: 95 °C 3′; 30×; 95 °C 45″, 60 °C 1′, 72 °C 1′; final elongation 72 °C 7′	Zhang et al. [25]

Detection of *mcr* genes by PCR

DNA isolation of strains was performed using bacterial DNA isolation kit (Canvax Biotech, Spain) for *mcr 1-5* screening by molecular method. PCR amplification was performed using the primers and PCR conditions listed in Table 1. Amplicons obtained after PCR were detected in 2% agarose (Canvax Agapure Agarose LE) gel electrophoresis using 50–1,000 bp (Canvax BrightMAX) DNA ladder. BigDye PCR (initial denaturation: 96 °C 1 min; 25×; 96 °C 10 s, 50 °C 5 s, 60 °C 4 min) using amplicon purification and Canvax Clean-Easy purification kit were performed on PCR products with positive results. PCR products were purified using Canvax DNA purification SPRI magnetic beads kit. Positive results were confirmed by sequencing with the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA).

Ethics Committee Approval: Ethics Committee approval was not taken as the study was performed from the collection material.

RESULTS

Sixty one of the strains were found to be resistant to colistin and 39 were susceptible with the reference method. All 61 colistin resistant isolates were identified as *K. pneumoniae*. All six *E. coli* isolates were colistin susceptible. The resistance rates of the isolates for carbapenems was 96%, for cephalosporins 100%, for fluoroquinolones 100% and for aminoglycosides was 93%. MIC values of the quality control strains were found in the expected range (ATCC 25922 MIC: 0.5 mg/L, NCTC 13846 MIC: 4 mg/L). The essential and categorical agreement of our Vitek-2 results and the reference method was determined as 100 and 99%, respectively. While no ME was detected with Vitek-2, a VME was detected in one strain (1.6%). The results are summarized in Table 2.

All 61 strains that were resistant to colistin with reference BMD grew on COLR medium. Colistin susceptible strains (n = 39) did not grow on the COLR medium except one. The MIC value of the *mcr-1* positive strain with false positivity in the medium was found to be 2 mg/L (sensitive) with the reference method. The sensitivity of the COLR medium was 100% and its specificity was 97.5%. The results are summarized in Table 2.

In our study plasmid-mediated *mcr 1-5* genes were investigated by PCR method, the expected (309 bp) band size for *mcr-1* gene region was obtained in 10 isolates (Fig. 2); no positivity was detected for other *mcr* (*mcr 2-5*) genes. The amplicons with positive results were confirmed by sequence analysis for eight isolates, and the sequence verification could not be made in two isolates with weak bands. Six positive isolates were resistant to colistin with reference BMD, and two isolates were susceptible to colistin. The results are summarized in Table 2.

DISCUSSION

The increasing use of colistin worldwide has led to an increase in polymyxin resistance, especially in countries where carbapenem resistant Enterobacterales strains are endemic [7, 8].

In order to phenotypically determine colistin resistance with alternative methods, rapid tests such as Polymyxin NP and the usability of various selective media in routine studies are being investigated extensively [9–11]. We found the results are highly compatible with the reference method in our study where we investigated the qualitative detection of

Table 2. Summary results of 94 *K. pneumoniae* and 6 *E. coli* strains

Isolate number	Causative bacteria	VITEK-2 MIC (mg/L)	BMD MIC (mg/L)	COL R agar reproduction	*mcr-1* gene
1	*K. pneumoniae*	R (≥16)	8	Positive	Negative
2	*K. pneumoniae*	R (=4)	4	Positive	Negative
3	*K. pneumoniae*	R (≥16)	8	Positive	Negative
4	*K. pneumoniae*	R (≥16)	8	Positive	Negative
5	*K. pneumoniae*	R (≥16)	8	Positive	Negative
6	*K. pneumoniae*	R (≥16)	8	Positive	Negative
7	*K. pneumoniae*	R (≥16)	8	Positive	Negative
8	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
9	*K. pneumoniae*	S (≤0.5)	0.25	Negative	Negative
10	*K. pneumoniae*	R (≥16)	8	Positive	**Positive**
11	*K. pneumoniae*	R (≥16)	8	Positive	Negative
12	*K. pneumoniae*	R (≥16)	8	Positive	Negative
13	*K. pneumoniae*	R (≥16)	4	Positive	Negative
14	*K. pneumoniae*	R (≥16)	4	Positive	Negative
15	*K. pneumoniae*	R (≥16)	8	Positive	Negative
16	*K. pneumoniae*	R (=8)	8	Positive	Negative
17	*K. pneumoniae*	R (>16)	8	Positive	Negative
18	*K. pneumoniae*	R (=4)	8	Positive	Negative
19	*K. pneumoniae*	S (≤0.5)	0.25	Negative	Negative
20	*K. pneumoniae*	R (>16)	8	Positive	**Positive**
21	*K. pneumoniae*	R (>16)	16	Positive	Negative
22	*K. pneumoniae*	R (>16)	8	Positive	Negative
23	*K. pneumoniae*	R (=4)	8	Positive	Negative
24	*K. pneumoniae*	R (=8)	4	Positive	Negative
25	*K. pneumoniae*	R (≥16)	8	Positive	Negative
26	*K. pneumoniae*	R (≥16)	8	Positive	Negative
27	*K. pneumoniae*	R (≥16)	16	Positive	Negative
28	*K. pneumoniae*	R (≥16)	16	Positive	Negative
29	*K. pneumoniae*	R (≥16)	16	Positive	**Positive**
30	*K. pneumoniae*	R (≥16)	8	Positive	Negative
31	*K. pneumoniae*	R (≥16)	4	Positive	Negative
32	*K. pneumoniae*	R (=4)	4	Positive	Negative
33	*K. pneumoniae*	R (≥16)	8	Positive	Negative
34	*K. pneumoniae*	R (≥16)	16	Positive	Negative
35	*K. pneumoniae*	R (≥16)	8	Positive	Negative
36	*K. pneumoniae*	R (≥16)	8	Positive	Negative
37	*K. pneumoniae*	R (≥16)	8	Positive	Negative
38	*K. pneumoniae*	R (≥16)	4	Positive	Negative
39	*K. pneumoniae*	R (≥16)	8	Positive	Negative
40	*K. pneumoniae*	R (≥16)	8	Positive	Negative
41	*K. pneumoniae*	R (=4)	8	Positive	Negative
42	*K. pneumoniae*	R (≥16)	32	Positive	Negative
43	*K. pneumoniae*	R (8)	4	Positive	Negative
44	*K. pneumoniae*	R (≥16)	8	Positive	Negative
45	*K. pneumoniae*	R (≥16)	8	Positive	Negative
46	*K. pneumoniae*	R (≥16)	4	Positive	Negative
47	*K. pneumoniae*	R (≥16)	8	Positive	Negative
48	*K. pneumoniae*	R (≥16)	8	Positive	Negative
49	*E. coli*	S (≤0.5)	0.12	Negative	Negative
50	*K. pneumoniae*	R (≥16)	8	Positive	Negative
51	*K. pneumoniae*	R (≥16)	32	Positive	Negative
52	*K. pneumoniae*	R (≥16)	4	Positive	Negative
53	*K. pneumoniae*	R (=8)	4	Positive	**Positive**
54	*K. pneumoniae*	R (=8)	8	Positive	**Positive**
55	*K. pneumoniae*	S (=2)	2	Positive	**Positive**
56	*K. pneumoniae*	R (=8)	64	Positive	**Positive**
57	*K. pneumoniae*	R (=8)	4	Positive	Negative
58	*K. pneumoniae*	S (≤0.5)	0.12	Negative	Negative
59	*E. coli*	S (≤0.5)	0.5	Negative	Negative

(continued)

Table 2. Continued

Isolate number	Causative bacteria	VITEK-2 MIC (mg/L)	BMD MIC (mg/L)	COL R agar reproduction	*mcr-1* gene
60	*K. pneumoniae*	R (≥16)	8	Positive	Negative
61	*K. pneumoniae*	R (≥16)	4	Positive	Negative
62	*K. pneumoniae*	R (≥16)	16	Positive	Negative
63	*K. pneumoniae*	R (≥16)	4	Positive	Negative
64	*K. pneumoniae*	R (≥16)	8	Positive	Negative
65	*K. pneumoniae*	R (≥16)	8	Positive	Negative
66	*K. pneumoniae*	R (≥16)	8	Positive	Negative
67	*K. pneumoniae*	S (≤0.5)	0.12	Negative	Negative
68	*E. coli*	S (≤0.5)	0.12	Negative	Negative
69	*K. pneumoniae*	S (≤0.5)	0.12	Negative	Negative
70	*K. pneumoniae*	S (≤0.5)	0.12	Negative	Negative
71	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
72	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
73	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
74	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
75	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
76	*K. pneumoniae*	S (≤0.5)	0.25	Negative	Negative
77	*K. pneumoniae*	S (≤0.5)	0.25	Negative	Negative
78	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
79	*K. pneumoniae*	S (≤0.5)	1	Negative	Negative
80	*E. coli*	S (≤0.5)	0.5	Negative	Negative
81	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
82	*K. pneumoniae*	S (=2)	4	Positive	Negative
83	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
84	*E. coli*	S (≤0.5)	0.5	Negative	Negative
85	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
86	*K. pneumoniae*	R (≥16)	8	Positive	Negative
87	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
88	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
89	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
90	*K. pneumoniae*	S (≤0.5)	1	Negative	Negative
91	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
92	*E. coli*	S (≤0.5)	0.5	Negative	Negative
93	*K. pneumoniae*	S (≤0.5)	2	Negative	Negative
94	*K. pneumoniae*	S (≤0.5)	0.5	Negative	**Positive**
95	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
96	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
97	*K. pneumoniae*	S (≤0.5)	0.12	Negative	Negative
98	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative
99	*K. pneumoniae*	S (≤0.5)	0.25	Negative	Negative
100	*K. pneumoniae*	S (≤0.5)	0.5	Negative	Negative

mcr-1 positive : bold

colistin resistance rates using the selective chromogenic screening medium COLR agar. Fernandez et al. in their screening study with 59 *Enterobacterales*, 20 of which were *mcr-1* positive, found the specificity and sensitivity of the COLR medium as 100 and 88.1%, respectively and commented that the medium could be used to screen colistin resistance for *Enterobacterales*, including strains carrying *mcr-1*, despite false negative results in an *E. coli* and a *K. pneumoniae* strains [9]. Girlich et al. compared performance of Superpolymyxin medium (ELITechGroup) and COLR medium, found the sensitivity (86.8%) and specificity (97.9–100%) of both media to be high and concluded that both media could be used as a useful method for screening colistin resistance in routine studies [10]. Roche et al. using 46 colistin resistant, 20 colistin susceptible strains and 61 stool samples, determined the sensitivity of the COLR medium as 95% and the specificity as 100% in their study and commented that the culture medium provides an advantage compared to traditional methods in the differentiation of species known to carry *mcr* and in the evaluation of mixed cultures [11].

Vitek-2 system is routinely used in our laboratory to detect antibiotic sensitivity results. Very different rates are reported regarding the performance of automated systems in detecting colistin sensitivity. Pfennigwerth et al. have compared the detection of colistin resistance with six different methods in 206 *K. pneumoniae* strains. While Vitek-2 system didn't detect any ME, in 12 strains VME's were detected; EA and CA were 81.7–94.2% [12]. In our study, the EA and CA (>90%) between the Vitek-2 and

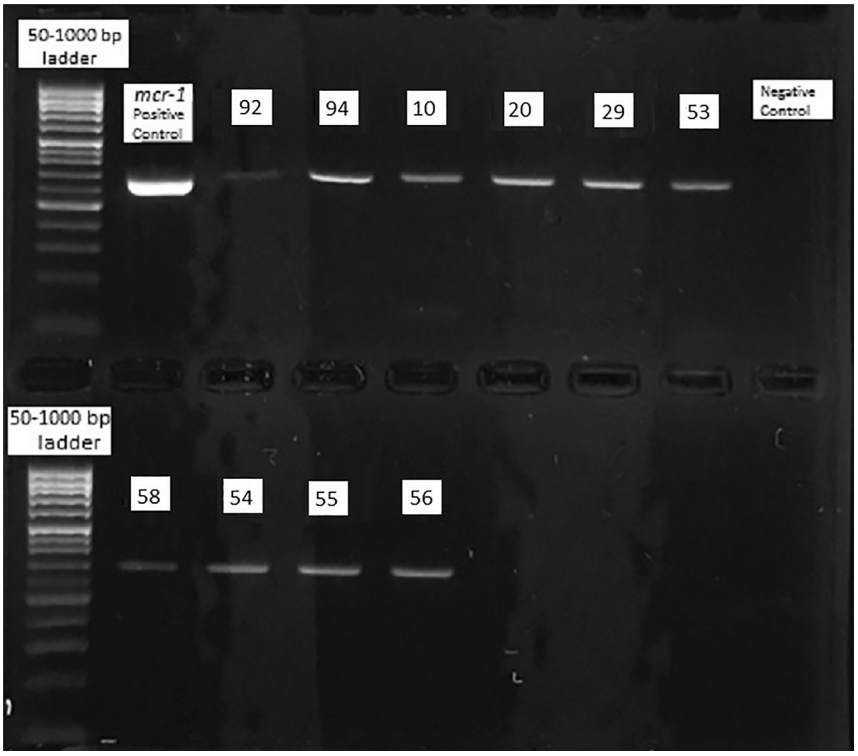

Fig. 2. Gel electrophoresis of isolates carrying the gene *mcr-1*

reference method, ME and VME (<3%) results also were found suitable according to ISO criteria. Despite the high agreement of the results we obtained with the Vitek-2 system with the reference method, there are also publications stating that the VME rates of Vitek-2 systems are unacceptable (VME: 36–57.4%) [4].

In a study conducted by Liu et al. in 2016, they reported that the *mcr-1* gene encoded by the plasmid was responsible for colistin resistance in an *E. coli* strain isolated from animal samples [13]. Since the first definition of the *mcr* gene region around the world, seven different members (*mcr 2-8*) of the *mcr* family have been identified [14, 15]. In our study, only plasmid-mediated colistin resistance genes (*mcr 1-5*) were investigated in isolates, most of which are multidrug resistant strains (94% *K. pneumoniae*, 6% *E. coli*) isolated from clinical samples. The expected (309 bp) band size was obtained for the *mcr-1* gene region in 10 isolates, and amplicons detected positive were confirmed by sequence analysis for 8 isolates (8%). No positivity was detected for other *mcr* genes (*mcr 2-5*). In addition to our colistin resistant isolates with *mcr-1* ($n = 6$), the detection of *mcr-1* in two strains found to be susceptible to colistin indicates the presence of non-functional *mcr* genes, as emphasized by other researchers and shows that the *mcr* genes should be screened epidemiologically in colistin resistant samples as well as in colistin sensitive samples [3].

Srijan A. et al. in their study in 2017 from Thailand, found *mcr-1* gene positive in 2 *K. pneumoniae* isolates which are member of a 118 multidrug resistant *Enterobacteriaceae* [16]. In the study of Zaki et al. investigating the *mcr 1-2* gene in 50 colistin resistant (MIC> 2 mg/L) *Enterobacteriaceae* strains, they detected *mcr-1* gene in one *E. coli* and one *K. pneumoniae* strain, but did not detect any *mcr-2* gene [17]. Although the *mcr-1* gene was not shown in the study conducted by Xavier et al. in 2016 with colistin resistant *E. coli* strains in Belgium, they identified the presence of *mcr-2* gene [18].

Özhelvacı in his study that included 40 colistin resistant *K. pneumoniae* strains isolated from various clinics, could not detect *mcr-1* gene, but they detected *mcr-2* gene in two isolates [19]. In a study investigating *mcr 1-2* gene regions in 2049 Gram-negative bacterial strains isolated from urine of patients with urinary infections in Switzerland, an *E. coli* strain was found to be *mcr-1* positive, while no strain with the *mcr-2* gene was found. The strain found to be *mcr-1* positive in the study was found to be susceptible to colistin, and it was interpreted that the spread of the *mcr* gene could be hidden [20].

Zhong et al. found the *mcr-1* positivity with the standard PCR method in 2.1% of 144 *E. coli* isolates isolated from patients with bloodstream infection. All strains found to be *mcr-1* positive ($n = 3$) were also found resistant to colistin using the reference BMD method [21]. Quan et al. in 2066 strains (1495 *E. coli* and 571 *K. pneumoniae*) isolated from bloodstream infections, *mcr-1* positivity was found as 1%. While 20 of the 21 *mcr-1* positive isolates were found to be colistin resistant (MIC: 4–32 mg/L) with BMD, one strain was found to be colistin susceptible (MIC: 0.06 mg/L) and this was interpreted that it may be due to the dysfunction of the gene in some species [22]. Arabacı et al. have investigated the *mcr-1* gene in 57 *K. pneumoniae* strains isolated from blood cultures and the *mcr-1* was detected in three

isolates (5.7%); all *mcr-1* positive strains were found resistant by BMD method [23]. In a study of Lee et al. conducted in 2019 in which the presence of *mcr 1-5* genes in different bacterial groups was investigated, *mcr-1* was detected in two (9.1%) of 22 colistin resistant *K. pneumoniae* isolates and in one of the two *E. coli* isolates. From these three isolates, two were found resistant and one sensitive (MIC 2 mg/L) with BMD [24]. Zhang et al. have investigated *mcr 1-5* genes in human vaginal samples and they detected high percentage of *mcr-4* (12.7% *mcr-4*, 1.5% *mcr-2*, 1.5% *mcr-3*, 0.7% *mcr-1*, 0.7% *mcr-5*). They also found that animal and human *mcr* genes in their cities are identical with phylogenetic studies [25]. In a comprehensive study investigating colistin *mcr 1-9* genes in carbapenem resistant clinical isolates collected between 2014 and 2019 in China, *mcr-1* positivity in *E. coli* strains was determined as 2.1%; It was also determined that it increased to 6.3% after 2017 [26]. In the study where other *mcr* variants were not detected, no interpretation was made for isolates *mcr-1* positive that were found to be susceptible to colistin.

As a result, the remarkable rate of *mcr-1* positivity detected in our study, which consisted of clinical isolates, although in a limited number, is an important epidemiological finding. In the future, the situation in our country will be revealed in real terms only with large series and multidisciplinary contribution and thus healthier results can be obtained regarding the spread, and source of resistance and measures can be taken. In addition, due to the high sensitivity and specificity of the COLR medium we tested, it has been concluded that it can be useful in the practical and rapid detection of colistin resistant strains and can be used in routine studies.

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Conflict of interest: No conflict of interest declared.

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