Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2023;48(3):1139-1147
DOI: 10.17826/cumj.1348548

RESEARCH

Misdiagnosis in molecular detection of colistin resistance: false mcr-1-PCR positivity among the colistin-susceptible Acinetobacter baumannii isolates

Kolistin direncinin moleküler tespitinde yanlış tanı: kolistine duyarlı Acinetobacter baumannii izolatlarında yanlış mcr-1-PCR pozitifliği

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Abstract

Purpose: The aim of this study was to investigate the presence of the mcr-1 gene, which is responsible for colistin resistance, in carbapenem-resistant Gram-negative bacteria that cause difficult-to-treat infections in a research hospital in Turkey.

Materials and Methods: The mcr-1 gene was examined using PCR in 103 carbapenem-resistant isolates, including 75 Acinetobacter baumannii, 19 Pseudomonas aeruginosa, and 9 Klebsiella pneumoniae. DNA sequencing was performed to confirm the mcr-1 positivity. Other antimicrobial resistance genes were investigated in isolates that were found to be mcr-1-positive by PCR and colistin-resistant isolates.

Results: Four (3.9% of the 103 carbapenem-resistant isolates and 5.3% of the 75 A. baumannii isolates) A. baumannii isolates, all susceptible to colistin, were found to be mcr-1-positive by PCR, whereas mcr-1 was not detected in four colistin-resistant isolates, one in A. baumannii and three in K. pneumoniae. DNA sequencing analysis determined that none of the amplification products was the targeted fragment, but they matched more than 70% with the chromosomal DNA fragments of A. baumannii strains. Therefore, these results were considered false-positive. Although these false-positive isolates were susceptible to colistin, they were extensively drug-resistant (XDR). Two of them were found to carry blaOXA23-like and blaTEM genes, another blaOXA23-like, blaTEM and blaOXA48-like genes, and the fourth one to have blaOXA23-like and blaCTXM genes.

Conclusion: Although the specificity of the primers used to detect the mcr-1 gene by PCR was reported as 100% in most studies, we concluded that PCR tests are insufficient yet to use alone or with antibiotic susceptibility tests in rapid routine diagnosis. Confirming at least PCR-positive

Öz

Amaç: Bu çalışmanın amacı Türkiye'deki bir araştırma hastanesinde tedavisi zor enfeksiyonlara neden olan karbapenem dirençli Gram negatif bakterilerde kolistin direncinden sorumlu mcr-1 geninin varlığını araştırmaktı.

Gereç ve Yöntem: 75'i Acinetobacter baumannii, 19'u Pseudomonas aeruginosa ve 9'u Klebsiella pneumoniae olmak üzere karbapeneme dirençli 103 izolatta mcr-1 geni PCR kullanılarak incelendi. Mcr-1 pozitifliğini doğrulamak için DNA dizi analizi yöntemi kullanıldı. PCR ile mcr-1 pozitif olarak saptadığımız izolatlarda ve kolistine dirençli izolatlarda diğer antimikrobiyal direnç genleri araştırıldı.

Bulgular: Tamamı kolistine duyarlı olan 4 (karbapeneme dirençli 103 izolatın %3,9'u ve 75 A. baumannii izolatının %5.3'ü) A. baumannii izolatı PCR ile mcr-1 pozitif olarak belirlenirken, biri A. baumannii ve üçü K. pneumoniae olmak üzere kolistine dirençli dört izolatta mcr-1 saptanmadı. DNA dizileme analizi amplifikasyon ürünlerinden hiçbirinin hedeflenen parça olmadığını belirledi, ancak bunlar A. baumannii suşlarının kromozomal DNA parçalarıyla %70'ten fazla eşleşti. Bu nedenle bu sonuçlar yanlış pozitif kabul edildi. Bu yanlış pozitif izolatlar kolistine duyarlı olmalarına rağmen genişlemiş ilaç dirençliydi (XDR). Bunlardan ikisinin blaOXA23 benzeri ve blaTEM genlerini, bir diğerinin blaOXA23 benzeri, blaTEM ve blaOXA48 benzeri genleri, dördüncüsünün ise blaOXA23 benzeri ve blaCTXM genlerini taşıdığı belirlendi.

Sonuç: PCR ile mcr-1 genini saptamak için kullanılan primerlerin özgüllüğü çoğu çalışmada %100 olarak bildirilse de PCR testlerinin hızlı rutin tanıda tek başına veya antibiyotik duyarlılık testleri ile birlikte kullanılmasında henüz yetersiz olduğu sonucuna vardık. En azından PCR pozitif örneklerin DNA dizi analizi

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Received: 24.08.2023 Accepted: 21.09.2023

samples using DNA sequence analysis would be appropriate for a certain period.
Keywords:. DNA sequence analysis, gram-negative bacteria, carbapenem resistance, mcr-1 gene, PCR.

kullanılarak doğrulanması belli bir süre için uygun olacaktır.
Anahtar kelimeler: DNA dizi analizi, gram-negatif bakteri, karbapenem direnci, mcr-1 geni, PCR.

INTRODUCTION

The use of last-choice reserve antibiotics such as colistin, which has nephrotoxic and neurotoxic effects in humans, against severe infections caused by carbapenem-resistant Gram-negative bacteria is increasing due to the lack of new antibiotics[1,2]. As a result of this common use in clinical applications, colistin resistance has also increased recently[2,3].

Until the plasmid-mediated *mcr-1* gene was identified in *Escherichia coli* isolates collected from hospitalised patients and animals in China in 2015, colistin resistance was supposed to be carried only by chromosomes[2,4]. In subsequent studies, ten main variants (from *mcr-1* to *mcr-10*) distributed in 5 continents of the transferable *mcr* gene, which can generally exist on different plasmids in *Enterobacteriaceae* isolates, were determined. It has been reported that the most common variant is *mcr-1*, and colistin resistance spreads rapidly owing to this gene[5-8]. In recent studies, the *mcr-1* gene has also been detected in non-fermentative bacteria, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, albeit rarely[9-11].

Detecting the *mcr-1* gene in various multidrug-resistant bacteria, including *Enterobacterales* that produce extended-spectrum beta-lactamases and carbapenemases and are even resistant to aminoglycosides, raises further concern. Accurate and rapid identification of this gene is of great importance in preventing the spread of colistin resistance. In research-oriented studies, when the *mcr-1* gene is detected by polymerase chain reaction (PCR), it is confirmed by DNA sequencing in this context[12-14]. Considering that *A. baumannii*, *P. aeruginosa*, and *Klebsiella pneumoniae* are the top three in the ranking of pandrug-resistant (PDR) Gram-negative bacteria, while *E. coli* is in the last place[15]; our study aimed to investigate the presence of the *mcr-1* gene in clinical isolates of these three bacterial species, which have a high potential to develop antibiotic resistance. We hypothesised whether PCR detects the *mcr-1* gene in these isolates and, if it is detected, whether it is confirmed by DNA sequence analysis. Although plasmid-mediated mcr genes have been widely investigated in *E. coli* strains, more research is required on these genes in highly pathogenic PDR Gram-negative bacteria. Therefore, our study will contribute to the literature on this subject.

MATERIALS AND METHODS

Identification of isolates

A total of 103 clinical strains of carbapenem-resistant Gram-negative bacteria, which were isolated in the Department of Medical Microbiology, Cukurova University Faculty of Medicine, Turkey, including 75 (72.8%) *A. baumannii*, 19 (18.5%) *P. aeruginosa*, and 9 (8.7%) *K. pneumoniae*, were enrolled in this descriptive study based on an in vitro research. All tests were conducted in the Department of Medical Microbiology, Cukurova University Faculty of Medicine. For this kind of research to obtain ethical approval was not an obligation, legally in Turkey and institutionally in Cukurova University.

Species-level identification of the isolates was confirmed phenotypically by conventional culture methods and biochemical tests (catalase, oxidase, Triple Sugar Iron Agar (TSI) and Indole, Methyl Red, Voges Proskauer, Citrate (IMVIC)) as well as BBL Crystal E/NF kit (Becton Dickinson, Australia) and genotypically by PCR. As previously described, the primer sets targeting the DNA sequences specific for *A. baumannii* (497 bp region in OXA-51 gene)[16], *P. aeruginosa* (222 bp region in gyrB gene)[17], and *K. pneumoniae* (130 bp region in 16S-23S internal transcribed spacer)[18] were F:5'-GACCGAGTATGTACCTGCTTCGACC-3' and R:5'-GAGGCTGAACAACCCATCCAGTTAACC-3', F:5'-CCTGACCATCCGTCGCCACAAC-3' and R:5'-CGCAGCAGGATGCCGACGCC-3', and F:5'-ATTTGAAGAGGTTGCAAACGAT-3' and R:5'-TTCACTCTGAAGTTTTCTTGTGTTC-3', respectively. Carbapenem resistance was confirmed using the carbapenem inactivation test[19]. *E. coli* ATCC 25922 reference strain was used as a control.

Determination of colistin resistance

The broth microdilution method was performed for antimicrobial susceptibility testing (AST) of colistin,

and minimum inhibitory concentration (MIC) was determined according to the EUCAST criteria[20].

Detection of *mcr-1* by PCR

DNA was extracted by the mechanical lysis principle using the MICKLE device (The Mickle Lab. Engineering Co. Ltda, Gomshall, Surrey, UK). PCR was applied to DNA samples with concentrations between 0.1-1.2 μg/μl, measured at 260nm using a spectrophotometer (CHEBIOS s.r.l. Optimum-One UV-VIS Spectrophotometer). The primer set (CLR5-F:5'-CGGTCAGTCCGTTTGTTC-3' and CLR5-R:5'-CTTGGTCGGTCTGTAGGG-3') targeting the specific 309 bp region of the *mcr-1* gene was used to determine the colistin resistance[4]. Amplification was performed in a total of 50 μl PCR mix containing 25 μl of PCR Master Mix (2X) reagent (Thermo Fisher Scientific, Waltham, MA, USA), 4 μl of template DNA, 0.50 μl of each primer, and water using a thermal cycler (APPLIED BIOSYSTEMS 2720 Thermal Cycler). Initial denaturation at 94 °C for 15 min was followed by 25 cycles at 94 °C for 30 s, 58 °C for 90 s, and 72 °C for 1 min. The final extension step was 10 min at 72 °C. The ATCC 25922 (MIC=0.5 mg/L) and NCTC 13846 (MIC=4 mg/L) strains of *E. coli* were used as negative and positive controls, respectively. Amplification products were visualised on 1.5% agarose gel using the Gel Logic 1500 imaging system (discrimination power: 1708x1280 pixels, Kodak Company, NY, USA).

Antimicrobial resistance profiles and other antimicrobial resistance genes

In isolates that we detected as *mcr-1*-PCR positive and in colistin-resistant isolates, antimicrobial resistance profiles were determined by the agar dilution method on Mueller-Hinton agar media according to the EUCAST criteria and other antimicrobial resistance genes were investigated by PCR, as we previously performed[20,21].

DNA sequencing

PCR results were verified by DNA sequence analysis. PCR-positive DNA products were purified using ExoSAP-IT™ Express PCR Product Cleanup Reagent (Applied Biosystems™, USA) and Sefadex G-25 Superfine (Sigma-Aldrich, USA) according to the manufacturer's instructions. They were sequenced using the same primer sets and BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). DNA sequence analysis was performed in ABI Prism 310 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions and principles of "F. Sanger's dideoxy chain termination method"[22,23].

Statistical analysis

IBM SPSS Statistics Version 20.0 statistical software package (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.) was used for the statistical analyses. Categorical variables were summarised using numbers and percentages. The data obtained by DNA sequencing were aligned and analysed with the GenBank database using the BLAST (nucleotide-nucleotide blast) program on the National Center for Biotechnology Information (NCBI) web page (htpp://www.ncbi.nlm.nih.gov/BLAST/).

RESULTS

In 4 of the 103 carbapenem-resistant isolates (3.9%), DNA fragments of about 309 bp targeted to detect the *mcr-1* gene were observed and were identified as *mcr-1*-PCR-positive (Figure 1). All four isolates were *A. baumannii* and were phenotypically susceptible to colistin. The incidence of *mcr-1*-PCR-positivity among the *A. baumannii* was 5.3% (4/75). Only four isolates, including one *A. baumannii* and three *K. pneumoniae*, were phenotypically resistant to colistin, but they all were *mcr-1*-PCR-negative (Table 1).

Although the amplification products of *mcr-1*-PCR positive isolates matched the sizes (approximately 309 bp), DNA sequence analysis revealed that they were partially similar to the targeted region of the *mcr-1* gene but were not the same region. Namely, while one of these four isolates did not match any *Acinetobacter* spp., two showed more than 70% similarity with different chromosomal DNA fragments belonging to *A. baumannii* and one with those of *A. pitti* (Figure 2,3,4).

We determined that, among the colistin-resistant strains, the *A. baumannii* strain harboured $bla_{\text{OXA23-like}}$ gene, two *K. pneumoniae* strains had bla_{CTXM}, bla_{CTXM9}, $bla_{\text{OXA24-like}}$ and *rmtH* genes, and other *K. pneumoniae* strain harboured the bla_{CTXM}, bla_{CTXM9} and $bla_{\text{OXA48-like}}$ genes. Among the *mcr-1*-PCR-positive *A. baumannii* strains, two harboured $bla_{\text{OXA23-like}}$ and bla_{TEM} genes, one harboured $bla_{\text{OXA23-like}}$, bla_{TEM} and $bla_{\text{OXA48-like}}$

genes, and one had bla_{CTXM} and $bla_{OXA23\text{-like}}$ genes (Table 1). The *mcr-1*-PCR positive isolates were resistant to cefepime, ceftazidime, gentamicin, imipenem, meropenem, ampicillin/sulbactam, ciprofloxacin, and tetracycline. Three of these isolates were additionally resistant to amikacin (Table 1).

All PCR tests and sequencing analyses were repeated at least once, and the same results were obtained.

Table 1. Antimicrobial resistance status of the colistin-resistant or *mcr-1*-PCR-positive isolates

Isolate[a]	Clinical data				AST[c] result for colistin (MIC)	*mcr-1*-PCR result	Resistance profile for other antimicrobials[e]	Other antimicrobial resistance genes
	Age	Gender	Source of specimen	Type of specimen				
AB02	50	Female	Oncology Service	urine	S (<=0.5)	positive[d]	AK, FAM, CEF, CAZ, GEN, IPM, MEM, CIP, TET, TZP	bla_{CTXM}, $bla_{OXA23\text{-like}}$
AB36	48	Male	Burn Unit	wound	S (<=0.5)	positive[d]	FAM, CEF, CAZ, GEN, IPM, MEM, CIP, TET, TZP	bla_{TEM}, $bla_{OXA23\text{-like}}$
AB48	63	Male	Reanimation	blood	S (<=0.5)	positive[d]	AK, FAM, CEF, CAZ, GEN, IPM, MEM, CIP, TET, TZP	bla_{TEM}, $bla_{OXA23\text{-like}}$
AB51	23	Male	Reanimation	tracheal aspiration	S (<=0.5)	positive[d]	AK, FAM, CEF, CAZ, GEN, IPM, MEM, CIP, TET, TZP	bla_{TEM}, $bla_{OXA23\text{-like}}$, $bla_{OXA48\text{-like}}$
AB14	42	Male	Internal Medicine ICU[b]	blood	R (>=16)	negative	AK, FAM, CEF, CAZ, GEN, IPM, MEM, CIP, SXT, TZP	$bla_{OXA23\text{-like}}$
KP01	3	Female	Pediatric ICU[b]	blood	R (=8)	negative	AK, AMC, CEF, CAZ, GEN, IPM, MEM, CIP, SXT, CP, TZP, LEV, PIP	bla_{CTXM}, bla_{CTXM9}, $bla_{OXA48\text{-like}}$
KP07	86	Female	Chest Diseases Service	urine	R (>=16)	negative	AK, AMC, CEF, CAZ, MEM, CIP, CP, TZP, LEV, PIP, TET	bla_{CTXM}, bla_{CTXM9}, $bla_{OXA24\text{-like}}$, *rmtH*
KP08	38	Male	Internal Medicine ICU[b]	wound	R (>=16)	negative	AK, AMC, CEF, GEN, MEM, CIP, SXT, CP, TZP, LEV, PIP	bla_{CTXM}, bla_{CTXM9}, $bla_{OXA24\text{-like}}$, *rmtH*

[a] AB: A. baumannii, KP: K. pneumoniae. [b] Intensive Care Unit. [c] Antimicrobial susceptibility testing [d] DNA Sequence analysis did not determine sequences belonging to the mcr-1 gene, and the result was assessed as false-positive. [e] AK: amikacin, FAM: ampicillin/sulbactam, CEF: cefepime, CAZ: ceftazidime, CP: cefoperazone, GEN: gentamicin, IPM: imipenem, MEM: meropenem, CIP: ciprofloxacin, TET: tetracycline, LEV: levofloxacin, AMC: amoxicillin/clavulanic acid, SXT: trimethoprim/sulfamethoxazole, TZP: piperacillin/tazobactam, PIP: piperacillin.

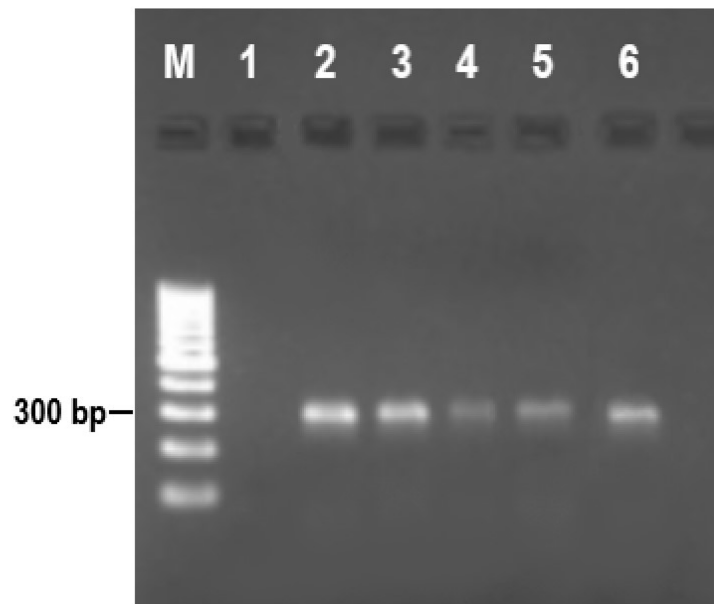
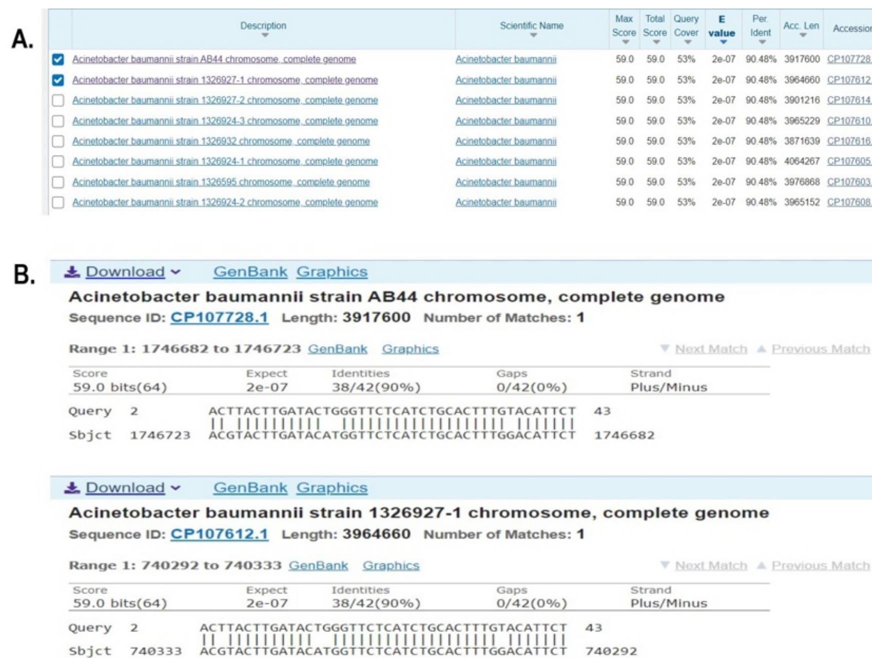


Figure 1. Images of mcr-1-PCR-positive amplification products (~309bp) on 1.5% agarose gel. M: Marker (100-bp DNA ladder); 1: Negative control; 2: Positive control; 3-6: mcr-1-PCR-positive isolates.



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Acinetobacter baumannii strain AB44 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3917600	CP107728.1
Acinetobacter baumannii strain 1326927-1 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3964660	CP107612.1
Acinetobacter baumannii strain 1326927-2 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3901216	CP107614.1
Acinetobacter baumannii strain 1326924-3 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3965229	CP107610.1
Acinetobacter baumannii strain 1326932 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3871639	CP107616.1
Acinetobacter baumannii strain 1326924-1 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	4064267	CP107605.1
Acinetobacter baumannii strain 1326595 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3976868	CP107603.1
Acinetobacter baumannii strain 1326924-2 chromosome, complete genome	Acinetobacter baumannii	59.0	59.0	53%	2e-07	90.48%	3965152	CP107608.1

Figure 2. Alignment of the *mcr-1*-PCR-positive gene fragment of isolate AB51 using the BLAST (nucleotide-nucleotide blast) databases. A: Sequences producing significant alignments, B: Alignment views of the top two sequences.

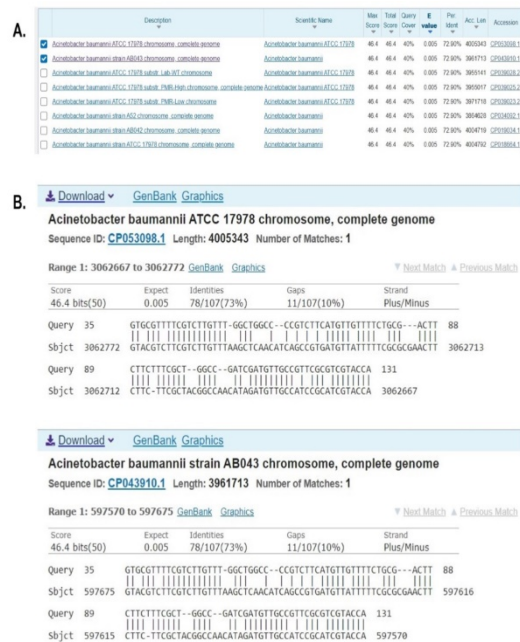


Figure 3. Alignment of the mcr-1-PCR-positive gene fragment of isolate AB36 using the BLAST (nucleotide-nucleotide blast) databases. A: Sequences producing significant alignments, B: Alignment views of the top two sequences.

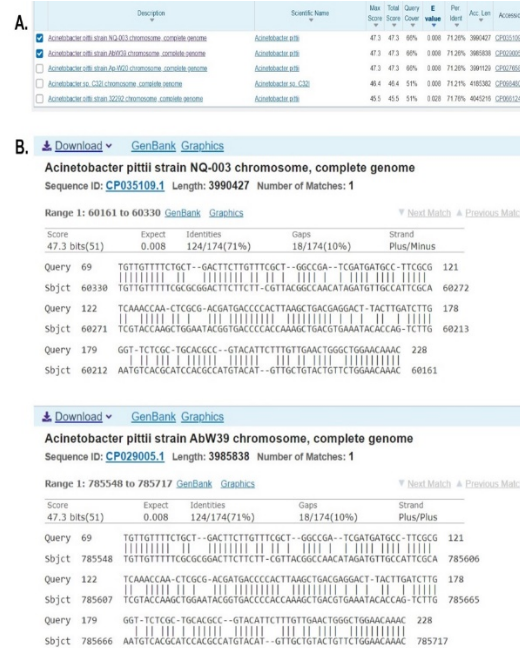


Figure 4. Alignment of the mcr-1-PCR-positive gene fragment of isolate AB48 using the BLAST (nucleotide-nucleotide blast) databases. A: Sequences producing significant alignments, B: Alignment views of the top two sequences.

DISCUSSION

Since colistin resistance gene *mcr-1* harboured on a plasmid was first identified in China in 2016, it has been observed that this gene is predominant among the ten reported *mcr* gene variants and is most frequently isolated from *E. coli* isolates[5-7,13]. The *mcr-1* gene has been identified in *E. coli* and *K. pneumoniae* isolated from food and humans worldwide[24-28]. We investigated the presence of this gene in *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* isolates and detected that four *A. baumannii* isolates were *mcr-1*-PCR positive. DNA sequencing analysis to confirm these results determined that the gene fragments we detected were not mcr-1. Three of the PCR-positive samples showed more than 70% similarity with the chromosomal DNA fragments of *Acinetobacter* spp., whereas the other did not match. Therefore, we identified these samples as false *mcr-1*-PCR positive. As far as we searched, *mcr-1* detection in *A. baumannii* isolates has been previously reported from Turkey[29], South Africa[30], Iraq[31], Pakistan[11] and India[32]. In the studies conducted in Turkey, South Africa and Iraq, *mcr-1* positivity was detected in *A. baumannii* isolates by PCR, but DNA sequence analysis did not confirm this[29-31]. On the other hand, *mcr-1*-PCR positivity in *A. baumannii* isolates was confirmed by DNA sequence analysis in the studies from Pakistan and India[11,32].

It has been reported that the *mcr-1* gene, which has been determined to be harboured in several plasmids and to have a variable genetic background as a result of various studies conducted in recent years[3], is localised not only on plasmids but also on chromosomes in *E. coli* and *K. pneumoniae* isolates[33,34]. The fact that the insertion sequences exist in some strains harbouring the *mcr-1* gene but not in others[3] supports this information. The study reporting the *mcr-1* gene in *A. baumannii* isolates from India determined that this gene could not be horizontally transferred by conjugation, so it was thought to be localised in the chromosomes[32]. However, using the same primers as us, Hameed F et al. from Pakistan

detected the *mcr-1* gene in the plasmid in one *A. baumannii* and one *P. aeruginosa* isolate[11].

There are some limitations in our study. Since we did not perform plasmid extraction, conjugation experiment and whole genome sequence analysis for the *mcr-1*-PCR positive four isolates, we could not determine whether the gene fragments we detected were on the plasmid or chromosome. Their partial matching with chromosomal DNA by sequencing analysis initially suggested possible chromosomal localisation. However, these gene fragments might also be associated with antibiotic resistance, although this is very unlikely. Although many studies[28,35,36] reported that colistin-resistant *mcr-1*-positive isolates were resistant to a small number of antibiotics, in our research, colistin-susceptible *mcr-1*-PCR-positive isolates were XDR. In addition, the fragments detected approximately the same size as the targeted region of the *mcr-1* gene might also be related to *mcr* genes. Even if so, these fragments were considered non-functional as the isolates were phenotypically susceptible to colistin. Furthermore, we did not examine chromosomal mechanisms of colistin resistance.

Although the sensitivity and specificity of some primers used to detect *mcr* genes by PCR have been reported to be 100%[37], we have concluded that PCR tests are insufficient yet to be used alone or with antibiotic susceptibility tests in rapid routine diagnosis of the coding region of the *mcr-1* gene in *A. baumannii* that is underreported in the world. Confirming at least PCR-positive samples using DNA sequence analysis would be appropriate for a certain period. On the other hand, our findings provide data that will shed light on further investigation of plasmid-mediated *mcr* genes in *A. baumannii* strains. More studies should be done on this subject in order to make a definitive interpretation of the false positive results of the *mcr-1* gene region.

Author Contributions: Concept/Design : TN, TK; Data acquisition: TN, TK; Data analysis and interpretation: TN, FK, TK; Drafting manuscript: TN, TK, FK; Critical revision of manuscript: TN, TK, FK; Final approval and accountability: TN, TK, FK; Technical or material support: FK; Supervision: TN; Securing funding (if available): n/a.

Ethical Approval: None required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors have no competing financial or non-financial interests to disclose..

Financial Disclosure: Authors declared no financial support

REFERENCES

1. Prim N, Turbau M, Rivera A, Navarro JR, Coll P, Mirelis B. Prevalence of colistin resistance in clinical isolates of Enterobacteriaceae: A four-year cross-sectional study. J Infect. 2017;75:493–8.
2. Baron S, Hadjadj L, Rolain JM, Olaitan AO. Molecular mechanisms of polymyxin resistance: knowns and unknowns. Int J Antimicrob Agents. 2016;48:583–91.
3. Bakthavatchalam YD, Pragasam AK, Biswas I, Veeraraghavan B. Polymyxin susceptibility testing, interpretative breakpoints and resistance mechanisms: An update. J Glob Antimicrob Resist. 2018;12:124–36.
4. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16:161–8.
5. MacNair CR, Stokes JM, Carfrae LA, Fiebig-Comyn AA, Coombes BK, Mulvey MR et al. Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. Nat. Commun. 2018;9:458.
6. Caldes C, de Waard JH, Salgado MS, Villacís MJ, Coral-Almeida M, Yamamoto Y et al. Worldwide prevalence of mcr-mediated colistin-resistance escherichia coli in isolates of clinical samples, healthy humans, and livestock—a systematic review and meta-analysis. Pathogens. 2022;11:659.
7. Hussein NH, AL-Kadmy IMS, Taha BM, Hussein JD. Mobilized colistin resistance (mcr) genes from 1 to 10: a comprehensive review. Mol Biol Rep. 2021;48:2897–07.
8. Xiaomin S, Yiming L, Yuying Y, Zhangqi S, Yongning W, Shaolin W. Global impact of mcr-1-positive Enterobacteriaceae bacteria on "one health". Crit Rev Microbiol. 2020;46:565-77.
9. Pathak A, Singh S, Kumar A, Prasad KN. Emergence of chromosome borne colistin resistance gene, mcr-1 in clinical isolates of Pseudomonas aeruginosa. Int J Infect Dis. 2020;101:22.
10. Chen H, Mai H, Lopes B, Wen F, Patil S. Novel Pseudomonas aeruginosa Strains Co-Harbouring blaNDM-1 Metallo β-Lactamase and mcr-1 Isolated from Immunocompromised Paediatric Patients. Infect Drug Resist. 2022;15:2929-36.
11. Hameed F, Khan MA, Muhammad H, Sarwar T, Bilal H, Rehman TU. Plasmid-mediated mcr-1 gene in Acinetobacter baumannii and Pseudomonas aeruginosa: first report from Pakistan. Rev Soc Bras Med Trop. 2019;5:52.
12. Özkaya E, Buruk CK, Tosun İ, Toraman B, Kaklıkkaya N, Aydın F. Investigation of plasmid mediated mcr colistin resistance gene in clinical Enterobacterales isolates Mikrobiyol Bul. 2020;54:191-202.

13. Li A, Yang Y, Miao M, Chavda KD, Mediavilla JR, Xie X et al. Complete sequences of mcr-1-harboring plasmids from extended-spectrum-lactamase- and carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother. 2016;60:4351–4.
14. Xie J, Liang B, Xu X, Yang L, Li H, Li P et al. Identification of mcr-1-positive multidrug-resistant Escherichia coli isolates from clinical samples in Shanghai, China. J Glob Antimicrob Resist. 2022;29:88-96.
15. Karakonstantis S, Kritsotakis EI, Gikas A. Pandrug-resistant Gram-negative bacteria: a systematic review of current epidemiology, prognosis and treatment options. J Antimicrob Chemother. 2020;75:271-82.
16. Voets GM, Fluit AC, Scharringa J, Cohen Stuart J, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. Int J Antimicrob Agents. 2011;37:356-9.
17. Salman M, Aamir Ali A, Haque A. A novel multiplex PCR for detection of Pseudomonas aeruginosa: A major cause of wound infections. Pak J Med Sci. 2013;29:957-61.
18. Liu Y, Liu C, Zheng W, Zhang X, Yu J, Gao Q et al. PCR detection of Klebsiella pneumoniae in infant formula based on 16S-23S internal transcribed spacer. Int J Food Microbiol. 2008;125:230-5.
19. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One. 2015;10:e0123690.
20. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0, 2021. http://www.eucast.org. (cited 2023 January 10).
21. Guven Gokmen T, Nagiyev T, Meral M, Onlen C, Heydari F, Koksal F. NDM-1 and rmtC-Producing Klebsiella pneumoniae Isolates in Turkey. Jundishapur J Microbiol. 2016;9:e33990.
22. Sanger F, Nicklen S, Coulson AR. DNA Sequencing by chain termination with dideoxynucleotides. Proc Natl Acad Sci USA. 1977;74:5463-7.
23. Fawaz AS. Methods for Sanger Sequencing in Diagnostic and Research. (serial online) 2018 (cited 2023 January 27). https://www.researchgate.net/publication/326672981.
24. Kurekci C, Aydin M, Nalbantoglu OU, Gundogdu A. First report of Escherichia coli carrying the mobile colistin resistance gene mcr-1 in Turkey. J Glob Antimicrob Resist. 2018;15:169-70.
25. Adiguzel MC, Baran A, Wu Z, Cengiz S, Dai L, Oz C, Oz et all. Prevalence of Colistin resistance in Escherichia coli in Eastern Turkey and Genomic Characterization of an mcr-1 Positive Strain from Retail Chicken Meat. Microb Drug Resist. 2021;27:424-32.
26. Arabacı Ç, Dal T, Başyiğit T, Genişel N, Durmaz R. Investigation of carbapenemase and mcr-1 genes in carbapenem-resistant Klebsiella pneumoniae isolates. J Infect Dev Ctries. 2019;13:504-09.
27. Hazırolan G, Karagöz A. Emergence of carbapenemase-producing and colistin resistant Klebsiella pneumoniae ST101 high-risk clone in Turkey Acta Microbiol Immunol Hung. 2020;67:216-21.
28. Özkaya E, Buruk CK, Tosun İ, Toraman B, Kaklıkkaya N, Aydın F. Investigation of Plasmid Mediated mcr Colistin Resistance Gene in Clinical Enterobacterales Isolates. Mikrobiyol Bul. 2020;54:191-02.
29. Tarhan G, Şahin F, Cesur S. Investigation of the presence of OXA 48 and mcr-1 genes in carbapenem-resistant Acinetobacter baumannii, Klebsiella pneumoniae and Pseudomonas aeruginosa strains by in house-PCR method. J Med Palliat Care. 2021;2:118-23.
30. Mmatli M, Leshaba TMS, Skosana LB, Mbelle NM, Osei Sekyere J. Molecular screening of clinical multidrug-resistant gram-negative bacteria shows endemicity of carbapenemases, coexistence of multiple carbapenemases, and rarity of mcr in south africa. Microb Drug Resist. 2022;28:1028-36.
31. Al-Kadmy IMS, Ibrahim SA, Al-Saryi N, Aziz SN, Besinis A, Hetta HF. Prevalence of genes involved in colistin resistance in acinetobacter baumannii: first report from Iraq. Microb Drug Resist. 2020;26:616-22.
32. Rahman M, Ahmad S. First report for emergence of chromosomal borne colistin resistance gene mcr-1 in a clinical Acinetobacter baumannii Isolates from India. Open Forum Infect Dis. 2019;6:261–2.
33. Falgenhauer L, Waezsada SE, Gwozdzinski K, Ghosh H, Doijad S, Bunk B et al. Chromosomal locations of mcr-1 and bla CTX-M-15 in Fluoroquinolone-resistant Escherichia coli ST410. Emerg Infect Dis. 2016;22:1689-91.
34. Strepis N, Voor in 't holt AF, Vos MC, Zandijk WHA, Heikema AP, Hays JP et al. Genetic analysis of mcr-1-carrying plasmids from gram-negative bacteria in a dutch tertiary care hospital: evidence for intrapatient and interspecies transmission events. Front Microbiol. 2021;12:727435.
35. Zhong YM, Liu WE, Zheng ZF. Epidemiology and molecular characterization of mcr-1 in Escherichia coli recovered from patients with bloodstream infections in Changsha, central China. Infect Drug Resist. 2019;12:2069–76.
36. McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R et al. Escherichia coli Harboring mcr-1 and blaCTX-M on a Novel IncF Plasmid: First

Report of mcr-1 in the United States. Antimicrob Agents Chemother. 2016;60:4420-21.
37. Xiang Q, Hu S, Ke Y, Hu S. Multiplex PCR for detection of MCR genes in clinical fecal samples. E3S Web Conf. 2021;269:01019.

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