

DETECTION OF OXA-23 GENE  
IN AMOXICILLIN-CLAVULANIC ACID-RESISTANT  
*PROTEUS MIRABILIS* ISOLATES IN TURKEY

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**Abstract**

Class D carbapenemases, including OXA-23, OXA24/40, OXA-51, OXA-58, and OXA143, are typically associated with *Acinetobacter* species; OXA-48-like carbapenemases are usually found in *Enterobacterales* isolates. Although a few studies on *Acinetobacter*-type OXA genes in *Enterobacterales* have been reported, the presence of these *Acinetobacter*-type OXA genes in these isolates is quite limited. In contrast to other *Enterobacterales* isolates, studies have identified the presence of genes OXA-23, OXA-24, OXA-51, and OXA-58, which are typically associated with *Acinetobacter* species, in *Proteus mirabilis*. This study aimed to investigate the presence of carbapenemase genes in amoxicillin-clavulanic acid-resistant *P. mirabilis* isolates. Thirty-two *P. mirabilis* isolates isolated from samples sent from intensive care patients and resistant to amoxicillin-clavulanic acid were included in the study. Vitek MS was used for bacterial identification, and the antibiotic resistance profile was determined by the Vitek 2 automated system. The presence of *Acinetobacter* type OXA genes (OXA-23, OXA-24, OXA-51, and OXA-58) and OXA-48, KPC, NDM, VIM, and IMP genes in *P. mirabilis* strains were investigated by polymerase chain reaction (PCR) method. In addition, ESBL production was detected in carbapenemase gene-positive isolates. According to the PCR results, while the presence of the OXA23 gene was detected in 15.6% of the isolates, the presence of OXA-24, OXA-51, OXA-58, OXA-48, KPC, NDM,

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VIM and IMP genes was not detected in any of the isolates. OXA-23, OXA-24, OXA-51, and OXA-58 are common carbapenemases in *Acinetobacter* species, especially OXA-23 is very common. However, these genes are very rare in *Enterobacteriales* isolates. There are studies in which these genes were identified in *P. mirabilis* isolates. Similarly, in our research, the OXA-23 gene was detected in *P. mirabilis* isolates. To our knowledge, this is the first OXA-23 positivity reported in *P. mirabilis* isolates from our country.

**Key words:** *Proteus mirabilis*, amoxicillin-clavulanic acid resistance, class D carbapenemase, OXA-23

**Introduction.** *Proteus mirabilis* is widely isolated from various clinical specimens related to healthcare-associated infections, particularly urinary tract samples [1]. Class D carbapenemases, including OXA-23, OXA24/40, OXA-51, OXA-58, and OXA143, are predominantly associated with *Acinetobacter* species, while OXA-48-like carbapenemases are usually observed in *Enterobacteriales* isolates. Although several studies of *Acinetobacter*-type OXA genes have been reported in *Enterobacteriales*, the prevalence of actual *Acinetobacter*-type OXA genes in these isolates was underreported because most surveillance studies have focused on the most widespread “big five” carbapenemases (OXA-48, KPC, NDM, VIM, and IMP) [2,3]. Unlike other *Enterobacteriales*, *Acinetobacter*-type carbapenemases (specifically OXA-23, OXA-24, OXA-51, and OXA-58) have been reported in *P. mirabilis* [4]. This study aimed to evaluate the presence of *Acinetobacter*-type carbapenemase genes in amoxicillin-clavulanic acid-resistant *P. mirabilis* isolates.

**Materials and methods. Study design, bacterial identification, and antimicrobial susceptibility test.** *P. mirabilis* isolates resistant to amoxicillin-clavulanic acid and isolated from samples sent from intensive care patients to the Microbiology Laboratory of Ondokuz Mayıs University Medical Faculty Hospital between 2021–2022 were involved in the study. Only one of the isolates obtained from each patient was included in the study.

Bacterial identification of the isolates was determined using the Vitek MS (bioMérieux, Marcy l’Etoile, France) automated system. The antimicrobial susceptibility of these isolates was determined using Vitek 2 Compact (bioMérieux, Marcy l’Etoile, France) with European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

**Molecular methods.** DNA isolation was performed from the isolates by boiling method for the molecular determination of carbapenemase genes. The obtained DNAs were stored at  $-20^{\circ}\text{C}$  until used as template DNA for polymerase chain reaction (PCR).

Electrophoresis of PCR products was performed using 2% agarose gel, and DNA bands of the samples were compared with the GeneRuler 100 base pair (bp) DNA Ladder Plus (Thermo Fischer, USA) DNA marker and examined on a Bio-Rad gel imaging device.

**Detection of carbapenem resistance genes.** *Acinetobacter*-type carbapenem resistance genes (OXA-23, OXA24, OXA-51, and OXA-58) were investigated by multiplex PCR. The primers used were determined after the literature review (Table 1) [5]. Amplification of these resistance genes includes denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 25 s, primer annealing at 52 °C for 40 s, and elongation at 72 °C for 50 s followed by 6 min of final extension at 72 °C.

T a b l e 1  
Primers used in the study

| Gene   | Primer   | Sequence (5'-3')        | Amplicon size | Reference |
|--------|----------|-------------------------|---------------|-----------|
| OXA-23 | OXA-23-F | GATCGGATTGGAGAACCAGA    | 501 bp        | 5         |
|        | OXA-23-R | ATTTCTGACCGCATTTCCAT    |               |           |
| OXA-24 | OXA-24-F | GGTTAGTTGGCCCCCTTAAA    | 246 bp        | 5         |
|        | OXA-24-R | AGTTGAGCGAAAAGGGGATT    |               |           |
| OXA-51 | OXA-51-F | TAATGCTTTGATCGGCCTTG    | 353 bp        | 5         |
|        | OXA-51-R | TGGATTGCACTTCATCTTGG    |               |           |
| OXA-58 | OXA-58-F | AAGTATTGGGGCTTGTCTG     | 599 bp        | 5         |
|        | OXA-58-R | CCCCTCTGCGCTCTACATAC    |               |           |
| KPC    | KPC-F    | TGTCACTGTATCGCCGTC      | 900 bp        | 6         |
|        | KPC-R    | CTCAGTGCTCTACAGAAAACC   |               |           |
| NDM    | NDM-F    | GCAGCTTGTGCGCCATGCGGGC  | 782 bp        | 6         |
|        | NDM-R    | GGTCGCGAAGCTGAGCACCGCAT |               |           |
| OXA-48 | OXA-48-F | TTGGTGGCATCGATTATCGG    | 438 bp        | 6         |
|        | OXA-48-R | GAGCACTTCTTTTGTGAGGC    |               |           |
| VIM    | VIM-F    | GTTTGGTTCGCATATCGCAAC   | 389 bp        | 6         |
|        | VIM-R    | AATGCGCAGCACCAGGATAG    |               |           |
| IMP    | IMP-F    | GGAATAGAGTGGCTTAATTCTC  | 188 bp        | 7         |
|        | IMP-R    | CCAAACCACTACGTTATCT     |               |           |

bp: Base pair

Carbapenem resistance genes frequently encountered in *Enterobacteriales* isolates (NDM, VIM, KPC, IMP, and OXA-48) were investigated by multiplex PCR. The primers used were determined after the literature review (Table 1) [6,7]. Amplification of these resistance genes includes denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 45 s, primer annealing at 60 °C for 45 s, elongation at 72 °C for 60 s followed by 8 min of final extension at 72 °C.

The presence of specific genes was confirmed through sequence analysis of isolates that detected positive results in the PCR.

Additionally, a double-disc synergy test was performed to detect extended-spectrum  $\beta$ -lactamases (ESBL) production in carbapenem resistance genes-positive isolates. In this test, an amoxicillin-clavulanic acid disc was placed in the centre of the Mueller Hinton agar medium while ceftazidime, ceftriaxone, and cefotaxime discs were placed 20 mm away from the amoxicillin-clavulanic acid

disc. The plates were then incubated at 37 °C for 18–24 h, and zone diameters were evaluated after incubation. Enlargement of the zone of inhibition formed around the ceftazidime, ceftriaxone, and cefotaxime discs towards the amoxicillin-clavulanic acid disc was accepted as the presence of ESBL [8,9].

**Results.** In this investigation, 32 *P. mirabilis* isolates resistant to amoxicillin-clavulanate were collected from various patient samples. According to sample type distribution, 50% were exudate, 31.25% were urine, 9.4% were blood, and 9.4% were tracheal aspirate samples.

OXA-23 gene was determined in 5 (15.6%) isolates, while OXA-24, OXA-51, OXA-58, OXA-48, KPC, NDM, VIM, and IMP genes were not found in any of the isolates. The gel image of the OXA-23 gene-positive isolates is shown in Fig. 1. As a result of the sequence analysis, the presence of the gene region was confirmed in the OXA-23 suspected positive isolates.

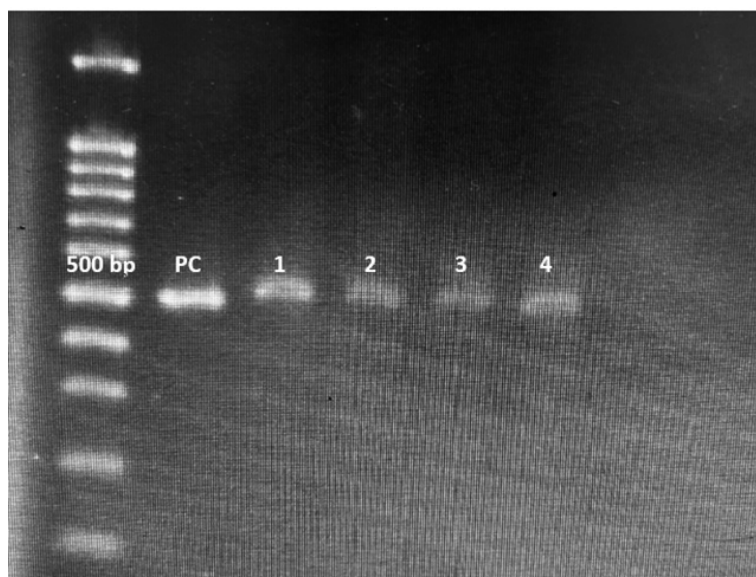


Fig. 1. Gel image of four of the OXA-23 positive isolates

ESBL production by double disc synergy test was observed in three of the OXA-23 positive *Proteus mirabilis* isolates. The antibiotic susceptibility test results of the isolates are presented in Table 2.

**Discussion.** As a member of the *Enterobacterales* order and the *Morganelaceae* family, *Proteus spp.* constitutes a component of the natural gut microbiota in both humans and animals. Within the *Proteus* genus, *P. mirabilis* emerges as the predominant species, notably those linked to healthcare-associated infections, particularly urinary tract specimens [10,11].

*P. mirabilis* intrinsically demonstrates resistance to polymyxins, nitrofurantoin, and tetracyclines. It does not produce any  $\beta$ -lactamases and exhibits sus-

T a b l e 2  
Antibiotic susceptibility of *Proteus mirabilis* isolates

|                               | All isolates              |                                               |                             | OXA-23 positive isolates  |                                               |                             |
|-------------------------------|---------------------------|-----------------------------------------------|-----------------------------|---------------------------|-----------------------------------------------|-----------------------------|
|                               | Resistant<br><i>n</i> (%) | Susceptible dose<br>dependent<br><i>n</i> (%) | Susceptible<br><i>n</i> (%) | Resistant<br><i>n</i> (%) | Susceptible dose<br>dependent<br><i>n</i> (%) | Susceptible<br><i>n</i> (%) |
| Amoxicillin-Clavulanic Acid   | 32 (100)                  | —                                             | —                           | 5 (100)                   | —                                             | —                           |
| Ampicillin                    | 32 (100)                  | —                                             | —                           | 5 (100)                   | —                                             | —                           |
| Amikacin                      | 7 (21.8)                  | 22 (68.75)                                    | 3 (9.35)                    | 2 (40)                    | —                                             | 3 (60)                      |
| Ertapenem                     | 4 (12.5)                  | 1 (3.1)                                       | 27 (84.4)                   | 1 (20)                    | —                                             | 4 (80)                      |
| Fosfomycin*                   | 6 (60)                    | —                                             | 4 (40)                      | 1 (50)                    | —                                             | 1 (50)                      |
| Gentamicin                    | 24 (75)                   | 3 (9.4)                                       | 5 (15.6)                    | 4 (80)                    | —                                             | 1 (20)                      |
| Imipenem                      | 4 (12.5)                  | 6 (18.75)                                     | 22 (68.75)                  | 1 (20)                    | 2 (40)                                        | 2 (40)                      |
| Meropenem                     | —                         | 3 (9.4)                                       | 29 (90.6)                   | —                         | —                                             | 5 (100)                     |
| Nitrofurantoin*               | 10 (100)                  | —                                             | —                           | 2 (100)                   | —                                             | —                           |
| Piperacillin/Tazobactam       | 5 (15.6)                  | 1 (3.1)                                       | 26 (81.3)                   | 5 (100)                   | —                                             | —                           |
| Cefepime                      | 12 (37.5)                 | 2 (6.2)                                       | 19 (59.3)                   | —                         | 2 (40)                                        | 3 (60)                      |
| Ceftazidime                   | 15 (46.8)                 | —                                             | 17 (53.2)                   | 3 (60)                    | —                                             | 2 (40)                      |
| Ceftriaxone                   | 18 (56.25)                | —                                             | 14 (43.75)                  | 2 (40)                    | —                                             | 3 (60)                      |
| Cefuroxime axetil             | 20 (62.5)                 | —                                             | 12 (37.5)                   | 5 (100)                   | —                                             | —                           |
| Ciprofloxacin                 | 24 (75)                   | 1 (3.1)                                       | 7 (21.9)                    | 3 (60)                    | —                                             | 2 (40)                      |
| Trimethoprim/sulfamethoxazole | 30 (93.8)                 | —                                             | 2 (6.2)                     | 4 (80)                    | —                                             | 1 (20)                      |

\*Fosfomycin and nitrofurantoin were evaluated for urinary tract specimens only.

ceptibility to all  $\beta$ -lactams except for imipenem. The reduced susceptibility to imipenem is linked to low-affinity penicillin-binding proteins. Acquired resistance to  $\beta$ -lactams predominantly originates from the extended-spectrum  $\beta$ -lactamases (ESBLs), cephalosporinases, and sporadic acquisition of carbapenemases [12,13]. These carbapenemases are generally detected in *Enterobacteriales*, such as KPC (Ambler class A), NDM, VIM, or IMP type metallo- $\beta$ -lactamases (Ambler class B), and carbapenem hydrolyzing Ambler class D  $\beta$ -lactamases [14].

Ambler class D carbapenemases, specifically OXA-23, OXA-24/40, OXA-51, OXA-58, and OXA-143, are prominently associated with *Acinetobacter* species; OXA-48-like carbapenemases are usually found in *Enterobacteriales* isolates. While several studies have reported the presence of *Acinetobacter*-type OXA genes in *Enterobacteriales* isolates, the actual prevalence of *Acinetobacter*-type OXA genes in these isolates may be underestimated. This potential underreporting is because most surveillance studies have primarily been focused on the five most common carbapenemases (KPC, NDM, OXA-48, VIM, and IMP) [14–16].

Despite their categorization as carbapenemases, the expression of OXA-23 and OXA-58 enzymes interestingly does not result in the development of multidrug resistance within *P. mirabilis*. Generally, strains of *P. mirabilis* producing OXA-23 exhibit an antibiotic susceptibility profile marked by resistance only to amoxicillin, ticarcillin, and piperacillin, with no improvement in susceptibility when combined with clavulanic acid or tazobactam. They remain susceptible to third-generation cephalosporins, and resistance to carbapenems is challenging to observe due to the weak carbapenem hydrolyzing activity and typical chromosomal localization of OXA-23, OXA-24/-40 and OXA-58 [11,13].

Based on the double disc synergy test results of the isolates harbouring the OXA-23 gene in our study, three isolates were ESBL-positive. Two ESBL-negative isolates were susceptible to 3rd and 4th generation cephalosporins. This result shows that amoxicillin-clavulanic acid resistance may be caused by OXA-23 gene positivity, as stated in previous studies [14].

OXA-23, OXA-24, OXA-51, and OXA-58 are common carbapenemases in *Acinetobacter* species. Although OXA-23 is very common, it is endemic to some regions. However, these genes are rarely encountered in *Enterobacteriales* isolates [4]. In contrast to *Enterobacteriales* other than *P. mirabilis*, studies have reported the presence of these genes in *P. mirabilis* isolates [14]. The results of previous studies show that OXA-23 and OXA-58 positivity was detected in *P. mirabilis* isolates, especially in Europe. OXA-23 genes in France and Finland, OXA-58 genes in Belgium and Germany, and OXA-24/-40 genes in Algeria were detected in *P. mirabilis* isolates [3,14,17–19]. Similarly, in our study, the OXA-23 gene was detected in *P. mirabilis* isolates. To our knowledge, this is the first OXA-23 positivity reported in *P. mirabilis* isolates from our country.

While OXA-23, OXA-24, OXA-51, and OXA-58 have not been extensively investigated in *Enterobacteriales* isolates, a limited number of studies, including

our research, indicate that these gene regions can also be present in *Enterobacterales* isolates. Comprehensive studies to assess the prevalence of these genes will significantly contribute to antimicrobial resistance surveillance.

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