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# Molecular Study of Carbapenem Resistance Genes in *Proteus mirabilis* Isolated from Clinical Samples in Baghdad Hospitals

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**Abstract*:*** The majority of *Proteu*s species are opportunistic pathogens that infect humans with a variety of illnesses including, infections of the respiratory system, nose, eye, ear, skin, and throat, as well as wounds and urinary tract infections. The aim of this study to isolate and identify of *Proteus mirabilis*; molecular screening of carbapenems Resistance genes (*blaOXA-48, blaOXA-58* and *blaOXA-23*) of *Proteus mirabilis* and analysis its prevalence among local isolates in Baghdad. Two hundred and fifty samples have been collected from urine, ear swabs, burn and wound swabs of patients of both sexes and different age from several hospitals in Baghdad. The time between October 2022 to until the end of March 2023, has been used for the collecting of samples. From inpatients and outpatients in four hospitals in Baghdad. Gram's staining, microscopic examinations, cultural on MacConkey agar and blood agar plates and biochemical properties were used to identify all isolates. The diagnosis was confirmed through VITEK-2 system. According to the diagnostic results, out of 250 clinical samples, 225 were found to be positive, with 65 (26%) of the 250 isolates being *Proteus mirabili*s, while the remaining 160 (64%) belonged to other bacterial species. Antibiotic sensitivity test against 16 different antibiotics showed that Proteus *mirabilis* was highest resistance Piperacillin (89.2%), Cefotaxime (87.6%), Ceftazidime (86.1%), Cefepime (81.5%), Trimethoprim / Sulfamethoxazole (78.4%), Gentamicin (76.9%), Tetracycline (75.3%), While there is less resistance at Doxycycline (69.2%). On the other hand, moderate resistance Ceftriaxone (58.4%), Amoxicillin-clavulanic acid (56.9%), Amikacin (53.8%), Aztreonam (41.5%), and Ciprofloxacin (40%). Based on the results of the current study, *P. mirabilis* had only low levels of resistance to Levofloxacina (33.8%), Imipenem (15.3%) and Meropenem (1.5%). Conventional PCR using *16S rRNA* housekeeping gene to Detection all isolates were *P. mirabilis*. In conclusion, Carbapneam resistance genes were identified by PCR. It was concluded that the existence of the *OXA-23* gene gave (15, 61, 40, 43, 31, 21) isolates to be 60% resistant to carbapenem, whereas the presence of the *OXA-58* gene in (6) isolates to be 60% resistant.

**Keywords*:*** *blaOXA-23, blaOXA-58*, PCR, *P .mirabilis*.

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## Introduction

*Proteus* species are common in the environment and are also found in the normal flora of the intestine. Because the pathogen has several modes of transmission, it is one of the most commonly involved bacteria in both nosocomial and community-acquired infections (1). *Proteus mirabilis* is an opportunistic pathogen, which means that it causes infections in people with reduced natural immunity (2). These bacteria possess several virulence factors, which are essential for the colonization of new areas, involving host tissues and organs (3). UTIs are the most prevalent clinical symptoms of

*Proteus* infection. *Proteus* accounts for one to two percent of all UTIs in otherwise healthy women, whereas it accounts for 5% of hospital acquired UTIs. *Proteus mirabilis* UTI patients often have bacteriuria, cystitis, kidney and bladder stones, catheter obstruction from stone encrustation, and acute pyelonephritis in addition to their UTI. (4, 5). The virulence factors expressed by *P. mirabilis* are many Flagella, quorum sensing molecules, efflux pumps, adhesion proteins, hemolysins, lipopolysaccharides, and IgA proteases, ability to acquire antibiotic resistance and urease enzyme are a few examples of such elements (6). This organism can be defined as a gram-negative rod which is which is a member of *Enterobacteriaceae* family, motile, urease-positive, lactose-negative, indole-negative and produces hydrogen sulfide (7). In addition to urinary tract infections, *P. mirabilis* has been associated with opportunistic pulmonary system infections, burns, wound, skin, eyes, nose, ears, and gastroenteritis, this also causes an autoimmune disease in human who is genetically susceptibility to develop rheumatoid arthritis (8). Otitis media happens after an acute infection of the upper respiratory tract. Otitis media is the fifth most prevalent disease worldwide and the second most important cause of hearing loss, with a higher prevalence in underdeveloped regions such as Africa and South Asia. In sub-Saharan Africa, *Pseudomonas aeruginosa* and *P. mirabilis* were found to be the most prevalent bacterial pathogens causing Otitis media (9). Additionally, *P. mirabilis* has been associated with foodborne disease (10). According to recent statistics, 700,000 patients die each year from diseases caused on by organisms that are resistant to antibiotics (11). It employs a number of resistance strategies against different classes of antibiotics, such as target modification, altered cell membrane permeability, synthesis of enzymes, the presence of efflux pumps, and altered metabolic pathways (12, 13). The beta-lactam antibiotics work by acting as an irreversible inhibitor of the transpeptidase enzyme, which bacteria employ to make their cell walls. The transpeptidase, also known as penicillin-binding proteins (14).

## Materials and methods

### Collection of samples

Baghdad hospitals were used to conduct this investigation. two hundred and fifty (250) samples have been collected from urine, swab from ear, burn and wound infections of patients of both sexes and different age from several hospitals in Baghdad. *P. mirabilis* orientation medium was employed for the rapid diagnosis together Blood agar and McConkey agar for *P. mirabilis* isolation. According to the manufacturer's recommendations, these isolates were identified using standard bacteriological techniques and biochemical testing using the VITEK 2 system (bioMerieux, France).

### Testing for antimicrobial susceptibility

A disc diffusion method antimicrobial susceptibility test was carried out. In a nutshell, overnight growth of *P. mirabilis* were generated on McConkey agar and then resuspended in Mueller-Hinton broth. The suspension's turbidity was adjusted to be equivalent to 0.5 McFarland, and Mueller-Hinton agar plates were inoculated with this suspension. The 16 various antibiotics included on the antibiotic discs utilized in this investigation were as follows: Imipenem (IMP), Meropenem (MER), Ciprofloxacin CIP), Levofloxacin (LE),

Amikacin (AK), Gentamicin (CN), Azetrenam (ATM), Cefepime (FEP), Cefotaxime (CTX), Ceftriaxone (CRT), Ceftazidime (CAZ), Amoxicillin/Clavulanate (AMC), Piperacillin (PRL), Tetracycline (TE), Doxycycline (DXO) and Trimethoprim / Sulfamethoxazole (SXT). The inhibition zone was assessed and interpreted by the percentage of susceptible, intermediate, or resistant isolates as defined by CLSI after the agar plates had been incubated at 37 °C for 24 hours (15), interpretative criteria for breakpoints (CLSI 2022).

**Extraction of bacterial DNA**

DNA extraction was performed; *Proteus mirabilis* isolates were cultured in brain heart broth for 24 hours at 37°C. Genomic DNA was extracted from the *P. mirabilis* isolates diagnosed by the VITEK 2 system according to the protocol of ABIOpure using the ready kits (promega, USA). Total DNA extraction kit. The concentration of extracted DNA was measured by Quantus Fluorometer, then detected by gel electrophoresis on 2% agarose and exposed to U.V light in which the DNA appears as compact bands.

**Detection of *P. mirabilis 16SrRNA* gene and *OXA*-gens using conventional PCR**

Genes encoding the Carbapneam resistance determinants, *blaOXA-58* and *blaOXA_ 23*, were examined using specific primers and PCR in (Table 1) In a 20 µl reaction, PCR amplification was completed. (Table 2), with the following actions for each primer: 30 cycles of 94°C for 30 sec, 50°C for 45 sec, and 72°C for 7 mins would follow an initial denaturation step at 94°C for 5 min. 1.5% agarose gel was electrophoresed at 150 volts for one hour. Fragments were stained with ethidium bromide after electrophoresis, and the results were seen under an ultraviolet light.

**Table (1): Primers sequences used in PCR.**

| Primers | Sequence of primer (5–3) | Size product | Reference |
|---|---|---|---|
| ***16SrRNA*** | **F:**CACACTGGAACTGAGACAC<br>**R:** CTTCTTCTGCGGGTAACG | 189 | 16 |
| ***OXA_ 23*** | **F:**GATCGGATTGGAGAACCAGA<br>**R:**ATTTCTGACCGCATTTCCAT | 501 | 17 |
| ***OXA-58*** | **F:** TGGCACGCATTTAGACCG<br>**R:** AAACCCACATACCAACCC | 945 | 17 |

**Table (2): Component of Master Mix for PCR Reaction.**

| Components of PCR reaction | | Volume |
|---|---|---|
| Master mix | | 10 |
| DNA template | | 3 |
| Primers | forward | 1 |
| Primers | reverse | 1 |
| nuclease-free H2O | | 5 |
| Total volume | | 20 |

**Results and discussion**

Two hundred and fifty samples have been collected from urine, ear swabs, burn and wound swabs from patients of both sexes and different ages from several hospitals in Baghdad for a period 5 months. The period between October 2022 to until the end of March 2023 has been used for the collecting of samples, in four hospitals in Baghdad (Teaching Laboratories Institute, Al Kindy Teaching Hospital, Ibn Al-Baladi Hospital, AL-Imam Ali General hospital).

**Isolation and identification of *Proteus mirabilis***

Following their morphology in Gram's staining, cultural traits, and biochemical features, isolates were isolated and identified. A total of 250 of urine, swab from ear, burn and wound infections specimens were cultured on MacConkey agar and Blood agar plates. The swarming phenomena on blood agar was used to first identify the *Proteus* isolates as belonging to the genus *Proteus*, while the bacteria on Macconkey agar showed signs of non-lactose fermentation and appeared pale. *P. mirabilis* isolates were subjected to a variety of biochemical tests to confirmed the presence of *P. mirabilis* characteristics, and the results of these determine their biochemical tests in 250 patients. All 65 *P. mirabilis* isolates tested positive for Urease, Citrate Utilization, catalase, H2S, Motility and Kliglar Iron Agar (KIA), but negative for oxidase or the synthesis of indole. Moreover, these isolates failed to ferment maltose and lactose.

**Distribution of samples among patients**

Clinical samples 250, 225 were found to be positive, with 65 (26%) of the 250 isolates being *P. mirabilis*, while the remaining 160 (64%) belonged to other bacterial species and 25 samples (10%) without any bacteria. In a local study which was carried out in Baghdad by Abed., (2020) (18). One hundred and ten of the 150 clinical samples yield positive results, with 19 (12.7%) isolates of *P. mirabilis* being among them. 65 bacterial isolates of *P. mirabilis* were distributed as 27 isolates from wound swab specimens (41.5%), 23 isolates from urine specimens (35.3%), 13 isolates from burn swab specimens ( 20 %) and 2 isolate from ear specimen (3%) (Table 3).

**Table (3): *Proteus* isolates are distributed according to the source of isolation.**

| Source of isolation | Sample number | Isolate number | % Percentage |
|---|---|---|---|
| **Wound swab** | 90 | 27 | 41.4% |
| **Urine** | 70 | 23 | 35.3% |
| **Burn swab** | 50 | 13 | 20% |
| **Ear swab** | 15 | 2 | 3% |
| **Total** | **225** | **65** | **100%** |
| **P-value** | **-** | **--** | **<0.001** |

These percentages were similar to that found by Almubarak (19), who have been shown a higher percentage of *Proteus* in wound swab (52%).As for age, the age groups of (11-20) years had the highest infection rates, while those from (21-30) years had the lowest infection rates. Show in (Table 4).

**Table (4): Percentages of *Proteus mirabilis* infection by age groups.**

| Age group (years) | Number | %Percentage |
|---|---|---|
| 1-10 | 6 | 9.2 |
| 11-20 | 15 | 23 |
| 21-30 | 13 | 20 |
| 31-40 | 8 | 12.3 |
| 41-50 | 13 | 18.4 |
| 51-60 | 12 | 9.2 |
| Over 60 | 5 | 7.6 |
| **Total** | **65** | **100** |
| **P-value** | **--** | ****0.001** |

Where it was found that the rate of infection in males (60%) more than females (40%). This study is similar to Bahashwan (20) an existing study in the

Kingdom of Saudi Arabia (KSA), where the infection rate was (75% males, 25% females in (Table 5)

**Table (5): Percentages of *Proteus mirabilis* infection by gender**

| Gender | Number | Percentage% |
|---|---|---|
| **Males** | 39 | 60 |
| **Females** | 26 | 40 |
| **Total** | 65 | 100 |

## Antimicrobial susceptibility test

Antimicrobial susceptibility was performed on all 65 *P. mirabilis* isolates to 16 antibiotics by disc diffusion method (See Table 6). Antimicrobial susceptibility was performed to 16 antibiotics represented by Amikacin, Gentamicin, Imipenem, Meropenem, Azetrenam, Cefepime, Cefotaxime, Ceftriaxone,Ceftazidime, Ciprofloxacin, Levofloxacin,Amoxicillin / Clavulanate, Piperacillin, Tetracycline,Doxycycline and Trimethoprim / Sulfamethoxazole.

**Table (6): Percentages of 65 *P. mirabilis* isolates' antimicrobial sensitivity to 16 antimicrobial agents.**

| Antibiotic (A.B) | Resistant | | Intermediate | | Sensitive | |
|---|---|---|---|---|---|---|
| | Number Sample | % | Number Sample | % | Number Sample | % |
| **Amoxicillin-clavulanic acid** | 37 | 56.9 | 9 | 13.8 | 19 | 29.2 |
| **Piperacillin** | 58 | 89.2 | 3 | 4.6 | 4 | 6.1 |
| **Ceftriaxone** | 38 | 58.4 | 10 | 15.3 | 17 | 26.1 |
| **Cefotaxime** | 57 | 87.6 | 0 | 0 | 8 | 12.3 |
| **Ceftazidime** | 56 | 86.1 | 0 | 0 | 9 | 13.8 |
| **Cefepime** | 53 | 81.5 | 3 | 4.6 | 9 | 13.8 |
| **Imipenem** | 10 | 15.3 | 2 | 3 | 53 | 81.5 |
| **Meropenem** | 1 | 1.5 | 0 | 0 | 64 | 98.4 |
| **Amikacin** | 35 | 53.8 | 13 | 20 | 18 | 27.6 |
| **Gentamicin** | 50 | 76.9 | 2 | 3 | 13 | 20 |
| **Ciprofloxacin** | 26 | 40 | 12 | 18.4 | 27 | 41.5 |
| **Levofloxacin** | 22 | 33.8 | 6 | 9.2 | 37 | 56.9 |
| **Tetracycline** | 49 | 75.3 | 3 | 4.6 | 13 | 20 |
| **Doxycycline** | 45 | 69.2 | 3 | 4.6 | 17 | 26.1 |
| **Aztreonam** | 27 | 41.5 | 10 | 15.3 | 28 | 43 |
| **Trimethoprim / Sulfamethoxazole** | 51 | 78.4 | 4 | 6.1 | 9 | 13.8 |

The antibiogram for studied isolates revealed that *P. mirabilis* clinical isolates were highly resistant to the majority of the tested antibiotics. The present study showed the highest resistance Piperacillin (89.2%), Cefotaxime (87.6%), Ceftazidime (86.1%), Cefepime (81.5%), Trimethoprim / Sulfamethoxazole (78.4%), Gentamicin (76.9%), Tetracycline (75.3%), While there is less resistance at Doxycycline (69.2%). On the other hand, moderate resistance Ceftriaxone (58.4%), Amoxicillin-clavulanic acid (56.9%), Amikacin (53.8%), Aztreonam (41.5%), and Ciprofloxacin (40%). The current study demonstrated that *P. mirabilis* possessed a low-level resistance against Levofloxacin (33.8), Imipenem (15.3%) and Meropenem (1.5%). Among 65 isolates of *P. mirabilis*, 52(80 %) were

resistant to more than 3 classes of selected antibiotics, in other words, Multi-Drug Resistant (MDR) *P. mirabilis.* In the present study, there was an obvious high resistance to beta-lactam antibiotics, β-lactamases of Ambler class D, known as OXA enzymes. This type of β-lactamases is frequently encoded on plasmids and/or integrons allowing for their wide dissemination. Carbapenem-hydrolyzing class D β-lactamases (CHDLs) are OXA enzymes that hydrolyze carbapenems (21).

A study from Iraq show that *P. mirabilis* isolates exhibited a higher percentage of resistance into Piperacillin (89.2%)Cefotaxime (87.6%), while, the lowest resistance with imipenem and Meropenem (15.3% and 1.5 %). These results are close to the results of Ghazi., (2021) (22) It has a high resistance to Cefotaxime (88.9%) and Piperacillin (86.6.4 %).

**Molecular identification of *P. mirabilis 16sRNA* gene by polymerase chain reaction (PCR)**

Using PCR to confirm the diagnosis, all Phenotypically positive isolates of *P. mirabilis* strains were submitted to molecular identification using the special initiator of the *16S rRNA* gene. The findings demonstrated that every isolate belonged to *P. mirabilis* (Figure 1).

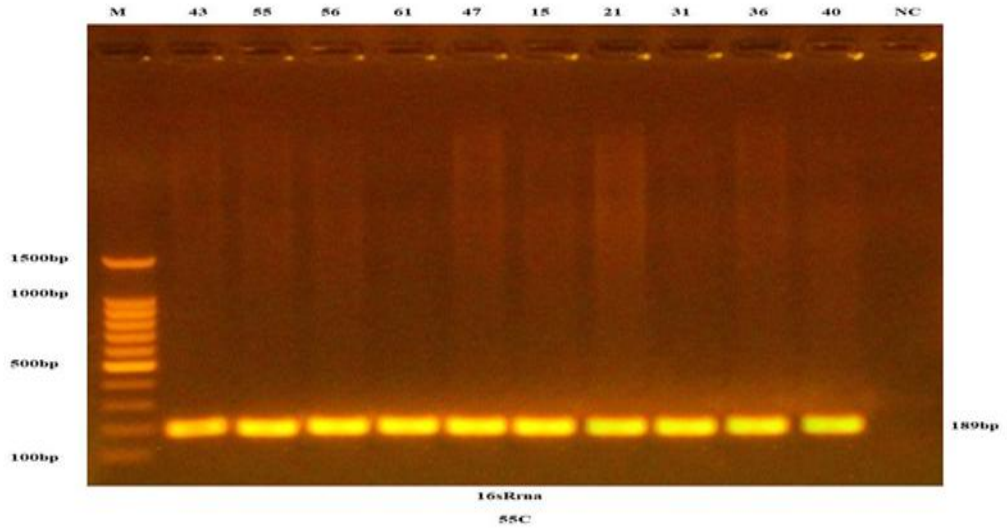


**Figure (1): *Proteus mirabilis* samples result of the amplification of *16S rRNA gene of* were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. Lane1:100bp DNA marker**

The polymerase chain reaction amplification test used to detect the *16S rRNA* gene produced a positive result for the positive isolates identified by the VITEK 2 system assay. All isolates were confirmed to be *P. mirabilis 16SrRNA*-PCR. Included (10 Isolates) tested positive for imipenem were positive (100%).

The present study's findings are in agreement with the results of the local study conducted by the researcher AL-Hamdani., (2019) (23) where the percentage of bacterial isolates possessing *16sRNA* is 100%.

**Detection of *P. mirabilis blaOXA-23* gene using conventional PCR**

Using a genomic DNA purification kit ABIO pure extraction, from *P. mirabilis* isolates, genomic DNA was extracted. Extraction DNA from (10 isolates) tested positive for imipenem resistance, and this resistance

was confirmed as bands by gel electrophoresis. The Quantus Fluorometer was used to measure the DNA extraction's concentration. The concentration of total DNA ranged from (35 to 128) ng/ µl , then detected by gel electrophoresis on 2% agarose and exposed to U.V light in which the DNA appears as compact bands.

In order to detect Carbapeneam resistance gene *blaOXA-23* for each sample of DNA taken from *P. mirabilis* clinical isolates, a uniplex polymerase chain reaction (PCR) was used to determine the prevalence of each gene. Total 10 isolates included were positive (60%). The positive result isolates are shown in (Figure 2).

The current results were similar to the study performed by Bonnin, *et al.* (24) in France and Belgium show 21 of the 61 isolates tested positive for the *blaOXA-23* gene, while one tested positive for the *blaOXA-58* gene. However, another study by Lombes, *et al*. (25) show that 26.9% (14/52) of amoxicillin-clavulanate-resistant *P. mirabilis* isolates gave a positive signal for *blaOXA-23*.

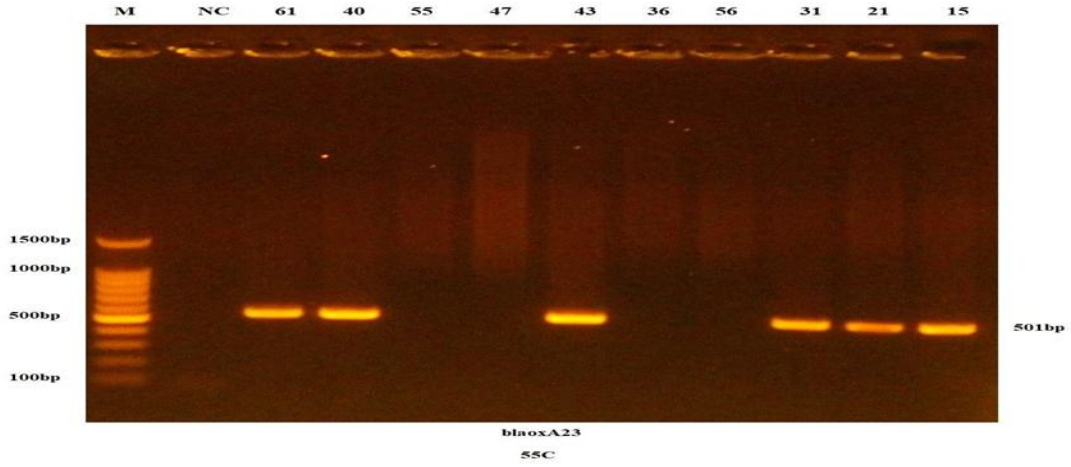


**Figure (2): Amplification of ' *blaOXA-23* gene of *Proteus mirabilis* samples results were separated on 2% agarose gel and stained with Eth.Br. Lane1:100bp DNA marker**

## Detection of *P. mirabilis blaOXA-58* gene using conventional PCR

The results showed that total 10 isolates were included positive (60%) containing the *OXA-58* gene. The positive result isolates are shown in the (Figure 3). The current results were similar to the results in Germany by Lange, *et al.* (26) between 37 isolates of *P. mirabilis* obtained (4 strains) discovered in various parts of Germany tested positive for *blaOXA-58*, indicating that a plasmid was harboring the resistance gene.

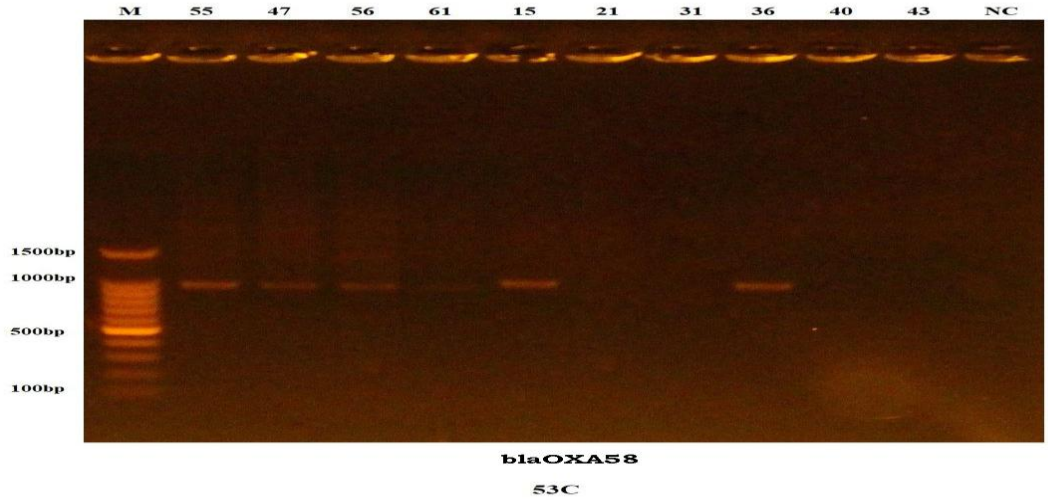


**Figure (3): Amplification of *blaOXA-58* gene of *Proteus mirabilis* samples' results were separated on 2% agarose gel and stained with Eth.Br. Lane1:100bp DNA marker**

## Conclusion

The current study shows that most *Proteus mirabilis* isolates have a high rate of antibiotic resistance. Imipenem is a broad-spectrum antibiotic of carbapenem that resistance to *Proteus* infection especially wound and burn infection. Identification of isolates containing *OXA-23* and *OXA-58* genes using conventional PCR. These samples are resistant to Carbapneam groups when compared to the molecular and phenotypical susceptibility test.

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