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Detection And Study Of *bla*$_{IMP}$ Gene *bla*$_{OXA}$ Gene In *Pseudomonas aeruginosa* Isolates From Diabetic Foot Ulcers In Diabetic Patients

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ABSTRACT

Background: *Diabetes mellitus is an acquired and/or inherited disease results from insulin deficiency or decrease effectively of the produced insulin; infection by Gram-negative bacteria in the wounds is common in patients with Diabetes mellitus, the wound dressing is very important for the treatment (P. aeruginosa) Pseudomonas aeruginosa which provided with factors OF genetic virulence. The objective of the current research is to identify the presence of the blaIMP and blaOXA genes in Pseudomonas aeruginosa bacteria that were isolated from diabetic individuals with diabetic foot ulcers. Approaches: A grand total of 120 skin grafts were harvested from patients with diabetic foot ulcers who visited Alsader medical city (September to January 2021) (males and females) at (20-70) years. Antibiotic resistance tests have been done for all specimens. Primary Identification depended on biochemical tests and Gram stain . Finally, Identification with Vitek 2 system was made. PCR for amplification required gene was performed, then gel electrophoresis to show amplified genes. Results: (83.3%) showed a positive culture of bacterial growth, while (16.7 %) showed a negative for culturing. So 17 (15.4%) Pseudomonas aeruginosa isolates . However, the percentage of blaOXA gene was (47%) in P. aeruginosa isolates while negative with blaIMP gene of P. aerginosa. Conclusion: Our investigation indicates that Pseudomonas aeruginosa is one of the primary causes of diabetic foot ulcers., and bacterial isolates showed the presence of blaOXA resistance gene, with the absence of blaIMP gene. These genes are one of the virulence factors and are partly antibiotic-resistant bacterium.*

Keywords: *Pseudomonas aeruginosa, blaOXA, and blaIMP gene.*

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INTRODUCTION

Diabetes mellitus is a chronic disease because deficiency of insulin or decreased effectiveness. Decreased insulin levels in the blood lead to increased glucose, damaging the body tissues, such as nerves and blood vessels [1]. When the bacteria are established in the wound, the bacterium is almost difficult to remove by the antibacterial results in biofilm production [2]. The wound with *P. aeruginosa* is associated with diabetes mellitus. The wound dressing are utilized in treating bacterial infections such as Gram-negative microbes associated with diabetes mellitus wounds, while Gram-

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positive microbes are associated with non-diabetic ulcers [3]. Mostly, the bacteria showed tolerance to antibiotics depending on the genetic material. The present work aims to make the molecular analysis of some resistance genes in *P. aeruginosa* to study antibiotic resistance patterns. 80 % of human foot ulcers have bacterial biofilms. The biofilms produced due to the bacteria's attachment to the surface produce microcolonies [3].

Hard to eradicate the grown bacteria in mature biofilm due to the development of many resistance ways [4]. *Pseudomonas aeruginosa* is one of the most bacteria which causes chronic wounds due to it can produce resistant biofilms. *P. aeruginosa* is often acquired in hospitals and is associated with burns, wounds, and urinary and respiratory infections (5).

Bacteria P. aeruginosa gain genes to adapt and persist in hostile settings, particularly in the presence of antibiotics [6]. MGE (Mobile Genetic Elements) and other Plasmids play a crucial role in this process. They act as carriers for spreading and acquiring the genes of resistance [7]. Sialidase, exotoxin A, exoenzyme S, and elastase are some of the identified factors that contribute to virulence. Cell-to-cell signaling networks carefully regulate all of these parameters. Hence, of *blaIMP* gene *bla OXA* genes of *Pseudomonas Aeruginosa* isolated from DM foot ulcers.

METHODS

Patients and the Collection of Clinical Specimens

In the conducted study, we obtained a total of 120 samples from patients with diabetic foot ulcers between September 2020 and January 2021. The samples were collected from Al-Hakeem General Hospital and Al-Sadder Medical City in Najaf Al-Ashraf. The clinical materials were conveyed to the laboratory, where swabs were introduced onto appropriate medium, such as blood agar plates and MacConkey's agar. Subsequently, the media were incubated at a temperature of 37°C for a duration of 18-24 hours. The isolates were mostly diagnosed based on the Gram's stain و colony morphology , and biochemical assays. Furthermore, the identification confirmed through biochemical analysis using the VITEK2-automated system. The swab collection tube provided by the JSHXRT Chinese company was used to collect samples from all patients [7].

Isolation and Identification of Bacterial Isolate

Following sample collection, clinical specimens inoculated into appropriate medium, such as (blood agar plates) and (MacConkey's agar). The incubated in a 37°C incubator for 18-24 hours. The main diagnosis using gram staining, colony morphology , and biochemical assays. Using the Vitek-2 system A total of 120 isolates were verified and identified .The system GN-underutilize ID cards for identifying Gram-positive bacteria GP-ID cards for identifying Gram-negative bacteria and . The identification message provided by the system had a confidence level ranging(95% to 99%) from good to outstanding. Furthermore, the VITEK2-automated system was utilized to ultimately biochemically validate the identification of bacterial isolates. The study investigated the antibacterial resistance of isolates of (*P. aeruginosa)* [8].

Amplification of PCR

PCR is performed on (5µL)DNA samples using a reaction mixture25µL. The text is referenced by the number 19. . The blaIMP gene was amplified using the heat specific cycling strategy described below: The protocol involves (30) cycles, with an initial denaturation step 10 minutes, followed by denaturation 40

seconds at 94 °C for, annealing at 55 °C for 40 seconds, extension at 72 °C for 1 minute, and 10 mminutes final extension step lasting . The blaOxa gene amplification was conducted according to the specified conditions: The first step denaturation at a temperature of 94°C for a duration of ten minutes. This is followed by (30 cycles) of denaturation at 94°C for forty seconds, annealing at for forty seconds 60°C, and extension for one minute at 72°C. Lastly, there isfinal stage last five minutes at 72 °C .

TABLE 1. Specifies the precise sequence primers utilized, which were supplied by (Canada) Company, Alpha DNA

Primer Type	Gene	Primer sequence (5'-3')	Amplicon size (bp)	Reference
IMP	blaIMP	F: TTGACACTCCATTTACG R: GATGAGAATTAAGCCACCT	139	[9]
OXA	BlaOXA	F: GGCACCAGATTCAACTTTCAAG R: GACCCCAAGTTTCCTGTAAGTG	564	[9]

The analysis of DNA fragments using electrophoresis involved the use of a 1% agarose gel. The electrophoresis was conducted at a voltage of 70 volts for a duration of 90 minutes. The gel was prepared using TBE 1X buffer and was stained with 4 µL of a 10mg/mL solution of ethidium bromide. A 100-bp DNA ladder from Korea, specifically from Bioneer, was used as a reference for determining the size of the DNA fragments. The band is visualized on a gel using a Cleaver company's ultraviolet light transilluminator in the UK. Photographs are taken to capture the image.

RESULTS

Characteristics and distributions of patients

TABLE 2. Shows the patient distribution according to age groups and gender.

Gender		Age group No.(%)				
	20-30	31-40	41-50	51- 60	61-70	Total
Female	1 (0.8)	8 (6.6)	18 (15)	19 (15.8)	0 (0)	46
Male	3 (2.5)	17 (14.2)	24 (20)	28 (23.3)	2 (1.6)	74
Total	6 (3.3)	25 (20.8)	42 (35)	47 (39.1)	2 (1.6)	120

Shows 120 patients, (74) (61.7 %) were male, and (46) (38.3%) were female, as in Table 1. The infection in males is more prevalent when compared with females. The frequency in males was 78.64%, while it was 21.36% in females in patients with DM foot ulcers.

Antibiotic Susceptibility of isolates *P. aeruginosa* to antibiotics.

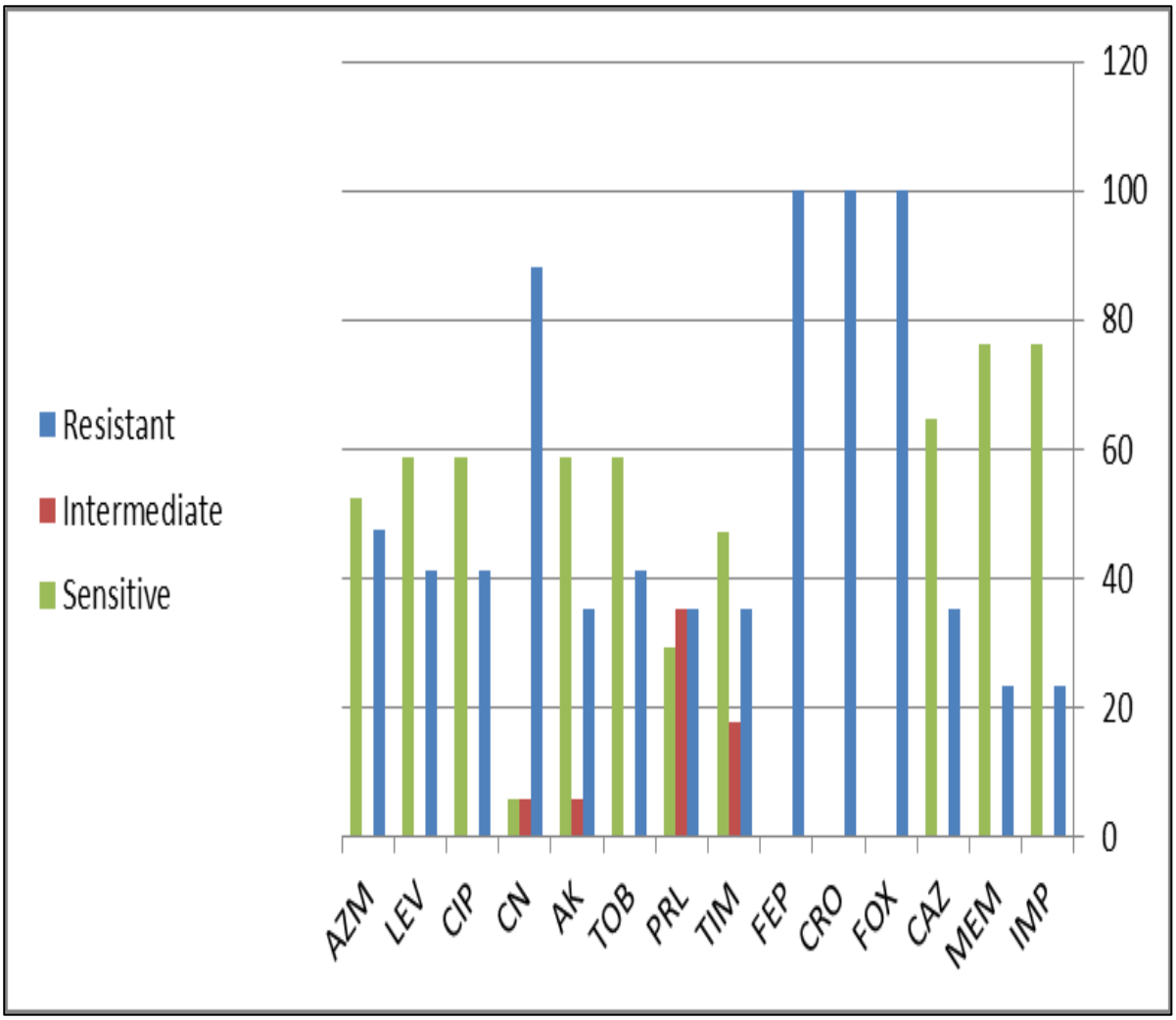

FIGURE 1 . Susceptibility of isolates *P. aeruginosa* to antibiotics.

Regarding P. aeruginosa, there were different levels of resistance observed. to; ceftriaxone, cefoxitin and Cefepime with a percentage (100%) agree with ; tobramycine, ciproflaxcin and levofloxacin with rates of (41.1%) agree with (23) *P.aerginosa* hight resistance antibiotic (33.96%); ticarcllin with clavulalnic acide, ceftazidime, piperacllin, Amikacin with percentage (35.2%); aztreonam with the percentage of (47.5%); meropenem and imipenem with percentage (23.5 %), so resistance to antibiotics under study (cefoxitin, Cefepime, ceftazidime, ceftriaxone, meropene, imipenem, azteronam, piperacllin, ticarclline/clav, Amikacin, gentamycin, tobramycin, ciprofloxacine, and levofloxacine).

Detection of the *blaOXA* gene

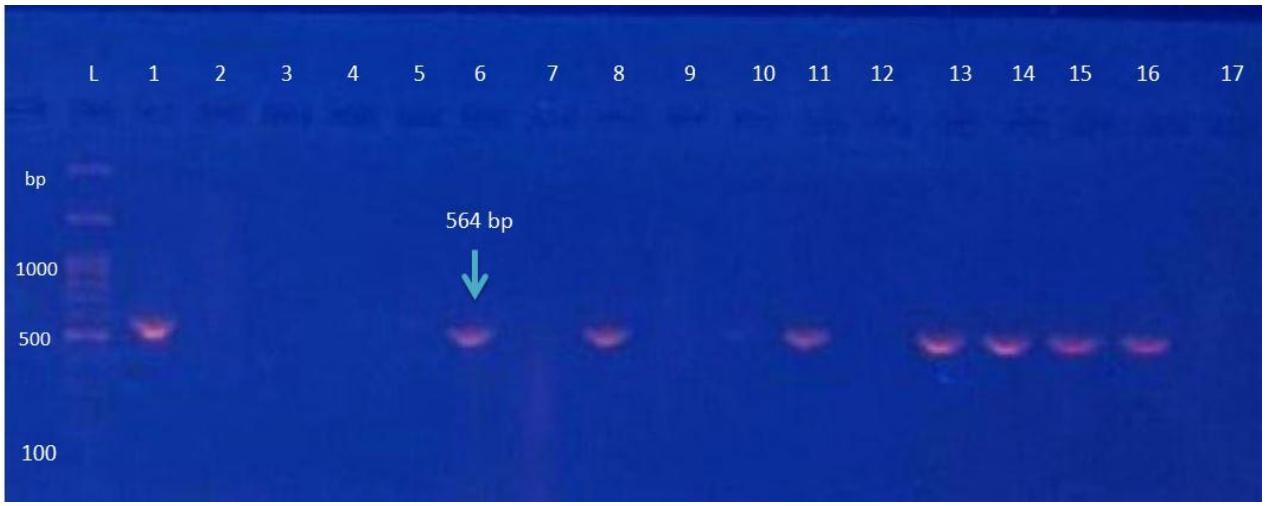

FIGURE 2.The result showed that the *blaOXA* gen (564 bp) in *P. aeruginosa* isolates was determined by amplification of PCR resistance gene was detected in 8/17(47%) *P. aeruginosa* isolates..

Detection of *blaIMP*

The findings indicated the presence of the *blaIMP* gene of P. aeruginosa, which was found to be negative.

DISCUSSION

The predominant observation in the investigation was that the majority of (P. aeruginosa) isolates obtained from patients with otitis media exhibited the presence of the blaOXA gene, in accordance with (24). Out of the isolates we examined, 8 out of 17 (47%) were determined to contain the blaOXA gene. Carbapenemases exhibit resistance to carbapenems, which are classified into three categories: A, B, and D. The OXA-β-lactamases are a type of Class D carbapenemases. They can be further classified into different sub-groups, including blaOXA-23, blaOXA-24/40, blaOXA-58, blaOXA-48, blaOXA-51, and blaOXA-143. The OXA-type β-lactamases, namely blaOXA-51, are frequently found in isolates of Pseudomonas aeruginosa. These enzymes are encoded by the species and provide resistance to carbapenems when their expression levels are elevated [20].

Integron-borne mobile gene cassettes contain class B carbapenemases, which can be horizontally transferred to various bacteria. Klebsiella pneumoniae carbapenemase (KPC) harbors Class A carbapenemases on a plasmid [21].

The results indicated the absence of the blaIMP gene in the Pseudomonas aeruginosa samples . Recent studies have revealed that the mobile gene cassettes incorporated into the integrons are still being transmitted by two families of imported metallo-β-lactamases, VIM and IMP [22].

Metallo-β-lactamases are enzymes that break down β-lactam, a type of antibiotic. These enzymes are produced by certain bacteria as a defense mechanism against β-lactam antimicrobials. The initial investigation into the presence of metallo-β-lactamase in P. aeruginosa was conducted in Japan in 1988. The dissemination of mobile genetic elements is responsible for the encoding of metallo-β-lactamases in P. aeruginosa [22].

CONCLUSION

According to our research, P. aeruginosa bacterial isolates from patients with diabetic foot ulcers carry the blaOXA gene. The bacteria's resistance to the medication is most likely partially explained by those genes.

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