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Research Article

Prevalence of *bla*$_{IMP-1}$ among *P. aeruginosa* isolated from patients with burn infections

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Abstract
Carbapenems are often used as antibiotics for treating infections by multidrug-resistant Gram-negative bacteria. Pseudomonas *aeruginosa* is considered a major cause of health care issues associated with higher mortality and morbidity rates worldwide. This study aimed to detect the prevalence of carbapenemase gene *bla*$_{IMP-1}$ that is produced by carbapenem-resistant *P. aeruginosa* isolated from burn infection patients. A total of 137 burn swabs were collected from burn patients in different hospitals in Babylon province from December 2021 to April 2022. All of the swabs were streaked on selective media cetrimide agar. Antibiotic susceptibility was performed for Imipenem and meropenem and polymerase chain reaction for species specific 16S rDNA gene and *bla*$_{IMP-1}$ gene was done for all isolates. 50 isolates were confirmed by amplicon of species-specific 16S rDNA for *P. aeruginosa*. Results of antibiotic susceptibility for imipenem and meropenem revealed that 5/50 (10%) and 13/50(26%) of *P. aeruginosa* isolates were resistant to imipenem and meropenem, respectively. Co-susceptibility (co-resistance or co-sensitive) for both imipenem and meropenem results showed 5/50 (10%) display resistance for both imipenem and meropenem, 8/50 (16%) display resistance for only meropenem while 37/50(74%) sensitive for both and none was resistant to imipenem and sensitive for meropenem. Phenotypic detection of Metallo-β-lactamase gave inverse result while the Mean±SD of inhibition zone (mm) for imipenem disc (10µg) =29.34±7.11 and for imipenem+EDTA (10 µg+750 µg) =21.8±4.98. Investigation of *bla*$_{IMP-1}$ gene revealed that 23/50 (46%) have *bla*$_{IMP-1}$ while 18/50 (36%) were sensitive for imipenem and meropenem but have *bla*$_{IMP-1.}$ The current study concludes the possibility of the presence of resistance gene among phenotypically sensitive isolates pushing great threat of antibiotic misuse and leaving the infection untreated.

Keywords: *bla*$_{IMP-1}$, burn patients, Carbapenems, Metallo-β-lactamase and *Pseudomonas aeruginosa*

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic human pathogen capable of causing a great threat to health, especially in immunocompromised patients) Farhan *et a*., 2019). It is the main cause of nosocomial infections and due to their ability to acquire resistance to a wide range of antibiotics making it a major global concern (Kumar and Illamani, 2021; Hashemi, *et al*., 2021; Jafari-Sales *et al*., 2020). It is responsible for a wide range of infections, including urinary tract, respiratory system, burn and wound infection and otitis media (Paudel *et al*., 2021). *P. aeruginosa* is one of the most dangerous burn wound infections pathogens causing 75% of all deaths in patients and is considered a major medical challenge because of its difficulty to treat (Farhan *et al*., 2019).

Carbapenems antibiotics are effective against multidrug-resistant bacteria, but their widespread use yields in the emergence of resistant strains. Carbapenem hydrolyzing enzymes are assigned into four groups according to Ambler classification A, B, C and D (Paudel *et al*., 2021; Vural *et al*., 2020). The metallo- β-lactamase (MBLs) belong to group B and they require divalent cations (such as zinc ion) as cofactors for enzyme activity, which is inhibited by the action of a metal ion chelator such as EDTA and thiol-based compounds. This behavior is considered as dependent phenotype detection. The MBLs efficiently hydrolyze all beta lactams, except aztreonam and in vitro MBLs con-

sist of several variants of enzymes such as IMP, VIM, SPM, GIM and SIM (Marshall *et al*., 2017). *P. aeruginosa* is often difficult to eradicate due to their resistant drug profile. Therefore, detection of MBL-producing *P. aeruginosa* is crucial for the optimal treatment of patients particularly in critically ill and hospitalized patients, and to control the spread of resistance (Safarirad *et al*., 2021). bla_{IMP-1} was the most prevalent MBL among MDR-*P. aeruginosa* in Iraq (Al-Charrakh *et al*., 2016; Alkhudhairy and Al-Shammari, 2020). This study aims to know the extent of the spread of bla_{IMP-1} in Babylon province, especially among burn patients.

MATERIALS AND METHODS

Samples collection

Babylon Health Directorate approved for the collection swaps from patients. Accordingly,t a total of 137 burn swabs (102 female with age mean±SD= 36.17±21.23 and 48 male with age mean±SD= 34.56±19.09) were collected during the study period from hospitalized burn patients who showed signs suggestive of infection from December 2021 to April 2022. The samples were transported to transport swab and then submitted to bacteriological investigation.

Bacterial diagnosis

All swabs were cultured on cetrimide agar (Khalil *et al*., 2021) plates at 37℃ overnight for selective growth of *P. aeruginosa* isolates. All positive isolates will submit for polymerase chain reaction (PCR) to confirm them as *P. aeruginosa* using species specific primer pair for *P. aeruginosa* 16S rDNA gene. Forward primer (16S-PA-F): 5′GGGGGATCTTCGGACCTCA3′, reverse primer (16S-PA-R): 5′TCCTTAGAGTGCCCACCCG3′, which annealed at 61℃ to give 956 bp amplicon (Spilker *et al*., 2004).

Antimicrobial susceptibility testing

All 50 confirmed *P. aeruginosa* isolates were tested for their antibiotic susceptibility patterns (for imipenem (10µg) and meropenem (10µg) according to CLSI-2021. The test was done on Mueller Hinton agar using the selected antibiotic discs. After 24 hours of incubation at 37°C, all samples were examined and inhibition zones were measured and interpreted (Clinical and Laboratory Standards Institute, 2021).

Phenotypic detection of MBL production

Imipenem–EDTA combination disc testing was used for the detection of MBL-producing isolates. A solution of 0.5 M EDTA was prepared by dissolving 18.61 g of EDTA in 100 mL distilled water with pH 8, (Also used an artificial disc of imipenem/EDTA 10/750 was used). The tested isolates were cultured on Muller–Hinton agar plates. Two 10 µg imipenem disks were placed on agar plates and 10 µL EDTA solution added to one imipenem disc. Inhibition zones around discs with EDTA were measured after 18-24 hours' incubation at 37°C and compared with others without EDTA. An increase in zone diameter of at least 7 mm around the imipenem–EDTA disc was considered positive results (Chanu *et al*., 2019).

Detection of bla_{IMP-1} MBL gene by PCR

DNA extraction was done by using FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction Mini Kit (FABGK100) (Favorgen/Tiwan). The extracted DNA was subjected to PCR amplification reaction using specific primers for MBL genes (bla_{IMP-1}). The DNA amplification was done using GoTaq® Green Master Mix, 2X (M7122) (Promega/USA). The primers were purchased lyophilized; (Macrogen/Korea) and reconstituted by the addition of sterile nuclease free water and stored at –20°C. Forward primer (blaIMP-F): 5′ TGAGCAAGTTATCTGTATTC3′ and reverse primer (blaIMP-R): 5′ TTAGTTGCTTGGTTTTGATG3′ which annealed at 51℃ to give 740 bp amplicon (Moosavian and Rahimzadeh, 2015).

RESULTS

The results of all 50 isolates grown on cetrimide agar submitted to confirmation by PCR for amplification of *P. aeruginosa* specific fragment of 16S rDNA revealed that all of them were *P. aeruginosa* (Fig. 1). Results of antibiotic susceptibility for imipenem and meropenem revealed that 5/50 (10%) and 13/50(26%) of *P. aeruginosa* isolates were resistant for imipenem and meropenem respectively (Fig. 2). Co-susceptibility (co-

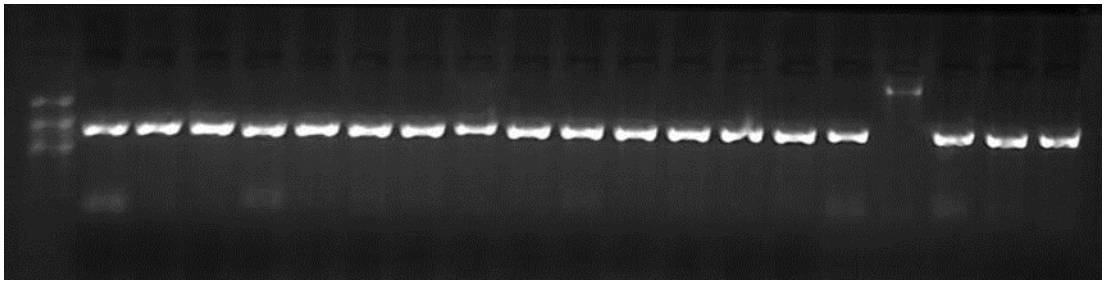

Fig. 1. *Agarose gel (1.5% in TBE) electrophoresis for P. aeruginosa specific amplicon of 16S rDNA (956bp)*

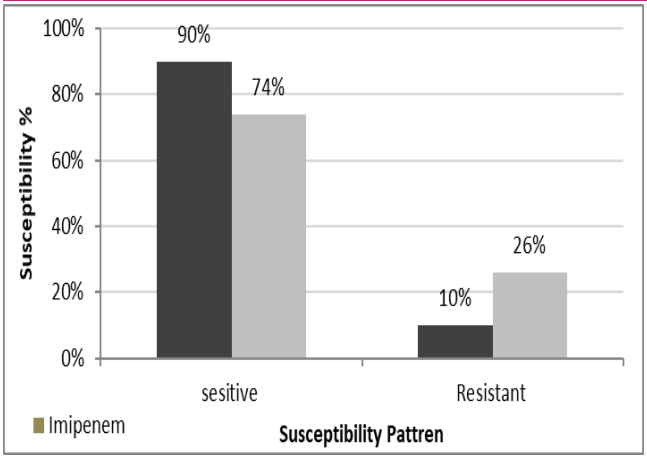

Fig. 2. *Antibiotic susceptibility of P. aeruginosa for imipenem and meropenem*

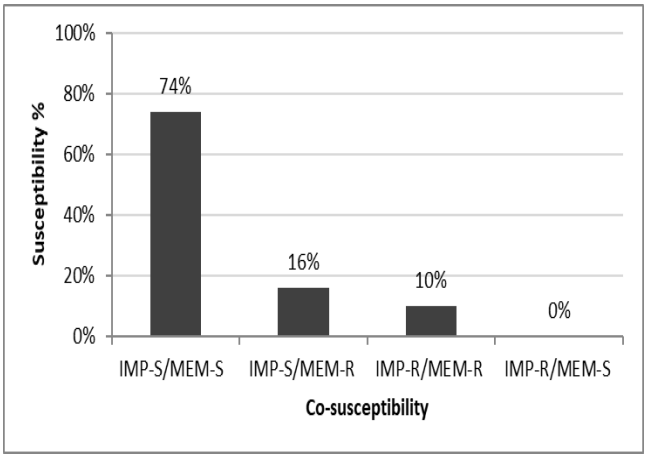

Fig. 3. *Co-susceptibility of P. aeruginosa for imipenem and meropenem. IMP-S=imipenem sensitive, IMP-R = imipenem resistant , MEM-S=meropenem sensitive, MEM-R=meropenem resistant*

resistance or co-sensitive) for both imipenem and meropenem are highlighted in Fig. 3. It was revealed that 5/50 (10%) display resistance for both imipenem and meropenem, 8/50(16%) displayed resistance for only meropenem while 37/50(74%) was sensitive for both and none was resistant to imipenem and sensitive for meropenem. Phenotypic detection of Metallo-β-lactamase gave inverse result while the Mean±SD of inhibition zone (mm) for imipenem disc (10µg) =29.34±7.11 and for imipenem+EDTA (10 µg+750 µg) =21.8±4.98 as mentioned in Table 1. Results of investigation of bla_{IMP-1} gene among 50 *P. aeruginosa* isolates revealed that 23/50 (46%) have bla_{IMP-1} (Fig. 4). Phenotypic resistance or sensitive isolate reflected the resistance gene content and so it was involved in routine work to predict resistance gene presence. The results of the relation of genotype to phenotype gave unexpected results when 18/50 (36%) were sensitive for imipenem and meropenem but had bla_{IMP-1} (Fig. 5) and this is the big issue when phenotypically sensitive isolates have a resistance gene.

Table 1. Phenotypic detection of Metallo-β-lactamase among *P. aeruginosa* isolates

Antibiotic	Inhibition zone (mm)
IMP	29.34±7.11
IMP+EDTA	21.8±4.98

DISCUSSION

The emergence of *P. aeruginosa* carbapenem-resistant represents a threat to the clinical approach because it decreased susceptibility to a range of antimicrobials and possesses ability to develop resistance to other classes (Morales *et al*., 2003). The current results reports that the percentages of *P. aeruginosa* in burns swabs are (36.49%), and this result is higher than in the Iraqi results of Ulabdeen in 2016 recorded (8.2%) (Ulabdeen, 2016), Al-Rubaye and colloquies in 2020 was (24.27%) (Al-Rubaye *et al*., 2020). This difference in rate may belong to the level of health care, the speed arrival of patient to the hospital, the patient had taken antibiotics in advance, or may be because of the long hospital hospitalisation. This percentage may increase within 72 hours due to an infection acquired from nosocomial infection due to contact with patients or through using of contaminated instruments that lead to spread these bacteria (Al-Rubaye *et al*., 2020). Other studies stated that resistance to imipenem 71% and meropenem was 63% (Al-Rubaye *et al*., 2020) and Azeez and Bakr 2019 showed resistant to imipenem and meropenem was 4% and 20%, respectively (Azeez

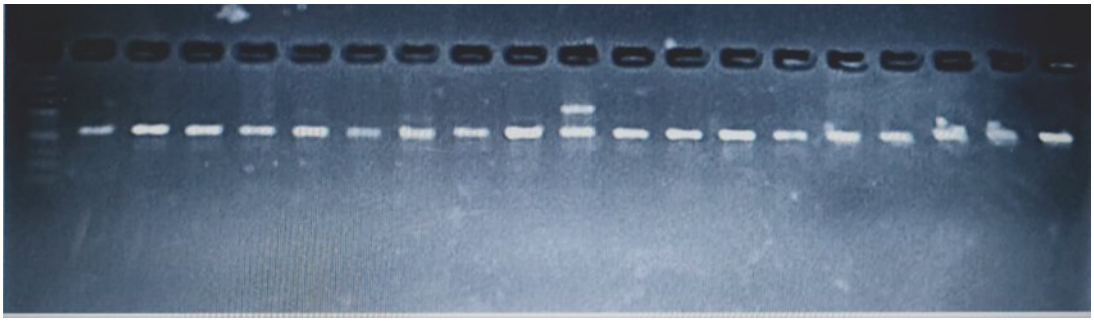

Fig. 4. *Agarose gel (1.5% in TBE) electrophoresis for P. aeruginosa* bla_{IMP-1} *amplicon* 40bp)

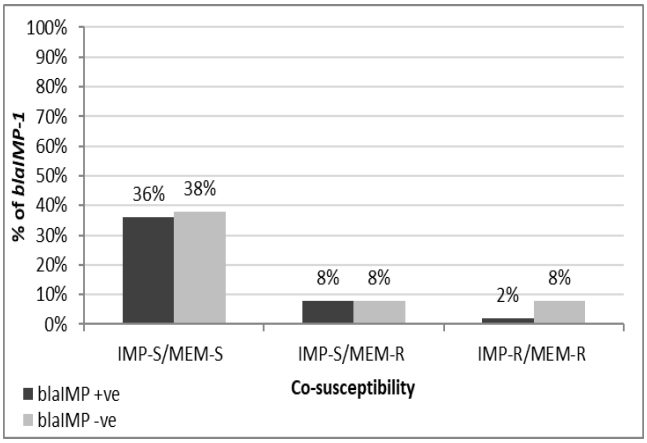

Fig. 5. *Presence of bla_{IMP-1} and Co-susceptibility among P. aeruginosa isolates*

and Bakr, 2019). This variant may have occurred because the random use of antibiotics had an important role in the emergence of resistance to these antibiotics. However, in other countries such as Pakistan, the most common bacterial isolate from burn patients was *P. aeruginosa* with (24.91%) and showed resistance to different antibiotics including imipenem and meropenem (Chaudhary *et al*., 2019). In United States, most *P. aeruginosa* isolates had resistance to Carbapenems (Jenny and Kingsbury, 2018). In Philippines 31.1% has been reported resistance to imipenem, in Thailand 28.7%, Japan 28.5%, Singapore 23.3%, Korea 22%, and Romania 48%, and in Germany, Austria, Denmark, Finland, French, Luxemburg < 20% (Vaez *et al*., 2017). In Iran the prevalence of imipenem-resistant *P. aeruginosa strains* was 57.1% in Ardabil (Safarirad *et al*., 2021). In this study, the results revealed that 5/50 (10%) displayed resistance for both imipenem and meropenem, 8/50(16%) displayed resistance for only meropenem while 37/50(74%) sensitive for both and none was resistant to imipenem and sensitive for meropenem. Furthermore, the results of the investigation of bla_{IMP-1} gene among 50 *P. aeruginosa* isolates revealed that 23/50 (46%) - have bla_{IMP-1}, and these results of genotype detection was unidentical with most phenotypic method when the results are little invers of expected when found that the bla_{IMP-1} present in 36% sensitive isolates to imipenem and meropenem and this issue may be explained as gene silencing and no production of bla_{IMP-1} enzyme. The additional unexpected results were the decreasing the inhibition zone upon addition of EDTA to imipenem and it can be explained may be due to decreasing the uptake of IMP-EDTA combination of diminishing the uptake by porins. However, previous studies revealed variant percentages of bla_{IMP-1} prevalence, in Erbil was 57% (Azeez and Bakr, 2019), in Ardabil city was 35.5% (Safarirad *et al*., 2021), Iranian burn centers 76.8% (Jabalameli *et al*., 2018) and in Minia, Egypt was 42.8% (Farhan *et al*., 2019).

Conclusion

The results concluded implication of bla_{IMP-1} gene in resistance to carbapenem and the possibility of presence of a resistance gene among phenotypically sensitive isolates may push a great threat of misuse of antibiotics and leave the infection untreated.

Conflict of interest

The authors declare that they have no conflict of interest.

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