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Dissemination and Molecular Characterization of 16s RNA Methylase and Aminoglycoside Modifying Enzymes- Producing *P. Aeruginosa* Isolated from Burn Specimens in Najaf City

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a significant species of bacteria connected with nosocomial infections involving, pneumonia, infections of urinary tract, and damage of the surgical site.

Aim of the Study: The aim of this thesis is to investigate the occurrence of genes encoding clinically important 16S rRNA methylases and AMEs enzymes in aminoglycosides resistance *P.aeruginosa* isolates recovered from Najaf hospitals and the characteristics of these isolates.

Methods: a total of 68 specimens were isolated from patients at burn center in Najaf City from the whole study period**.** The 24 isolates were screened for the sensitivity versus 3 various antibiotics disks of the one class by using Kirby-Bauer disk diffusion method.

Results: The resistance rate of 24 isolates of XDR *Pseudomonas aeruginosa* against aminoglycoside groups: gentamicin, amikacin, and tobramycin were 91.6 %, 83.3 %, and 66.6 %, respectively. However, the resistance profiles of the isolates show that 22 (91.6 %) were resistant to 1 or more antibiotics of aminoglycoside. Present study show that only 3 (12.5%) of XDR isolates harbored *ant(4')-IIb* gene isolates and none of the XDR isolates investigated were positive for *rmtA* and *aac(6')-IIb* gene.

Conclusions: This survey has showed for the initial time three isolates harbors the *ant(4')-IIb* aminoglycoside resistance genes in Iraq.

KEYWORDS: *Pseudomonas aeruginosa*, UTIs, Aminoglycoside Modifying Enzymes, 16S RNA Methylase, XDR, CLSI, VITEK-2 compact system.

1. INTRODUCTION

Pseudomonas aeruginosa is a significant species of bacteria connected with nosocomial infections involving, pneumonia, infections of urinary tract, and damage of surgical site. (Hayami *et al*., 2013). Aminoglycoside are important types of antibiotics utilized in the treatment of severe infections caused by bacteria of Gram-negative, including ESBL producing *P. aeruginosa* (Shokravi *et al*., 2015). They prevent protein synthesis of bacteria by linking irreversibly to the bacterial 30S ribosomal subunit, thereby leading to death of the cell. They are often used in combination with fluoroquinolones, especially in the sepsis treatment, as these drugs work synergically (Michalska *et al*., 2014a).

In Najaf, gentamicin and amikacin resistance has steadily increased during the last decade and has reached 61.5% and 2.9% among *P. aeruginosa*, respectively (Al-Hilali, 2015). Resistance of bacteria to aminoglycoside may be because of chromosomal mutation as such as acquisition of mobile genetic factors (transposons, integrons and plasmid) having genes of resistance. Aminoglycoside resistance is mediated by three various ways, including reduce accumulation of an intracellular antibiotic, alteration of an enzymatic drug and the substitution of ribosomal proteins (Ramirez and Tolmasky, 2010).

Production of AMEs is one of the most commonly happening way of resistance to the aminoglycoside among *P. aeruginosa*. Enzymes in the AAC groups inhibit

aminoglycoside antibiotics by acetylation, APH can phosphorylate aminoglycoside and ANT cause aminoglycosides adenylation (Lim *et al*., 2013). The genes encoding this enzyme usually are located on plasmids carrying genes for ESBLs. Additionally, a connection between development of resistance to aminoglycoside and utilize of ciprofloxacin, depend on the identification of a genes of the aminoglycoside modifying enzyme ACC(6′)-Ib named ACC(6′)-Ib -cr, which gives resistance to both fluoroquinolones and aminoglycoside (Xiao and Hu, 2012).

16S rRNA (ribosomal) methylase might be has as a recent resistant way versus aminoglycosides among pathogens of Gram-negative in the family of Enterobacteriacae, *Pseudomonas aeruginosa*, and *Acinetobacter* species (Doi and Arakawa, 2007).

2. MATERIALS AND METHODS

2.1. Samples collection

Najaf burn centers across sections procedure on patients visited in the period of September 2023 to December 2023. 68 specimens were isolated from patients from period of research and the specimens were taken to the laboratory.

2.2. Isolation and identification of *P. aeruginosa*

Microbiological standard diagnostic criteria were used to isolate and identify the clinical isolates of *P. aeruginosa*, which included colony morphology, Gram stain, and conventional biochemical tests. The VITEK-2 automated system has been utilized to diagnose *P.aeruginosa*.

2.3. Aminoglycoside susceptibility testing

The isolates were screened for the sensitivity versus 3 various antibiotics disks of the class by utilizing technique of Kirby-Bauer disk diffusion which utilized to measure inhibition zones in agreement with the recommendations of laboratory and clinical Standards system (CLSI, 2020).

2.4. Aminoglycoside Molecular Screening:

This thesis was carried out to discover the dissemination of the aminoglycoside modifying enzymes among the XDR *P.aeruginosa* isolates taken from center of burn in Najaf during period of 3 months. The early detection of aminoglycoside resistance genes may inactivate the dissemination of these isolates of XDR *Pseudomonas aeruginosa* on the future.

3. RESULTS

3.1. Aminglycosides-Resistant XDR *P. aeruginosa* Isolates

Present study showed that resistance rate of 24 isolates of XDR *Pseudomonas aeruginosa* against aminoglycoside groups: gentamicin, amikacin, and tobramycin were 22 isolates (91.6 %), 20 isolates (83.3 %), and 16 isolates (66.6 %), respectively. However, the resistance profiles of the isolates show that 22 (91.6 %) were resistant to 1 or more antibiotics of aminoglycoside. The summary of resistance profiles for all tested isolates is shown in Table (3-1). Overall, isolates were divided into five phenotype groups. First group (A1), 15 (62.5%) isolates exhibited resistant to all aminoglycosides tested. Second group (A2), 5(20.8%) isolates manifested resistant to gentamicin and amikacin but sensitive to tobramycin. Third group (A3), 1(4.1%) isolates were tobramycin and gentamycin resistance but susceptible to amikacin. Fourth group (A4), 1 (4.1%) isolate was resistant to gentamicin but susceptible to amikacin and tobramycin. In contrast, the fifth group (A5), 2 (8.3%) isolate was susceptible to all aminoglycosides tested.

Table (3-1): Aminoglycosides resistance patterns of 24 XDR *P. aeruginosa* isolates.

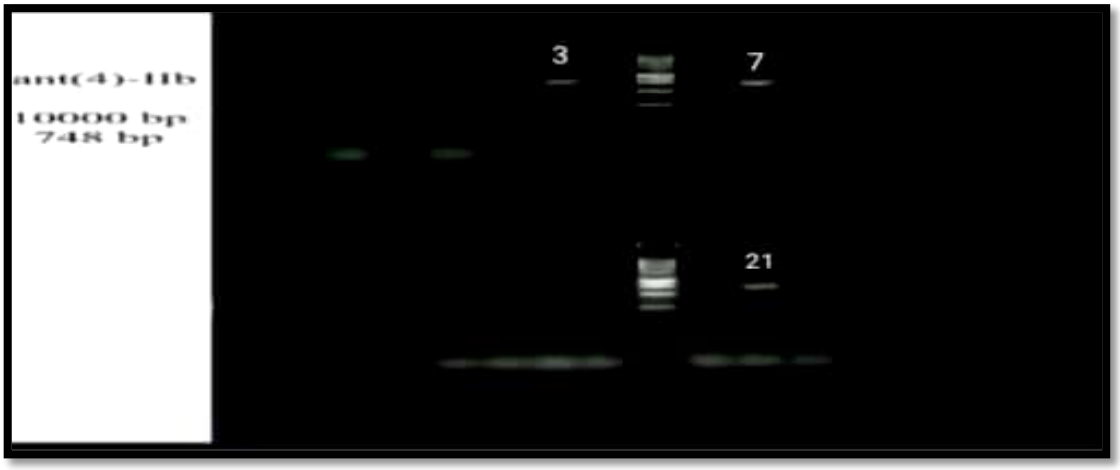
Phenotype	Aminoglycosides resistant pattern	No. (%) of isolates	Isolate code No.
A1	Amikacin, gentamicin, tobramycin	15 (62.5)	Pa7, Pa5, Pa9, Pa3, Pa8, pa11, Pa21, Pa31, Pa12, Pa14, Pa18, Pa16, Pa15, Pa22, Pa19
A2	Amikacin, gentamicin	5 (20.8)	Pa1, Pa17, Pa6, pa20, pa23
A3	Gentamicin, tobramycin	1(4.1)	Pa10
A4	Gentamicin	1 (4.1)	Pa2
A5	No resistance	2(8.3)	Pa4, pa24

In addition, PCR was used to screen all XDR isolates for the presence of the selected aminoglycoside acetyltransferases (AAC), *aac(6')-IIb*, aminoglycosides O-nucleotidyltransferase (ANTs), *ant(4')-IIb*, and *16rRNA* methylase, *rmtA* genes using specific primers. The occurrence of aminoglycoside resistance genes among the isolates are shown in Table (3-2). Compared with the sensitivity test of aminoglycosides in this study, the PCR results did not correlate well. PCR method showed the missing of genes resistance to aminoglycosides in vast majority of the isolates (21, 87.5%). However, present study found that one type of aminoglycoside resistant genes [*ant(4')-IIb*] were found in only 3 (12.5%) isolates and none of the XDR isolates investigated were positive for *rmtA* and *aac(6')-IIb* gene.

The correlation between the aminoglycoside sensitivity pattern of the isolates and the presence genes of aminoglycoside resistance is observed in table (3-2). The *ant(4')-IIb* positive isolates demonstrated resistance to three tested aminoglycosides, including amikacin, gentamicin and tobramycin.

Table (3-2): Distribution of aminoglycosides-resistance genes in 24 XDR isolates of *P. aeruginosa*

Amplified gene	No. (%) of isolates	Isolate code No.	Aminoglycosides resistant pattern
ant(4')-IIb	3(12.5)	Pa3	Amikacin, gentamicin tobramycin
		Pa7	Amikacin, gentamicin tobramycin
		Pa21	Amikacin, gentamicin, tobramycin



Picture (3-1): Gel of agarose with red save stained of mono-plex PCR amplified product isolates DNA of isolates with *ant(4')-IIb* primer. The electrophoresis used for 120 min at 65 volt. Lane (L) is marker of DNA molecular size (10,000 –bp ladder). Lanes 3, 7 and 21 show positive results with *ant(4')-IIb* gene (748 bp).

4. DISCUSSION

4.1. Aminglycosides Resistant among XDR *P. aeruginosa* Isolates

Although aminoglycosides represent a small fraction of antibiotic consumption in Iraqi hospitals as compared with β-lactam antibiotics and fluoroquinolones, they are still significant class of antibiotics in treating life threatening *P. aeruginosa* and other Gram negative bacterial infections. Therefore, detecting the level of aminoglycoside resistance is an important task. Some aminoglycoside remain active against many XDR *P.aeruginosa* isolates (Pfaller *et al.*, 2018).Then, a numbers of results have showed a moderate average of AR (gentamycin & amikacin) in *P. aeruginosa* infection cases in Iraqi hospitals (Abdul-Wahid, 2014; Al-Janahi, 2020). Present study also focused on resistance patterns to three antibiotics of aminoglycosides (gentamycin, tobramycin and amikacin) in isolates of XDR *P.aeruginosa*. According to the sensitivity testing, these result reported that 22 (91.6%) of XDR isolates of *Pseudomonas aeruginosa* were found to be resistant to at least 1 of aminoglycosides tested (Table 3-1). The occurrence phenotypes of aminoglycoside resistance in XDR *P. aeruginosa* isolates were divided into five groups: (A1) resistant to all aminoglycosides tested (62.5%), (A2) resistant to amikacin and gentamicin (20.8%), (A3) resistant to gentamicin and tobramycin (4.1%), (A4) resistant to gentamicin (4.1 %) and (A5) susceptible to all aminoglycosides tested (8.3%). An important striking feature found in present study is differences in aminoglycosides resistance phenotypes among isolates suggesting that aminoglycoside resistance in XDR *P. aeruginosa* isolates might be mediated by complex and multifactorial mechanisms. The elevated rate of pan-

aminoglycoside resistance found in this result (A1) would undoubtedly have an influence on illness and deaths of persons in both community and hospital. Despite these various resistance phenotypes, aminoglycosides are still valuable weapons in antimicrobial treatment, particularly in the management of life threatening infections of *Pseudomonas aeruginosa* in Najaf hospitals.

In this study, occurrence of the selected aminoglycoside acetyltransferases (AAC), *aac(6')-IIb*, aminoglycosides O-nucleotidyltransferase (ANTs), ant(4')-IIb, and 16rRNA methylase (16S-RMTases), *rmtA*, and their correlation with aminoglycoside resistance in XDR *P. aeruginosa* isolates were investigated. Present study show that only 3 (12.5%) of XDR isolates harbored *ant(4')-IIb* gene (Table 4-15). This is the initial report of determination of *ant(4')-IIb* gene from XDR isolates of *P.aeruginosa* in the region of study. Even in a similar previous study from Al-Nasseryia, *ant(4')-IIb* gene was not found in any of the aminoglycosides resistant *P. aeruginosa* isolates (Abdul-Wahid, 2014).

Genes encoding 16S RNA Methylase are responsible for an extremely elevated level of resistance to all aminoglycosides which administered parenterally that are commonly in clinical use (Tada *et al.*, 2017; Asghar and Ahmed, 2018). In contrast to these reports, none (0%) of the XDR isolates, which were aminoglycoside resistant, carried gene encoding *rmtA* 16S-RMTase. However, this data suggest that *rmtA* not play a significant role in XDR isolates that resistance to aminoglycoside, may be these isolates utilize an alternative resistance mechanism.

5. CONCLUSIONS

The study emphasizes the significance of aminoglycoside resistant *P. aeruginosa* isolates as a cause of burn infection in Najaf City. The vast majority of XDR isolates exhibited resistant to at least 1aminoglycoside. This result has showed for the initial time three isolates harbors the *ant(4')-IIb* aminoglycoside resistance genes in Iraq.

Clearance of ethical: Ethics committee show there is no wrong study and there is no plagiarism in this result.

Interest collision: this thesis showed there is no interest collision.

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