MJMR, Vol. 34, No. 4, 2023, pages (181-185).

Open Access
ISSN:2682-4558

Research Article

Detection of Multidrug resistance pattern of *Pseudomonas aeruginosa* clinical isolates in Minia University hospitals

Hadeer Ibrahim Yassin Ali[1], Wedad Mahmoud Abdelraheem[1], Shaimaa Hassouna Zaki[1] and Mona Abd Elmoneum Ismail[1]

[1] Department of Medical microbiology and Immunology Faculty of Medicine Minia University .

DOI: 10.21608/MJMR.2023.214391.1405

Abstract

Background*: Pseudomonas aeruginosa (P. aeruginosa*) infection shows a higher morbidity and mortality rates especially in immunosupperessed individuals. It has a high level of resistance through various mechanisms to most antimicrobials **Methods**: From Febuarary 2022 to May 2023, 48 samples collected from various infected surgical wound specimens were used in this work. Then, antimicrobial susceptibility tests were done to the isolates by the disc diffusion method .PCR used for detection of gene responsible for antibiotic resistence. **Results**: Out of 48 samples, 25 isolates were positive for *P. aeruginosa* and (18/25) 72% were MDR. **Conclusion**: *P. aeruginosa* Infections have become a severe problem that requires a precise diagnosis and effective treatment. To replace the current unsuccessful therapies, new alternative treatment plans should be taken into consideration and researched.

Keywords: MDR, *P.aeruginosa*, Antibiotic resistence.

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is considered a non- lactose-fermenting Gram-negativerod shaped bacilli [(1).]

P. aeruginosa can cause severe nosocomial infections, manifesting as pneumonia, surgical site infections, urinary tract infections (UTI) and It is one of the most common causes of bacteremia [(2)] .

P. aeruginosa infection shows a higher morbidity and mortalityrate especially in immunosupperessed individuals with neutropenia. Rates of

This bacterial pathogen has a significant number of virulence factors in its core genome that enable it to colonise and infect a wide range of hosts, including unicellular organisms and people. However, These virulence factors are not the only crucial components for spreading an infection to hospital patients [(4).]

Due to the ability of *P. aeruginosa* to resist many of the currently available drugs, treating infections caused by this *P. aeruginosa* tolerates many of the therapies that are currently on the market therefore treating infections induced on by this Bacterium has become extremely difficult. Intrinsic, acquired, and adaptive resistance are the three types of resistance that *P. aeruginosa* develops to fight off antibiotic attacks, acquired and adaptive[(5).(6)]

It shows a high level of intrinsic resistance to m ost antibiotics through reduced outer membrane permeability, the development of antibiotic-inactivating enzymes like β-lactamases, and efflux systems that evacuate antibiotics from the cell [(5)] .

Newly generated genetic mutations result in acquired resistance, frequently manifests by overexpression of efflux pumps and β-lactamases, Acquired resistance because of horizontal gene transfer frequently takes the form of synthesis of transferable modifying enzymes and β-lactamases [(7).]

Adaptive antibiotic resistance of *P.aeruginosa* includes Resistance mediated by biofilms and

the development of multidrug-tolerant persister cells. Due to bad administering cultures for antibiotics. Significant resistance to cephalosporins, carbapenems, and aminoglycosides has been found in isolates from critical care units [8].

Aim of the work

1- Isolation of *P.aeruginosa* from outpatients and inpatients in Minia University hospitals.
2- Determination of antimicrobial susceptibility pattern of *P.aeruginosa.*
3- Genotypic detection of Genes responsible for Antimicrobial resistance.

Material and methods

Subjects:

This study was carried out between February 2022 and May 2023. 48 patients with infected surgical wounds who had clinical symptoms and indicators of wound infection were included in this study. They were obtained from the outpatient and inpatient plastic surgery department at Minia University hospital.

Patients' complete personal and medical histories, including gender, age, reason for admission, and past usage of antibiotics, were obtained.

Ethical approval:

The study was approved by the Ethical Committee of Minia University, Faculty of Medicine.
Consent: Written informed consents were obtained from all patients**.**

Collection and transport of samples:

All samples were delivered within an hour to the Medical Microbiology and Immunology Department, Faculty of Medicine Minia University, using sterile cotton swabs and transport media.
Samples were processed as quickly as possible.

Isolation and identification *P. aeruginosa*:

All isolates undergo the subsequent procedures:

1- culture on cetramide agar media (Oxoid, England) for 24-48 hours of incubation at 37°C
2- All isolates that give Characteristics green color on cetramide agar are subjected for biochemical reactions as oxidase test, TSI and citrate test.

After complete identification, *P. aeruginosa* isolates were incubated in nutrient broth and then preserved on glycerol and then frozen at -20°C. Glycerol was obtained by Al-Nasr Company (Egypt).

Antimicrobial susceptibility pattern

All *P. aeruginosa* isolates tested by Disc diffusion method to detect Antibiotic susceptibility patterns. The results were interpreted**. CLSI 2022** defines MDR as the presence of at least one agent resistance among three or more classes of antibiotics[9].
The following antibiotics were tested: Cefipime (30µg), Ceftazidime (30µg), Pipracillin (100µg), Gentamycin (10µg), Amikacin (10µg) and Levofloxacin (5µg). Those antibiotics obtained from Oxoid Company.
- 3-5 well-isolated colonies from the organism's pure culture were dissolved in sterile saline using a sterile loop.
By comparing the inoculum tube and the 0.5 McFarland standards visually to a card with a white background and sharp black lines in favourable lighting, the turbidity of the suspension was matched to the turbidity of the standards.
- To ensure equal distribution, a sterile swab was immersed in the inoculum suspension, the excess liquid was squeezed off by pressing the swab against the inside of the tube, and the plate of Muller-Hinton (MH) agar was then rubbed with the swab in three different directions.
- Antibiotic discs were applied to the cultured plate using sterile forceps, with enough space between each disc.
- Results were calculated by measuring the zone of inhibition size in mm following an overnight incubation. The CLSI recommendations were followed in the interpretation of the antimicrobial susceptibility results [9].

Molecular

DNA extraction:

DNA extraction was done using boiling method. *P.aeruginosa* subcultured on nutrient agar , and incubated for 24 hrs. 3 to 5 colonies from each sample were inoculated into 1.5ml Luria broth (LB) and incubated for another 24 hrs.
- The isolates were centrifuged for 3 minutes at 5,000 g. The pellet was resuspended in 200 l of

molecular biology-grade water; the mixture was pipetted. Supernatant was removed (Eppendorf, Hamburg, Germany).
- Then, They stayed 8 minutes in the bath of water at 100°C boiling.
- Sudden cooling in ice for 20 min. Followed by centrifugation for 3 min at 5,000 × g.
- Two hundred µl of the supernatant containing DNA was put in a newly labeled Eppendorf for PCR using.

PCR

PCR was used to check all *p. aeruguinosa* isolates for the presence of the mex B gene, which expresses efflux pump resistance [(10)]. As shown in table (1) .

Results

Out of 48 sample collected from surgical wound infection 25 samples were positive for *P.aeruginosa*. It was identified by its green growth in cetramide agar, positive oxidase test (blue color in oxidase strip), positive Citrate test (blue color) and alkaline red/red in TSI.
Ages of patients who participated in the study ranged from 5 to 67 years including the 33 males and 15 women.

The proportion of *P. aeruginosa* isolates in samples of all wounds samples was 52% (25/48). Out of the positive 25 strain 18 strains (72%) were MDR which have at least one agent resistance across three or more different antibiotic families. As shown in table (2).
MexB gene positive in 21/25 (84%) strains. As shown in Figure (1).

Table (1): primer sequences size and annealing temperature for mexB [10].

Sequence	F: GTGTTCGGCTCGCAGTACTC R : AACCGTCGGGATTGACCTTG
Size	244 bp
Annealing	55 C

Table (2): Antibiotic resistance pattern of *P. aeruginosa* isolates.

Antibiotic	ResistantNo. (%)	SensitiveNo. (%)
Cefepime	11 (44%)	14 (56 %)
Amikacin	18(72%)	7(28%)
Gentamycin	15 (60%)	10 (40 %)
Levofloxacin	10 (40%)	15 (60%)
Ceftazidime	16 (64%)	9 (36%)
Pipracillin	12(48%)	13 (52%)

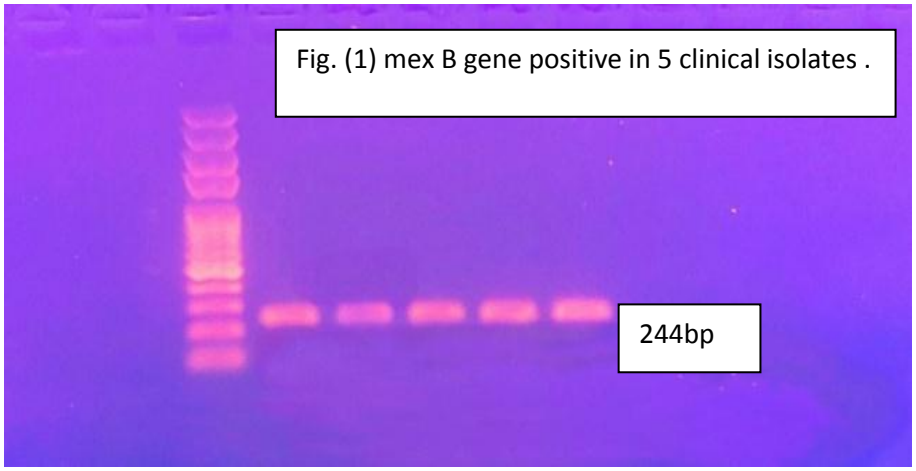

Figure 1: Result of gel electrophoresis for PCR products of mex b gene (5 positive samples for MexB gene size 244bp)

Discussion

P. aeruginosa is thought to be an extremely opportunistic human pathogen, with attachment, colonisation, local invasion, and dissemination as a systemic illness comprising the final infection.

P. aeurginosa are being isolated from nosocom ial infections more and more frequently. so, The resistance of these isolates to routinely used antibiotics should be continuously researched in order to properly select antimicrobials for management of these infection [(8) (11).]

25 isolates from 48 patients who were admitted to Minia University Hospital's plastic surgery department were used in this study. Management of *P. aeruginosa* infections are challenging due to the organism's natural resistance to numerous antibiotics as well as its capacity to develop newer resistant mechanisms.

In our research, 72% of *P. aeuroginosa* (18/25) are MDR; whereas, Mohamed et al., study found a higher proportion of MDR of 78% [(12).]
In this study prevalence of males was 72% (18/25) and females represented 28% (7/25).
The highest level of resistance was to Amikacin (72%, 18/25); however, this result was a little lower than that seen by El- Far et al., who noted a 79% resistance to Amikacin [(13)].
Gentamycin resistance was 68% (17/25) which is higher than that obtained by Kishk, et al., it was 65%.

Ceftazidime had a significant percentage of resistance that reached up to 64% (16/25), which is lower than the 68% obtained by Kishk, et al.,[(14)]

Cefipime had resistence of 44% (11/25) and Levofloxacin had resistence of 40% (10/25) which both were higher than result reported by Saderi et al, it was 34% for both drugs [(15)]
Pipracillin resistence 48% (12/25) it was higher than that obtained by Bonyadi, et al., it was 40% [(16).]

Mex B gene was detected in 21 strains out of 25 (84%) this result is higher than that detected by Yoneda, et al.,[(10)]. In this study, the proportion of genes that express antibiotics and the rates of antibiotic resistance were different numerous factors, including severe sociodemographic disparities, could be the cause.

In this study, the proportion of genes that express antibiotics and the rates of antibiotic resistance were different numerous factors, including severe sociodemographic disparities, could be the cause.

Conclusion

P. aeruginosa Infections have become a severe problem that requires a precise diagnosis and effective treatment. To replace the current unsuccessful therapies, new alternative treatment plans should be taken into consideration and researched.

References

1- Liu T, Zhang Y, Wan Q. Pseudomonas aeruginosa bacteremia among liver transplant recipients. Infection and Drug Resistance. 2018;11:2345.

2- Zhang Y, Li Y, Zeng J, Chang Y, Han S, Zhao J, et al., Risk factors for mortality of inpatients with Pseudomonas aeruginosa bacteremia in China: impact of resistance profile in the mortality. Infection and Drug Resistance. 2020:4115-23.

3- Laborda P, Hernando-Amado S, Martínez JL, Sanz-García F. Antibiotic Resistance in Pseudomonas. Pseudomonas aeruginosa: Biology, Pathogenesis and Control Strategies: Springer; 2022. p. 117-43.

4- Subedi D, Vijay AK, Willcox M. Overview of mechanisms of antibiotic resistance in Pseudomonas aeruginosa: an ocular perspective. Clinical and Experimental Optometry. 2018; 101(2):162-71.

5- Meletis G, Bagkeri M. Pseudomonas aeruginosa: Multi-drug-resistance development and treatment options. Infection Control. 2013; 2:34-45.

6- Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al., Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clinical microbiology reviews. 2019;32(4): e00031-19.

7- Al-Orphaly M, Hadi HA, Eltayeb FK, Al-Hail H, Samuel BG, Sultan AA, et al., Epidemiology of multidrug-resistant Pseudomonas aeruginosa in the Middle East and North Africa Region. Msphere. 2021; 6(3):e00202-21.

8- Liew SM, Rajasekaram G, Puthucheary SA, Chua KH. Antimicrobial susceptibility and virulence genes of clinical and environmental isolates of Pseudomonas aeruginosa. PeerJ. 2019;7:e6217. CLSI. 2022.

9- Yoneda K, Chikumi H, Murata T, Gotoh N, Yamamoto H, Fujiwara H, et al., Measurement of Pseudomonas aeruginosa multidrug efflux pumps by quantitative real-time polymerase chain reaction. FEMS Microbiol Lett.2005;243(1):125-31.

10- Abdel-Rhman SH, Rizk DE. Serotypes, antibiogram and genetic relatedness of pseudomonas aeruginosa isolates from urinary tract infections at urology and Nephrology Center, Mansoura, Egypt. Advances in Microbiology. 2018; 8(8): 625-38.

11- Mohamed MA, Mohamed HA, Afifi MM. Prevalence of MDR Pseudomonas aeruginosa in Intensive care units and burned patients. Journal of Environmental Studies. 2022; 27(1):10-5.

12- El-Far A, Samir S, El-Gebaly E, Omar M, Dahroug H, El-Shenawy A, et al., High rates of aminoglycoside methyltransferases associated with metallo-beta-lactamases in multidrug-resistant and extensively drug-resistant pseudomonas aeruginosa clinical isolates from a tertiary care hospital in Egypt. Infection and Drug Resistance. 2021:4849-58.

13- Kishk RM, Abdalla MO, Hashish AA, Nemr NA, El Nahhas N, Alkahtani S, et al., Efflux MexAB-Mediated Resistance in P. aeruginosa Isolated from Patients with Healthcare Associated Infections. Pathogens. 2020;9(6):471.

14- Saderi H, Owlia P. Detection of Multidrug Resistant (MDR) and Extremely Drug Resistant (XDR) P. Aeruginosa Isolated from Patients in Tehran, Iran. Iran J Pathol. 2015;10(4):265-71.

15- Bonyadi P, Saleh NT, Dehghani M, Yamini M, Amini K. Prevalence of antibiotic resistance of Pseudomonas aeruginosa in cystic fibrosis infection: a systematic review and meta-analysis. Microbial Pathogenesis. 2022;165:105461.