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Characterization Of Extremely Drug-Resistant Pseudomonas Aeruginosa Isolates From Burn Center In Najaf, Iraq

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Abstract

Background:P. aeruginosa is indeed a prominent Gram-negative bacteria and opportunism pathogen that has been associated in several of the life-threatening diseases in recent years. Carbapenems, in specific imipenem and meropenem, have a broadest spectrum of activity. Resistance to hydrolyzed by most β-lactamases provides them significant assets in the combat against severe nosocomial infections

Aim of the study: Examinations the incidence of extensive-drug resistant (XDR) and detecting of carbapenems antibiotic resistant genes among susceptibility profile in P. aeruginosa which isolated from Najaf burn Center.

Methods: overall of 73 samples were taken from patients at the Najaf Burn Center from the whole research period. A Kirby-Bauer disk diffusion technique using 17 antimicrobial agents has been used to examine P. aeruginosa specimens for bacterial resistance. Carbapenems production was initially screened by using the disk diffusion method , according to the CLSI (2020) and potential Carbapenems producing isolates were further determined by using disk diffusion method based on the MIC.

Polymerase chain reactions (PCR) were used to identify Carbapenemase genes with in specimens.

Result: There were 24 (32.8%) P. aeruginosa specimens confirmed.The distribution of antimicrobial sensitivity revealed extensive drug resistance in 7 of the isolates (29%). Based on the findings of antibiotic susceptibility, Most of these samples were shown to be resistant to at least one carbapenem, and 6 (25) % of P. aeruginosa were shown to have considerable resistance to carbapenems, extensive resistance was found in all of these specimens.3 (50%) types of genes encoding carbapenem-inactivating enzymes (bla_{NDM} , bla_{SPM-1} and bla_{OXA-50}) were detected in this study

Conclusions: Carbapenem resistance has increased, demonstrating the significance of such a challenge as in management of severe infections with P. aeruginosa which is extensive-resistant.

Keywords: Pseudomonas Aeruginosa, Resistance , extensive-drug resistant, Antibiotics

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INTRODUCTION

Pseudomonas aeruginosa is among the most important microorganisms inside the body,Gram-negative bacteria with wide-ranging pathogenic potential, considered the most important cause of an ever-expanding spectrum of possibly serious diseases (Rashid et al., 2020). One of most concerning aspect of this bacteria is that it is resistant to most antibiotics, this may be attributed to the limited accessibility of a microbial organisms membranes and the activity of efflux pumps (Pang et al., 2019). In additional toward this intrinsic resistance, Pseudomonas aeruginosa may acquire resistance from mutations within chromosomal genes or through the transfer of resistant determinants through horizontal transmission. These pathways can often be seen working in concert, consequently giving multi-drug resistance on various antibiotics (Eichenberger and Thaden, 2019). Obviously, Multiple drug resistance has become more common in P. aeruginosa, making treatment more problematic due to fewer possible treatment alternatives (Palavutitotai et al., 2018). The words multidrug resistance (MDR), extensive drug resistance (XDR), and pan drug resistance (PDR) have been used to explain the various types of multidrug resistant strains that P. aeruginosa demonstrates. (Magiorakos et al., 2012).

For the most severe situations of multiple drug resistance, carbapenems are just the first medication. The widespread usage of carbapenems with in hospitals has resulted in the production of resistant bacteria . Regardless of that, carbapenem-resistant Worrisome P. aeruginosa samples are expanded fast over the globe, especially Iraq (Alshara et al., 2014) ,(Sedighi et al., 2014)found a significantly greater separation rate (41.5 %), in Iran, where such a total of 106 P. aeruginosa isolates were screened among 255 clinical isolates analysed.In Egypt, Abaza et al. (2014), stated indicated 175 (11.3 %) P. aeruginosa specimens were detected. Ambler class A, class B, and class D carbapenemases have been identified and categorized(McCracken et al., 2019) . The purpose of this study is to explore the prevalence, drug resistance profiles, the occurrence of selective antibiotic resistant genes as well as molecular epidemiology of clinical isolates of XDR P. aeruginosa at Najaf burn center. through

Materials And Method

Collecting of Samples

Najaf's burn center's across sections procedures on patients admitted and visited during September 2020 to December 2020.73 specimens were obtained from patients from the research period and the samples were taken to the laboratory .

Identification of Bacterial Isolates

In accordance with MacFaddin, (2000)'s standard approach, suspect P.aeruginosa colony are identified and isolated by standard laboratory assays, In order to identify P.aeruginosa, the VITEK-2 compact system has been used ,which made use of GN AST 222 card.

Susceptibility Testing for Antibiotics

The isolates were screened for their susceptibility against 17 different antibiotic discs of 9 classes by using Kirby-Bauer agar disk diffusion method (Hudzicki, 2012), which was used to measure zones of inhibition in accordance with the recommendations of clinical and Laboratory Standards Institute (CLSI 2020) . E. coli ATCC 25922 was used as control strain in all susceptibility assays.

Carbapenemase Molecular Screening :

This study was carried out to detect the diversity and distribution of the main carbapenemase genes amongst the XDR P. aeruginosa isolates obtained from Najaf burn center during 3-months period. the main carbapenemase-encoding genes including; Ambler class B MBLs (bla_{VIM}, bla_{NDM}, bla_{SPM-1}, and bla_{GIM}), Ambler class A carbapenemases (bla_{IMI} and bla_{GES}), and Ambler class D oxacillinases (bla_{OXA-40}, bla_{OXA-48}, bla_{OXA-50}).The early detection of carbapenemase producing P. aeruginosa may avoid the future spread of these XDR isolates.

Result

Among 73 specimens that collected from burn center found that 24 (32.8%) of isolates were P.aeruginosa ,and 6/24(25%) isolate were extensively drug resistant carbapenems P.aeruginosa ,were only 1/24(4%) was XDR but not resistant to carbapenems as shown in table (1) .All six P. aeruginosa specimenswhich were resistant to meropenem and/or imipenem, A minimum inhibitory concentration (MIC) was also confirmed by VITEK-2 compact system.

Table 1: Antibiotic susceptibility test results from P.aeruginosa patients (n=24) isolated

Antimicrobial agent	Total of Clinical resistant (n=24)		
	R	I	S
Piperacillin	14 (58.3)	3 (12)	7 (29)
Ticarcillin- clavulanic acid	9 (38)	/	15 (62)
Cefotaxime	17 (71)	3 (13)	4 (17)
Ceftriaxone	14 (58)	/	10 (42)
Ceftazidime	21 (88)	1 (4.1)	2 (8.3)
Cefepime	11 (46)	3 (12)	10 (42)
Aztreonam	7 (29)	/	17 (71)
Imipenem	5 (21)	2 (8.3)	17 (71)

Meropenem	5 (21)	/	19 (79.1)
Tobramycin	12 (50)	2 (8.3)	10 (42)
Gentamicin	7 (29)	4 (17)	13 (54.1)
Amikacin	7 (29)	/	17 (71)
Ciprofloxacin	8 (33.3)	2 (8.3)	14 (58)
Levofloxacin	7 (29)	1 (4.1)	16 (66.6)
Norfloxacin	13 (54.1)	/	11 (46)
Colistin	2 (8.3)	/	22 (92)
Fosfomycin	16 (66.6)	/	8 (33.3)

Molecular screening of carbapenemase poducers:

All 6 carbapenem resistant P. aeruginosa samples were detected by PCR for potential genes determinants encoding carbapenemase using a specific primers for Ambler class A carbapenemases, class B MBL and class D. Three types of genes encoding carbapenem-inactivating enzymes (bla_{NDM} , bla_{SPM-1} and bla_{OXA-50}) were detected. According to PCR findings ,Carbapenem-resistant genes were detected in 50 % of specimens screened in this investigation as shown in Figures (1,2 and 3).

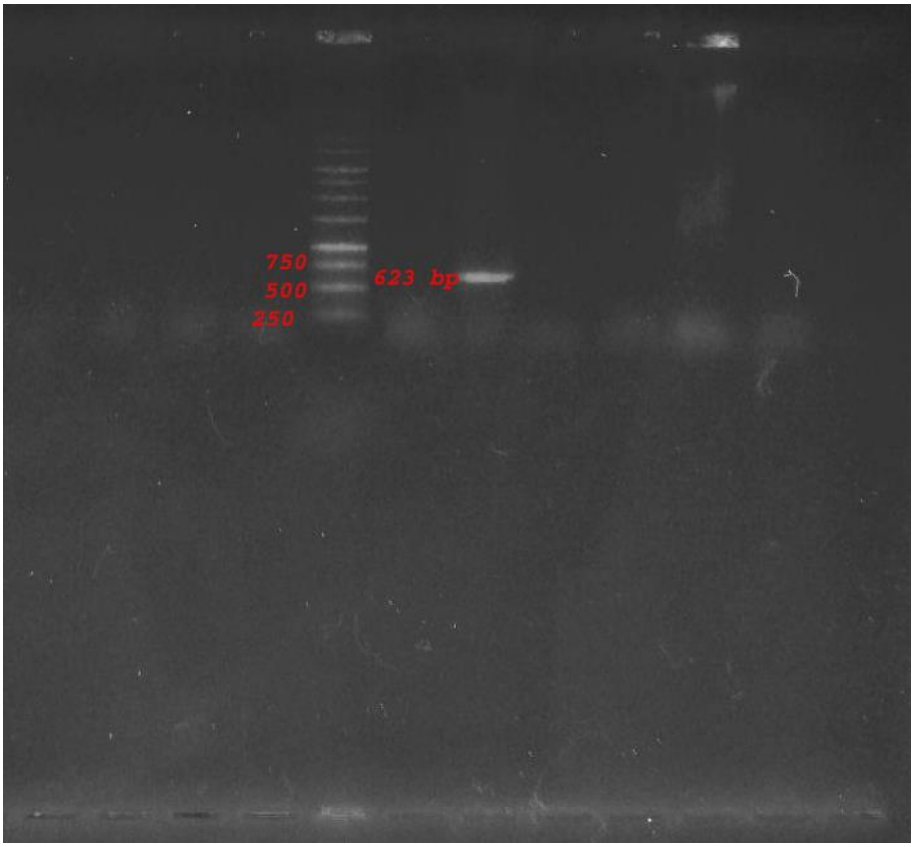

Figure (1): Agarose gel with red save stained of mono-plex PCR amplified product from extract DNA of P. aeruginosa isolates with blaNDM gene primer. The electrophoresis performed at 65 volt for 120 minutes. Lane (L) is DNA molecular size marker (10,000-bp ladder). Pa1 show positive results with blaNDM gene (623 bp).

Figure (2): Agarose gel with red save stained of mono-plex PCR amplified product from extract DNA of P. aeruginosa isolates with bla SPM-1 gene primer. The electrophoresis performed at 65 volt for 120 minutes. Lane (L) is DNA molecular size marker (10,000-bp ladder). Pa4 show positive results with blaSPM-1 gene (650 bp).

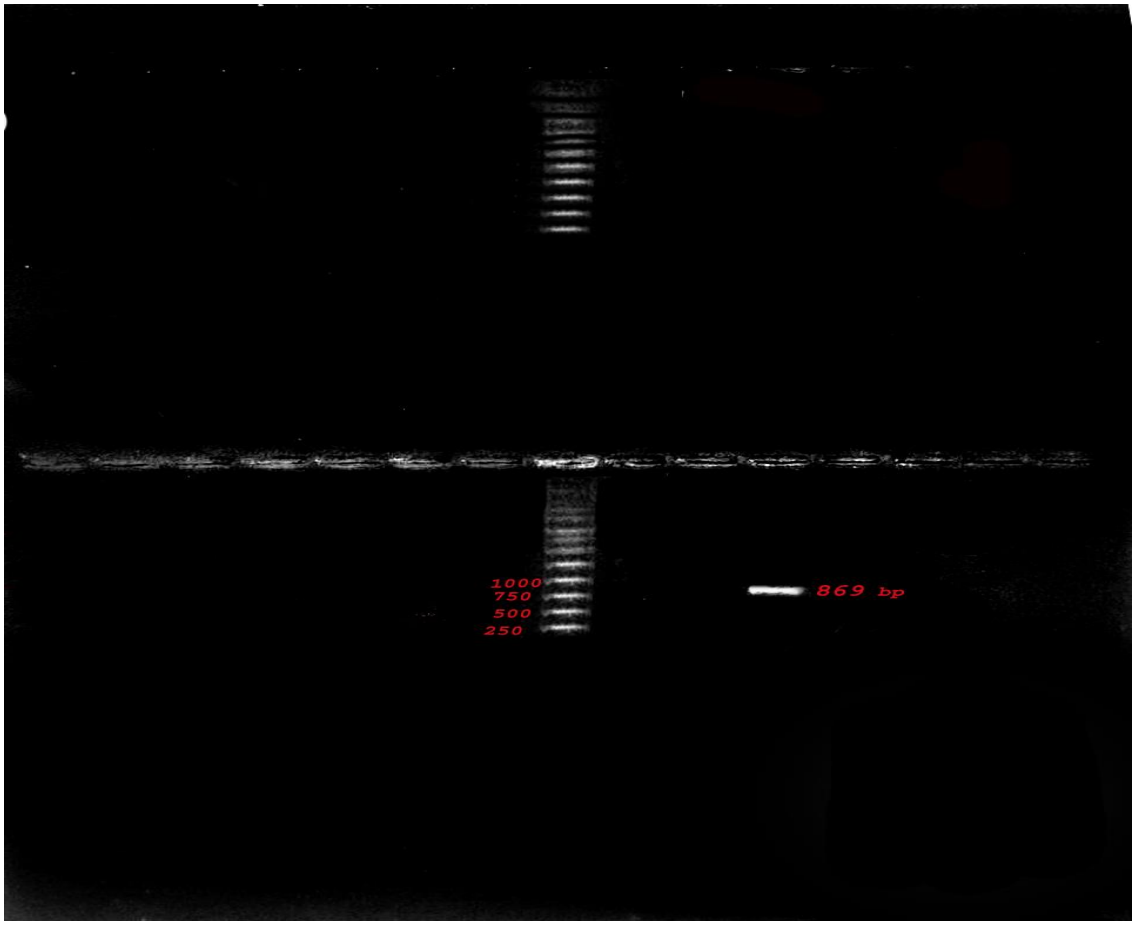

Figure (3): Agarose gel with red save stained of mono-plex PCR amplified product from extract DNA of P. aeruginosa isolates with blaOXA-50 gene primer. The electrophoresis performed at 65 volt for 120 minutes. Lane (L) is DNA molecular size marker (10,000-bp ladder). Lanes pa5 show positive results with blaOXA-50 gene (869 bp).

Discussion

Pseudomonas aeruginosa was among the most common bacteria responsible with healthcare-associated illnesses(Litwin et al., 2021).The huge load of sickness is caused by this bacterium, which represents a severe health concern,mortality rates including both developed and emerging countries(Khosravi et al., 2019; Pang et al., 2019). Such significant pathogenicity of P. aeruginosa was due in part towards the bacteria's inherent resistance to a variety of commonly prescribed antibiotics, and also the emergence of multiple-drug resistance (Langendonk et al., 2021). This cross-sectional research, which occurred from September 2020 to November 2020, collected 73 clinician specimens . According to the outcomes of this

investigation, 24 (32.8%) P. aeruginosa was isolated and detected in a wide range of clinical specimens collected from Najaf burn center. According to some research, including those conducted in India, 55% of the population is affected (Rajput et al., 2010) and 50% in Palestine(Al Laham et al., 2013). That variation in prevalence rates across the preceding research might be attributable to variations in the sorts of samples examined, geographic region and sanitary conditions. Extensively drug resistant isolatesin specifically those that are resistant towards carbapenems, a serious problem for healthcare facilities(Meletis & Bagkeri, 2013).Unfortunately, P. aeruginosa demonstrating resistance toward carbapenems are growing because of multifactorial and can be caused by many causes, involving carbapenemase synthesis, excess supply of efflux pump, and decrease of cellular membranes porins (Doi, 2019). Carbapenemase genes were found in a large percentage of specimens with in present study, Carbapenem-resistant genes were found in both class B and D, Three of such XDR specimens (50%) that received PCR screening,demonstrating that carbapenem resistance among XDR P. aeruginosa samples was caused in quantity by the synthesis of carbapenemase. In Najaf, there are different rates of carbapenemase genes blaoxa-50(16.6%), according to (El-Shouny et al., 2018), 42.8 % of the P. aeruginosa samples detected in Egypt exhibited OXA-50 type providers. The rate of bla_{NDM-1} in najaf was (16.6%) . Currently, the occurrence of NDM-1 had already risen significantly from the world, and has been identified mostly in Asia (Tada et al., 2019; Honda et al., 2019), in Europe (Urbanowicz et al., 2019), and in Africa (Yoon and Jeong, 2021). Also bla_{SPM-1} was demonstrated as (16.6%) rate in this investigation , bla_{SPM-1} likewise has been detected in Switzerland (Salabi et al., 2010), Salabi et al. (2010), the United Kingdom (Azim et al. 2010). Consequently, the discovery of (50%) carbapenemase resistant genes in this research recommended potentials of dissemination through its high rate of genetic transfer among pathogenic bacteria in Najaf hospitals, or possibilities to human factors such as hygiene and international tourists.

CONCLUSIONS

The results of this research confirmed that Carbapenem resistance has increased among P. aeruginosa isolates. A high rate of carbapenem resistance was observed among XDR isolates and bla_{OXA-50}, bla_{NDM}, bla_{SPM-1} genes were detected in three isolates. Colistin is still the most potentate antibiotic for P. aeruginosa . However, this study found that 29% resistant to P. aeruginosa.

REFERENCES

1. Al Laham, N. A., Elmanama, A. A., & Tayh, G. A. (2013). Possible risk factors associated with burn wound colonization in burn units of Gaza strip hospitals, Palestine. Annals of Burns and Fire Disasters, 26(2), 68–75.

2. El-Shouny, W. A., Ali, S. S., Sun, J., Samy, S. M., & Ali, A. (2018). Drug resistance profile and molecular characterization of extended spectrum beta-lactamase (ESβL)-producing Pseudomonas aeruginosa isolated from burn wound infections. Essential oils and their potential for utilization. Microbial Pathogenesis, 116, 301–312. https://doi.org/10.1016/j.micpath.2018.02.005

3. Hudzicki, J. (2012). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. American Society For Microbiology, December 2009, 1–13. https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro

4. Khosravi, A. D., Taee, S., Dezfuli, A. A., Meghdadi, H., & Shafie, F. (2019). Investigation of the prevalence of genes conferring resistance to carbapenems in pseudomonas aeruginosa isolates from burn patients. Infection and Drug Resistance, 12, 1153–1159. https://doi.org/10.2147/IDR.S197752

5. Langendonk, R. F., Neill, D. R., & Fothergill, J. L. (2021). The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Frontiers in Cellular and Infection Microbiology, 11(April), 1–22. https://doi.org/10.3389/fcimb.2021.665759

6. Litwin, A., Rojek, S., Gozdzik, W., & Duszynska, W. (2021). Pseudomonas aeruginosa device associated – healthcare associated infections and its multidrug resistance at intensive care unit of University Hospital: polish, 8.5-year, prospective, single-centre study. BMC Infectious Diseases, 21(1), 4–11. https://doi.org/10.1186/s12879-021-05883-5

7. Meletis, G., & Bagkeri, M. (2013). Pseudomonas aeruginosa: Multi-Drug-Resistance Development and Treatment Options. Infection Control. https://doi.org/10.5772/55616

8. Pang, Z., Raudonis, R., Glick, B. R., Lin, T., & Cheng, Z. (2019). Antibiotic resistance in Pseudomonas aeruginosa : mechanisms and alternative therapeutic strategies. Biotechnology Advances, 37(1), 177–192. https://doi.org/10.1016/j.biotechadv.2018.11.013

9. Rajput, A., Saxena, R., Singh, K. P., Kumar, V., Singh, S., Gupta, A., & Singh, R. K. (2010). Prevalence and antibiotic resistance pattern of metallo-β-lactamase- producing pseudomonas aeruginosa from burn patients-experience of an Indian tertiary care hospital. Journal of Burn Care and Research, 31(2), 264–268. https://doi.org/10.1097/BCR.0b013e3181d0f4bf

10. Sedighi, M., Safiri, S., Pirouzi, S., Jayasinghe, H., Sepidarkish, M., & Fouladseresht, H. (2014). Detection and Determination of the Antibiotic Resistance Patterns in Pseudomonas aeruginosa Strains Isolated From Clinical Specimens in Hospitals of Isfahan, Iran, 2012. Scimetr, 3(1). https://doi.org/10.5812/scimetr.21133

11.Rashid, A., Akram, M., Kayode, O.T. and Kayode, A.A. (2020). Clinical features and epidemiological patterns of infections by multidrug resistance Staphylococcus aureus and Pseudomonas aeruginosa in Patients with Burns. Biomed. J. Sci. Tech. Res. 25(4): 19272-19278.

12. Eichenberger, E.M. and Thaden, J.T. (2019). Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. Antibiotics, 8(37): 1-21.

13. Palavutitotai, N., Jitmuang, A., Tongsai, S., Kiratisin, P. and Angkasekwinai, N. (2018). Epidemiology and risk factors of extensively drug-resistant Pseudomonas aeruginosa infections. PLoS ONE. 13: 1-13.

14. Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E. and Giske C.G. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18: 268-281.

15 Alshara, J.M., Alsehlawi, Z., Aljameel, D.S., Al-Zubbedy, Z.S. and Almohana, A.M. (2014). First report of New Delhi metallo-β-lactamase (NDM-1) producing Pseudomonas aeruginosa in Iraq. J. Bio. Agricul. Healthc., 4: 40-47.

16. MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott Williams and Wilkins, USA.

17. Doi1, Y. (2019). Treatment options for carbapenem-resistant Gram negative bacterial infections. Clin. Infect. Dis., 69 (7): 565-575.

18. Tada, T., Hishinuma, T., Watanabe, S., Uchida, H., Tohya, M. and Kuwahara-Arai, K. (2019). Molecular characterization of multidrug-resistant Pseudomonas aeruginosa isolates in hospitals in Myanmar. Antimicrob. Agents Chemother., 63: e02397-18.

19. Honda, N.H., Aoki, K., Kamisasanuki, T., Matsuda, N., To, M. and Matsushima, H. (2019). Isolation of three distinct carbapenemase-producing Gram negative bacteria from a Vietnamese medical tourist. J. Infect. Chemother. 25: 811-815.

20.Urbanowicz, P., Izdebski, R., Baraniak, A., Zabicka, D., Ziolkowski, G. and Hryniewicz, W. (2019). Pseudomonas aeruginosa with NDM-1, DIM-1 and PME-1 beta-lactamases, and RmtD3 16S rRNA methylase, encoded by new genomic islands. J. Antimicrob. Chemother., 74: 3117-3119.

21.Yoon, E. and Jeong, S.H. (2021). Mobile carbapenemase genes in Pseudomonas aeruginosa. Frontiers Microbiol., 12: 1-21.

22.Salabi, A.E., Toleman, M.A., Weeks, J., Bruderer, T., Frei, R. and Walsh, T.R. (2010). First report of the metallo-beta-lactamase SPM-1 in Europe. Antimicrob. Agents Chemother., 54: 582.

23.Azim, A., Dwivedi, M., Rao, P.B., Baronia, A.K., Singh, R.K. and Prasad, K.N. (2010). Epidemiology of bacterial colonization at intensive care unit admission with emphasis on
24.extended-spectrum beta-lactamase- and metallo-betalactamase-producing Gram-negative bacteria-an Indian experience. J. Med. Microbiol., 59: 955-960.