Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17672
DOI URL: http://dx.doi.org/10.21474/IJAR01/17672

RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF PATHOGENIC *PSEUDOMONAS* SPECIES FROM URINARY TRACT INFECTION (UTI)AND WOUNDS IN SAUDI ARABIA AT MAKKAH REGION[1,2,]

Faisal Al-Zahrani[1] and Anas S. Dablool[2]

1. M.Sc. Umm Al-QuraUniversity Faculty of Applied Sciences Department of Biology.
2. Public Health Department, Health Sciences College at Al-Leith, Umm Al-QuraUniversity, Makkah, Saudi Arabia.

Manuscript Info

Manuscript History
Received: 05 August 2023
Final Accepted: 09 September 2023
Published: October 2023

Key words:-
P. Aeruginosa, Multiple Drug Resistance, Urinary Tract Infection

Abstract

Background: *Pseudomonas aeruginosa*(*p.aeruginosa*) is an opportunistic infection that causes severe urinary tract infection, it is well established That its common species in wound infection (chronic wound) is *aeruginosa*, here we studied the clinical isolates in urine and wound and their sensitivity pattern.

Materials and Methods: This was a cross-sectional study aimed to isolate and characterizing the *p. aeruginosa* from urine and wound samples, 106 participants were enrolled for this study, patients under antibiotic and unconsented were excluded, bacteria were isolated with automated instrument, data was analyzed by SPSS V26, p.value<0.050 was considered significant data was expressed as percentage.

Result: One hundred sex participants were enrolled for this study, n=77(72.64%) Saudi and n=29(27.35) was non-Saudi nationality. The male was n=56(**52.83%)**, while female n=50**(47.16%)** study participants. We included the wound sample which comprises 49.05% and urine samples 50.94% table (1). Of 106 isolates of *P. aeruginosa* enrolled for this study, the overall drug resistance was moderate ranging from 6(5.66%) to 27(25.47%) to the all 13psudomonal antibiotic tested. Piperaquine is the lowest resistance in comparison to other antibiotics (5.66% p.value<0.050 while the ipeneme is the most resistant in comparison to the other 12 antibiotics 25.47% p.value<0.050. finally, the overall multiple drug resistance of *P. aeruginosa* was 16.11%.

Conclusions: The clinical isolates of *P. aeruginosa* in urine and wound samples were moderate resistance further hospitals survey was required.

Introduction:-

Pseudomonas aeruginosa (*P. aeruginosa*) is gram-negative aerobic bacteria measure0.5 to 3µm, with complex structures and the largest species, it accounts for 80 % of opportunistic infections, and it causes serious health problems in a hospitalized patients with cystic fibrosis, cancer, and burns (1). In 2015 there is 144 species of *pseudomonas* and 10subspecies, a French pharmacist CreleGessard first reported pseudomonas in 1882 which produce several pigments in media, the word comes from the Greek words pseudo(false) monas (single unit

Corresponding Author:- Faisal Al-Zahrani
Address**:-** M.Sc. Umm AlQura University Faculty of Applied Sciences Department of Biology.

),*aeruginosa* greenish blue its Latin words mean aerugo (rusted copper)(2). The genome is 6.3Mbp in size which is related to PO 01 isolated from wound infection and commonly use strain in laboratories (3). Urinary tract infection UTIs are prevalent in the community and among health workers UTIs result in 8 million7 visits to physicians1.5 million emergency units ER 300000 hospital admission in the US (4). The global death from UTI236,790 in 2019.The prevalence increase with age (4). The prevalence in 65 years women reaches 20% while in the general population of 11% women, 50-60% of adult women will have at least one urinary tract infection in their life, and 10% of postmenopausal women that had UTI in the previous years (5)(6). In a cross-sectional study in Saudi Arabia with 3- month periods the prevalence of UTI was 1449 patients diagnosed with UTI (7), with female predominance and a total cost per patient 1.7 US dollars. Another study in our community mainly in children's study demonstrated that the prevalence of UTI was 25.8% in a sample size of 1083 study participants, the females more affected than females(8).In diabetic Saudi citizens, the prevalence was 25.3% out of 1000 diabetic patients with UTI, the female has a higher risk OR 6.102(9).In pregnant Saudi women's the prevalence was 20% out of 200 pregnant have UTI (10) *P. aeruginosa* is non-fermenter gram-negative bacilli with large intrinsic resistance to multiple antibiotics, its quick ability to acquire new antimicrobial resistance this feature make pseudomonas as growing health concern especially when it is nosocomial(11)(12). *P. aeruginosa* is an opportunistic infection that causes severe UTI (13). Pseudomonas comprises 7-10% of hospital-acquired infections (14)(15). The infected patients with pseudomonas are low survival due to many causes, respiratory failure, thrombocytopenia, mechanical ventilators, bedsore, cirrhosis, shock, steroid use, AIDS, resistance to carbapenem, and cancers (16)(17)

Materials and Methods:-

This was a cross-sectional study conducted at MAKKAH region (from January2021-to March 2023, all patients not on the antibiotic course last week were included, 106 patients were enrolled for this studywithwritten informed consent which was obtained from every study participant, and accordance with university ethical considerations. Demographic and laboratory data were obtained by the principal investigator and transferred to the questionnaire prepared for this study**,** questioners on, gender, nationality, bacteria, and antibiotic resistance were prepared, and mid-stream urine samples were collected in a sterile wide mouth container in the early morning, wound swabs were collected from the surface of the wound. Bacteria (*P. aeruginosa*) was isolated with culture and automated instruments (Vitek) The antibiotic includes Ipeneme, ciprofloxacin, ceftazidime, meropenem, cefepime, levofloxacin (piperacillin-tazobactam, piperaquine, amikacin, colistin, tobramycin, gentamycin, and aztreonam. The ethical clearance was obtained from the ethics review committee at the Faculty of Medical Laboratory Science at Um Alqura University. The data entry was entered and analyzed with SPSS version (20) Data was checked for normality for continuous variable mean and SD is used for normally distributed data. Median, interquartile for non-normally distributed data. We compared the antibiotic resistance between different groups with a p. value considered as significant as less than 0.050. while we considered the odds ratio of more than 1 as significant.

Results:-

One hundred six patients were enrolled for this study, n=77(72.64%) Saudi and n=29(27.35) was non-Saudi nationality. The male was 56, while the female had 50 study participants. We included the wound sample which comprises 49.05% and urine samples 50.94% table (1). Of 106 isolates of *P. aeruginosa* enrolled for this study, the overall drug resistance was moderate ranging from 6(5.66%) to 27(25.47%) to all 13 psudomonal antibiotic tested. Piperaquine is the lowest resistance in comparison to other antibiotics (5.66% p.value<0.050 while the ipeneme is the most resistant in comparison to the other 12 antibiotics 25.47% p.value<0.050. Considering a high-resistance antibiotic the most resistance was showed in ipeneme 25.47% followed by ciprofloxacin 23.58%, ceftazidime, meropenem (21.69), cefepime, levofloxacin (18.86%) piperacillin-tazobactam 17.92%. the others with less resistance showed lower resistance in piperaquine 5.66%, amikacin, colistin 9.43%, tobramycin 11.32%, gentamycin 12.26%, and aztreonam 13.20% Table (2). the resistance pattern is not affected by gender the odds ratio was Odds ratio for males and females = 0.8 p.value =0.124 while its slightly affected by sample type the urine is more drug resistance than wound samples the odds ratio was 1.399 and p.value 0.017. Finally, the overall multiple drug resistance of *P. aeruginosa* was 16.11%.

Table (1):- Sociodemographic characteristics of the participants.

Parameter	Number	Percentage
Saudi	**77**	**72.64%**
Non-Saudi	**29**	**27.35%**
Male	**56**	**52.83%**

Female	**50**	**47.16%**
Wound sample	**52**	**49.05%**
Urine sample	**54**	**50.94%**

Figure (1) Figure (4) Distribution of different susceptibility patterns of *P. aeruginosa* (AMK = amikacin TZP= Piperacillin-Tazobactam, ATM= AZETRONAM, CAZ = CEFAZIDEME, CIP=CIPROFLOXACIN, FEB= CEFIPENE, COL= COLISTIN, GEN= GENTAMYCIN, IPM= IPENEM, LVX= LEVOFLOXACIN, MEM= MEROPENEM, TOB TOBRAMYCIN, PIP =PIPERACYCLIN

Figure (2) resistance pattern of *P. aeruginosa* according to sample types and gender

Discussion:-

Our study revealed that the prevalence of multiple drug resistance *P. aeruginosa* was 16.11% in clinical isolates from urine and wound samples. The piperacillin resistance was less among all antibiotics this is very low in comparison with another finding of pseudomonas isolate which is 70% (18) and 73% (19) in India. The most resistant forms are shown in anti-pseudomonal carbapenems (Ipeneme and meropenem) our result is less than the internationally established range but agreed that the carbapenems are higher resistance which reaches 100% resistant and 80% (18) (20-21) This is a serious and bad news for loss of valuable drugs in pseudomonas and other infections higher resistance may be due to overuse of carbapenem drugs in the ICU clinics and transmission of resistance strains to the patients inside hospitals and ICU. Regarding fluoroquinolones (levofloxacin and ciprofloxacin) are slightly less resistant than carbapenem but are addressed in our results as a high resistance group in comparison to others which reach 100% in some study (22). A recent study revlead that the genetic mutations and drug efflux are contributed to ciprofloxacin resistance (23) while another study concludes that the use of levofloxacin was the cause of fluoroquinolones resistance (24), details of resistance recently emerging with new ciprofloxacin, ofloxacin, and levofloxacin demonstrated that change of drug concentration is bactericidal to MDR *P. aeruginosa* especially levofloxacin (25).fourth group aminoglycosides (gentamycin, amikacin) are light resistance 9 and 12% whereas it less resistance in comparison to other studies which revealed that 80 to 88% percent in Indian study(18) and 72to79% in another study (26)(27). Finally, cephalosporines (cefepime ceftazidime) are considered with high resistance in our study but with less resistance than international resistance (19), while another study concluded that cephalosporins reduced mortality than meropenem and a good drug of choice in MDR *pseudomonas* (28). Comparing our finding of overall MDR in Makkah regions there is a rising prevalence which is 10.7% in the previous studies (29-35) and here was 16% this is defined as the risk of spreading and transmission of drug-resistant *P. aeruginosa* in Saudi Arabia.

Data source and availability

The primary source of this data is a questionnaire, any other data is available upon request to the corresponding author.

Disclosure

Authors disclose no competing interest

Author contributions

F.A. and A.D., Conceived a study supervised the methodology of the work, F.A. analyzed data and write the final finding, all authors contributed to the writing of the manuscript, supervised data collection, critically reviewed the manuscript, and all authors read the final draft of the manuscript.

Acknowledgments:-

Great thanks to our study participants for their sharing in the interview, and many thanks to the Microbiology departments for their assistance.

Funding

This study didn't get any funds from the governmental or private sectors.

Future research points:

Conduct larger studies to confirm the findings of this study and to generalize the results to other regions of Saudi Arabia and to other countries.

Investigate the risk factors for Pseudomonas aeruginosa infections. This information could be used to develop targeted prevention strategies.

Investigate the clinical outcomes of patients with Pseudomonas aeruginosa infections. This information would be useful for understanding the impact of antibiotic resistance on patient outcomes and for developing more effective treatment strategies.

Investigate the mechanisms of antibiotic resistance in Pseudomonas aeruginosa isolates. This information could be used to develop strategies to overcome antibiotic resistance.

Evaluate the effectiveness of different antibiotic treatment regimens for Pseudomonas aeruginosa infections. This information would be useful for developing more effective treatment strategies.

Develop and evaluate new strategies for the prevention and treatment of Pseudomonas aeruginosa infections, especially multidrug-resistant infections. This could include developing new antibiotics, vaccines, or other therapeutic strategies.

In addition to these general research points, here are some specific research questions that could be investigated:

What is the role of Pseudomonas aeruginosa in the development of hospital-acquired infections in Saudi Arabia?

What are the most effective ways to prevent Pseudomonas aeruginosa infections in high-risk settings, such as hospitals and nursing homes?

Can vaccines be developed to prevent Pseudomonas aeruginosa infections?

Can new therapeutic strategies be developed to target Pseudomonas biofilms?

Study limitations:
Small sample size: The study was conducted with a relatively small sample size of 106 patients. This limits the statistical power of the study and makes it difficult to generalize the findings to a larger population.

Single-center study: The study was conducted at a single hospital in Saudi Arabia. This means that the results may not be generalizable to other regions of Saudi Arabia or to other countries.

Lack of clinical outcome data: The study did not collect data on the clinical outcomes of patients with Pseudomonas aeruginosa infections. This information would have been useful for understanding the impact of antibiotic resistance on patient outcomes.

Lack of risk factor assessment: The study did not investigate the risk factors for Pseudomonas aeruginosa infections. This information would have been useful for developing targeted prevention strategies.

References:-

1. Iglewski BH. Pseudomonas. In: S B, editor. Medical Microbiology [Internet]. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8326/
2. Diggle SP WM. Microbe Profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Microbiology. 2020;126:30-33.
3. Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, et al. Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. Nature. 2000;406:959–964.
4. Schmiemann G, Kniehl E, Gebhardt K et al. The diagnosis of urinary tract infection: a systematic review. DtschArztebl Int. 2010;107:361–367.
5. Chu CM LJ. Diagnosis and treatment of urinary tract infections across age groups. Am J Obs Gynecol. 2018;219:40–51.

6. La AJE y etiología de. Epidemiology and etiology of urinary tract infections in the community. Antimicrobial susceptibility of the main pathogens and clinical significance of resistance. EnfermInfeccMicrobiol Clin. 2005;23(Suppl 4).
7. MQ A. An evaluation of community-acquired urinary tract infection and appropriateness of treatment in an emergency department in Saudi Arabia. Ther Clin Risk Manag. 2015;5:2363–73.
8. Alrasheedy M, Abousada HJ, Abdulhaq MM, Alsayed RA, Alghamdi KA, Alghamdi FD, Al Muaibid AF, Ajjaj RG, Almohammadi SS, Almohammadi SS, Alfitni WA, Homsi AM, Alshelawi MM, Alshamrani HA, Tashkandi AA, Mannan SM AS. Prevalence of urinary tract infection in children in the kingdom of Saudi Arabia. Arch Ital UrolAndrol. 2021;28:206–10.
9. Al-Rubeaan KA, Moharram O, Al-Naqeb D, Hassan A RM. Prevalence of urinary tract infection and risk factors among Saudi patients with diabetes. World J Urol. 2013;31:573–8.
10. Faidah H. S, Ashshi A. M, Abou El-Ella G. A, Al-Ghamdi A. K MAM. Urinary Tract Infections among Pregnant Women in Makkah, Saudi Arabia. Biomed Pharmacol J. 2013;6:1.
11. Horino T, Chiba A, Kawano S, Kato T, Sato F, Maruyama Y et al. Clinical characteristics and risk factors for mortality in patients with bacteremia caused by *Pseudomonas aeruginosa*. Intern Med. 2012;51:59–64.
12. Schechner V, Gottesman T, Schwartz O, Korem M, Maor Y, Rahav G et al.*Pseudomonas aeruginosa* bacteremia upon hospital admission: risk factors for mortality and influence of inadequate empirical antimicrobial therapy. DiagnMicrobiol Infect dis. 2011;71:38–45.
13. Narten M, Rosin N, Schobert M TP. Susceptibility of Pseudomonas aeruginosa urinary tract isolates and influence of urinary tract conditions on antibiotic tolerance. CurrMicrobiol. 2012;64:7–16.
14. Bouza E, San Juan R, Muñoz P, Voss A KJ. A European perspective on nosocomial urinary tract infections I. Report on the microbiology workload, etiology and antimicrobial susceptibility (ESGNI-003 study). European Study Group on Nosocomial Infections. Clin Microbiol Infect. 2011;7:523–531.
15. Djordjevic Z, Folic MM, Zivic Z, Markovic V JS. Nosocomial urinary tract infections caused by *Pseudomonas aeruginosa* and Acinetobacter species: sensitivity to antibiotics and risk factors. Am J Infect Control. 2013;41:1182–7.
16. Morata L, Cobos-Trigueros N, Martínez JA, Soriano A, Almela M, Marco F et al. Influence of multidrug resistance and appropriate empirical therapy on the 30-day mortality rate of *Pseudomonas aeruginosa* bacteremia. Antimicrob Agents Chemother. 2012;56:4833–37.
17. Chamot E, Boffi El Amari E, Rohner P VDC. Effectiveness of combination antimicrobial therapy for *Pseudomonas aeruginosa* bacteremia. Antimicrob Agents Chemother. 2003;47:2756–64.
18. Gill JS, Arora S, Khanna SP KK. Prevalence of Multidrug-resistant, Extensively Drug-resistant, and Pandrug-resistant *Pseudomonas aeruginosa* from a Tertiary Level Intensive Care Unit. J Glob Infect Dis. 2016;4:155–9.
19. Javiya VA, Ghatak SB, Patel KR PJ. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, India. Indian J Pharmacol. 2008;40:230–4.
20. Rodríguez-Martínez JM, Poirel L NP. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2009;53:4783–8.
21. Tam VH, Chang KT, Abdelraouf K, Brioso CG, Ameka M, McCaskey LA et al. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2010;54:1160–4.
22. Kim J, Kang CI, Baek JY, Cho SY, Kim SH, Ko KS et al. Treatment failure due to induction of ciprofloxacin resistance during combination therapy with colistin and ciprofloxacin in multidrug-resistant *Pseudomonas aeruginosa*bacteraemia. Int J Antimicrob Agents. 2014;43:391–3.
23. Xu C, Liu H, Pan X, Ma Z, Wang D, Zhang X, Zhu G, Bai F, Cheng Z, Wu W JY. Mechanisms for Development of Ciprofloxacin Resistance in a Clinical Isolate of *Pseudomonas aeruginosa*. Front Microbiol. 2021;11:598291.
24. Lee YJ, Liu HY, Lin YC, Sun KL, Chun CL HP. Fluoroquinolone resistance of *Pseudomonas aeruginosa* isolates causing nosocomial infection is correlated with levofloxacin but not ciprofloxacin use. Int J Antimicrob Agents. 2010;53:261–4.
25. Sihotang TSU, Widodo ADW, Endraswari PD. Effect of Ciprofloxacin, Levofloxacin, and Ofloxacin on *Pseudomonas aeruginosa*: A case control study with time kill curve analysis. Ann Med Surg. 2022 Oct 1;82:104674.
26. Vahdani M, Azimi L, Asghari B, Bazmi F, Rastegar LA. Phenotypic screening of extended-spectrum ß-lactamase and metallo-ß-lactamase in multidrug-resistant *Pseudomonas aeruginosa* from infected burns.

Ann Burns Fire Disasters [Internet]. 2012 Jun 6 [cited 2023 Apr 3];25(2):78. Available from: /pmc/articles/PMC3506211/

27. Kettner M, Kallová J, Hletková M, Milošovič P. Incidence and mechanisms of aminoglycoside resistance in *Pseudomonas aeruginosa* serotype O11 isolates. Infection [Internet]. 1995 Nov [cited 2023 Apr 3];23(6):380–3. Available from: https://pubmed.ncbi.nlm.nih.gov/8655211/
28. Li S, Jia X, Li C, Zou H, Liu H, Guo Y, et al. Carbapenem-resistant and cephalosporin-susceptible *Pseudomonas aeruginosa*: a notable phenotype in patients with bacteremia. Infect Drug Resist [Internet]. 2018 Aug 20 [cited 2023 Apr 4];11:1225–35. Available from: https://www.dovepress.com/carbapenem-resistant-and-cephalosporin-susceptible-pseudomonas-aerugin-peer-reviewed-fulltext-article-IDR
29. Naveed M, Makhdoom SI, Ali U, Jabeen K, Aziz T, Khan AA, Jamil S, Shahzad M, Alharbi M, Alshammari A. Immunoinformatics Approach to Design Multi-Epitope-Based Vaccine against Machupo Virus Taking Viral Nucleocapsid as a Potential Candidate. Vaccines. 2022; 10(10):1732.
30. Khan MA FA. Antimicrobial resistance patterns of Pseudomonas aeruginosa in tertiary care hospitals of Makkah and Jeddah. Ann Saudi Med. 2016;36::23-8.
31. Naveed M, Mughal MS, Jabeen K, Aziz T, Naz S, Nazir N, Shahzad M, Alharbi M, Alshammari A, Sadhu SS. Evaluation of the whole proteome to design a novel mRNA-based vaccine against multidrug-resistant Serratia marcescens. *Front Microbiol.* 2022, 18;13:960285.
32. Naveed M, Sheraz M, Amin A, Waseem M, Aziz T, Khan AA, Ghani M, Shahzad M, Alruways MW, Dablool AS, et al. Designing a Novel Peptide-Based Multi-Epitope Vaccine to Evoke a Robust Immune Response against Pathogenic Multidrug-Resistant Providencia heimbachae. Vaccines. 2022; 10(8):1300.
33. Naveed M, Shabbir MA, Ain N-u, Javed K, Mahmood S, Aziz T, Khan AA, Nabi G, Shahzad M, Alharbi ME, et al. Chain-Engineering-Based De Novo Drug Design against MPXVgp169 Virulent Protein of Monkeypox Virus: A Molecular Modification Approach. Bioengineering. 2023; 10(1):11.
34. Muhammad N, Ali U, Tari A, Rasool MJ, Ijaz A, Alharbi M, Alharbi ME, Alshammari A, Alasmari AF. A reverse vaccinology approach to design an mRNA-based vaccine to provoke a robust immune response against HIV-1. *Acta Biochim Pol*. 2023 17;70(2):407-418.
35. Naveed M, Hassan J, Aziz T, Ali U, Rana IK, Ali Khan A, Fakhrul H, Alharbi M, Alshammari A, Alasmari AF. A one-health approach to design an mRNA-based vaccine candidate against the lumpy skin disease virus as an alternative to live-attenuated vaccines. *Eur Rev Med Pharmacol Sci.* 2023 ;27(13):6401-6413.