Journal of Pharmaceutical Research International

32(32): 26-31, 2020; Article no.JPRI.62258
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

A Study of Isolation and Identification of Multidrug Resistant *Pseudomonas aeruginosa* from Wound Specimen

Maria Muddassir[1], Sadaf Munir[2], Almas Raza[1], Adeel Iqbal[3], Muddassir Ahmed[4], Usama Basirat[5], Arif Malik[1] and Syed Zeeshan Haider Naqvi[1*]

[1]Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore, Pakistan.
[2]Department of Pathology, Sharif Medical & Dental College Lahore, Pakistan.
[3]DHQ Hospital Kasur, Pakistan.
[4]M. Islam Medical & Dental College, Pakistan.
[5]Lahore Medical & Dental College, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. Author MM designed the study, wrote the first protocol and wrote the first draft of the manuscript. Authors SM and AR managed the analyses of the study and review literature. Authors AI and MA contributed in manuscript writing. Authors UB and AM performed the data analysis and formatting. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i3230930
Editor(s):
(1) Dr. Wenbin Zeng, Xiangya School of Pharmaceutical Sciences, Central South University, China.
Reviewers:
(1) Mowna Karthick, Dr. Sulaiman Al Habib Hospitals Riyadh, Saudi Arabia.
(2) Tahmeena Khan, Integral University, India.
(3) Balasubramanian Sathyamurthy, Royale Concorde Pu College, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62258

Original Research Article

Received 25 August 2020
Accepted 31 October 2020
Published 01 December 2020

ABSTRACT

Background: *Pseudomonas aeruginosa* is a clinically important pathogenic microbe in hospitalized patients. It is a major cause of mortality and morbidity having a number of mechanisms that make it antibiotic resistant. Considering the dearth of antimicrobial drugs to treat infection with this pathogen, it has become a necessity to open up new arena for treatment with this organism. Recently, there has been an up rise in the number of multidrug resistant pathogenic strains of *Pseudomonas aeruginosa*.
Objective: Isolation and identification of multidrug resistant *Pseudomonas aeruginosa* from wound specimens and to evaluate the antibiotic resistant strains of this microbe.

**Corresponding author: E-mail: zeeshan.haider@imbb.uol.edu.pk;*

Methodology: One hundred and fifty clinical samples of wound were taken from hospitalized patients at Jinnah hospital Lahore during the period of October 2019 to April 2020. In total, twenty (20) isolates of *Pseudomonas aeruginosa* were identified using the cultural features, morphological characteristics and various biochemical tests plus the Vitek 2 system. Blue/green, brown /blue and yellow/green pigment production showed the presence and growth of *Pseudomonas aeruginosa*.
Results: Percentage of *Pseudomonas aeruginosa* in females came out to be 15% as compared to 11.42% in males. This was followed by testing susceptibility of isolates of *Pseudomonas aeruginosa* to various antimicrobial drugs. Piperacillin/tazobactam and meropenem showed the highest efficacy against *Pseudomonas aeruginosa*. Highest resistance was exhibited against trimethoprim/sulfamethoxazole which was 75%.
Conclusion: Most isolates showed multidrug resistance to four or more drugs. Development of multidrug resistance has emerged as a global problem with pathogens commonly causing infections becoming increasingly resistant to antimicrobial agents.

Keywords: Pseudomonas aeruginosa; multidrug resistance; wound infection.

1. INTRODUCTION

Pseudomonas aeruginosa is gram negative and belongs to phylum proteobacteria [1]. It marks as the most pathogenic microbe that is causative for opportunistic infections as well as nosocomial infections [2]. It has been reported to be a major cause of mortality in burn patients [3]. In accordance with Center for Disease Control and Prevention (CDC), incidence of infections caused by *Pseudomonas aeruginosa* in United States Hospital averages 0.4% (4/1000 discharges). It marks as the fourth most commonly isolated bacterium accounting for approximately 10.1% of the total hospital acquired infections. According to another estimate by CDC, *Pseudomonas aeruginosa* accounts for 10% of all nosocomial infections increasing the mortality rate in immunocompromised individuals from 20% to 70% [4].

A number of virulence factors are released when pathogenic bacteria enter the host. These factors are toxic for host tissue plus they cause damage by invasion. *Pseudomonas aeruginosa* likewise produces many virulence factors that can be extracellular or intracellular associated products. The major phenazine pigment produced by *Pseudomonas aeruginosa* is pyocyanin whose presence is relatively easier to detect because of the blue colour that has the ability to become green upon remaining in stationary phase. This pigment is causative for staining pus, tissue and dressings that have been infected with *Pseudomonas aeruginosa*. An infected wound is the one whereby the invading microbes have led to significant impairment of wound healing. Virulence factors help establish bacteria in the host tissue. Response of the host towards bacterial invasion is through increased production of inflammatory cells like neutrophils that release oxygen radicals, cytotoxic enzymes as well as inflammatory mediators leading to more damage to host. This mechanism of host response also contributes to non-healing stage of wound infection [5]. Biofilm formation makes the elimination of bacteria from wound almost impossible [6]. These wounds are then contaminated with microbes that are present in environment, surrounding skin, by the hands of healthcare personnel or microbes from the gastrointestinal tract [7]. The cardinal signs of infection include redness, swelling, heat as well as impairment of function. Chronic wounds in addition may develop necrotic tissue, wound deterioration, foul odour, discoloration and deterioration of wound [7]. Amongst the most common pathogenic microbes in chronic wounds is *Pseudomonas aeruginosa* that has the ability to form resistant biofilms [8]. Burns and wounds destroy anatomical barriers leading to weakened immune system and allowing opportunistic pathogens like *Pseudomonas aeruginosa* to avail the opportunity. Hospital environment leads to the cultivation of multidrug resistant *Pseudomonas aeruginosa* increasing the emergence of complications that are caused by MDR microbes. *Pseudomonas aeruginosa* likewise is an opportunistic bacteria that is mostly acquired by hospital environment being causative for urinary and respiratory infections as well as leading to chronic wound formation [1]. The mechanisms by which *Pseudomonas aeruginosa* develops resistance to various antimicrobial drugs is based either on intrinsic resistance that is due to non-mutational reasons or acquired resistance that is mutational. Aminoglycoside and fluoroquinolone are the two major classes of antibiotic drugs that are in common use to treat infection by *Pseudomonas*

aeruginosa. This microbe quickly gains resistance against aminoglycoside and fluoroquinolone [8]. As reported by Haleem et al. [9,10], 48 isolates of *Pseudomonas aeruginosa* were obtained from burn injuries and wounds and all these isolates were 100% resistant to penicillin, ampicillin, chloramphenicol, cefotaxime, erythromycin and doxycycline while exhibiting 35.5% resistance to amikacin, 31.26% resistance to ciprofloxacin and 40% resistance to polymyxin. Resistance in *Pseudomonas aeruginosa* has also been attributed to plasmids. This study has been conducted to evaluate the antibiotic resistant strains of this organism.

2. MATERIALS AND METHODS

This cross-sectional study was conducted at pathology department of Jinnah Hospital Lahore. Sample collection was done from October 2019 till April 2020. A total of 150 samples were taken from wounds of the patients who were admitted at Jinnah hospital, Lahore in province of Punjab after proper consent. Swabs taken from specimens were plated on MacConkey medium and blood agar for phenotypic identification of the microbe. A colourless single non-lactose fermenting colony was then sub-cultured on appropriate medium. Initially, gram staining was performed upon which gram negative rods were seen. *Pseudomonas aeruginosa* produces pyocyanin that is a bluish green pigment. Colonies that appeared were flat, oval and large. A characteristic fruity smell was present. This microbe was provisionally identified as *Pseudomonas aeruginosa*, which was again sub-cultured on nutrient agar slant, incubated for 24 hours at 37°C and stored at 4°C in refrigerator [11]. The redesigned colorimetric Vitek 2 compact system (bioMerieux) helps to accurately and rapidly identify clinical isolates and detects antimicrobial resistance [12]. Vitek 2 is an automated microbiology system that utilizes growth-based technology. Vitek 2 gave 95.8% compatibility results with the reference API strips (bioMerieux) in identifying the gram negative rods plus the accuracy was finally approximated to 98.3% using additional confirmatory tests. Most resistant isolates were identified within 12 hours of incubation. This was followed by testing the antimicrobial susceptibility using disc diffusion method which is also known as Kirby Bauer method. This was carried out in accordance with the Clinical and Laboratory Standard Institute guidelines (CLSI) formerly known as National Committee for Clinical Laboratory Standards (NCCLS) [13].

- Mueller Hinton agar was prepared, sterilized, cooled to 45°C and poured into sterilized Petri dish.
- Inoculum suspension was then prepared by standardization to match the turbidity to McFarland 0.5 standard.
- This was followed by inoculating medium plates with sterile cotton swab dipped in bacterial suspension.
- The antibiotic disc was then placed on surface of inoculum using sterile forceps.
- Inoculated plates were incubated at 37°C for 18-24 hours.
- Following incubation, diameter of zone of inhibition by every antibiotic was measured.
- Zones of inhibition were then interpreted by using chart table recommended by NCCLS.

All the data was recorded in the study proforma. Data was analyzed by using SPSS Version 20.

3. RESULTS

A total of 150 samples were collected from patients admitted to Jinnah hospital Lahore during October 2019 to April 2020. Our results showed that out of 150 samples, 20 (13.33%) came out to be culture positive with *Pseudomonas aeruginosa*, while 130 (86.66%) came out to be negative as shown in Table 1.

Table 1. Frequency of *Pseudomonas aeruginosa* isolated from wound samples

Wound infection	Number	%
Infected	20	13.33
Non-infected	130	86.66
Total	150	100

Infections caused by *Pseudomonas aeruginosa* in females were 12(15%), and this was higher than the count in male patients 8(11.42%) as shown in Table 2.

Antimicrobial sensitivity testing was then performed on all isolates of *Pseudomonas aeruginosa*. The results were interpreted according to CLSI. *Pseudomonas aeruginosa* was most sensitive to piperacillin/tazobactam (85%), tobramycin (80%), ceftazidime (80%), (amikacin (75%) and imipenem (75%) while largely resistant to trimethoprim/ sulphamethoxazole (25%) as shown in Table 3.

According to Table 4, nine of the *Pseudomonas aeruginosa* isolates from wound samples was found to be multidrug resistant showing resistance to four or more antimicrobial drugs. Only 1 isolate was found to be sensitive to all of the 10 antibiotic drugs.

Table 2. *Pseudomonas aeruginosa* according to gender (n=150)

Gender	Wound infection	
	Infected	Non-infected
Male	8(11.42%)	62(88.57%)
Female	12(15%)	68(85%)
Total	20(13.33%)	130(86.66%)

Table 3. Antimicrobial susceptibility testing for *Pseudomonas aeruginosa*

Antibiotic	Sensitive	Resistant
Piperacillin/Tazobactam	17 (85%)	3 (15%)
Ceftazidime	16 (80%)	4 (20%)
Cefepime	13 (65%)	7 (35%)
Imipenem	15 (75%)	5 (25%)
Meropenem	14 (70%)	6 (30%)
Amikacin	15 (75%)	5 (25%)
Gentamicin	12 (60%)	8 (40%)
Tobramycin	16 (80%)	4 (20%)
Ciprofloxacin	14 (70%)	6 (30%)
Trimethoprim/Sulfamethoxazole	5 (25%)	15(75%)

Table 4. Number of antibiotics (sensitive and resistant) for *Pseudomonas aeruginosa*

P	PT	COZ	CF PM	IMI	MEM	AMI	G	TOB	CIP	SXT	Number of resistant	Number of sensitive
P.A1	S	S	R	R	S	S	R	S	R	R	5	5
P.A2	S	S	S	S	S	R	S	R	S	R	3	7
P.A3	R	S	S	S	R	S	R	S	S	S	3	7
P.A4	S	R	S	R	S	S	R	S	S	R	4	6
P.A5	S	R	R	S	S	R	S	S	S	R	4	6
P.A6	S	R	S	R	S	S	S	R	R	S	4	6
P.A7	R	S	S	S	R	S	S	R	R	S	4	6
P.A8	R	S	S	S	S	R	R	S	S	R	4	6
P.A9	S	R	R	S	S	R	S	S	S	S	3	7
P.A10	S	S	S	S	S	S	S	S	S	S	0	10
P.A11	S	S	R	R	S	S	R	S	S	R	4	6
P.A12	S	S	R	S	R	S	S	S	R	R	4	6
P.A13	S	S	S	S	S	R	S	S	S	R	2	8
P.A14	S	S	S	S	S	S	R	S	S	R	2	8
P.A15	S	S	R	S	S	S	S	S	S	R	2	8
P.A16	S	S	R	R	S	S	S	S	R	R	4	6
P.A17	S	S	S	S	S	S	R	R	S	R	3	7
P.A18	S	S	S	S	S	S	R	S	S	R	2	8
P.A19	S	S	S	S	S	S	S	S	S	R	1	9
P.A20	S	S	S	S	S	S	S	S	S	R	1	9

P.A: Isolated pathogen, S: Sensitive, R: Resistant, PT: Piperacillin/tazobactam, COZ: Ceftazidime, CFPM: Cefipime, IMI: Imepenem, MEM: Meropenem, AMI: Amikacin, G: Gentamicin, TOB: Tobramycin, CIP: Ciprofloxacin, SXT: Trimethoprim/sulphamethoxazole

4. DISCUSSION

In this study out of 150 samples, 20(13.33%) came out to be culture positive with *Pseudomonas aeruginosa*, while 130(86.66%) came out to be culture negative. *Pseudomonas aeruginosa* was isolated and identified utilizing microscopic, morphological characteristics as well as the biochemical tests along with Vitek 2 system. Production of yellow/green pigment indicated presence of pyocyanin while brown/blue pigment production indicated presence of pyomelanin. Biochemical tests that were employed included catalase test, urease production, indole test and citrate utilization. Motility of the microbe was also checked.

In this study, infections caused by *Pseudomonas aeruginosa* in females were 12(15%), and this was higher than the count in male patients i.e 8(11.42%). These results are in consent with reports by Langeotz et al. [14] from Berlin, Germany who stated that the rate of infection by *Pseudomonas aeruginosa* in surgical wound in females was 258(6.9%) and this was more as compared to surgical wound in male 182(5.3%).

In this study, according to the antimicrobial sensitivity testing, *Pseudomonas aeruginosa* was most sensitive to piperacillin/tazobactam (85%), tobramycin (80%), ceftazidime (80%), amikacin (75%) and imipenem (75%) while largely resistant to trimethoprim/sulphamethoxazole (25%). Twenty isolates of *Pseudomonas aeruginosa* have been screened in the current study for resistance against ten commonly employed antibiotics (piperacillin/tazobactam, ceftazidime, imipenem, cefepime, meropenem, tabromycin, ciprofloxacin, amikacin, trimethoprim/sulfamethoxazole and gentamicin). Resistance gained by *Pseudomonas aeruginosa* has been seen to increase by leaps in last few years and this markedly decreases the treatment options. It has been reported by Henwood et al. [15] that the resistance developed by *Pseudomonas aeruginosa* is attributed to impermeability of the drugs as well as the multidrug efflux pump. Othman et al. [16] has reported that more than 50 isolates of *Pseudomonas aeruginosa* isolated from clinical specimens exhibited 98% resistance to amikacin, 96% resistance to cefotaxime, 80% resistance to rifampicin, 70% to ampicillin while 70% resistance was exhibited to amoxicillin and 60% resistance to doxycycline. Clinicians often initiate empirical therapy before culture reports are available. This excessive usage of antimicrobial agents has been known to lead to antibiotic resistance.

In this study, nine of the *Pseudomonas aeruginosa* isolates from wound samples were found to be multidrug resistant showing resistance to four or more antimicrobial drugs. Only 1 isolate was found to be sensitive to all of the 10 antibiotic drugs. The development of multidrug resistance in *Pseudomonas aeruginosa* can be largely attributed to reduced cell permeability of the drugs, modification of the targeted enzymes as well as inactivation of antimicrobial agents plus the presence of efflux pumps [17]. Over the counter use of antibiotics has caused the development of multidrug resistant bacteria. The exposure of bacteria to indiscriminate use of antimicrobial drugs leads to development of resistance by various mechanisms.

5. CONCLUSION

Piperacillin, tazobactam and meropenem showed the highest efficacy against *Pseudomonas aeruginosa*. Highest resistance rate was exhibited against trimethoprim/sulfamethoxazole which was 75%. Development of multidrug resistance has emerged as a global problem with pathogens commonly causing infections becoming increasingly resistant to antimicrobial agents. Future studies are suggested on this subject.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Madigan MT, Martinko JM. Microorganisms and microbiology. Brock biology of microorganisms. 11th ed. Upper Saddle River, New Jersey (NJ): Pearson Prentice Hall. 2006;1-20.

2. Engel, J. *Pseudomonas aeroginosa* internalization by non-phagocytic cells. American Journal of Medicine and Microbiology and Immunology. 2007;5: 343-368.
3. Mahboobi. M. Shahcheraghi, F. and Feizabadi.M.M. Bactericidal Effects of Essential Oils from Clove, Lavender and Geranium on multi drug resistance isolates from *Pseudomonas aeruginosa*. Iranian Journal of Biotechnology.2006;4(2):137-140.
4. Parsons J.F, Greenhagen B.T, Shi K, Calabrese K, Robinson H, and Ladner J.E. Structural and functional analysis of the pyocyanin biosynthetic protein PhzM from *Pseudomonas aeruginosa*. Biochemistry. 2007;469(7):1821-28.
5. Bjarnsholt T, Kirketerp-M K, Jensen PØ, Madsen K.G, Phipps R. Why chronic wounds will not heal: a novel hypothesis. Wound repair and regeneration. 2008;16(1): 2-10.
6. Kirketerp-M K, Jensen PØ, Fazli.M, Madsen K.G, and Pedersen J. T. Distribution, organization, and ecology of bacteria in chronic wounds. Journal of clinical microbiology. 2008;46 (8):2717-22.
7. Sibbald R.G, Orsted H, Schultz G.S, Coutts P, Keast. Preparing the wound bed Focus on infection and inflammation. Ostomy Wound Manage. 2003;49; 11.
8. Schweizer HP. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria. Unanswered questions. Genetic molecular research. 2003;2:48:62.
9. Thomsen T.R, Aasholm M.S, Rudkjøbing V.B, Saunders A.M, Bjarnsholt T, Givskov. The bacteriology of chronic venous leg ulcer examined by culture-independent molecular methods. Wound repair and regeneration. 2010;18(1):38-49.
10. Haleem H, Tarrad Jk, Banyan IA. Isolation of *Pseudomonas aeruginosa* from clinical cases and environmental samples, and analysis of its antibiotic resistant spectrum at Hilla teaching hospital. Medical Journal of Babylon. 2011;8(4):45-52.
11. Kleyn B. Microbiology Experiments: A health science Perspective. 4th Ed. The McGraw-Hill Companies. New York.2003.
12. Nakasone I, Kinjo T, Yamane N, Kisanuki K and Shiohira C M. Laboratory- based evaluation of the colorimetric VITEK 2 Compact System for species identification and of the Advanced Expert System for detection of antimicrobial resistances: VITEK 2 Compact System identification and antimicrobial susceptibility testing. Diagnosis Microbiology Infection Disease Journal. 2007;58:191-8.
13. Wayne P A. Clinical and laboratory standards institute. Performance standard for antimicrobial susceptibility testing. 15th ed. Informational Supplement. CLSI / NCCLS M 2005;100-S15.
14. Langelotza C, Mueller-Raua, Terziyskia S, RauaB, Petra A, Gastmeierc,d Geffersc. Gender-Specific Differences in Surgical Site Infections: An Analysis of 438,050 Surgical Procedures from the German National Nosocomial Infections Surveillance System. 2014;30:114–117.
15. Hen Wood C, Livermore D, James D, Waner M. Antimicrobial susceptibility of *Pseudomonas aeruginosa*: results of a UK survey and evaluation of the British. Journal of Antimicrobial Agents and Chemotherapy. 2001;47:789-799.
16. Othman A. Anti plasmid (curing) effect of alcoholic extract of *Rosmarinus officinalis* on resistant isolate of *Pseudomonas aeruginosa*. M.Sc thesis in sciences in medical Microbiology College of medicine at Hawler Medical University.2011
17. Matsuo Y, Eda S, Gotoh N, Yoshihara E, Nakae T . Mex Zmedited regulation of mexXY multidrug efflux pumps expression in membrane protein profiles of *Xanthomonas maltophilia* isolates displaying temperature. Dependent susceptibility to gentamicine. Antimicrobial agents and Chemotherapy. 2004;33: 663-666.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62258