Antibiotic Effect on Pseudomonas Aeruginosa Isolated From Patient In Hila City

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Abstract

In this study, a total of 400 clinical samples were collected from patients different hospitals in hilla city((13.51%) Margin teaching hospital, (27.03%)Al-Hilla teaching hospital,(43.24%) Babylon hospital for maternity and pediatric and (16.22%)Chest Disease Center) , (37) isolates were identified as to P. aeroginosa, from 9 (18%) isolates from burn specimen, 7 (14%) isolates from otitis , 5 (10%)isolates from UTI , 4 (8%) isolates from wound , 3(7.5%) was isolated from blood specimen where as 6 (6%)was isolated sputum and only 3 (5%)isolates were detected from skin. The period of collection was extended from January 2019 to June 2019. Also antibiotic susceptibility were preformed using disk employed diffusion and agar utilizing dilution processes. Furthermore , the resistance of isolate to a variety of antibiotics has been investigated and found that these isolate have been resistance to more than one antibiotic : 37(100%) resistance to Cloxacilin , 37(100%) to Amoxicillin /Clavulanic acid, 37(100%) to cefotaxime, 36(97%) to ceftizoxone, 34(92%) to Ceftazidime, 14(37%) to Levofloxacin, 18(49%) to Gentamicin, , 3(8%) to Norfloxacin and 1(3%) to Azetronam.

Keywords: *Pseudomonas aeruginosa, Different sources, Antibiotic susceptibility, Resistant, Gram-negative bacteria.*

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium, an aerobe rod belongs to the Pseudomonadaceae bacterial family and so taxonomic classification relies on preserved substances, including 16srRNA which is a range of members of the Pseudomonas genus, divided into eight classes [17]. *Pseudomonas aeruginosa* infection allowed the incorporation of the constituents of the outer membrane, namely secretary toxins like Lipopolysaccharides (LPs), general endotoxin, which is a protein, toxoids, mutant reacting toxins or nontoxic substances, Pilli, and Flagella [23,35]. *P. aeruginosa* allows β-Lactamases; enzymes to deactivate the antibiotics by hydrolyzing the peptide attachment of the Ring β-Lactam. P.aeruginosa is capable of producing numerous β-lactamases, such as extended-spectrum β-lactamases (ESBL), chromosomal cephalosporinase (AmpC), and metallo-β-lactamases (MBL). Different kinds of MBLs were previously identified and brought on integrated products. Earlier researches have shown that high levels of morbidity and mortality are connected with MBL-generating P.aeruginosa in bloodstream diseases. the ampC gene mutation generates such resistance which unlike other β-lactamases [1,9,10,13].

Material and Methods

1- **Patients**:

A total of 37 Pseudomonas aeruginosa isolates were collected from different clinical specimens in Babylon province during the period from January to June 2019. These specimens were collected from 400 specimens obtained from inpatients suffering from different infections by taking swabs from Burn, wound, ear, UTI, blood, urine ,sputum and skin. MacConkey and Nutrient agars were utilized in aerobic conditions with a temperature at 42ºC for 24 to 48hrs. Later, the bacteria were characterized using traditional biochemical tests depending on protocols from MacFaddin, (2000).

2- Antibiotic susceptibility tests:

The susceptibility of P. aeruginosa isolates were determined by disk diffusion method (Kirby–Bauer standardized disk method).

The Mueller–Hinton medium was employed for this test. The medium was cooled to 45–50°C and with a sterile wire loop, the 4–5 pure colonies were transferred to a tube containing broth at 5ml of BHI 37°C-incubated until its turbidity standard. This usually required at least 4–6 hours incubation. The cells density was compared with McFarland standard tube No. 0.5. Sterile swab made of wood and cotton was standardized-suspension-dipped streaking it onto the Mueller–Hinton medium dry surface with three different directions to obtain an even distribution of the inoculums. Using a flat and even surface, the plates were let to stay without distribution for 3 to 5mins for better dryness. The disks were inserted on the surface using gentle pressing with a sterile forceps within 15mins and incubated for 18hrs at 37°C with inverted position. The inhibition zones later were ruler-measured (millimeter) using CLSI (2012) as guidelines for deciding the sensitivity or resistance of the bacteria to the antibiotics used.

Result and Discussion

1) Isolation of Pseudomonas aeruginosa

During the period of study, 400 hospital-patient-based specimens were collected in hilla city, only 37 (9.25%) isolates from P. aeroginosa bacterium, 9 (18%) isolates burn specimen, 7 (14%) from otitis , 5 (10%) isolates, 4 (8%) isolates from wound ; 3 (7.5%) was isolated from blood specimen whereas 6 (6%) was belong to sputum and only 3 (5%) isolates were detected from skin . The outcomes were identified, table (1).

Table (1): P. aeruginosa isolate frequency from different clinical specimens.

Specimen source	No. of Specimen	No. (%) of P.aeruginosa
Burn	50	9 (18%)
Wound	50	4(8%)
Sputum	100	6(6%)
UTI	50	5(10%)
Otitis	50	7(14%)
Blood	40	3(7.5%)
Skin	60	3(5%)
Total	400	37(9.25%)

The second Gram negative HAP bacterium isolated from health care centers is P. aeruginosa with leveled up incidence and fatality rates [22,27] with highly risky illnesses, such as septic burns, in patients with low defense of the immune system [11,34].

The P. aeruginosa incidence findings, here, assured 9.25 % (37/400) of the hospital samples with lower rates than that, 39.1%, uncovered by Okon et al. ,2009 from Nigerian patients with infected wounds and, 25.5%, revealed by Ndip et al., (2005) in Cameron, in Al-Sulaimania City Hospital, Iraq, at 17.85% , and in Egypt at 18.6%. Sample size and geographical distribution may have induced variations in the collected findings [30,14,15].

P. aeruginosa is well-revealed as HAP antimicrobial and chemical resistant gene carrying as unveiled of those genetically transferred materials such as via plasmids Nordmann (1993).

2. Properties of P. aeruginosa

Smooth and irregular looking colonies on nutrient agar with aromatic sweetish odor. Pseudomonas aeruginosa appear on Pseudomonas base agar Shiny, opaque, shiny, convex smooth, greenish-yellow colony Medium turned light blue.as shown in figure (1), a wide collection of the bacterium isolates unveiled colonies with green-blue fluorescent color with pyocyanin of medium diffusion in nature [25]

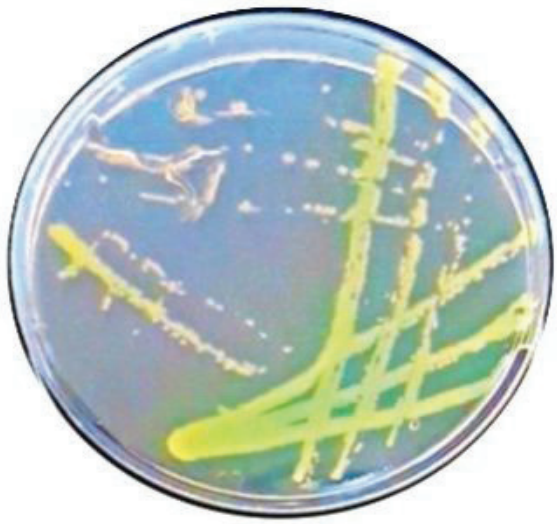

Figure (1) Pseudomonas aeruginosa on Pseudomonas base agar .

β-hemolysin was recorded to be released by certain isolates on blood agar. The growing on MacConkey agar was the dominant feature of all isolates; however, a limited number claimed positive lactose fermentation properties. Positive oxidase with Gram negative non-fermenting bacterial rods was uncovered for the survey isolates [8]

3. Antibiotic susceptibility test of P. aeruginosa:

Disk diffusion method:

The results of presents study showed resistant and sensitive isolates, so the effects of different antibiotics on P. aeruginosa isolates were investigated, and the results were similar to locally and worldly studies and the drug susceptibility patterns of P. aeruginosa isolates were varied as shown in table (2) .

Table (2): P. aeruginosa based susceptibility-to-antibiotics profile

Antibiotic	Sensitive No.(%)	Resistance No.(%)
Cloxacilin	0(0%)	37(100%)
Amoxicilin / Clavulan acid	0(0%)	37(100%)
Cefotaxime	0(0%)	37(100%)
Ceftazidime	3(8%)	34(92%)
Ceftriaxone	1(2%)	36(97%)
Azetronam	36(97%)	1(3%)
Gentamicin	19(51%)	18(49%)
Levofloxacin	23(62%)	14(37%)
Norfloxacin	34(91%)	3(8%)

As shown above antibiotic susceptibility to P.aeruginoasa results showed 97% resistance to ceftizoxone (30 µg), 63% to Norfloxacin , 100% to Cloxacilin , 100% to Amoxicilin /Clavulan acid, 37% to Levofloxacin, 49% to Gentamicin (10 µg), 92% to Ceftazidime (30 µg) , 100 % to cefotaxime (30 µg) and 71% to Azetronam , the results of presents study in line with results of were locally studies conducted by Al-Maamori (2011), Al-saffar (2005), Al-Gibouri (2006) and Zeki (2006). In presents study P. aeruginosa isolates showed resistant to aminoglycoside antibiotics, gentamicin (49%) to be disagreed with Lee et al in (2007) who was detected aminoglycoside-resistant in (47%) of aeruginosal reported variants and Alammary**,** (2013) declared resistant to aminoglycoside antibiotics, gentamicin (45%) in Hilla teaching hospital because the appearance of aminoglycoside resistant as acquired [6].

Resistance of P. aeruginosa to antibiotics of aminoglycosidic nature shows geographical variation as highest in Southern Europe with Greece, 49.8%, and the United Kingdom, 96.6%, of non-susceptibility to gentamicin [36]

The greatest antibiotic resistance rates were observed in ceftizoxime (100%),Cloxacilin (100%) , Amoxicilin /Clavulan acid (100%) followed by Ceftriaxone (97%) which was agreement with other studies in ceftizoxime (99.2%), Cloxacilin (91%) and Amoxicilin /Clavulan acid (94%) (Amini and Mobasseri, 2017) and Kerman (95%) ceftizoxime, Cloxacilin (88%) and Amoxicilin /Clavulan acid (95%) (Moosavian and Rahimzadeh, 2015) This can make ceftizoxime , Cloxacilin and Amoxicilin /Clavulan acid a bad choice for further prescription against P. aeruginosa.(ICA, 2016). Show in Figure (2).

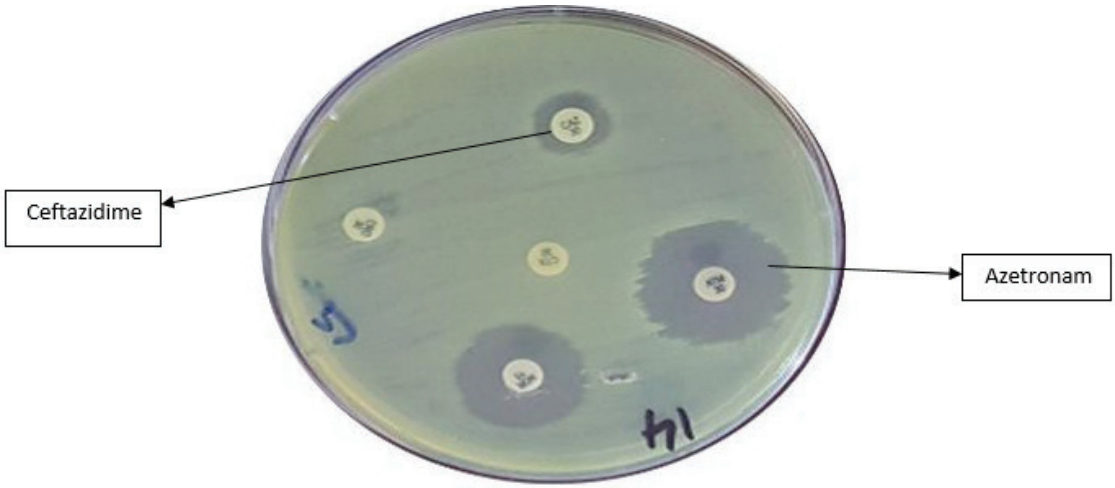

Figure (2) Antibiotics on Pseudomonas aeruginosa

Strains of P. aeruginosa have evolved increased resistance rates against in ceftizoxime (100%),Cloxacilin (100%) , Amoxicilin /Clavulan acid (100%) and Ceftriaxone (97%) (Sofianou et al.,1997 and Snydman, 2010) , The super bacterial resistance may be due the incidence of genetic modifications via mutations and gene moving between strains of P. auroginosa. [7, 32]

The result of present study was disagreement with Babaeekhou et al., (2018) who showed 78.9% resistance to ceftizoxime, 68.1% to Amoxicilin /Clavulan acid ,67.3% to Ceftriaxone 67.3% to carbenicillin, 65.2% to Cloxacilin 63.7% to ceftazidime 63.04% to gentamicin, 53.6% .

Azetronam and Norfloxacin had been recorded the lowest rate of resistance in the present study (3%) and (8%) respectively . It shows relatively low frequencies in other studies, the current findings from this work in line with Saderi and Owlia (2015) findings in Tehran (10.4%) resistance to Azetronam and (13.6%) resistance to Norfloxacin.

Levofloxacin had been recorded resistance rate in present study (37%) was agreement with study concluded by Peymani et al., (2015) in iran showed resistance of p. aeruginosa to levofloxacin (33.7%).

Geographical and time criteria may apply critical alterations in the status of resistance to those drugs. However, MDR of the bacterium is a featured property narrowing the antibiotic options available for managing related diseases. De novo occurrence of elevated rates of MDR was ensured via drug exposure with circulating of MDR strains between individuals. [2, 18, 21]

Conclusion

Pseudomonas aeruginosa recovering from patients' infected burns constitute a serious a serious therapeutic problem.Pseudomonas aeruginosa isolates were resistant to Cloxacilin, Amoxicilin /Clavulanic acid and Cefotaxime, where as Pseudomonas aeruginosa isolates were susceptible to Azetronam.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science for women and all experiments were carried out in accordance with

approved guidelines.

References

1. Al Otaibi F E. and Al-Hulaily F F. Imipenem-resistant Psedomonas aeruginosa: Epidemiology and susceptibility patterns at a Teaching Hospital in Riyadh, Saud. Arab.Afric. J. of Microbiol. Res. 2012;6(7): 1527-1533.
2. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug–resistnat Pseudimonas aeruginosa: Risk factors and clinical impact. Antimicrob Agents Chemother. 2006;50(1):143-8.
3. Amini K, Mobasseri P. Detection Rate of Metallo-β-Lactamase- Expressing Genes; blaVIM-1, blaVIM-2 and blaSPM-1 in Pseudomonas aeruginosa Isolates. Int J Basic Sci Med. 2017;2(1):41-5.
4. Babaeekhou, Laleh, Hossein Karshenasan, Leila Pishkar. Antibiotic Resistance in Clinical Isolates of Pseudomonas aeruginosa: A New Viewpoint for Antibiotic Prescription. Avicenna Journal of Clinical Microbiology and Infection. 2018;5(3):55-60
5. Bahadur A, Chaudhry Z, Jan G, Danish M, Rehman A, Ahmad R. et al. Nutritional and elemental analyses of some selected fodder species used in traditional medicine. Afr J Pharm Pharmacol. 2011;5:1157-61.
6. Bhatt P, Rathi K R, Hazra S, Sharma A, Shete V. Prevalence of multidrug resistant Pseudomonas aeruginosa infection in burn patients at a tertiary care centre. Indian J Burns. 2015; 23:56-59.
7. Chander A, Shahid R M. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa clinical isolates at a tertiary care hospital in Kathmandu, Nepal,Asian/ Pharm Clin Res. 2013;6:235-238.
8. Collee J G, Fraser A G, Marmion B P, and Simmons A. Mackie and Mecartney. Practical Medical Microbiology. 14th ed., USA. 1996; 413-424.
9. Cornaglia G M, Akova G, Amicosante R, Canton R, Cauda J D, Docquier M. et al. Metallo-β-lactamases as emerging resistance determinants in Gram-negative pathogens. Int. J. Antimicrob. Agents. 2007;29:380-388.
10. Crespo M P, Woodford N, Sinclair A, Kaufmann M E, Turton J, Glover J, Velez J D, Castaneda C R, Recalde M, and Livermore D M. Outbreak of carbapenem-resistant Pseudomonas aeruginosa producing VIM-8, a novel metallo-β-lactamase, in a tertiary care center in Cali, Colombia. J. Clin. Microbiol. 2004;42:5094-5101.
11. Dale R M, Schnell G, and Wong J P. Therapeutic efficacy of "Nubiotics" against burn wound infection by Pseudomonas aeruginosa.Antimicrobial agents and chemotherapy, 2004;48(8): 2918-2923.
12. De A. Simit S, Kumar H, and Sujata M. Prevalence of metallo-β-lactamase producing Pseudomonas aeruginosa and Acinetobacter species in intensive care areas in a tertiary care hospital. Indian J Crit Care Med. 2010;14(4): 217-219.
13. Docquier J D, Mugnaioli C, Luzzaro F, Amicosante G, Toniolo A, and G M, Rossolini G M. Diversity of IMP-type metallo-β-lactamases in carbapenem-resistant clinical isolates of Pseudomonas spp. from Italy. Clin. Microbiol. Infect. 2003; 9(1):121.
14. Ekrem K. and Rokan D K. Antibiotic susceptibility patterns of Pseudomonas aeruginosa strains isolated from various clinical specimens. Sky J. Microbiol. Res. 2014;2(2):13-17.
15. Gad G F, El-Domany R A, Zaki S, and Ashour H M. Characterization of Pseudomonas aeruginosa isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. Journal of antimicrobial chemotherapy, 2007;60(5): 1010-1017.
16. ICA. Central Asian and Eastern European Surveillance of Antimicrobial Resistance. Annual report. World Health Organization. 2016.
17. Japion A S, Farshed and A. Alborzi Pseudomonas aeruginosa; Burn infection, treatment and antibacterial resistance. Shiraz university of Medical sciences, Shiraz, Iran, 2009; 11:244-253
18. Javiya V A, Ghatak S B, Patel K R, Patel J A. Antibiotic susceptibility patterns of Pseudomonas aeruginosa at a tertiary care hospital in Gujarat, India. Indian J Pharmacol. 2008;40(5):230-4.
19. Lee J H, Kim Y G, Lee J. Carvacrol-rich oregano oil and thymol-rich thyme red oil inhibit biofilm formation and the virulence of uropathogenic Escherichia coli. J. Appl. Microbiol. **2017;** 123: 1420–1428.
20. Mac Fadden J F. Biochemical tests for Identification of Medical Bacteria 3rded. The Williams and Wilkins Co., USA. 2000; 689 - 691.
21. Mahmoud A B, Zahran W A, Flindawi G R,

Lebib A, Galal R. Prevalence of multidrug – resistant Pseudomonas aeruginosa in patients with nosocomial infection at a university hospital in Egypt, with special reference to typing methods. J Virol Microbiol. 2013;153.

22. Meenakumari S, Verma Sh, Absar A, Chaudhary A. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in an Indian cardiac hospital. Int. J. Eng. Sci. Technol. 2011;3:7117-7124.
23. Moore B, and Forman A. An outbreak of urinary Pseudomonas aeruginosa infection acquired during urological operations. Lancet, 1996;29: 929-931.
24. Moosavian M, Rahimzadeh M. Molecular detection of metallo-beta-lactamase genes, bla IMP-1, bla VIM-2 and bla SPM-1 in imipenem resistant Pseudomonas aeruginosa isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. Iran J Microbiol. 2015;2:7(1):2-6.
25. Mowat E, Paterson S, Fothergill J L, Wright E A, Ledson M J, Walshaw M J, and Winstanley C. Pseudomonas aeruginosa population diversity and turnover in cystic fibrosis chronic infections. American journal of respiratory and critical care medicine, 2011;183(12): 1674-1679.
26. Ndip R N, Dilonga H M, Ndip L M, Akoachere J F K, and Nkuo Akenji T. Pseudomonas aeruginosa isolates recovered from clinical and environmental samples in Buea, Cameroon: current status on biotyping and antibiogram. Tropical Medicine & International Health, 2005;10(1): 74-81.
27. Okon K, Agukwe P C, Oladosu W, Balogun S T, and Uba A. Antibiotic resistance pattern of Pseudomonas aeruginosa isolated from clinical specimens in a tertiary hospital in Northeastern Nigeria. J Microbiol, 2009;8(2): 5-7.
28. Owlia P, Saderi H, Rasooli I, Sefidkon F. Antimicrobial characteristics of some herbal Oils on Pseudomonas aeruginosa with special reference to their chemical compositions. Iranian J Pharm Res. 2010;107–14.
29. Peymani A, Naserpour Farivar T, Mohammadi Ghanbarlou M, Najafipour R. Dissemination of Pseudomonas aeruginosa producing bla IMP-1 and bla VIM-1 in Qazvin and Alborz educational hospitals, Iran. Iran J Microbiol. 2015;7(6):302-9.
30. Savaş L, Duran N, Savaş N, Önlen Y, and OCAK S. The prevalence and resistance patterns of Pseudomonas aeruginosa in intensive care units in a university hospital. Turkish Journal of Medical Sciences, 2005;35(5): 317-322.
31. 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.
32. Snydman D R, Clinical implications of multi-drug resistance in the intensive care unit. Scand J Infect Dis. 2010; 78: 54S-63S.
33. Shajeela P S, Kalpanadevi V, Mohan V R. Potential antidiabetic, hyperlipidaemic and antioxidant effects of Nymphaea pubescens extract in alloxan induced diabetic rats. Journal of Applied Pharmaceutical Science. 2012; 2:83-88.
34. Sule A M, Thanni L O A, Odu O S, and Olusanya O. Bacterial pathogens associated with infected wounds in Ogun state University Teaching Hospital, Sagamu, Nigeria. African Journal of Clinical and Experimental Microbiology, 2002;3(1): 13-16.
35. Tzong-Yueh Chen. Chia Po Lin. Chien-Chang Loa. Tso- Ling, Huey-Fang Shang; Jaulang Hwang, and Cho-Fat Hui. A nontoxic Pseudomonas Exotoxin A induces active immunity and passive protection antibody against Pseudomonas Exotoxin A intoxication. J. Biomed. 1999.
36. Van Landuyt H W, Boelaert J, Glibert B, Gordts B, Verbruggen A M. Surveillance of aminoglycoside resistance. European data. Am J Med. 2015; 80(6B): 76-81.
37. Vilcheze C, Hartman T, Weinrick B, Jacobs W R, Jr. Mycobacterium tuberculosis is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Nat Commun. 2013;4:1881.