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Pseudomonas aeruginosa: Antibiotic resistance pattern to different isolates in Al-Hillah city, Iraq

Ali Hussein Al-Marzoqi[1*] (Corresponding author), and Zahraa Mohammad Al Taee[2]

1. College of Science for women, Babylon University, PO box 435, Al-Hillah city, Babylon, Iraq. Tel: 009647710336121 E-mail: ali_almarzoqi@yahoo.co.uk
2. College of Science, Babylon University

* E-mail of the corresponding author: ali_almarzoqi@yahoo.co.uk

Abstract

Aim:

Pseudomonas aeruginosa (*Ps. aeruginosa*) considered as most important bacteria which can isolated from various kinds of infection. This study tries to survey the infections caused by *Ps. aeruginosa* especially medical and surgical care units and try to reveal the antimicrobial agents susceptibility against *Ps. aeruginosa.*

Material and Method:

This study was conducted during September 2012 to February 2013. During this period total of **285** samples were tested and showed growth of bacteria. The isolates of *Pseudomonas aeruginosa* were selected on the basis of their growth on Nutrient agar pigmented and non-pigmented colonies with oxidase positive and on routine MacConkey medium which showed lactose Non-fermenting pale colonies. Antimicrobial susceptibility of all the isolates was performed using disc-diffusion (Modified-Kirby Baur method) according to CLSIs guidelines.

Result:

In present study, maximum isolates of *Ps. aeruginosa* isolated from various samples. The isolates were obtained from different clinical specimens, including pus, urine, respiratory fluids, blood, tissue, and genitalia. All the clinically isolated samples were identified as *P. aeruginosa*. Out of 285, 74.04% are males and 25.96% are females. Most of patients were aged between 27-48 years. Approximately half the isolates tested were from community patients, mostly from infections of the Wound/Pus (22.46%), urinary tract (22.11%), Swab (18.6%) and Respiratory Tract (15.09%). *P. aeruginosa* strains screened showed sensitivity to AK \Amikacin, E \Erythromycin and P\Penicillin while showed resistance to penicillin, erythromycin, and norfloxacin, AX \Amoxicillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin.

Conclusion:

To prevent the spread of the resistant bacteria, it is critically important to have strict antibiotic policies while surveillance programs for multidrug resistant organisms and infection control procedures need to be implemented.

Introduction

Pseudomonas aeruginosa is inherently resistant to many antimicrobial agents owing to impermeability, multi-drug efflux and a chromosomal AmpC β-lactamase [1]. Pseudomonas aeruginosa is gram negative rod, an aerobic, motile bacterium which belongs to pseudomonadaceae family [2].

It had the ability to cause nosocomial infections, especially among patients who are admitted to intensive care units (ICU). Many infections like severe burns, nosocomial pneumonias, urinary tract infections (UTIs), skin and soft tissue infections and in infections of immunocompromised individuals were caused by this bacterium. Of particular concern is the limited number of effective Anti-Pseudomonal agents which are used in the therapeutic practice, due to the constitutive low level resistance to several agents and the multiplicity of the mechanisms of resistance in *P. aeruginosa* [3].

Its general resistance is due to a combination of factors [4]. It is intrinsically resistant to antimicrobial agents, due to the low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanisms. It can become resistant through mutations in the chromosomal genes which regulate the resistance genes. It can acquire additional resistance genes from other organisms via plasmids, transposons and bacteriophages. In recent years, a considerable increase in the prevalence of multidrug resistance (MDR) in *P.*

aeruginosa has been noticed, which is related to high morbidity and mortality [3 and 5].

Regional variations in the antibiotic resistance exist for different organisms, including *P. aeruginosa* and this may be related to the difference in the antibiotic prescribing habits. Over the past few years, a notable increase in antibiotic resistance among gram negative bacteria recovered from hospitalized patients has been reported, especially for critically ill patients [6].

Infections caused by multidrug resistant (MDR) gram negative bacteria, especially MDR *P. aeruginosa* have been associated with increased morbidity, mortality and costs [7]. Multidrug-resistant strains of *P. aeruginosa* are often isolated among patients suffering from nosocomial infections particularly those receiving intensive care treatments [8].

The aim of this study was to assess the current levels of antimicrobial susceptibility and to evaluate the resistance mechanisms to Anti-Pseudomonal antimicrobial agents among the clinical isolates of *P. aeruginosa* isolated from patients admitted to Educational Al-Hillah hospital and Babylon Maternity and Children hospital in Iraq.

Materials and Methods

Two hundred and eighty five clinical isolates of *P. aeruginosa* strains were collected from different patients who were admitted to Educational Al-Hillah hospital and Babylon Maternity and Children hospital as well as out clinic from September 2012 to February 2013. The isolates were obtained from different clinical specimens, including pus, urine, respiratory fluids, blood, tissue, and genitalia. All the clinically isolated samples were identified as *P. aeruginosa* by the hospital personnel. The study was therefore carried out using both manual (Kirby-Bauer method) as well as automated (Vitek2 system) method to determine the Antimicrobial susceptibility pattern of pseudomonas aeruginosa isolates from in-patients and out-patients attending the microbiology section of the hospitals. We have identified all the isolates again at our Laboratory by the conventional biochemical tests i.e., gram staining, catalase test, oxidase test , motility test, Triple Sugar Iron Assay, citrate test, urease test and indole test etc. [9].

Also using VITEK 2, which is a 64-well plastic card containing 41 fluorescent biochemical tests, including 18 enzymatic tests for aminopeptidases and osidases. Substrates used for detection of aminopeptidases are usually coupled with 7-amino methylcoumarin (7AMC); substrates for detection of oxidases are usually coupled with 4-methylumbelliferone (4MU). In addition there are 18 fermentation tests, 2 decarboylase tests, and 3 miscellaneous tests. There are two negative control wells, and the remaining wells are empty. Results are interpreted by the ID-GNB database after a 3-hr incubation period.

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was performed to determine the antibiotic susceptibility [10]. The antibiotics tested were:

AK \Amikacin, AX \Amoxicillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, , B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol, CLR \Clarithromycin, DA \Clindamycin, E \Erythromycin, CN\Gentamycin, K\Kanamycin, L\Lincomycin, ME\Methicillin, F\Nitrofurantoin, NOR\Norfloxacin, OFX \Ofloxacin, OX\Oxacillin, T\Oxytetracyclin, P\Penicillin G, PRL \Piperacillin, RA\Rifampim. Results of disk diffusion method were interpreted in accordance to the Clinical and Laboratory Standards Institute (CLSI, 2009)

Results and Discussion

Two hundred and eighty five clinical isolates of *P. aeruginosa* strains were collected from different patients who were admitted to Educational Al-Hillah hospital and Babylon Maternity and Children hospital as well as out clinic from September 2012 to February 2013. The isolates were obtained from different clinical specimens, including pus, urine, respiratory fluids, blood, tissue, and genitalia. All the clinically isolated samples were identified as *P. aeruginosa.*

P. aeruginosa is one of the important causes of morbidity among hospital patients; it is emerged as an important pathogen and responsible for the nosocomial infection as showed in table1.

Out of 285, 74.04% are males and 25.96% are females as showed in table 2. Most of patients were aged between

27-48 years. Most of samples were collected from surgical wards, followed by medical ward, pediatrics ward; obstetrics ward orthopedic, gynecology and ICU. Maximum resistant isolates of Pseudomonas aeruginosa were isolated from wounds and pus/swab samples.

Approximately half the isolates tested were from community patients (Table 3), mostly from infections of the Wound/Pus (22.46%), urinary tract (22.11%), Swab (18.6%) and Respiratory Tract (15.09%).

Some studies have shown that males were more susceptible than females in the ratio of 8:3 [11]. Previous studies have shown that males were more susceptible than females in the ratio of 2:1, which is in accordance with the current study. Predominance of male over female patients as shown in the study can be explained by the fact that in our province males are exposed more to the outside environment because of their mobility as compared to females.

Some studies reported the prevalence of *Pseudomonas* species to be 18.79% from a diabetic center in Chennai [12]. In a similar study conducted in a private hospital in Chennai, 29.8% strains among diabetic foot ulcer patients were *P. aeruginosa* [13]. This finding shows the high prevalence of Pseudomonas species and *P. aeruginosa* among diabetes patients with foot ulcers.

The unique feature of *P. aeruginosa* is its resistance to a variety of antibiotics, which is attributed to a low permeability of the cell wall, the production of inducible cephalosporinsaes, an active efflux and a poor affinity for the target (DNAgyrase) [14].

The Mueller Hinton agar based anibiograms pattern study of *P. aeruginosa* isolated from different sources is shown in Figure 1. Some of the *P. aeruginosa* strains screened showed sensitivity to AK \Amikacin, E \Erythromycin and P\Penicillin while showed resistance to penicillin, erythromycin, and norfloxacin, AX \Amoxicillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, , B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol, CLR \Clarithromycin, DA \Clindamycin, CN\Gentamycin, K\Kanamycin, L\Lincomycin, ME\Methicillin, F\Nitrofurantoin, NOR\Norfloxacin, OFX \Ofloxacin, OX\Oxacillin, T\Oxytetracyclin, G, PRL \Piperacillin, RA\Rifampim.

Because of the increasing resistance to fluroquinolone in many hospitals, its empirical usage is either banned or restricted, to bring the developing resistance rates under control9. Ceftazidime and cefepime are the most frequently prescribed third and fourth generation cephalosporins respectively. The resistance to Cefodizime was reported as 4-18%, but in our study, it was more than 85%. These high values of resistance which were observed were comparable to those of the reports from Gujarat, with a resistance value of 75% [15]. The increased prevalence of ceftazidime resistant *P. aeruginosa* is related to the increased use of beta lactam antibiotics such as amoxicillin and ceftazidime. Selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains.

In conclude; although multidrug resistance has commonly been reported in nosocomial *P. aeruginosa* infections, community acquired data have less frequently been reported. For this reason, epidemiological studies on the prevalence and antimicrobial susceptibility pattern of the resistant isolates in different geographical settings would provide useful information in order to guide clinicians in their choice of therapy and to contribute to the global picture of antimicrobial resistance. Rigorous monitoring of the MDR in *P. aeruginosa*, the restriction of the inappropriate use of antimicrobial agents and adherence to infection control practices should be emphasized in order to delay the emergence of clinically significant *P. aeruginosa*.

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Table 1: Total number of isolates in different specimen

Total No. of samples	586
Positive for *P. aeruginosa*	280

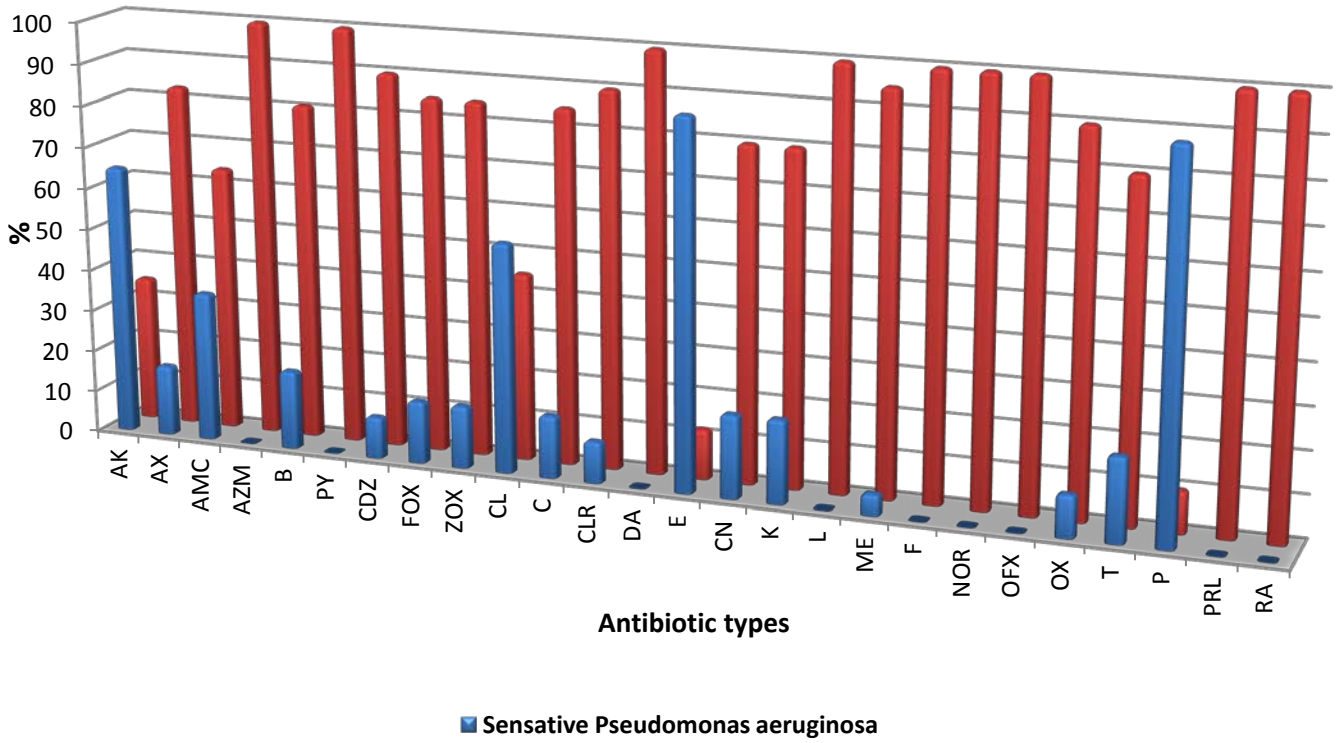
Table 2: Sex wise distribution of cases

Sex	Total no	Percentage (%)
Male	211	74.04
Female	74	25.96
Total	280	100

Table 3: Isolation of Pseudomonas aeruginosa from different clinical samples

Name of sample	No. of Sample in which Pseudomonas aeruginosa Isolated	%
Pus	3	1.05
Sputum	12	4.21
Urine	63	22.11
Swab	53	18.60
Stool	16	5.61
Respiratory Tract	43	15.09
Wound/Pus	64	22.46
I/V Line Tips	14	4.91
Ear	6	2.11
Eye	3	1.05
Genitalia	8	2.81
Total	285	100

Figure 1 : Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa*



AK \Amikacin, AX \Amoxicillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, , B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol, CLR \Clarithromycin, DA \Clindamycin, AK \Amikacin, AX \Amoxicillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, , B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol, CLR \Clarithromycin, DA \Clindamycin

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