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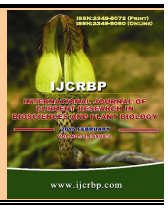

Original Research Article

Detection of Antibiotic Resistance Patterns of *Pseudomonas aeruginosa* Isolated from Ear Infection

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Abstract

Out of 70 specimens (ear swabs) collected from patient suffering from CSOM, *P. aeruginosa* was the dominant bacteria with 28% (21 isolates). *P. aeruginosa* was the commonest bacterial isolated in adult ages group in larger than 10 years old and the proportion of these isolates were increased with the increasing of age and the highest percentage of isolates were seen in the age groups 51 – 60 years (50%) and 31 – 40 years (35%). *P. aeruginosa* isolated in female (60%) higher them in male (40%). Most of isolates show (100%) multiple – resistance to Cefotaxime, Amoxicillin, Naldixic acid, Rifampin and Cephalothin, and highly resistance to Gentamycin, Ceftraxone and Cefotaxime (90.4, 90.4, 95.2) % respectively. While resistance of isolates to Ciprofloxacin and Azithromycin append in (2.3 and 4.7) % respectively. The patterns (Ciprofloxacin, Naldixic acid, Cephalothin, Ceftraxone, Cefotaxime, Cefotaxime, Amoxicillin, Rifampin, Gentamycin) represent the highest degree of multiplicity (42.8%) and the patterns (Naldixic acid, Cephalothin, Ceftraxone, Cefotaxime, Cefotaxime, Amoxicillin, Rifampin) represent the lowest degree of multiplicity (9.5%). The resuts of MIC for chemical disinfactants showed that sekulyse inhibit the bacterial growth at the concentration 2 (76.2 %) and 4 (57.1 %), while minudes stopped the bacterial growth at concentration 1 (38.1 %), 2 (47.6 %) and 4 (71.4 %). Skin sept and detolin inhibit bacterial growth at the concentration of 4 (6.3, 42.9) % and 8 (9.5, 14.3) %, respectively. These results showed that minudes is the best detergent for killing *P. aeruginosa* since it inhibit growth at the MIC (1).

Keywords

Antibiotic Resistant

Ear Infection

MIC

Pseudomonas aeruginosa

Introduction

Pseudomonas aeruginosa is widely distributed in nature (soil, water, plants, animals). It may colonize healthy humans without causing disease, but is also a significant opportunistic pathogen, and a major cause of nosocomial infections (Harvey et al., 2007). *P. aeruginosa* is a classic opportunist

pathogen with innate resistance to many antibiotics and disinfectants (Govan, 1996). *P. aeruginosa* has always been considered to be a difficult target for antimicrobial and chemotherapy (Stover et al., 2000).

This organism shows a remarkable capacity to resist antibiotics, either intrinsically (because of constructive expression of ß-lactamases and efflux pumps, combined with low permeability of the outer – membrane) or following acquisition of resistance genes (e.g: genes for ß-lactamases, or enzymes inactivating amino glycosides or modifying their target), over – expression of efflux pumps, decreased expression of poring, or mutations in quinolone targets (Mesaros et al., 2007).

P. aeruginosa known for many years to be a cause of serious wound and surgical infection, but often regarded as a secondary or opportunistic invader rather than a cause of primary infection in healthy tissues, *P. aeruginosa* has now clearly emerged as a major nosocomial pathogen in immuno-compromised and debilitated patients, as well as in cystic fibrosis patients (Pier et al., 2005).

P. aeruginosa is regularly a cause of nosocomial pneumonia, nosocomial infections of the urinary tract, surgical sites, severe burns and of infections patients with cystic fibrosis, or those who are undergoing either chemotherapy for neoplastic diseases or antibiotic therapy for other infections (Harvey et al., 2007).

Due to the important of *P. aeruginosa* in any time and the higher pathogenesty of it this study was aimed for isolate and identification of *Pseudomonas aeruginosa* from CSOM patients, Determine the antibiotic resistance patterns of isolates to different antibiotic, and Determine the sensitivity of isolates to some disinfectants.

Materials and methods

Patients and specimen collection

A total of 70 sample (ear swabs) were collected from 70 consecutive out patients female & male with CSOM during the period with a different age groups at ENT department in Al-Sader teading Hospital in Najaf governorate. They included both sex with a different age groups.

Isolation and identification of bacteria

Each specimen (ear swab) was immediately inoculated on the Blood agar and MacConky agar plate at 37°C for 24-48 h. The primitive diagnoses of bacterial types according to the color of colony (blue green), and then siy colony from each specimen purred on nutrient agar medium and the cultured on slants. The bacteria identify according to the diagnostic procedures recommended by Macfadden (2000).

Identification of bacteria depending on doing Microscopy examination(gram stain),Growth at (42°C), Catalase test, Oxidase test, Sugar fermentation test, Pyocynin production test, Triple sugar Iron Agar test and H_2S, Urease production test, Hemolysin production test, Citrate utilization test, Motility test,Indol test, Methyl Red Test, Voges Proskauer Test.

Api 20 –E test

According to the manufacture company (Bio Meurienx) – France. Api (20 E) test is biochemical system used for diagnostic Enterobacteracea

Antibiotic sensitivity (Kirby-Bauer method)

Two-ml of brain heart infusion broth have been inoculated with an isolated colony of test bacteria and incubated for 24 h at (37°C). After that, the turbidity of bacterial suspension has been adjusted turbidity of McFarland (0.5) standard tube. 0.1 ml of bacterial suspension has been spread on the surface of Mueller Hinton medium plate and left to dry; antimicrobial disks have been placed and incubated for 24 h at (37°C) (Bauer et al., 1966). The resulting zone of inhibition have been measured by using a ruler and compared with zones of inhibition determined by CLSI (2012) and to decide the susceptibility of bacteria to antimicrobial agent, whether being resistant or susceptible.

MIC of chemical disinfectants

Minimal Inhibitory Concentration (MIC) for four important chemical disinfectants (sekulyse, minudes, Skin sept and detolin) that widely used in all hospitals units were determined by broth microdilution procedure according to the method of Abdul-Rahman (2002).

Results and discussion

Isolation and identification of *P. aeruginosa*

Out of 70 specimens (ear swabs) collected from patient suffering from CSOM, P. aeruginosa was the dominant microorganism which isolates in (28%). The diagnosis was doing according to the morphological character (Gram–negative, non-sporing, non–capsulate) and cultural characters of colonies when growing on blood agar, nutrient agar (circular, flat with irregular surface, translucent edge, produce diffusible pigment (piocyanin) with a sweet grape–like odor) as presented in Fig. 1. The results of biochemical reactions and Api2o E of 20 *P. aeruginosa* isolates are shown in Table 1 and Fig. 2.

Fig. 1: *Pseudomonas aeruginosa* on nutrient agar.

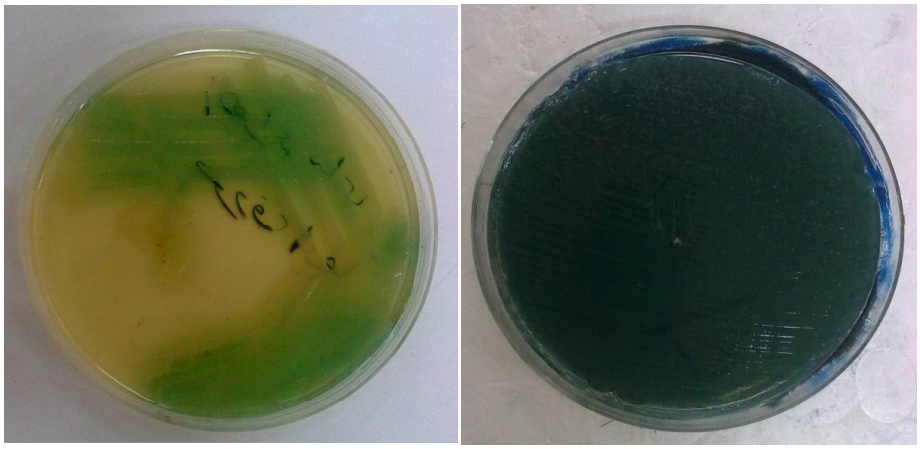
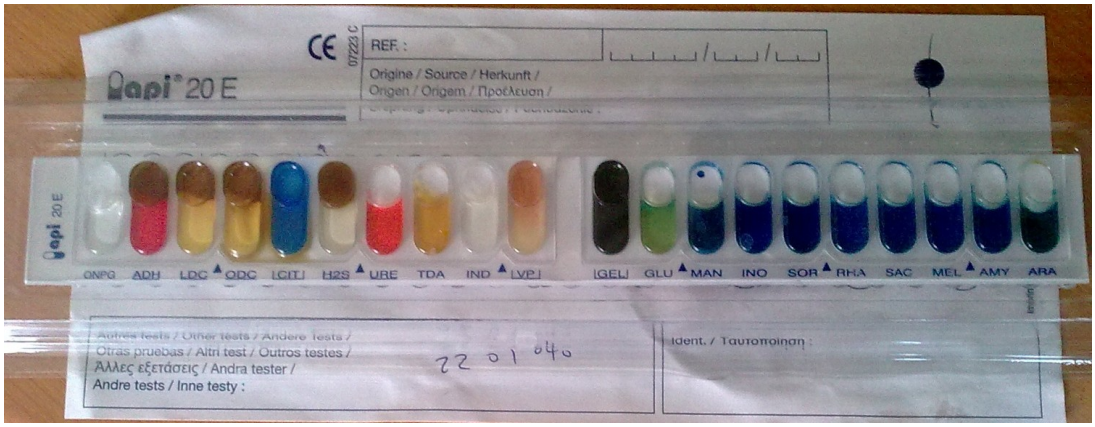


Table 1. The results of biochemical tests used for the diagnosis of *Pseudomonas aeruginosa.*

Test	Result
Catalase	+
Oxidase	+
Growth on TSI	K / K
H_2S	-
Indole production	-
Methyl – Red	-
Voges Proskaur	-
Citrate utilization	+
Urease	-
Gelatin	+
Pyocianin production	+
Growth at 42°C	+
Hemolycin	+

Fig. 2: The results of biochemical tests of *Pseudomonas aeruginosa* using Api2o E.



Our results in agreement with result of Ad-Dahhan (2001) and Indudharan et al. (1999) who stated the *P. aeruginosa* was the commonest pathogen of CSOM and isolated in 21.6% and 27.2% respectively. The result was expected for this organism may be due to many reasons, firstly, *P. aeruginosa* is the most secondary invaders when the resistance of the middle ear is lowered. Secondary, it easily spreads to the compromised patients via external auditory canal (EAC) from healthy carriers and thirdly, *P. aeruginosa* indicates more general antibiotic resistance and it resist to phagocytosis by producing a large number of extra cellular products such as, alkaline protease, elastases and exotoxin A (Stenfors and Raisanen, 1993).

Distribution of *P. aeruginosa* isolates according to the sex and age group

According to the age group, *P. aeruginosa* was the commonest bacterial isolated in adult ages group in larger than 10 years old and the proportion of these isolates were increased with the increasing of age and the highest percentage of isolates were seen in the age groups 51 – 60 years (50%) and 31 – 40 years (35%). As shown in Table 2.

Table 2. *Pseudomonas aeruginosa* isolates with reference to the age group of people.

Age	Male			Female			Total		
	No. of patients	No. of isolate +ve	%	No. of patients	No. of isolate +ve	%	No. of patients	No. of isolate +ve	%
11 - 20	6	2	33 %	13	3	23 %	19	5	26 %
21 - 30	9	3	33 %	16	4	25 %	25	7	28 %
31 - 40	10	3	30 %	7	3	42 %	17	6	35 %
41 - 50	3	-	0%	2	1	50 %	5	1	20 %
51 - 60	2	-	0%	2	2	100 %	4	2	50 %

Table 2 also shows the percentage of *P. aeruginosa* isolated in female (60%) higher them in male (40%) of all isolates these may be due to anatomical and physiological differences between male and female and Immunological response which make barriers that prevent the microorganism to reach the specific area of the body.

Antibiotic resistance *of P. aeruginosa*

Table 3 shows the results of antibiotic susceptibility test of *P. aeruginosa* to 10 types of antibiotics. Usually used for treatment different infections. Most of isolates show (100%) multiple – resistance to CFM, AX, NA, RA and KF, and highly resistance to CN, CRO and CTX (90.4, 90.4 and 95.2%) respectively. While resistance of isolates to CIP and AZM append in (2.3 and 4.7%) respectively. The highly resistance of *P. aeruginosa* to the common antimicrobial agents may be attributed to it has a high intrinsic resistance or acquired resistance resulting from many factors such as structural or and genetic factors which protect it from the action of antimicrobial. For this reason, the organism has been described to be widely distributes through staff and patients in hospital (Mesaros et al., 2007). Masuda et al. (2010). Suggest that at least three different outer membrane proteins; OPrM, OPrJ and OPrN, are associated with multiple drug resistance in *P. aeruginosa*.

Table 3. Antibiotic resistance of *P. aeruginosa* isolate from CSOM.

Antibiotic	Symbol	No. of isolates	Percent %
Azithromycin	AZM	1	4.7
Ciprofloxacin	CIP	11	52.3
Ceftraxone	CRO	19	90.4
Gentamycin	CN	19	90.4
Cefotaxime	CTX	20	95.2
Cefotaxime	CFM	21	100
Amoxicillin	AX	21	100
Naldixic acid	NA	21	100
Rifampin	RA	21	100
Cephalothin	KF	21	100

P. aeruginosa represents a phenomenon of bad trial resistance, since particularly all known mechanisms of antimicrobial resistance can be seen in it and it is responsible for 10–15% of the nosocomial infections worldwide. Often these infections are hard to treat due to the natural resistance of the species, as well as to its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents (Strateva and Yordanov, 2009).

Antibiotic resistance patterns

Table (4) shows the antibiotic resistance patterns of *pseudomonas aeruginosa* isolated in which the patterns (CIP, NA, KF, CRO, CFM, CTX, AX, RA, CN) represent the highest degree of multiplicity (%) and the patterns (NA, KF, CRO, CFM, CTX, AX, RA) represent the lowest degree of multiplicity (%).

In the study of Abdul-Rahman (2002). AMP, CN, Py, FM, KF, CXT is the most frequent patterns of *p. aeruginosa* isolates (30.4 %). The change in the antibiotic resistance patterns (especially between hospital isolates) may attribute to the high transferred of plasmids and selective pressure.

Resistant to disinfectants and detergents

Table (5) shows that sekulyse inhibit the bacterial growth at the concentration 2 (76.2 %) and 4 (57.1 %), while minudes stopped the bacterial growth at concentration 1 (38.1 %), 2 (47.6 %) and 4 (71.4 %). Skin sept and detolin inhibit bacterial growth at con. 4 (6.3, 42.9) % and 8 (9.5, 14.3) %, respectively. These results shows that minudes is the best detergent for killing *P. aeruginosa* since it inhibits growth at least MIC (1).

Table 4. *Pseudomonas aeruginosa* antibiotic resistance patterns

Frequency of resistance	Dominant patterns	Resistant isolate	
		No.	%
9	CIP NA KF CRO CFM CTX AX RA CN	9	42.8
8	NA KF CRO CFM CTX AX RA CN	8	38
7	NA KF CRO CFM CTX AX RA	2	9.5

Table 5. The MIC of chemical detergent to *P. aeruginosa* isolates.

MIC / Types of detergent	% of isolate inhibited MIC (Mg/ml)							
	0.5	1	2	4	8	16	32	64
Sekulyse	0	0	16 (76.2)	12 (57.1)	0	0	0	0
Minudes	0	8 (38.1)	10 (47.6)	15 (71.4)	0	0	0	0
Skin sept	0	0	0	3 (6.3)	2 (9.5)	0	0	0
Detolin	0	0	0	9 (42.9)	3 (14.3)	0	0	0

In Abdul-Rahman (2002) study Habitane have the high activity as detergent for wound and burns due to Russell (1999) reported that the resistance of Antimicrobial agents (Antibiotic & Disinfectant) due to plasmids may include change in cellular permeability against these compounds, degradation of inhibitors to drug exclusion, tolerance, modification in antimicrobial components, alteration of target binding site. Masuda et al. (2010) suggested that the outer membrane in Gram-negative bacteria may be acting as barrier preventing the interning the many inhibitor factors and he also elucidated that the mutant stain (losing OMPs and LPs) are more susceptible to the disinfectants and detergent at the same concentration than wild strains. From all above, the detergents and disinfectants may not be considered as disinfectant solution, and may be a source of infection unless it prepared and used in correct scientific methods.

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