



Antibiosis resistant of *Pseudomonas aeruginosa* isolated from different clinical specimens

Siham Sh. AL-Salihi¹, Braihan H. Hameed², Braihan H. Hameed³

¹ Technical College\Kirkuk

Suham2011@yahoo.co.uk

² Technical College\Kirkuk

Pr_ha84@hotmail.com

³Azadi Teaching Hospital\Kirkuk

Adil777ali@yahoo.com

Received date 1/9/2013 Accepted date 18/6/2014

ABSTRACT

Objective: Detection of antibiotic sensitivity of Pseudomonas aeruginosa isolated from different clinical specimens in Kirkuk, Iraq.

Material and Methods: The present study included (3138) samples collected from different clinical specimens from outpatients and inpatients of both sexes with different ages who were attended Azadi Teaching Hospital/ Kirkuk during the period from October/2007 until May/ 2013.

Results: From a total of 3138 Specimens, 1485 gave positive cultured and 1653 Specimens showed no bacterial growth, 319/3138 (10.17%) isolates were identified as P. aeruginosa. These isolates were identified according to morphological, cultural and biochemical characteristics. Wound and ear swab were important source for P. aeruginosa and isolated more frequently in inpatients than outpatients. The rate of isolation in females 169/319 (52.97%) was higher than males 150/319 (47.01%). Antibiotic susceptibility test of these isolates was performed, and the results showed that all Pseudomonas isolates (100%) were resistant to ampicillin, cephradine and trimoxazole, followed by gentamycin (97.3%), Amoxicillin (97.3), cephalexin (92.3%), neomycin (91.4%), nalidixic acid (89%), nitrofurantoin (87.5%), tobramycin (87.5%) and ciprofloxacin (84%), and the resistance to amikacin was (75%).



Conclusions: *P. aeruginosa* is multiresistant isolated in a high frequency from wound swabs followed by ear swabs.

Key words: Pseudomonas aeruginosa, Antibiotic sensitivity.

مقاومة المضادات الحيوية للزوائف الزنجارية المعزولة من عينات سريرية مختلفة

سهام شكور عبيد الصالحي¹ ، بريهان حمدي حميد²، ساجدة حسن درويش³

¹ الكلية التقنية/ كركوك

Suham2011@yahoo.co.uk

² الكلية التقنية/ كركوك

Pr_ha84@hotmail.com

³ مستشفى آزادي التعليمي/ كركوك

Adil777ali@yahoo.com

تاريخ قبول البحث 2014/6/18

تاريخ استلام البحث 2013 / 9 /1

الملخص

الهدف: الكشف عن الحساسية للمضادات الحيوية للزوائف الزنجارية المعزولة من العينات السريرية المختلفة في مدينة كركوك-العراق.

المواد وطريقة العمل: شملت هذه الدراسة (3138) عينة تم جمعها من العينات السريرية المختلفة للمرضى الراقدين والوافدين من كلا الجنسين وبأعمار مختلفة، الذين حضروا إلى مستشفى آزادي التعليمي / كركوك خلال الفترة من تشرين الاول / 2007 وحتى مايس / 2012.

النتائج: من مجموع 3138 أعطت 1485 نتيجة زرع موجبة و1653 عينة لم تظهر نمو بكتيري. شخصت 319 (10.17%) من مجموع 3138 عذلة كالزوائف الزنجارية وقد تم تحديد هذه العزلات وفقاً لخواصها الشكلية والمزرعية والكيموحيوية. مسحة الجرح والأذن كانا مصدرا هاما للزوائف الزنجارية وكانت أكثر تكرارا في المرضى الراقدين من



المرضى الوافدين. كانت نسبة الزوائف الزنجارية في الإناث 319/169 (52.97%) وهي أعلى من الذكور 319/150 (49.01%) تم إجراء اختبار الحساسية للمضادات الحيوية لهذه العزلات، وأظهرت النتائج أن جميع العزلات الزوائف الزنجارية (100%) كانت مقاومة للأمبيسيلين، سيفرادين، ترايموكسازول، تليها جنتاميسين (97.3%)، أموكسيسيلين (97.3%)، السيفالكسين (92.3%)، النيوميسين (91.4%)، وكان حمض الناليديكسيك (89%)، نتروفورانتوين (87.5%)، توبراميسين (87.5%) وسيبروفلوكساسين (84%)، والمقاومة لأميكاسين كانت (75%).

الاستنتاج: الزوائف الزنجارية متعددة المقاومة للمضادات الحيوية وعزلت بتردد عالي في عينات مسحات الجرح تليها مسحات الأذن.

الكلمات المفتاحية: الزوائف الزنجارية ، الحساسية للمضادات الحيوية.



1. INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an aerobic, motile, gram negative rod bacterium that belongs to the family pseudomonadaceae, [1] usually found in soil, water, plants, animals and humans [2] and is commonly present in moist environments in hospitals. It can colonize normal humans, in whom it is a saprophyte [3]. *Pseudomonas aeruginosa* is one of the most frequent and dangerous pathogens involved in the etiology of severe nosocomial infections [4]. It has been implicated in diverse nosocomial infections like nosocomial pneumonias, urinary tract infections (UTIs), skin and soft tissue infections, in severe burns and in infections in immunocompromised individuals [5]. Infections caused by *P. aeruginosa* are often life threatening and difficult to treat because of its primary limited susceptibility to commonly used antimicrobial agents [6]. Most strains of *P. aeruginosa* are multidrug resistant [7]. The development of bacterial resistance is a major worldwide problem complicating the use of chemotherapeutic agents and the control of infectious diseases [8].

Thus, the aims of the present study were to isolate and identify of *P. aeruginosa* from outpatients and inpatients from different clinical specimens (blood, urine, wound and ear infections) and assess of the antibiogram profile of the isolates.

2. MATERIALS AND METHODS

2.1 Sample collection

The study was conducted in the Medical Microbiology Laboratory of the Azadi Teaching Hospital/Kirkuk between October/2007 to May/2012. Approximately (3138) samples were collected from different clinical specimens (blood, urine, swabs from wounds and ear infections) from outpatients and inpatients of both sexes with different ages.

2.2 Bacterial isolation and identification

Collected samples were cultured onto nutrient, blood and MacConkey's agar for primary isolation. Non-lactose fermented colonies were selected and cultured onto Cetrimide



agar, then incubated overnight at 37°C for refreshment and demonstration of their ability for blood hemolysis [9, 3].

2.3 Antibiotic sensitivity (disc diffusion test)

McFarland standard solution was used in antimicrobial susceptibility test, tube No. (0.5) was used which prepared by adding 0.05 ml of BaCl₂.2H₂O con. (1.175%) to 9.95 ml of H₂SO₄ con. (1%). The tube No. (0.5) of McFarland standard was used to compare the bacterial cell in suspension which gives a cell density 1.5x10⁸ cell/ml [10].

Several colonies (2-5) were picked off from nutrient agar and inoculated into 4 to 5 ml of nutrient broth. The broth was incubated for 24 hours at 37°C until a good visible growth was obtained. The growth was adjusted to 0.5 McFarland turbidity standard using saline or broth 100 µl of diluted bacterial suspensions was poured onto the surface of Muller-Hinton agar plates. The bacterial suspension was spread evenly over the agar surface using flamed L-shape glass rod. The plates were allowed to dry and then discs (the Manufacturer Company were Bioanalyse /Turkey & Himedia/ India) were placed at least 22 mm from each other and at least 14 mm from the edge of the Petri dish using sterile forceps. The plates were incubated at 37°C for 18 to 24 hours. After incubation, zone of inhibition was recorded in millimeter using metric ruler [11, 12] and translated into predetermined categories as resistant and sensitive [13].

3. RESULTS AND DISCUSSION

3.1 Isolation and identification of *P.aeruginosa*

In the current study, all bacterial isolates were cultured selectively using cetrimide medium and according to microscopic characteristics, cultural and biochemical tests (**Table.1**). These colonies were identified as *P. aeruginosa*. The selected colonies were tested for oxidase and catalase production and sub-cultured on MacConkey's agar to obtain pure culture for further diagnosis investigations. All the isolates that produced pyocyanin (blue pigment), and are able to grow at 42°C but not at 4°C, these criteria were depended in our study for identification of *P. aeruginosa* from other species not possess these criteria which



neglected and do not included in the study results [3]. Under microscope it appears as gram negative, rod-shape, and occurs as single, pairs, or in short chains [14].

Table 1: Biochemical tests used for identification of *P. aeruginosa* .

Biochemical tests	Result
Indole production	Negative
Methyl red test	Negative
Voges-Proskauer	Negative
Citrate utilization	Positive
Oxidase test	Positive
Catalase test	Positive
Triple sugar iron	Alkaline slant, Alkaline butt, No H ₂ S and No gas production

In this study, the prevalence of *P. aeruginosa* isolates in clinical specimens examined over the study period was (10.17%) (**Table.2**), this level is relatively low when compared with similar studies with higher prevalence level. In India, Savaş *et al.* reported a level of (20.3%) [15], while (30%) was reported in a study conducted in Pakistan [16], and (18.25) in Egypt [17]. However, this finding is higher than a study in northeastern Nigeria which found that a level of (2.1%). Comparison of epidemiological data of bacterial pathogens as in this study might be difficult as there are other variables that influence the outcome of results such as, clinical specimens received for examination, studied population, type of hospitals and geographical locations [15].



Table 2: Number and percentage of *P. aeruginosa* isolates.

Cultured result samples	Number of isolates	%
<i>Pseudomonas aeruginosa</i>	319	10.17
Other bacteria	1166	37.16
Negative	1653	52.67
Total	3138	100

In the (Table.3), the rate of isolation in female 169/319 (52.97%) was higher than male 150/319 (47.01%), the reason may be that female more infected with urinary tract infection and exposed to burning and wounded during cooking more than male.

Table 3: Distribution of *P. aeruginosa* according to gender.

Gender	No.	%
Male	150	47.02
Female	169	52.98
Total	319	100%

According to source of infection, the present study revealed that *P. aeruginosa* were most common 128/319 (40.13%) in wound infection, followed by 87/319 (27.27%) ear infection, 53/319 (16.61%) urinary tract infection and 51/319 (15.98%) blood (bacteremia) (Table.4). There are many studies and researches that have been conducted on *P. aeruginosa*. A study reported that *P. aeruginosa* is responsible for (12%) of hospital acquired urinary tract infections, (8%) of surgical wound infections, and (10%) of bacteremia [18].

In this study, the distribution of isolates also differs with other studies, a study done by Latif in Iraq who showed that *P. aeruginosa* were most common (44.4%) in burn infection, followed by (38.1%) ear infection, (16.6%) wound and (6.6%) urinary tract infection, while *P. aeruginosa* cannot be isolated from eye infections [19].

However, in India which found that isolated *P. aeruginosa* was (30%) of wound and (30.5%) of urine [20] and in northeastern Nigeria which found that Significant proportion of



isolates were recovered from wounds specimen (39.6%), followed by ear swabs (30.2%), and urine (7.5%) [21]. In another Iraqi study, the results showed that all burned cases infected with *P. aeruginosa* (100%), followed wound (41.7%) and (28%) from ear swab [22]. While, Hayder *et al* who found that most isolates were obtained from burns (8.55%), wound (3.95%), ear swab (3.30%) and (1.97%) isolates from each urine and blood [23].

There are differences in the percentage of infections between our results and others, and the reasons for these variations in all studies may be due to the percentage of distribution of isolates which varied according to the place of clinical samples collection, environmental factors, nutrition requirements and virulence factors [24].

Table 4: Distribution of *P. aeruginosa* according to source of isolates.

Types of specimens	Specimens (n=1485)	<i>P. aeruginosa</i> (n=319)	
		N	%
Wound swab	258	128	40.13
Ear swab	164	87	27.27
Urine	897	53	16.61
Blood	166	51	15.98
Total	1485	319	100%

3.2 Antibiotic susceptibility testing

The present study showed that all wound samples isolates were resistant nearly to all 12 tested antibiotics (**Table.5**). Antibiotic susceptibility test of these isolates was performed, and the results showed that all *Pseudomonas* isolates (100%) were resistant to ampicillin, cephadrin and trimoxazole, followed by gentamycin (97.3%), Amoxicillin (97.3), cephalexin



(92.3%), neomycin (91.4%), nalidixic acid (89%), nitrofurantoin (87.5%), tobramycin (87.5%) and ciprofloxacin (84%), and the resistance to amikacin was (75%). A major problem in *P. aeruginosa* infection is the resistance to relatively high levels of most antibiotics in use [25]. It has been emphasized that there is a remarkable increase in the incidence of infection by antibiotic resistance microorganisms in different parts of the world. A study indicated that *P. aeruginosa* infections are difficult to treat as this organism displays a high level of intrinsic antibiotic resistance [26].

Table 5: *P. aeruginosa* susceptibility to (12) antibiotics.

Name of antibiotics	symbol	Concentration µg/disc	Resistance		sensitive	
			No	%	No	%
Amoxicillin	AX	25	310	97.3%	9	2.7%
Ampicillin	AM	10	319	100%	0	0.0%
Gentamicin	GM	10	310	97.3%	9	2.7%
Amikacin	AK	30	238	75%	81	25%
Tobramycin	TOB	10	279	87.5%	40	12.5%
Ciprofloxacin	CIP	5	268	84%	51	16%
Neomycin	N	30	292	91.4%	27	8.6%
Cephalexin	CL	30	294	92.3	25	7.7%
Cephradin	CE	30	319	100%	0	0.0%
Nalidixic Acid	NA	30	284	89%	35	11%
Nitrofurantoin	F	300	279	87.5	40	12.5
Trimoxazole	CO	5	319	100%	0	0.0%

Until the end of the 1980s, fluoroquinolones had excellent activities against *P. aeruginosa* but extensive use of the antimicrobial, in particular ciprofloxacin, had led to an increasing incidence of ciprofloxacin-resistant isolates [27]. Whereas at 1984 almost (100%) of *P. aeruginosa* isolated in the USA, Europe and Japan were susceptible to ciprofloxacin [28]. The current study showed that ciprofloxacin resistance was (84%). This finding is higher than that in a study in India which found that the resistant of ciprofloxacin against *P. aeruginosa* was (73.2%) [29].



The current study revealed that antipseudomonasal effect of amikacin is higher than gentamycin. This finding correlates with other studies conducted by Smitha *et al* and Poole *et al* who found that resistance to amikacin of *P. aeruginosa* was still lower than to gentamicin [30, 31]. Other study reported that *P. aeruginosa* showed higher levels of resistance to gentamicin and imipenem [32].

Another study found a group of *P. aeruginosa* strains lacked β -lactamase activity but were resistant to penicillins, ceftazidime, ciprofloxacin and amikacin which is due to impermeability or multidrug efflux (intrinsic mechanism of resistance) [33].

The present study is in agreement with a study which found that *P. aeruginosa* isolated from patients demonstrated resistance to ampicillin, gentamycin and nalidixic acid [34]. However, disagrees with other study which observed that all *P. aeruginosa* isolates were resistant to ampicillin, cephalexin and nalidixic acid while they were sensitive to ciprofloxacin and tobramycin [19].

Antibiotic resistance is now generally accepted as a major public health issue. *P. aeruginosa* infection is considered a major problem because of its resistance to relatively high levels of most antibiotics in use [35], particularly due to the combination of the following mechanisms: betalactamase production, a strong barrier to diffusion at the outer bacterial membrane and bacterial efflux. Selective pressure of antimicrobial drugs has an important impact on the development of bacterial resistance [36].

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AUTHOR



Siham Shakoor Obid AL-Salihi is lecturer in Medical Laboratory Sciences Department in Technical College\Kirkuk. She received her B. Ed. in 1996, M. Sc. In Ecology of Microbiology in 1999 and Ph.D. in Microbiology in 2012 from Tikrit University/Iraq. She has taught a variety of courses in microbiology including diagnostic microbiology and medical bacteriology. She has conducted many studies on the virulence factors produced by opportunistic bacteria and published 5 researches in Iraqi journals.