

Prevalence study of *Pseudomonas aeruginosa* in Teaching Tikrit Hospital from different sources.

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Abstract

In the current study, it was isolated 117 samples from different sources, the isolates were identified using bacteriological and biochemical tests (bacteria swabs uptaken from wound, otitis media, stool and urine and), out of 117 samples, only 18 specimens were positive for *P.aeruginosa* (14%) in the otitis media, (14%) in the stool, (3%) in the wound and (0%) in the urine. All bacterial isolates identified through the use of bacterial and biochemical tests. *P.aeruginosa* isolates showed high resistance to most used antibiotics especially Ceftriaxone (CRO), Erythromycin (E), Cefixime (CFM) 100% followed by Cefepime (FEP), Carbenicillin (PY) 44%, Tobramycin (TOB) 22% and Piperacillin (PRL) 16.66% but Norfloxacin (NOR) showed attractive activities against all *P.aeruginosa* isolates, the resistance was 0%.

Conclusion: *P.aeruginosa* isolated in low prevalence in the Teaching Tikrit Hospital only in 15.38%, all bacterial isolates were multi drug resistance (MDR), and resist to most used antibiotics except Norfloxacin (NOR).

Introduction

Pseudomonas aeruginosa, it is an opportunistic pathogen that causes extensive morbidity and mortality in individuals who are immunocompromised or have underlying medical conditions such as, urinary tract, respiratory tract and skin infections and primarily causes of nosocomial infections (1,2). Its non sporulating, gram negative, oxidase positive motile bacterium with a polar flagellum (3). It produces many numbers of extracellular toxins, which include phytotoxic factor, pigments, hydrocyanic acid, proteolytic enzymes, phospholipase enterotoxin, exotoxin and slime (1). *P.aeruginosa* grows well on media and most strains elaborate the blue phenazine pigment pyocyanin and fluorescein (yellow), which together impart the characteristic blue-green coloration to agar cultures (4). A major obstacle to effective control of *P.aeruginosa* infections is its intrinsic resistance to most antibiotic classes, which results from chromosomally encoded drug-efflux systems and multiple acquired resistance mechanisms (5). The aim of the current study is to evaluate the prevalence of *P.aeruginosa* in the Teaching Tikrit Hospital from different sources and test its antimicrobial resistance.

Material and methods

samples collection:

This study lasted for 9 months from September 2013 to June 2014. One hundred seventeen specimens isolated from Teaching Tikrit Hospital, from different sources (wound, urine, otitis media, stool).

Isolation and identification of bacteria:

The collected specimens were inoculated on MacConkey agar, then incubated at 37°C for 24 hr, the pale non lactose fermenting colonies were selected then a single colony was inoculated on blood agar for the activation and detection of bacterial ability to lysis red blood cells. The large flat colonies with grape like odor were inoculated on cetramide medium, incubated at 37°C for 24 hr. Then single colony that grows on cetramide media was inoculated on King A and King B agar to determine the ability to produce pigments. Single colony was inoculated on brain heart infusion agar to carry out other biochemical tests that confirm the identification of bacterial isolation (6).

Antibiotic susceptibility test:-

Antibiotic susceptibility test by disk-diffusion method was performed according

To Bauer and his coworkers (7), and the results were compared with standard inhibition zone. Eight antibiotics type used in this experiment (Erythromycin, Norfloxacin, Piperacillin, Carbenicillin, Cefixime, Cefepime, Ceftiaxone) table (1).

Table (1) show antibiotic disks used in the present study.

Antimicrobial agent *	Symbol	Concentration µg/disk	Diameters of inhibition zones (mm)		
			resistant	intermediate	susceptible
Penicillins					
Piperacillin	PRI	100	≤17	-	≥18
Carbenicillin	PY	100	≤13	14-16	≥17
Cephalosporins					
Ceftriaxone	CRO	30	≤14	15-19	≥20
Cefixime	CFM	5	≤15	16-18	≥19
Cefepime	FEP	30	≤14	15-17	≥18
Aminoglycosides					
Tobramycin	TOB	10	≤12	13-14	≥15
Fluoroquinolone					
Norfloxacin	NOR	10	≤13	14-16	≥17
Macrolides					
Erythromycin	E	15	≤13	14-22	≥23

*Suggested ranges from (Collee *et al*,1996).

**Standard values recommended by (NCCLS,2003)(Murray *et al*,2003).

***all above disks from bioanalyse company (Turkey).

Result and discussion

Isolation and identification of specimens:

Out of 117 specimens from various clinical source, (wound, otitis media, urine, stool) collected from Teaching Tikrit Hospital ,only 18 isolates were successfully diagnosed as *P.aeruginosa* (15.38%) ,as shown in table (3) ,diagnosis based on assessment of bacteriological and standard biochemical test as shown in table(2).

The preliminary cultural diagnosis for bacterial isolates exhibited that all the thirty 18 isolates characterized by circular rough colonies with large size relatively with long axis in the line of the inoculums streak surrounded by a serrated strict of growth. All the identified isolates produced the diagnostic diffusible pigments on nutrient agar, cetramide agar and king A agar except 3 isolates (2 from wound,1from otitis media), although the pigments varied according to the isolates between bluish–green in wound and stool isolates to yellowish–green in color in otitis media isolates and all those isolates produced a sweet grape–like odor which was easily recognized (8, 9,5). All the isolates grew on 7% human blood agar. All those isolates grew on MacConkey’s agar, but did not ferment lactose sugar(10).Under the microscope, the bacterial cells appeared as short bacilli arranged in single or short chain, negative for Gram–stain reaction, motile, non–spore forming and without capsule (6). In this study some biochemical tests, table (2) were carried out and the results were compared with standard results documented by (11,10,6,12). Accordingly, results revealed that the characteristic closely related to the *P.aeruginosa* were in agreement with referential results. For the practical purposes, isolates on cetramide agar that exhibited the characteristic

colonial morphology and odor oxidase–positive can be presumptively identified as *P.aeruginosa* (13). Moreover, all isolates are able to grow at (42°C),an ability which regarded as confirmative character *pseudomonas aeruginosa* (11).All these observations were considered through the identification at *P.aeruginosa* isolates diagnosed in this study.

Table (2)Biochemical Tests for Bacterial Isolates in this Study

Test	Result
Catalase test	+
Gram-stain	-
Growth at 42°C	+
Hemolysis(β-hemolysis)	+/-
H ₂ S production	-
Indole test	-
Kligler’s iron agar	K/no change
Methyl-red	-
Growing on cetramide agar	+
Oxidase test	+
Pigments production	+/-
Urease test	+

Proportion of *Pseudo.aeruginosa* in different sources:

Pseudo.aeruginosa cause abroad spectrum of infections which are associated with urinary, respiratory, gastrointestinal tract. Burn, wound, eyes, ears, as well as with other sites (14) .Table (3) show the distribution of *Pseudo.aeruginosa* according to the site of infection. The Results show that the otitis media are the most accessible site by *P.aeruginosa* in the ratio 42 % ,followed by stool 14% and wound 10% whereas distribution of *P.aeruginosa* in urine sample was 0% .

Table (3) Frequency of *P.aeruginosa* according to the site of infection.

Type of sample	Total	<i>P.aeruginosa</i> isolates n (%)
Otitis media	33	14 (42%)
Urine sample	37	0 (0%)
wound	30	3 (10%)
stool	7	1(14%)
Total	117	18(15 %)

In this study the distribution of the isolates differ from other studies. The study done by Latif (14) in Iraq showed that *P.aeruginosa* were most common (44%) in burn infection, (16.6%) in wound and (6.6%) in urinary tract infection, while *P.aeruginosa* cannot be isolated from eye infections. While Hayder (15) found that most isolates were obtained from burn (8.55%), wound (3.95%), ear swab (3.30%) and (1.97%) isolates from each urine and blood. Also our study contrary to Alsalihi and his coworker (16) who found that *P.aeruginosa* were most common (40.13%) in wound infection, (16.16%) from urinary tract infection and (15.98%) from blood. There are differences in the percentage of infections between our results and others, and the reason of the variation in all studies may be due to the percentage of the distribution of isolates which varied according to the place of clinical samples collection, environmental factor, nutrition requirements and virulence factor (17).

Antibiotic susceptibility test:-

The present results show highly antibiotic diseffective against *P.aeruginosa* is (erythromycin, Ceftriaxon, Cefixime) followed by (carbenicillin), whereas (norfloxacin) show highly effectiveness against *P.aeruginosa* followed by Cefepime (piperacillin) and (tobramycin) **table(4)**

Table (4) show antibiotic resistance percentage of *P.aeruginosa*.

Antibiotic type	R %	Antibiotic type	R %
Norfloxacin	0	Erythromycin	100
Tobramycin	22.22	Cefixime	100
Piperacillin	16.66	Cefepime	5.8
Carbenicillin	44.44	Ceftriaxon	100

Concerning (norfloxacin), our present study is in agreement of Bhat and his coworkers (18), who found *P.aeruginosa* resist norfloxacin in the percent (0%), about tobramycin our result are in harmony with Jaafar (19) who found *P.aeruginosa* resist tobramycin in the percent 24% and disagree with Alsalihi and his coworkers (16) who found *P.aeruginosa* resist tobramycin in the percent (87.5%), respectively. Concerning ceftriaxone, the present results agree with Jouda (20) who show all *P.aeruginosa* resist ceftriaxone in the percent 100%, whereas disagree with Flayyih and his coworkers (21) who showed *P.aeruginosa* resist

ceftriaxone in the percent (33.4%) only. Concerning cefixime, Bhat and his coworkers (18) who showed all *P.aeruginosa* isolates resist cefixime in the percent (100%) whereas Naher (22) showed *P.aeruginosa* isolates resist cefixime in the percent (80%). Concerning piperacillin our results agree with Araj and his coworkers (23) who showed *P.aeruginosa* isolates resist piperacillin in the percent (16%) whereas disagree with Naher and his coworkers (22) who found *P.aeruginosa* isolates resist piperacillin in the percent (30%). Concerning carbenicillin, current results show there is a remarkable disagree with Radi and his coworkers (24) who found his isolates resist carbenicillin in the percent (100%). Concerning erythromycin our results resemble Radi and his coworkers (24) who showed all their isolates resist erythromycin in the percent (100%). Concerning cefepime, current results disagree with Naher and his coworkers (22) who showed his isolates resist cefepime in the percent (70%). The difference in antibiotic resistance pattern observed in the isolates indicate that the organisms use several mechanisms of resistance simultaneously, and that all isolates do not necessarily use the same mechanisms for resistance to particular class of antibiotics (25,26). *P.aeruginosa* resistance can be conferred by the outer membrane which provides an effective intrinsic barrier in the cell wall (or) cytoplasmic membrane (or) within the cytoplasm and modifications in outer membrane permeability via alternations in porin protein channel represent a component of many resistance mechanisms. In addition, inactivating enzymes released from the inner membrane can function more efficiently within the confines of the periplasmic space, the mechanisms by which intracellular concentrations of drugs are limited include decreased permeability through the outer membrane and active efflux back out across the cytoplasmic membrane (27).



Figure (1) susceptibility testing of *P.aeruginosa* on muller-hinton agar (the used antibiotics (FEP, CRO, PRL, E)).

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دراسة نسبة انتشار بكتريا ألزوائف الزنجارية في مستشفى تكريت التعليمي المعزولة من مصادر مختلفة

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الملخص

تم جمع 117 عينة من مناطق مختلفة (الجروح، التهاب الاذن الوسطى، البراز، الادراج) وشخصت العينات باستخدام الفحوصات البكتريولوجية والبايو كيميائية، من بين 117 عينة معزولة تم عزل 18 عذلة فقط لبكتريا الزوائف الزنجارية تضمنت اعلى نسبة في التهاب الاذن الوسطى بنسبة 42% والبراز بنسبة 14% فقط تلتها الجروح في حين لم يعزل من عينات الادراج اي عذلة ، اما نتائج الحساسية للمضادات الحيوية اظهرت جميع العزلات مقاومة ملحوظة لمعظم انواع المضادات المستخدمة ، حيث كانت المقاومة بنسبة 100% لكلا من (السفترياكسون، الايرثرومايسين، السيفكسيم) وتلتها مضادات ، السيفيبيم، الكاربينيسيلين) حيث كانت المقاومة بنسبة 44.44% أما المقارنة لمضاد (التوبراميسين) كانت بنسبة 22% واخيرا (البايبيراسيلين) بنسبة 16.66% ولكن مضاد (النورفلوكساسين) اظهر فعالية عالية ضد جميع العزلات حيث كانت المقاومة بنسبة 0%. الاستنتاج:- انتشار بكتريا الزوائف الزنجارية بنسبة ضئيلة جدا في مستشفى تكريت التعليمي حيث كانت نسبة الانتشار فقط 15.38%، جميع العزلات اظهرت مقاومة متعددة للمضادات الحياتية المستخدمة، اذ اظهرت مقاومة لمعظم المضادات المستخدمة ماعدا مضاد (النورفلوكساسين).