Detection of Multidrug Resistant Pseudomonas spp. in Clinical Cases and Hospital Environments at Thi-Qar Province

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Abstract

A total of 260 swabs from clinical and hospital environments were collected during September 2011 to March 2012. Primary culturing on blood agar and MacConkey agar revealed that 25 (9.6%) were gave positive growth for *pseudomonas* spp., 14 (56%) was from clinical cases as follow: 10 (71.42%) from burns, 2 (14.3%) from wounds, 1 (7.14%) from urinary tract infections, 1 (7.14%) of respiratory tract infections, and 11 (44%) from hospital environments . All the 25 isolates were screened for their resistance against 13 antibiotics of different classes by disk diffusion method . All the tested isolates were appeared resistant to at least seven of antibiotics , hence the isolates are considered to be multidrug resistant .

Keywords : Multidrug , Resistant , Pseudomonas

الملخص:

جمعت 260 مسحة من حالات سريرية وعينات بيئية خلال الفترة من أيلول 2011 ولغاية آذار 2012 . أظهرت نتائج الزرع الأولي على وسط أكار الدم وأكار الماكونكي بان 25 (9.6%) أعطت نموا موجبا لبكتيريا الزوائف وكانت :14 (56%) من الحالات السريرية وكالاتي , 10 (71.42%) من الحروق , 2 (14.3%) من الجروح, 1 (7.14%) من مرضى التهاب المجاري البولية , 1 (7.14%) مرضى التهاب القناة التنفسية, و 11 (44%) من بيئة المستشفى . اختبرت حساسية جميع العزلات اتجاه 13 مضادا حيويا من أصناف مختلفة باستخدام طريقة انتشار القرص , وقد أظهرت النتائج إن جميع العزلات كانت مقاومة على الأقل لسبعة أصناف من المضادات لذلك اعتبرت هذه العزلات متعددة المقاومة.

Introduction

Pseudomonas spp. is frequently present in small numbers as normal intestinal flora and on the humans skin and is consider major pathogen of the group (Jawetz *et al* ., 2007). Because of their widespread occurrence in water and on plant seeds such as dicots, the pseudomonads were observed early in the history of microbiology. The generic name *Pseudomonas* created for these organisms was defined in rather

vague terms by Walter Migula (1894) and (1900) as a genus of Gram-negative, rod-shaped and polar-flagella bacteria with some sporulating species. Despite the vague description, the type species, *Pseudomonas pyocyanea* (basonym of *P. aeruginosa*), proved the best descriptor (Palleroni and Norberto, 2010). *P. aeruginosa* is one of the most important nosocomial pathogens, while other *Pseudomonas* spp. are occasional cause of infections. *Pseudomonas* spp. are often multiresistant to many classes of potent antimicrobial agents, including β-lactams, aminoglycosides, and fluoroquinolones (Kyungwon *et al.,* 2009). *P. aeruginosa* is responsible for outbreaks of nosocomial infections in different parts of the world. These isolates have also been responsible for serious infections such as septicemia and pneumonia. *P. aeruginosa* resistance is increasing to carbapenem (Pitout *et al.,* 2005) Treatment of infections due to *P. aeruginosa* is becoming increasingly complicated by its tendency to acquired resistance to multiple classes of antimicrobials (Rossolini and Mantengoli , 2005). The production of metallo-β-lactamases (MBLs) contributes substantially to panresistant phenotypes in *P. aeruginosa* because they confer resistance to all classes of β-lactam antimicrobials except aztreonam (Nordmann and Poirel , 2002). Multidrug-resistant isolates were defined as those resistant to three or more classes of antipseudomonal agents (i.e., penicillins/cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides). *Pseudomonas aeruginosa* causes infections in healthy individuals and those who are hospitalized or have a compromised immune system as a result of other diseases. A variety of human infections are commonly associated with this bacterium: urinary tract infections, ventilator-associated pneumonia, surgical site infection, respiratory infections, ocular infections, ear infections (external otitis, malignant external otitis) , skin and soft tissue infections, including hot tub folliculitis, and osteomyelitis, burn sepsis . Individuals with compromising conditions, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes have an increased risk of acquiring an infection and developing complications (Trautmann *et al.,* 2008). *P. aeruginosa* is gram negative opportunistic pathogen and often cause infections due to contamination in operating theater such as the event of Thi-Qar 2012 were 9 cases of infections during eyes surgery leading to loss of their eyes (www.iraqws.com.2012) . The aim

of this study is to collection of clinical samples from patients and hospital environments ,isolation and identification of *pseudomonas* spp. and determination of antimicrobial susceptibility against different types of antibiotics.

Materials and Methods

Collection of specimens for isolation and identification:

Two hundred sixty swabs were collected from different sites , 210 clinical swabs including (80) burns, (32) wounds, (34) urine, (30) otitis media, (2) sputum, (11) stool, (12) vagina, (9) nose and 50 swabs from hospital environment including (20) Bed of patient, (6) The operations surgical arena, (5) Transport Vehicle of patients, (5) Sinks, (4) Patients room floor, (4) Vehicle of the bandaging, (2) Tools for examination of ear, nose, throat (ENT), (2) Suckers, (2) Bacterial incubator. All swabs were labeled and transported to laboratory within one hour then streaked on blood agar and MacConkey agar. All plates were incubated aerobically in incubator at 37ºC for 24 hrs.

Identification

The grown colonies on the culture media with characterized diffusible pigments were selected for further diagnostic tests. according to(MacFaddin , 2000). Diagnosis of the isolates was confirmed by API 20 E system.

Antibiotic susceptibility:

The susceptibility of *Pseudomonas* isolates to 13 antibiotics were determined by disc diffusion method (Bauer *et al*., 1966). The antibiotics (content per disc) used in the study are Ax: Amoxycillin (25 µg); Ak: Amikacin (30 µg); CTX: Cefotaxime (30 µg); CRO: Ceftriaxone (30 µg); CAZ: Ceftazidime (30 µg); CIP: Ciprofloxacin (5 µg); CN: Gentamicin (10 µg); KF: Cephalothin (30 µg); IMP: Imipenem (10 µg); LEV: Levofloxacin (5 µg); MEM: Meropenem (10 µg); NA: Nalidixic acid (30 µg); TE: Tetracycline (30 µg). The antibiotic discs were purchased from Bioanalyse, Turkey. The results were recorded according to (CLSI, 2007).

Results

Out of 260 swabs, only 25(9.6%) were gave positive growth for *Pseudomonas* spp. Out of 80 burn swabs, 10 (12.5%) were gave *p. aeruginosa* . Out of 32 wound swabs, 2 (6.25%) were identified as (1) of each of *p. aeruginosa* and *p. fluorescens* . Out of 34 urine samples, 1 (2.9%) were gave *p. aeruginosa* . Finally, from 2 sputum specimens, 1 (50%) were gave *p. aeruginosa* .(Table 1). A total of 50 hospital environmental swabs were collected from different regions of main hospitals at Thi-Qar province and cultured. The results showed that 11 (22%) were gave positive growth and identified as: 9 *P. aeruginosa* and 2 *P. fluorescens* (Table2). Colony shape of *pseudomonas* isolates appeared as flat , mucoid , pigmented , non lactose fermented . Cells were G^{-ve} , motile , bacilli. All the isolate grew on Muller- Hinton agar and produced diagnostic pigment. The pigment varied from yellowish to green. Identification of the isolates was confirmed by API 20 E system to be *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* .

Table(1): Type of samples & numbers of *Pseudomonas* spp. isolates from clinical specimens.

Sample type	No. of Swabs	Isolation of *pseudomonas* spp.		*p. aeruginosa*	*p. fluorescens*
		No.	%		
Burn swab	80	10	12.5	10	0
Wound swab	32	2	6.25	1	1
Urine	34	1	2.9	1	0
Sputum	2	1	50	1	0
Ear swabs	30	0	0	0	0
Nasal swabs	9	0	0	0	0
Stool	11	0	0	0	0
Vagina swabs	12	0	0	0	0
Total	210	14	6.6	13	1

Table(2): Type of samples & numbers of *Pseudomonas* spp. isolates from environmental samples.

Site of swab	No. of swabs	Isolation of *pseudomonas*		*P. aeruginosa*	*P. fluorescens*
		No.	%		
Bed of patient	20	3	15	3	0
The operations surgical arena swabs	6	0	0	0	0
Transport Vehicle of patients	5	2	40	2	0
Sinks	5	1	20	1	0
Patients room floor	4	3	75	2	1
Vehicle of the bandaging	4	0	0	0	0
Tools of ENT	2	0	0	0	0
Suckers	2	1	50	0	1
Bacterial incubator	2	1	50	1	0
Total	50	11	22	9	2

Antibiotic susceptibility of *Pseudomonas* spp.

Susceptibility of 25 isolates of *Pseudomonas* spp. isolated during the present study against 13 antimicrobial agents from different classes using Kirby-Bauer disk diffusion method . Table (3) showing that 100% of the isolates were resistant to amoxicillin , 96% for cephalothin and nalidixic acid , 92% for tetracycline , 84% for each of ceftazidime and ceftriaxone and 80% for cefotaxime, while the lower percentage of resistance was for imipenem and meropenem (24%). The results also showed that the isolates considered as multidrug resistant .

Table (3): Antibiotics resistance percentage of *pseudomonas* spp. according to CLSI 2007. (n=25)

Type of antibiotic	No.(%) of Resistant Isolates		No. (%) of Intermediate Isolates		No. (%) of Sensitive Isolates	
	No.	%	No.	%	No.	%
Amikacin (AK)	9	36	3	12	13	52
Amoxicillin (AX)	25	100	0	0	0	0
Cefotaxime (CTX)	20	80	5	20	0	0
Ceftazidime (CAZ)	21	84	4	16	0	0
Ceftriaxone (CRO)	21	84	4	16	0	0
Cephalothin (KF)	24	96	1	4	0	0
Ciprofloxacin (CIP)	2	8	9	36	14	56
Gentamicin (CN)	15	60	1	4	9	36
Imipenem (IMP)	3	12	0	0	22	88
Levofloxacin (LEV)	5	20	7	28	13	52
Meropenem (MEM)	9	36	3	12	13	52
Nalidixic acid (NA)	24	96	1	4	0	0
Tetracycline (TE)	23	92	1	4	1	4

Discussion

P. aeruginosa infections are major causes of mortality and morbidity in hospitalized patients of developing countries (Rastegar and Alaghebandan, 2000). *P. aeruginosa* is an opportunistic pathogen bacteria that causes human infections; it can be isolated from different environments (Adel and Sabiha, 2010).

In the present study, a total of 25 *pseudomonas* spp. were isolated: 11 (44%) from hospital environmental samples, and 14 (56%) from clinical specimens. Our study showed high prevalence of bacterial infections among burn patients as compared to another study in Iran (Askarian and Hossseini, 2004). Though control of invasive bacterial burn wound infection, strict isolation techniques and infection control policies have significantly minimized the occurrence of burn wound infection (Amin and Kalantar, 2004).

In the present study, from 260 samples only 25 gave *Pseudomonas* spp. These results in disagreement with Blanc *et al.,* (1998)Even if the overall rate of *P. aeruginosa* colonization is not significantly reduced, it is important to recognize cross-infecting strains, especially if they exhibit resistance to a variety of antibiotics and give rise to severe infections. Colonized patients represent a continuous reservoir of (epidemic) strains to which other patients can be colonized via cross-acquisition.

The results showed high resistance against amoxicillin , cephalothin, nalidixic acid , tetracycline , ceftazidime, ceftriaxone and cefotaxime . While the lower percentage of resistance was for imipenem and meropenem. These results are in agreement with Deeba *et al.,* (2011) ; Hammami ***et al.,*** **(2011) and Yousefi** *et al.,* (2010).

P. aeruginosa is naturally resistant to β-lactams, including broad-spectrum cephalosporins, quinolones, chloramphenicol and tetracyclines, mainly because of the very low permeability of their cell wall. Moreover, *P. aeruginosa* is characterized by the production of inducible cephalosporinase, active efflux and poor affinity for the target (DNA gyrase), three mechanisms that synergize with poor cell wall permeability (Li *et al.,* 1994).

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