Isolation of *Pseudomonas aeruginosa* from Clinical Cases and Environmental Samples, and Analysis of its Antibiotic Resistant Spectrum at Hilla Teaching Hospital

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Abstract

The present study included 152 samples collected from different clinical cases and environmental samples in Hilla General Teaching Hospital during the period from October 2010 to January 2011. The age of the patients ranged from (6 to 65) years. The samples were immediately inoculated on pseudomonas isolation agar plates. All plates were incubated aerobically at 37ºC for 24-48 hrs. Results of morphological and biochemical characterization tests revealed that only 48(31.57%) isolates were *Ps. aeruginosa*, while other 104(68.43%) represented other bacterial genera. Antibiotic susceptibility test for all the isolates was determined by using disc diffusion test (DDT) and rapid iodiometric methods. The results showed that, the Ps. aeruginosa isolates were fully resistant (100%) for each the following antibiotics; ampicillin, Cefotaxime, chloramphenicol, penicillin, doxycycline, and Erythromycin, while they exhibited moderate resistance to Amikacin 19(39.5%), ciprofloxacin 15(31.26%) and Polymyxine 29(40 %), and were sensitive to Pipracillin, Ticarciline in a percentage rate(20.08%)for each antibiotic. The bacterium was found to carry beta lactamase enzymes with high rate of resistance (66.6%).

الخلاصه

تضمنت هذه الدراسة ١٥٢ عينه ،شملت عينات سريرية وبيئية من مستشفى الحلة التعليمي العام خلال الفترة من تشرين الاول٢٠١٠ الى كانون الثاني ٢٠١١ ، وتراوحت اعمار المرضى من ٦-٦٥ سنه. زرعت جميع هذه العينات على وسط اكار السيدوموناس، وحضنت جميع الاطباق هوائيا وبدرجة حرارة ٣٧ درجة مئوية لمدة تتراوح من ٢٤ الى ٤٨ ساعة. اشارت اختبارات الشكل العام والصفات الكيميائية الى ان ٤٨ عزله فقط مثلت بكتريا *Ps. aeruginosa*، بينما العينات الاخرى ١٠٤ كانت تمثل اجناس بكتيرية اخرى. اجريت اختبارات المضادات الحياتيه على عزلات بكتريا السيدوموناس بطريقة انتشار الاقراص فوجد انها كانت مقاومه بنسبه (١٠٠%) لكل من مضادات الامبسلين، السيفوتاكزيم، الكلورمفينيكول، البنسلين، الدوكسيسايكلين والارثرومايسين، بينما كانت مقاومه للاميكاسين بنسبه (٣٩,٥%)، سيبروفلوكساسين بنسبه (٣١,٢٦%) والبولي مكزين بنسبه (٤٠%). كما ان هذه العزلات كانت حساسة للبيبراسيلين والتيكارسيلين بنسبة(٧٩.92). و وجد ايضا ان هذه البكتريا تمتلك مجموعه البينالاكتام الانزيميه بنسبه عاليه للمقاومه (٦٦,٦%).

Introduction

Ps. aeruginosa is a bacterial pathogen that causes a substantial portion of hospital infections. It is frequently multi drug resistant, which contributes to the high morbidity and mortality of patients in intensive care units (ICUs), burn units and surgery wards[1]. It is also the common cause of chronic lung infections contributing to the death of patients with cystic fibrosis [2]. The major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics, such that even for the most recent antibiotics, a modest change in susceptibility can thwart their effectiveness [1,2]. Nosocomial isolates of *Ps. aeruginosa* were found in high proportion of resistance to a

new advanced – generation fluoroq-uinolone with a high potency and a broad spectrum of antimicrobial activity, Sitafloxacin, in European hospitals [2,3]. There are three classes of antibiotic resistance in *Ps. aeruginosa*: intrinsic resistance, acquired resistance and genetic resistance(3). Another part of the resistance appears to be caused by two recently discovered multidrug efflux systems[4]. In addition, in some cases, enzymes that specifically inactivate antibiotics lead to antibiotic resistance, e.g. The inducible β – lactamase of *Ps. aeruginosa* acquired resistance involves the induction of unstable resistance without any observable change in genotype because of exposure of a strain to a set of inducing conditions that can include antibiotic exposure [1,3].

Materials and Mmethods

Patients

The study included 152 samples were taken at Hilla General Teaching Hospital during a period of four months from (October 2010 to January 2011). The patient's age ranged from (6-65) years.

Specimen collection:

One hundred fifty two swab specimens were collected from different sites , 107 specimens from infected patients with infections (burns, wounds, urine, blood, otitis media, seminal fluid) and 45 samples from hospital environment like(patient beds, disinfectants and catheters) . Information about their age, and antibiotic usage was taken into consideration. Each swab taken carefully from the site of infection and placed in tubes containing readymade media to maintain the swab wet during transferring to laboratory. Each specimen was inoculated on *Pseudomonas* isolation agar[5]. 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. The *Ps. aeruginosa* isolates were identified according to biochemical tests that recommended by [5].

Antibiotic susceptibility tests:

The susceptibility of *Ps .aeruginosa* isolates were determined by two methods: antibiotic disk diffusion method and rapid iodiometric method as follows:

Antibiotic disk diffusion method:

The standardized antibiotic disk method was carried out [6]. The end point, measured to the nearest millimeter was compared with zones of inhibition determined by CLSI[7] and to decide the susceptibility of bacteria to antimicrobial agents, whether being resistant or sensitive.

Detection of β-Lactamase production:

This test was performed for all isolates that were resistant to β-lactam antibiotics, according to Rapid Iodiometric Method [8].

Results and Discussion

Isolation and identification of *Pseudomonas aeruginosa*:

Out of the 152 samples, only 48(31.57%) isolates were belonged to *Ps. aeruginosa*, while other 104(68.43%)isolates represented other bacterial genera. The most isolates were obtained from burns 13(8.55%), catheters 7(4.61%), wound 6(3.95%), ear swab 5(3.30%), beds 4(2.63%), disinfectant 3(1.97%), 3(1.97%) isolates from each urine and blood, and 2(1.31%) from each sputum and seminal fluid. The results are shown in table (1). The preliminary cultural diagnosis selective media is Pseudomonas isolation agar (PIA) for

Pseudomonas aeruginosa which appear circular mucoid smooth colonies with emits sweat grape odor , and then cultured on blood agar . Most isolates appear β-hemolysis on blood agar while others isolates were non hemolysis. All isolates grew on MacConkey agar, but did not ferment lactose sugar. All the isolate grew on the Muller- Hinton agar which produce the diagnostic pigment. The pigment varied from yellowish-green to bluish-green and also the isolates produced a sweat grape-like odor. In this study the biochemical tests were carried out and the result compared with standard result documented by [5]

Table1 Type of samples & numbers of *Ps. aeruginosa* isolates.

Sources of S.	Types of S.	Sample No.(%)	Positive No.(%)
Clinical Samples	Burn	27(17.76)	13(8.55)
	Wound	22(14.47)	6(3.95)
	Urine	15(9.87)	3(1.97)
	Blood	7(4.61)	3(1.97)
	Sputum	7(4.61)	2(1.31)
	Ear	19(12.50)	5(3.30)
	Seminal	10(6.58)	2(1.31)
	Total	107(70.40)	34(22.36)
Environmental Samples	Disinfectant	14(9.21)	3(1.97)
	Beds	14(9.21)	4(2.63)
	Catheter	17(11.18)	7(4.61)
	Total	45(29.60)	14(9.21)
	Total No.	152(100)	48(31.57)

Antibiotic susceptibility of *Ps. aeruginosa*:

Disk diffusion method

Regarding the antibiotics it has been found that *Ps. aeruginosa* was fully resistance (100%) to Cefotaxime, chloramphenicol, penicillin, ampicillin, doxycycline, erythromycin, tetracycline, cloxacillin 100%, while, some isolates showed less resistance to Azithromycin (81.25%), Gentamicin (70.83%), Polymyxine B (60.41%), Amikacin (39.58%), Norfloxacine (37.5%), Ciprofloxacin (31.25%) , Pipracillin and Ticarcillin (20.08%) as shown in table (2). All isolates revealed resistance to β-lactam group penicillin and cephalosporin. The results showed that (100%) of isolates were resistant to Ampicillin and penicillin, these results were nearly compatible with that of [9,10] that found all isolates were resistant to amoxicillin, also all isolate revealed resistance to Cefotaxime (100%). These results agreement are in with that results obtained by [11] who found that (95%) of isolates were resistant to Cefotaxime. High resistance of these isolates to the β-lactam antibiotics may due to their production of beta-lactamase enzymes that breakdown the beta-lactam ring and render it inactive. This is mediated by extra-chromosomal piece of DNA (plasmid), or by decreasing membrane permeability towards the antimicrobial agents [12].

Table 2 Antibiotic resistance and sensitivity of 48 isolates of *Ps. aeruginosa.*

Antibiotic	Clinical		Environmental		Total No. resistant isolates	Resistance %
	R	S	R	S		
Cefotaxim	34	0	14	0	48	100
Chloramphenicol	34	0	14	0	48	100
Azithromycin	26	8	13	1	39	81.25
Gentamicin	24	10	10	4	34	70.83
Ciprofloxacin	10	24	5	9	15	31.25
Penicillin	34	0	14	0	48	100
Ampicillin	34	0	14	0	48	100
Doxycycline	34	0	14	0	48	100
Erythromycin	34	0	14	0	48	100
Tetracyclin	34	0	14	0	48	100
Norfloxacin	12	22	6	8	48	37.5
Cloxacillin	34	0	14	0	48	100
Polymyxin B	21	13	8	6	29	60.41
Amikacin	13	21	6	8	19	39.58
Pipracillin	9	25	4	10	13	20.08
Ticarcillin	10	24	3	11	13	20.08

Resistance mediated by *P. aeruginosa* can be attributed both to an inducible, chromosomally mediated beta-lactamases that can render broad–spectrum cephalosporin inactive, and to a plasmid–mediated beta- lactamases that can lead to resistance to several penicillin and older cephalosporin [13]. The results also showed resistance to Aminoglycoside in different percentage. These results are in agreement with those results obtained by [14]. The mechanisms of bacterial resistance to aminoglycoside antibiotics in clinical isolates is usually controlled by enzymatic inactivation of the antibiotic, since nine different enzymes that catalyze the phosphorylation, acetylation, coradenylylation of aminoglycosides have now been identified in bacteria [15]. The synthesis of aminoglycoside modifying enzymes is often controlled by R-factors, permitting rapid spread of resistance among genetically compatible populations of bacteria exposed to appropriate selective conditions [16]. An independent mechanism for resistance to aminoglycosides, impermeability of the cell to the antibiotic, has also been reported in some cases of resistance to gentamicin in *Ps. aeruginosa.* It has been reported to have an innate resistance to several antibiotics due to the presence of lipopolysaccharides in the outer membrane, but persistent administration of antimicrobial agents, has resulted in the emergence of multi-resistant strains of *Ps. aeruginosa* [17]. This acquired resistance is characteristic of high-level resistance to almost all aminoglycosides but more importantly to the clinically used Tobramycin, Netilimicin and specifically gentamicin [18] and in the ciprofloxacin and norfloxacine. The result is agreement with result obtained by [19], who reported the flour quinolones have more effective on *Ps.*

aeruginosa. The main mechanisms of resistance are mutations in the target genes [20]. All isolates also were resistant to tetracycline and these result agreements with result obtained by [21] who found that all isolates were resistant to tetracycline. All isolates (100%) were resistant to macrolides but in low degree, but these result do not agree with the result obtained by [22] who found high percentage of isolates sensitive to macrolides. The evolution of multi-resistant *Ps. aeruginosa* and its mechanisms of antibiotic resistance have been examined. Primary mechanisms include reduced cell permeability, efflux pumps, changes in the target enzymes and inactivation of the antibiotics [23, 24].

Detection of β-Lactamase production:

In the present study (table -3) 66.66% of isolates have β-lactamase and this result does not agree with [25] who found that only (11.66%) of *Ps. aeruginosa* have β-lactamase enzyme, but agreement with [26] who found that of the isolates (84.4%) have this enzyme.

This method depends on the detection of penicilloic or cephalospoic acid, resulted from breakdown of amide bond in β-lactam ring for each of Penicillins or Cephalosporins [27,28]. Iodine reacts with starch to form dark blue complex, which stays without changes in the absence of β-lactamase enzymes. In the case of β-lactamase-producing bacteria, the resulting penicilloic or cephalospoic acid will reduce iodine into iodide; consequently, decolorization of starch-iodine complex occurs (changing the color directly to white) if an isolates a β-lactamase producer [28].

Table 3 Beta-lactamase enzyme in 48 isolates of *Ps. aeruginosa*.

Type of samples	Positive No.(%)	Negative No.(%)	Total No.(%)
Clinical	22(64.71)	12(35.29)	34(100)
Environmental	10(71.43)	4(28.57)	14(100)
Total	32(66.67)	16(33.33)	48(100)

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