Isolation and Diagnosis of Multi Drug Resistance *Pseudomonas Aeruginosa* from Wound and Burnpatients in Baghdad City

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Abstract

Background: *Pseudomonas* is a common bacteria found all over the world; in soil,water, and plants, and it is one of the most common pathogens in hospital-acquired infections.

Aims: The aims of this study were isolation of *P. aeruginosa* bacteria from patients with inflammation of burns, Diagnosis and identification of *P. aeruginosa* using chemical tests and VITEK2 system and alsostudy of antibiotic resistance in *P. aeruginosa* using the VITEK2 system.

Method: (206) swabs were collected from wounds and burns; (139) samples from burns and (67) samples from wounds; from different clinical cases for both sexes and ages (1-70) years, the patients coming and sleeping in Baghdad Teaching Hospital and Burns Hospital in the City of Medicine at Baghdad city; the duration from January to the end of March 2019. Samples were cultured on the variety of culture media (MacConkey agar, Blood agar and Cetrimide agar) in order to obtain the bacterial isolates of *P. aeruginosa* depending on their phenotypic characteristics.VITEK2 system were used for identification *P. aeruginosa*and to study their resistance to the antibiotics.

Results: Out of the 206 samples, 50 *Pseudomonas aeruginosa* were isolated from swabs. 31(62%) isolates were isolated from burn and 19 (38%) from wound swabs. The isolates were subjected to a series of biochemical tests as diagnosed with Api 20E; and VITEK2 system to increase confirmation of isolation yield for *P. aeruginosa* bacteria. The results showed that the majority of isolates were (92%) resistant to Amoxicillin while the isolates differed between sensitive and moderate sensitivity and resistance to other types of antibiotics.

Conclusion: The study showed that the percentage of isolation of *Pseudomonas aeruginosa* bacteria from wound infections is relatively higher than the rate of isolation from burn swabs. Most of *Pseudomonas aeruginosa* isolates showed high resistance to most types of antibiotics used in the present study, especially the antibiotic Amoxicillin where the resistance rate was 92%.

Keywords: *Pseudomonas aeruginosa; VITEK 2 system; resistance to antibiotics.*

Introduction

P. aeruginosa is a Gram-negative and ubiquitous environmental bacterium. It is an opportunistic human pathogen capable of causing awide array of life-threatening acute and chronic infections, particularly inpatients with compromised immune defense. It has been of particular importance since it is the main cause of morbidity and mortality in cysticfibrosis (CF) patients and one of the leading nosocomial pathogens affecting hospitalized patients while being intrinsically resistant to a widerange of antibiotics[(1)].

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This Gram negativebacteria structure includes a 0.5 – 0.8 μm by 1.5-3 μm rod shape, and has one flagellum for mobilization. Individualizing itself from most Gram-negative bacteria, *P. aeruginosa* is positive foran oxidase reaction. Moreover, it is permanently unable to fermentlactose. *P. aeruginosa* is found in water, plants, soil, and on theepidermis of animals. In nature, it is commonly found as a plankton swimming through water or as a biofilm, clusters of bacteria sharing thesame phenotype and common chemical properties[2]. Uniquely, *P. aeruginosa* can thrive and survive in a variety of temperature and infrequent nutrition. The bacterium has been observed in previous studies to grow in distilled water giving *P. aeruginosa* an advantage in adapting to changing environments[3]. Being an opportunistic pathogen, *P.aeruginosa* requires a lack of immunity to infect its host[4]. Moreover, this is the explanation as to why *P. aeruginosa* is such asizeable nosocomial threat for patients with ventilation machines,cancers, and burns. Colonization of *P. aeruginosa* in the respiratory tractis associated with sepsis and death. Any patient that is immunosuppressed or has experienced significant amounts of trauma are at risk for thecolonization of an infection. The mortality rate approximates 50% forsaid patients who obtain an infection[5][6]. The aims of this study were isolation of *P. aeruginosa* bacteriafrom patients with inflammation of burns, diagnosis and identification of *P. aeruginosa* using biochemical tests and VITEK2 system and alsostudy of antibiotic resistance in *P. aeruginosa* using the VITEK 2 system.

Materials and Method

I. Collection of Samples: 206 swab samples were collected from clinical sources including 67 burns and 139 wounds from patients suffering from burns and wounds infections and under the supervision of a medical specialist at City Medicine Hospital/ department of burns in Baghdad city, in the period beginning from January to the end of March 2019.

II. Identification of bacterial isolates:

A. Morphological examination: Blood agar, MacConkey agar and Cetrimide agar were used to study the phenotypes of *P. aeruginosa* colonies which including colonial form, shape and color, size, and aroma. Readymade media were used, Nutrient Broth, Mueller- Hinton agar medium, Brain heart infusion broth, *Pseudomonas* agar, Voges - Proskauer reagent, Urea agar medium, King A medium, King B medium and Indole test medium[7].

B. Microscope examination: The Gram stain was used for identification of *P. aeruginosa* in the samples[8].

C. Chemical Tests: The following biochemical tests were performed for the diagnosis of isolated *P. aeruginosa* bacteria: Catalase test, Oxidase test, Motility test, IMVIC test (Indole test, MR-VP, Simmons' citrate agar, Citrate utilization test)[7][8], and API 20E identification system.

D. API 20E identification system: API 20E is standardized system for identification the Enterobacteriaceae and other non- fastidious, by depending on using 20 biochemical tests. It was done according to the instructions of BioMerieux**.**

E. Determination of minimal inhibitory concentration of antibiotics using the VITEK2 compact system: The isolates were identified as *P. aeruginosa* by conventional method as well as by the VITEK2 system[9]. The VITEK2 is a fully automated system that performs bacterial identification[10].In VITEK2 compact system, the Antibiotic Sensitivity Test Card (AST C) Supplement 2 was used to determine the values of the (MICs) for *P. aeruginosa* isolates. There are 18 to 20 antibiotics distributed over 64 holes in the Antibiotic Test Card. The device recorded the turbidity changes following the growth of the bacteria, this is done according to the instructions of BioMerieux.

F. Antibiotic susceptibility test (AST): The sensitivity of bacterial isolates to many antibiotics was studied according to the Kirby-Bauer method and WHO[11][12].

Results and Discussion

The study included the collection of 206 swabs of burn injuries and wounds of both sexes, males and females, ranging in age from 1-70 years for the period from January to the end of March 2019, from burns hospital in the city of medicine and Baghdad Teaching Hospital. After the final diagnosis of samples obtained 50 isolates of *P. aeruginosa* (24.27%) were shown in Table 1.

Table 1: Number and percentages of *P. aeruginosa* isolates according to the source of isolation by using different culture media

Number of P. aeruginosa isolates (%)	Number of samples (%)	Source of isolate
19 (38)	67 (32.52)	Wounds
31 (62)	139 (67.47)	Burns
50 (100)	206 (100)	Total

On MacConkey agar, the bacterial colonies appeared pale yellow because they had not fermented lactose, and this compatible with the results of previous researches[10]. On Nutrient agar, the growing of *P. aeruginosa* colonies were identified depending on the pigments and odor production (grape like odor). On the blood agar medium, the bacterial colonies gave Beta-hemolysis (β). On the Cetrimide agar, the bacterial colonies appeared in greenish yellow, on the King A agar, they produced a blue and green pigment (pyocyanin), while all isolates grow on King B agar did not produce pyocyanin.

Microscopic examination showed that they were Gram negative bacilli. In the biochemical tests, all isolates showed positive results for Catalase test, which explained the bacterium's ability to break down hydrogen peroxide into water and oxygen gas. The score IMViC tests was Indole (-), Methyl red (-), Voges-Proskauer (VP) (-), positive (+) result for Citrate consumption, as shown in Table 2. Kligler's Iron Agar (KIA) have alkaline interaction (cannot ferment glucose and lactose), H_2S production (-). Growth temperature is 37°C and 42°C, and negative results were obtained for urease test; they were positive for motility test because the bacteria have flagella, these results corresponds positive for motility test because the bacteria have flagella, these results corresponds to[11][12].

Table 2: Morphological, physiological and biochemical results for *P. aeruginosa* identification

Tests		Test	Result
Microscopic		Gram stain	-Ve, bacilli
Growth	Selective media	MacConkey agar	Non Lactose fermented
		Cetrimide agar	+
		King A agar	pyocyanin
	Enriched media	Blood agar	β-hemolysis
Physiological		Grow at 37 °C	-
		Grow at 42 °C	+
Biochemical		Catalase	+
		Oxidase	+
		Urease	+/- (V)
		Indole	-
		Methyle red	-
		Voges proskauer	-
		Citrate utilization	+
		Kligler iron agar	k/k (slant and bottom)

+: positive; - : Negative; V: Variable; K: alkalin

All isolates of *P. aeruginosa* showed a clear variation in the resistance of the antibiotics used in this study. The resistance rate determined against Gentamicin was 81%, Ciprofloxacin was 79%, Tetracycline was 74%, and Nalidixic acid and Norfloxacin were 72%, Imipenem 66%Figure 1. The results showed that the minimum inhibitory concentration (MIC) of the antibiotic: the VITEK2 Compact system was used to determine the values of the lowest inhibitory concentrations (MICs) for 13 antibiotics namely Ticarcillin, Piperacillin/ Tazobactum, Ticarcillin/clavulanic acid, Piperacillin, Cefepime, Gentamicin, Imipenem, Meropenem, Tobramycinin, Ciploxidacin, Ciprofycin. The results showed that the value of the MIC of Ticarcillin antagonist is 32 µg/ml at 87%, anti-Piperacillin/Tazobactum 8-16 µg/ml at 85.5%, Ticarcillin/clavulanic acid 16-64 µg/ml at 85.5%, anti-Pipracillin ≥ 4 -16 µg/ml 85.5%, Anti-Cefepime 2-8 µg/ml 76.8%, Gentamicin ≤ 1-4 µg/ml 73.9%, Imipenem 0.5-2µg/ml 71%, and anti-Meropenem ≤ 0.25 µg/ml 63.8%, anti-Tobramycin ≤ 1-2 µg/ml 71%, anti-ciprofloxacin ≤ 0.5-0.25 µg/ml 68.1%, anti-Amikacin ≤ 8-2 µg/ml 60.9%, anti-ceftazidime 2 -8µg/ml 60.9% were shown in Table 3.

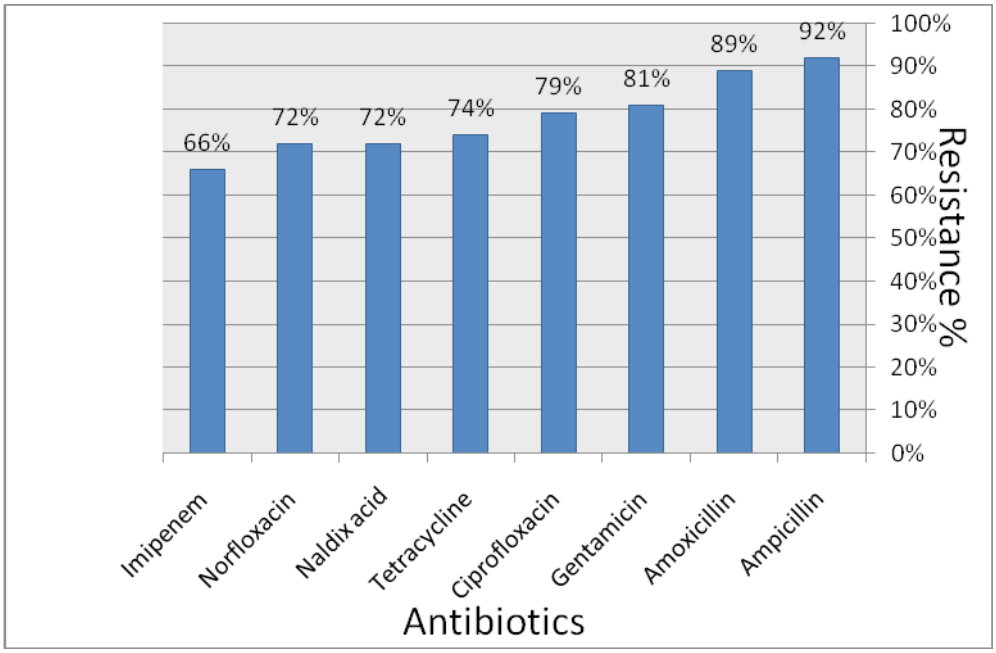

Figure 1: Resistance of *P. aeruginosa* isolates to antibiotics

Table 3: Minimum Inhibitory Concentration Values (MIC) (µg/ml) for some antibiotics used against *P.aeruginosa* bacteria isolated from wounds and burns using VITEK 2

Antibiotics	Resistance		Intermediate		Sensitive	
	No. of sample	%	No. of sample	%	No. of sample	%
Ticarcillin	30	87	0	0	5	13
Pipracillin/Tazobactam	29	85.5	1	1.5	4	13
Ticarcillin/Clavulanic acid	29	85.5	0	0	5	14.5
Pipracillin	29	85.5	1	1.5	5	13
Cefepime	26	76.8	1	1.4	8	23.1
Gentamicin	26	73.9	1	1.4	9	26
Imipenem	25	71	0	0	10	29
Meropenem	22	63.8	1	2.9	12	33.3
Tobramycin	25	71	0	0	10	29

Antibiotics	Resistance		Intermediate		Sensitive	
	No. of sample	%	No. of sample	%	No. of sample	%
Ciprofloxacin	23	68.1	0	0	11	31.9
Amikacin	21	60.9	0	0	14	39.1
Ceftazidime	22	63.8	2	7.2	10	29

A percentage (%) of the total isolates were calculated.*

The current results reports that the percentages of *P. aeruginosa* in both wounds and burns swabs are 50 isolates of (24.27%), and this result is higher than in other Iraqi results of researchers. Zainulabdeen in 2016[13]recorded that the percentage of *P. aeruginosa* in patients suffering from burn and wound infections in AL-Samawah General and teaching Hospitals in the South of Iraq was (8.2%) between 2014 and 2015. Whereas, in the North of Iraq, Kirkuk governorate, the percentage of *P. aeruginosa* in burns was (32.55%)[14].

The difference in isolation rate may be due to the degree of health care, which includes the arrival of the patient to the hospital before bacterial growth or the possibility that the patient had taken antibiotics in advance, and on the other hand may lead to a long hospitalization in the hospital increase the rate of isolation, especially in patients with burns. The intensity may increase to 20% within 72 hours as a result of an infection acquired from hospital infection and nosocomial infection directly as a result of contact with patients or indirectly through the use of contaminated surgical instruments and tools that help to spread these bacteria[14].Also, the reasons for the difference in isolation rates are due to the difference in the sample source, number of samples, geographical location, and method of antisepsis of wounds or burns, frequency of antisepsis, as well as the common and random use of antibiotics which had a significant role in the emergence of resistance to these bacteria.

Recent study showed that *P. aeruginosa* isolates were 100% resistant to (Amoxicillin, Ampicillin), 93% to the (Cefotaxime, Carbenicillin and Imipenem)[15]; this compatible to the current results. In Baghdad city, AL-Shamaa et al. 2016 [16] were isolated 31 *P. aeruginosa* isolates from 111 burn and wound swabs. All isolates were resistance 100% to Carbenicillin, 61% Ticarcillin, and Colistin to 84%. In Pakistan, the most common bacterial isolate from burnt patients was *P. aeruginosa*; it was 41 isolates (24.91%). All isolates showed highly resistance to different antibiotics including Augmentin, ceftazidime, cefotaxime, ceftriaxone, meropenem, and piperacillin+tazobactam [17]. In United States, most *P. aeruginosa* isolates had resistance to Penicillins, Cephalosporins, and Carbapenems[18].

It is noted that the results of the present study recorded high resistance of bacterial isolates towards the group Penicillin- β -lactam including Ampicillin and Amoxicillin as these antagonists work to prevent the synthesis of the bacterial cell wall by binding to special sites in the bacterial cell and thus inhibits the work of the enzyme transpeptidase which forms peptide bridges in the peptidoglycan layer, which is an important component of the cell wall. The results of the present study showed that the resistance of *P.aeruginosa* bacteria to the antibiotic Ampicillin and Amoxicillin, which isa medium-spectrum antibiotic, was very high (92 and 89%, respectively), this ratio is high and this result is consistent with the result obtainedby Al-Qasi, 2012[19]. The results of antibiotic resistance were consistentwith those of Reish*et al*., 1993[20]; it was 90.47%. In another study, allisolates of *P. aeruginosa* were resistant to penicillin and amoxicillin[21].It is a clear that the most important characteristic of *P. aeruginosa*bacteria is their low sensitivity to antibiotics. In addition to the lowpermeability of the cellular membranes of bacteria and the ability of *P.aeruginosa* bacteria to develop their readily acquired resistance either bymutation in coded genes or by horizontal transmission of antibiotic-resistant gene markers. The multiple resistance of manyantibiotics may be attributed to different virulence factors of bacterialisolates that increase resistance to different antibiotics[21].

Conclusion

The study showed that the percentage of isolation of *Pseudomonasaeruginosa* bacteria from wound infections is relatively higher than the rate of isolation from burn swabs, reaching 28.35%. Most isolates of *P. aeruginosa* showed high resistance to most types

of antibiotics used in the present study, especially the antibioticAmoxicillin where the resistance rate was 92%. Also Imipenem was the most effective antibiotics against isolates of *P. aeruginosa* bacteria with a resistance rate of 66%followed by Nalidixic and Norfloxacin with a rate of 72%.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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