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Study of the resistance of *P. aeruginosa* isolated from wounds and burns for some disinfects and antiseptic from some Baghdad hospitals

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Abstract

A total of 69 isolates of *P. aeruginosa* were obtained after morphological and microscopical investigation, and these isolates were collected from different clinical sources including wounds 24 (35%) and burns (65%). The resistance of *P. aeruginosa* isolates was tested for 10 antibiotics and the results showed a resistance to Ceftazidime (81%), Ceftatzxime (78%), Pipracillin (75%), Tobramycin and Ciprofloxacin (74%), Gentamicin (72%), Amikacin Meropenem (70%), Ofloxacin (66%) and Imipenem (65%). Minimum inhibition concentration (MIC) for antibiotics was determined using the phytic device; MIC value of Ticarcillin was 32 (88%), Piperacillin/Tazobactum 8-16 (85%), Ticarcillin/Clavulanic Acid 16-64 (85%), Pipracillin ≥4-16 (85%), Cefepime 2-8 (77%), Gentamicin ≥1-4 (74%), Imipenem 0.5-2 (71%), Meropenem ≥0.25-2 (71%), Tobramycin ≥1-2 (71%), Ciprofloxacin ≥0.25-0.5 (68%), Amikacin ≥2-8 (61 %), Ceftazidime 2-8 (49%) and Colistin ≥0.5-2 (7%).

The minimum inhibitor concentration (MICs) for the disinfectants was determined using dilution with agar method. The isolates in this study exhibit high resistance to quaternary ammonium compounds such as Cetrimide (128-512) µg/ml, Chlorohexidine disinfectant (64-256 µg/ml), Povidone-iodine disinfectant (32 µg/ml) and low resistance was seen with Acetic acid disinfectant (16 µg/ml). Moreover, the current study observed that QacE is an important gene that responsible for the resistance of *P. aeruginosa* to hospital disinfectants; it is found in the isolates with 98%.

Keywords: - *P. aeruginosa*, resistance to antiseptics disinfectants.

INTRODUCTION

The skin in the human is the main layer that provides the natural protection of the body tissues from the invasion of various microorganism, and the occurrence of burns or injury in the skin can lead to damage and destruction of these tissues and may occur infections of bacteria transmitted to the blood and internal tissues, which is a proteins-rich environment and encourage the growth and reproduction of microorganisms that play a major role in the pathogenicity and mortality [1].

P. aeruginosa is an opportunistic bacteria and one of the top five most important Noscomial pathogens worldwide. It represents as a major risk to the patients with critical conditions, especially patients with burns, cancer and immunodeficiency diseases, and also associated with high incidence of mortality with 75% [2]. *P. aeruginosa* has a high ability to invade the tissues and produce the toxins, and thus causes a complicated infection [3]. The presence of *P. aeruginosa* on the surface of the skin making it easy to penetrate through it and thus cause many inflammation of the burns, wounds, and urinary tract infection, Otitis media, eye infection, inflammation of the brain membranes (Meningitis), endocardinig, respiratory infection such as Pneumonia especially in people with cystic fibrosis patients, bacteraemia, bonic and joints infection, gastrointestinal infection and skin and soft tissue infection, [4, 5].

P. aeruginosa is known with high resistance to multiple antibiotics and disinfectants, and this resistance has become a common problem especially in the patients who are in the hospital [6]. The resistance of *P. aeruginosa* to disinfectants and antibiotics is due to the presence of Qac genes including QacE gene, which gives resistance to these bacteria via Efflux system, as well as to the a reciprocal relationship between QacE gene and the coded genes to resist bacteria for multiple antibiotics [7]. This gene is located in the integron class 1 which allows it to transport between the plasmid and the chromosome [8].

The aim of this study is to isolate and diagnose *P. aeruginosa* from wounds and burns samples with the study of the sensitivity of bacteria to several antibiotics, and also to determine MICs for both antimicrobials and disinfectants that used in this study; moreover detection of QacE gene that responsible for the resistance of these bacteria to disinfectants.

MATERIALS AND METHODS

1. Specimen collection of patients

A total of 100 samples of burns and wounds were collected from both males and females aged 1-70 years from Al-Kindi Teaching Hospital, Al-Yarmouk Teaching Hospital, Al-Harouq Hospital,Baghdad Teaching Hospital (Educational Laboratories) from the period 16/10/2017 to 30/12/2017.

2. Isolation and diagnosis of *Pseudomonas aeruginosa*

The samples were culture on the Cetrimide agar medium, MacConkey agar, and the blood agar medium. Biochemical tests (oxidase and catalase) were performed for the final detection of isolates using API20E system according to the instruction by BioMerieux Company.

3. Antibiotic Susceptibility Test

The sensitivity test was done using 10 antibiotics according to Kirby Bauer method using Muller-Hinton agar according to [9]. This test used tablets of the following antibiotics:-

Amikacin (10mg), Ceftazidime (30mg), Cefotaxime (30mg), Ciprofloxacin (10mg), Gentamicine (10mg), Imipenem (10mg), Meropenem (10mg), Ofloxacin (10mg), Pipracillin (100mg), and Tobramycin (10mg).

The measurement of the inhibition diameter (mm) zone around the disks od antibiotics tablets and were compared to the global scale of measurement according to [10].

4. Determination of MICs using the phytex system

Using Vitek 2 Compact system, minimum inhibitory concentrations (MICs) was determined for 13 antibiotics through AST card of sensitivity test according to [11] method, and following the instruction of BioMeriex Comparin. Antibiotics that included:-

Amikacin, Cefepime, Ceftazidime, Ciprofloxacin, Colistin, Gentamicin, Imipenem, Meropenem, Pipracillin, Pipracillin\Tazobactam, Ticarcillin, Ticarcillin\ Clavulinic acid and Tobramycin.

5. Determination MICs for chemical disinfectants

The Agar Dilution Method was used to calculate MICs of the following disinfectants [12]:-

Acetic acid (4-6)% Al-Badawy-Iraq, Cetrimide (0.06%) Steritech-Lebanon, Chlorhexidine (4%), AlFayhaa-Iraq,

Povidone-Iodin (10%), BETADIX- Turkey and Sodium Hypochlorite (6%) FAS-Iraq.

6. Detection of QacE gene that responsible for the resistance of *P. aeruginosa* to disinfectants using PCR

Primers were designed using the Primer 3 plus program from the NCBI website.

Table 1: The primers used in the study

Gene		primer sequence(5´-3´)	Product size	Source
QacE	F	ATGACCAACTATCTCTACCT	311	The primer was designed in this study
	R	AACAACTGGATCACCAGCA		

PCR procedure was done by AccuPower® PCR PreMex (Bioneer, Korea). The optimum conditions for the detection of this gene were one cycle for 5 minutes at 95°C for primary DNA denaturation and 30 cycles for 30 seconds at 95 °C for DNA denaturation, 30 s at 55° C so for primers annealing to DNA, 45 s at 72°C to elongate bounded primers and then only one cycle for 5 minutes and at 72°C for final elongation of DNA strand, then 5µl of multiplied DNA was transferred to the gel electrophoresis system using 2% of agaros gel with 100 volts voltage for 60 minutes.

RESULTS AND DISCUSSION

Insulation and diagnosis

After the laboratory diagnosis, 69 isolates of *P. aeruginosa* were obtained from 100 samples (69%) from clinical sources including wounds 24 (35%) and burns 45 (65%) as shown in Table 2.

Table 2: the Number and percentage of *P. aeurginosa* isolated from wound and burns infections.

Source of isolates	No. of isolates	No. of *P. aeurginosa* isolates	Percentage (%)
Wounds	35	24	35
Burns	65	45	65
Total	100	69	100

P. aeurginosa bacteria are a common cause of burns inflammation because they live in the humid environment; it is found in the water and soil, in the skin and intestines with small numbers, and in the wet environment in the hospitals. Therefore, they are the main causes of both burns and wounds [13]. The current study is similar to the studies of [7, 14, 15].

The results of this study showed a resistance of isolates to antibiotics with different rates as following (Table 3):-

Ceftazidime (81%), Cefotaxime (78%), Pipracillin (75%), Ciprofloxacin, Tobramycin (74%), Gentamicin (72%), Amikacin, Meropenem (70%), Ofloxacin (66%) and Imipenem (65%).

Table 3: The resistance of *P. aeruginosa* that isolated from wounds and burns to antibiotics

Antibiotics	No. of resistant isolates	Resistance percentage (%)
Amikacin	49	70
Cefotaxime	55	78
Ceftazidime	57	81
Ciprofloxacin	52	74
Gentamicin	51	72
Imipenem	46	65
Meropenem	49	70
Piperacilin	53	75
Ofloxacin	47	66
Tobramycin	52	74

The results of the present study are in agreement with previous study of [16] which identified the resistance of *P. aeruginosa* that isolated from the burns hospital in Isfahan (Iran) to the antibiotics Amikacin, Gentamicin and Imipenem in similar rates which was 73.3%, while ciprofloxacin was 50% and ceftazidime was 16.7%. Our results were in agreement with results of [17] that identified the resistance percentage of cefotaxime 84.36% and Gentamicin 83%. Moreover, the study of [13] in Egypt found that the resistance rate of cefotaxime was 86% which is consistent with the results of current study; although the resistance of ofloxacin was 34%, gentamicin 18%, while the isolates were completely resistant to piperacillin and imipenem (100%).

The study of [18] has been found that the isolates were resitand to Ciprofloxacin (74.62%), Gentamicin (71.43%) and was consistent with our results that shown a resistance to Piperacillin (91.30%), Tobramycin (56.52%). Additionally, the current results are in agreement with study of [19] which found that the resistance to Cefotaxime was 82%, and with [20] that observed the resistance to Amikacin was 64%. This study found that resistance to Piperacillin was 78.9% which is in consistent with [21] study and also with [22] that the resistance was to Gentamicin 74% and Tobramycin 79.3%.

The resistance of *P. aeruginosa* bacteria is due to antibiotics is due to the β-lactamase enzymes (Cephalosporinase, Penicillinase), which attacks the ring of β-lactam in the nucleus of the penicillins and cephalosporins that lead to the conversion of antibiotic into an inactive compound, these enzymes are encoded by genes found on the chromosome or plasmid . The random use of antibiotics increases bacteria resistance to antibiotics [23].

P. aeruginosa isolates were resistant to Aminooglycoside group, and this is due the production of modified enzymes by these bacteria such as Phosphotransferace and N-acetlytransferace, and the encoded genes of these enzymes are present on the chromosome or plasmid [24]. Moreover, the resistance is also occurs due to the changes in the membrane permeability and chromosomal mutations in the receptors of antibiotics on the ribosome [25].

The resistance of *P. aeruginosa* to quinolones and Ciprofloxacin that inhibits the action of DNA gyrase (Topoisomerase III) result in inhibition of DNA biosynthesis [26]. In addition, the structural changes in the membrane a of bacteria that causes absence of outside membrane porins, which transport the antibiotics inside the cells, may explain its resistance to antibiotics, as well as the presence of β-lactase, efflux system pumps and plasmid resistance [27].

MIC was investigated for the studied antibiotic and the results that MIC was for Pipracillin (2-16), Pipracillin\Tazobactam (8-16), Cefepime (2-8), Gentamicin (≥1--4), Meropeneme (0.25-2), Imipenem (0.5-2), Ciprofloxacin (≥0.25-0.5), Amicacin (≥2-8), Ceftazidime (2-8), Ticarcillin (32), Ticarcillin/clavulanic acid (16-64), Tobramycin (≥1-2) and Colistin (≥0.5-2).

This results in agreement with [28] study that identified the resistance of *P. aeurginosa* isolates isolated from wounds and burns for (Pipracillin/Tazobactam) and Gentamicin was 80%, Ciprofloxacin 75%, Meropenem and Imipenem 70% and Amikacin 65%. Furthermore, the results of the current study is consistent with [29] study that showed an increases in the resistance ratio of Ceftazideme (73.3%), Cefepime (61.6%), and also is consistent with [30] study, which was conducted in England and determined the resistance percentage of Ceftazideme (39%), and with [31], which identified the resistance of Tobramycin (79.6%) and Amikacin (65%). However, the difference in the rates of isolation of *P. aeurginosa* in local and global studies is due to several reasons including the source of isolation, geographical locations, dates of samples collection,

number of samples and the widespread and random using of antibiotics that significantly contributed to their distribution [32].

MICs for disinfectants was determined and the results were for Cetramide (128-512) µg/ml, Chlorhexidine (64 -256) µg/ml, Sodium Hypochloride (64-128) µg/m, Povidone-iodine (32) µg/ml, Acetic acid (16) µg / mL. As shown in Table (4).

Table 4: MICs value for chemical disinfectants used in the study

Disinfectants	MICs (µg/ml)
Cetramide	128-512
Chlohexidine	64-265
Sodium Hypochlorite	64-128
Povidone-iodine	32
Acetic acid	16

In recent years, there has been increasing the interest of resistant bacteria to antibiotics and disinfectants because of its importance in the hospital infections, including *P. aeruginosa*. The results of current study were in agreement with findings of [33] study, which used Acetic acid as an alternative to disinfectants to control the infection of these bacteria in the inflammation of wounds and burns because it is non-toxic, inexpensive and very effective in the concentration (0.5-5)%, as well as with [34].

Our results of chlorhexidine were consistent with [35, 16] in MICs value which was 256 µg/ml, and with [36] in Turkey which found that Chlorhexidine 4 % was effective against the strains of these resistant bacteria. For Sodium hypochlorite disinfectant, the result was in agreement with [37] that concluded to the importance of using sodium hypochlorite (5.25%) as a potent oxidative agent, a broad-spectrum disinfectant against bacteria, as well with [38, 39] in the using of this disinfect in the Killing these bacteria, which make up the biological membranes and their role in the high rates of pathogenicity and mortality.

Cetrimide, a quaternary ammonium compound (QAES), and its results were similar to results of [7, 37] which confirmed that the resistance of *P. aeruginosa* to quaternary ammonium compounds. Povidone-Iodin disinfectant showed efficacy against these bacteria; it is consistent with [40], which found a high efficiency and wide spectrum of this disinfectant in the killing of *P. aeruginosa* isolated from wounds and burns. The isolates of these bacteria exhibit different resistance to disinfectants that used in this study and the reason is to the type and concentration of disinfectant; the resistance of these isolates for chemical disinfectants is represented by mutations that occur during metabolism or to the acquisition of resistance genes from plasmides, Transposns or Efflux system [36]. In addition, the intrinsic resistance of these bacteria represented by external layer containing lipopolysacchrides that prevent the entry of these disinfectants into the bacterial cell or due to a defect in the mechanism of disinfecting dilution result in the failure of disinfect action and then promote the growth of resistant bacteria to antibiotics, which makes the hospitals and health centers as sites to spread infection [41].

QacE gene expression, a responsible gene for the resistance of *P. aeruginosa* to disinfectants, was detect by using PCR technique in *P. aeruginosa* isolates, and the results detected that 97.1% of collected isolates were positive to QacE gene expression. These results were consistent with the findings of [7, 1] studies that confirmed QacE gene was found in *P. aeruginosa* bacteria and it is the responsible gene for the resistant of bacteria to antibiotics and disinfectants with percentage of 42.3% and 50% respectively (Figure 1).

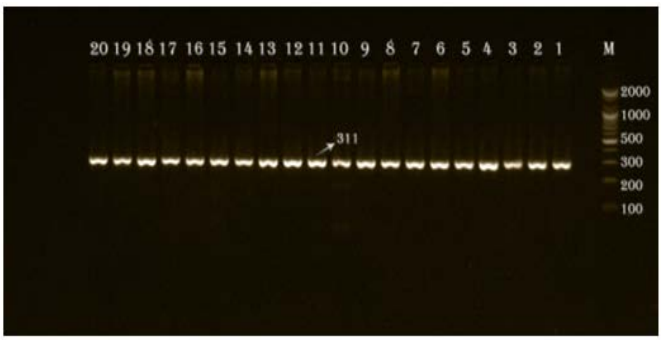

Figure 1: the electrophoresis of QacE gene produced by PCR technique. The product size is 311 bp of *P. aeruginosa.*

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