PLASMID ISOLATION FROM PSEUDOMONAS AEUREGINOSA THAT ISOLATED FROM BURNING PATIENT

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ABSTRACT

This study lasted for eight months. The main objectives of this study were to isolate the bacteria from burned patient. The relationship between their resistance to antibiotics and their plasmid contents were also studied. Seventy burn wound swabs were taken from burned patients, who presented invasive burn wound infection, from both sex, and average age 3-58 years, admitted to burn unit of teaching medical Al-Kendi hospital. The most preferred areas were the upper and lower extremities. Data revealed that 47.17% were contaminated with Pseudomonas aeruginosa. Antibiotic sensitivity test for different antibiotics revealed that most Pseudomonas aeruginosa isolates were moderately resistant to ciprofloxacin (68.18%), and (54.55%) resistant to Amikacin, whereas the resistance was more marked with other antimicrobials like Doxycycline (72.3%), Oxytetracycline (72.3%), Ticarcilin (68.18%) and Gentamicin (68.18%). The extraction of DNA-Plasmid revealed that all Pseudomonas aeruginosa isolates contained large, individual plasmid.

KEYWORDS: Pseudomonas aeruginosa, Burn Patient, Plasmid Isolation, Antimicrobial Resistant

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic human pathogen characterized by an intrinsic resistance to a variety of antimicrobial agents [1]. The clinically important opportunistic pathogen Pseudomonas aeruginosa exhibits intrinsic multiple-antibiotic resistance, which has been assumed to result from the low permeability of its outer membrane [2,3], so that Pseudomonas aeruginosa is a leading cause of nosocomial infections that are difficult to eradicate [4]. Infections due to P. aeruginosa are seldom encountered in healthy adults, but in the last two decades the organism has become increasingly recognized as the etiological agent in a variety of serious infections in hospitalized patients, especially those with impaired immune defenses [5]. The indiscriminate use of antimicrobial drugs, particularly in hospitalized patients, leads to the suppression of drug-susceptible organisms in the gut, oral and favors the persistence and growth of drug-resistant bacteria including P. aeruginosa. The closed environment of hospitals favors transmission of such resistant strains through personnel, vomits as well as by direct contact. In addition to its innate resistance, acquired additional resistance due to plasmids is also a problem in P. aeruginosa.

Plasmid-mediated resistance involving modifying enzymes is particularly associated with topical antibiotic use and with sites where high levels of antibiotics are achieved.

The present study was designed to determine resistance in P. aeruginosa isolates obtained from hospitalized burn patients in a teaching medical Al-Kendi hospital. We also isolated plasmids from the Multidrug-resistant isolates.
MATERIAL AND METHODS

- **Sample Collection**

70 burn wound swabs were taken from burned patients, who presented invasive burn wound infection, from both sex, and average age 3-58 years, admitted to burn unit of teaching medical Al-Kendi hospital in Iraq. The most preferred areas were the upper and lower extremities. The specimens were transport in sterile, leak-proof container to zoonotic disease unit. All specimens were inoculated on 5% blood agar and incubated overnight at 37 °C aerobically. Conventional biochemical methods according to standard microbiological techniques [6]

- **Cultural Media**

Media used for bacterial isolation and identification are ordinary media such as Nutrient agar, Blood agar, and special media such as pseudomonas agar.

- **Antimicrobial Susceptibility**

Antimicrobial susceptibility was performed on Muller-Hinton agar by the standard disk diffusion method [7]. The antibiotics tested for bacterial isolates were: Ciprofloxacin (Cip5), Amikacin (AK30), Ticarcilin (Tic 75), Oxytetracycline (t30), Gentamicin (CN10) and Doxycycline (Do30).

The zone of inhibition of bacterial isolates for individual antibiotics was measured in mm by applying ordinary ruler.

- **Plasmid Isolation**

Plasmid isolation by using Pure Yield™Miniprep System were performed on the seven isolates showing the most common drug resistance pattern. All experiments were performed in duplicate.

RESULTS

**Bacterial Isolates and Sensitivity Test**

The results showed that *Pseudomonas aeruginosa* was the commonest isolate (33 isolates: 47.17%), and only 37 samples (52.86%) were negative in bacterial growth.

Table (1) showed that *P. aeruginosa* isolates were moderately resistant to Amikacin (54.55%), and (68.18%) resistant to Ticarcilin, Gentamicin and ciprofloxacin, whereas the resistance was more marked with other antimicrobials like Doxycycline (72.3%), Oxytetracycline (72.3%).

**Table 1: The Percentage (%) of Resistance Showed by Pseudomonas aeruginosa to Different Antibiotics**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Symbol of Antibiotics</th>
<th>Total Number of Examined Isolated</th>
<th>Number of Isolates Resistant to Antibiotic</th>
<th>Percentage %</th>
<th>Number of Isolates Sensitive to Antibiotic</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>(Cip5)</td>
<td>22</td>
<td>15</td>
<td>68.18</td>
<td>8</td>
<td>36.36</td>
</tr>
<tr>
<td>Amikacin</td>
<td>(AK30)</td>
<td>22</td>
<td>12</td>
<td>54.55</td>
<td>10</td>
<td>45.55</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>(Do30)</td>
<td>22</td>
<td>16</td>
<td>72.3</td>
<td>7</td>
<td>31.82</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>(t30)</td>
<td>22</td>
<td>16</td>
<td>72.3</td>
<td>7</td>
<td>31.82</td>
</tr>
<tr>
<td>Ticarcilin</td>
<td>(Tic 75)</td>
<td>22</td>
<td>15</td>
<td>68.18</td>
<td>8</td>
<td>36.36</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>(CN10)</td>
<td>22</td>
<td>15</td>
<td>68.18</td>
<td>8</td>
<td>36.36</td>
</tr>
</tbody>
</table>

Seven isolates giving the most common resistance pattern were examined for the presence of plasmids by the
procedure described above. The results showed that all of the isolates harboured a larger plasmid (Figure 1) which was found to be responsible for Ciprofloxacin, amikacin, Doxycycline, Oxytetracycline, Ticarcillin and Gentamicin resistance.

Figure 1: Agarose (0.7%) Gel Electrophorasis of Plasmid Isolation of the Seven Isolates of Pseudomonas aeruginosa, 5V/cm, 90min

DISCUSSIONS

P. aeruginosa plays an important etiological role in human infections as many clinical isolates are resistant to common antibiotics. Early identification of infections due to this organism is necessary as the prompt institution of appropriate treatment might reduce the mortality in hospitalized patients [8, 9].

All the isolates tested in the present study for antimicrobial susceptibility were multidrug-resistant. The mechanisms of resistance to antibiotics include reduced cell wall permeability, production of chromosomal and plasmid mediated b-lactamases,[10] aminoglycoside-modifying enzymes[11] and an active multidrug efflux mechanism [12].

An alarming increase in resistance of Pseudomonas spp. to various antimicrobial agents has been reported by many workers [13, 14] studies regarding the isolation and characterization of plasmids from multidrug-resistant P. aeruginosa strains are still elementary in our country. In the present study we have isolated a plasmid from MDR P. aeruginosa isolates, which was found responsible for amikacin resistance. An important striking feature found in this study was increased resistance to amikacin and ciprofloxacin whereas the strains were sensitive to doxycycline and oxytetracycline (31.36% for each). To conclude, the presence of plasmid-multidrug resistance in P. aeruginosa isolates from burn patients was notable. The occurrence of large plasmid was noted in all seven of isolates.

To the best of our knowledge this report is among the first reports from Iraq regarding the emergence of resistance to amikacin, isolation and phenotypic characterization of the plasmids responsible for multidrug resistance including amikacin from the clinical isolates of P. aeruginosa from hospitalized burn patients.

This is a preliminary study on plasmid mediated amikacin resistance in P. aeruginosa isolates, however, there is a need for a large scale study to find out the plasmid mediated drug resistance in P. aeruginosa along with isolation and characterization of the plasmid(s).

REFERENCES


