INTRODUCTION:

Pseudomonas aeruginosa was ranked as the most frequent pathogen in surgery [1]. Opportunistic diseases brought about by P. aeruginosa are a genuine medicinal issue, and quinolone resistant P. aeruginosa could be recovered from clinical cases [2]. It is most harmful to individuals whose immune systems have been compromised similar to those with in AIDS, cancer, burns, cystic fibrosis, and neutropenia. Several infections can be acquired in the hospital such as wound, burn, urinary tract, and eye and outer ear infections, as well as meningitis and necrotizing pneumonia [3]. The diversity of P. aeruginosa strains has been frequently investigated through molecular typing methods, including ribotyping, repetitive-element-based PCR (rep-PCR), arbitrarily primed PCR (AP-PCR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphic (RFLP) DNA analysis, random amplified polymorphic DNA (RAPD) assay, and pulsed-field gel electrophoresis (PFGE) [4]. PCR has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism [5]. L and I lipoproteins are two outer membrane proteins of P. aeruginosa responsible for inherent resistance of P. aeruginosa to antibiotics and antisepsis. As these proteins are found only in this organism, they could be a reliable factor for rapid identification of P. aeruginosa in clinical samples [6]. The pathogenesis of Pseudomonas aeruginosa opportunistic infections is multifactorial, as suggested by the large number of cell-associated and extracellular virulence determinants; some of these determinants help colonization, whereas others facilitate bacterial invasion. The virulence of P. aeruginosa depends mainly on two types of virulence determinants: (i) virulence factors involved in acute infection, they being usually secreted and membrane bound factors. P. aeruginosa considered a large number of virulence factors such exoenzyme S, exotoxin A, elastase and sialidase [7] addition to several of the other extracellular virulence factors such as exoenzyme S, exotoxin A, elastase and sialidase [7], including ribotyping, repetitive-element-based PCR (rep-PCR), arbitrarily primed PCR (AP-PCR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphic (RFLP) DNA analysis, random amplified polymorphic DNA (RAPD) assay, and pulsed-field gel electrophoresis (PFGE) [4]. PCR has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism [5]. L and I lipoproteins are two outer membrane proteins of P. aeruginosa responsible for inherent resistance of P. aeruginosa to antibiotics and antisepsis. As these proteins are found only in this organism, they could be a reliable factor for rapid identification of P. aeruginosa in clinical samples [6]. The pathogenesis of Pseudomonas aeruginosa opportunistic infections is multifactorial, as suggested by the large number of cell-associated and extracellular virulence determinants; some of these determinants help colonization, whereas others facilitate bacterial invasion. The virulence of P. aeruginosa depends mainly on two types of virulence determinants: (i) virulence factors involved in acute infection, they being usually secreted and membrane bound factors. P. aeruginosa considered a large number of virulence factors such exoenzyme S, exotoxin A, elastase and sialidase [7] addition to several of the other extracellular virulence factors such as exoenzyme S, exotoxin A, elastase and sialidase [7].

The aim of this study was to isolate P. aeruginosa and detect oprL and toxA virulence genes.

MATERIALS AND METHODS:

Bacterial strains collection and identification test:

Totally 120 swap were collected from 90 patients (26.6%) isolates of Pseudomonas aeruginosa (28 (31%) from burns and 4 (13%) from wounds) were shown in the following table (2).

Table 2. number and percentage of Pseudomonas aeruginosa.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Total samples</th>
<th>Positives isolates</th>
<th>Negatives isolates</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns</td>
<td>90</td>
<td>28</td>
<td>62</td>
<td>31%</td>
</tr>
<tr>
<td>Wounds</td>
<td>30</td>
<td>4</td>
<td>26</td>
<td>13%</td>
</tr>
</tbody>
</table>
A total of %43.2 (n=14) and %56.2 (n=18) of Pseudomonas aeruginosa strains were isolated from Female and Male patients, respectively, table (3).

Table (3): Distribution and percentages of P. aeruginosa according to gender.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive sample</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Percentage</td>
<td>56.2</td>
<td>43.7</td>
</tr>
</tbody>
</table>

Also, the patients ages ranged between 5 to 64 years, the majority between 23-28 years. table (4).

Table (4): Frequency (%) of the Burn and wound Patients Involved in P. aeruginosa According Different Age Groups.

<table>
<thead>
<tr>
<th>Age groups(years)</th>
<th>Rate(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-10</td>
<td>11</td>
</tr>
<tr>
<td>11-16</td>
<td>15</td>
</tr>
<tr>
<td>17-22</td>
<td>22</td>
</tr>
<tr>
<td>23-28</td>
<td>30</td>
</tr>
<tr>
<td>29-34</td>
<td>5.6</td>
</tr>
<tr>
<td>35-40</td>
<td>6.4</td>
</tr>
<tr>
<td>41-46</td>
<td>0</td>
</tr>
<tr>
<td>47-52</td>
<td>5.5</td>
</tr>
<tr>
<td>53-58</td>
<td>4.5</td>
</tr>
<tr>
<td>59-64</td>
<td>0</td>
</tr>
</tbody>
</table>

The result revealed that the concentration of all DNA samples of the thirty two Pseudomonas aeruginosa isolates were between 65-100 ng/ul and the purity was between 1.8-2, figure (1).

Results showed the distribution of virulence genes in Pseudomonas aeruginosa which are 31 (96.8 %) isolates were positive for oprL and 1 (3.1%) were PCR negative while the toxA gene were detected in all of the 32 (100%) Pseudomonas aeruginosa isolates collected, table (5).

For detection of virulence genes of Pseudomonas aeruginosa (oprL and toxA) PCR reactions were done and the following results were obtained. PCR results of oprL gene (500bp) and toxA gene (352bp) expression are shown in Figure 2 and 3 respectively.

Figure (1): Agarose gel electrophoresis of DNA samples.

Figure (2): Agarose Gel electrophoresis of PCR product for the detection of oprL gene (504bp) using 1%agarose for 90 min at 70 volt, stained with ethidium bromide, M: 100bp DNA Ladder, Lanes 1 negative control. Lanes (2-18): Positive for oprL gene (504bp).

Figure (3): Agarose Gel electrophoresis of PCR product for the detection of toxA gene (352bp) using 1%agarose for 90 min at 70 volt, stained with ethidium bromide, M: 100bp DNA Ladder, Lanes 1 negative control. Lanes (2-18): Positive for toxA gene (352bp).
DISCUSSION:

Pseudomonas aeruginosa is a leading cause of nosocomial infections. Infections caused by it are often severe and life threatening and difficult to treat because the organism is inherently resistant to many drug classes (MDR) and is able to acquire resistance to all effective antimicrobial drugs. Over the years, P. aeruginosa contributes substantially to morbidity and mortality related to surgical site infection (SSI) worldwide, the third most commonly reported nosocomial infection[12] also, Burn patients are more liable to get infections in comparison with other patients because of their damaged skin barrier and suppressed immune system, in addition to extended hospital stay and invasive therapeutic and diagnostic procedures [13]. The findings come with matching results almost with others in Karbala city, Iraq, [14], which showed that the highest percentage of isolated bacterial in burn patients had the bacteria pseudomonas aeruginosa, (45%) and (49%) with Alharmy [15]. Many burn patients die as a result of infection during their hospital courses. The rate of infection in burn cases is extremely high in developing countries. [16][17]. This may be due to the prevalence of low level socioeconomic groups of patients in whom poor hygienic conditions prevail [18]. Also disparity in prevalence rate among several studies may be attributed to differences in hygienic practices and geographical location. According to gender and age group, the result of this study shows the rate of P. aeruginosa in the male (56.2 %), and 30% in the young patients (ages 23 to 28 years ) compared to the elderly, agree with [19] indicates that males in this age group are more active and involve in different clinical hygiene practices, even in hospital environment. This result is comparable with the study of Okon et al in Nigeria , which recorded that male patients showed a record of 52.8% and the highest frequency of this bacteria (20.7%) was found in age group of 29 years and above. In the other hand, these results disagree with results in Karbala city, Iraq, [14], studies of Shewatake et al. [21] in Ethiopia and Ekrem and R okan in Al-Sulaimania city, Iraq, where results of the studies showed higher occurrence of the bacterium in female and elderly patients [22].

Pseudomonas aeruginosa produces many of virulence factors whose expression is arranged by different systems [23]. A recent studies reveal P. aeruginosa is most frequent pathogen that formed many of virulence factors example ToxA, exoA, oprL and oprI genes [24] .

PCR results showed that, 31 of 32 P. aeruginosa isolates was positive for the oprI gene with amplified size (500 bp) in a percentage (98%). Similarly in this study, all of the isolates (100%) were remarkably positive for both oprI and oprL genes [25]. P. aeruginosa possesses a variety of virulence factors that may contribute to its pathogenicity. Our results showed that toxA gene were detected in all 32 tested strains of P. aeruginosa , The differences in the distributions of virulence factor genes in the populations strengthen the probability that some P. aeruginosa strains are better adapted to the specific conditions found in specific infectious sites [26] that may returned to the different environmental and geographical sources. The prevalence of pseudomonas aeruginosa and percentage of virulence factors genes depend on several causes including nature of places, immune status of patients, degree of contamination and type and virulence of strain [27].

CONCLUSION:

The outcome conclusions of this study show that high rate of infected wounds and burns of P. aeruginosa probably occur as a result of wide use and abuse of antibiotics. Therefore the result of this study may be as a recommendation to the correct use of antibiotics in treatment of patients. PCR seems that simultaneous use of specific primers different virulence factors genes as (oprL and toxA) of P. aeruginosa provides more confident detection of P. aeruginosa. Also its differences in the distributions of virulence factor genes in the isolated strains need further studies for finding out the actual role of these genes of P. aeruginosa from different sources. PCR showed that all P. aeruginosa strains do not necessarily have similar virulence genes.

REFERENCES:

