Genetic Identification of *Pseudomonas aeruginosa* Virulence Genes among Different Isolates

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**Abstract**

**Background and objectives:** *Pseudomonas aeruginosa* possesses a variety of virulence factors that may contribute to its pathogenicity. *P. aeruginosa* also has a large number of virulence factors such as exotoxin A, exoenzyme S, nan1 and Las genes. The aim of this study was to evaluate oprL and oprI as reliable factors for rapid identification of *P. aeruginosa* and to detect toxA, exoS, nan1 and LasB genes by Polymerase Chain Reaction (PCR).

**Materials and methods:** In this study 30 isolates of *P. aeruginosa* were recovered from burn, pulmonary tract and blood infections.

**Results and conclusions:** The oprL and oprI genes were detected in all of 30 *P. aeruginosa* isolates collected. The presence of toxA gene in isolates from burn and pulmonary tract was significantly higher than that from blood. All tested isolates harbored LasB gene. However, difference between exoS prevalence in isolates from pulmonary tract and burn isolates was statistically significant higher than that from blood. The prevalence of nan1 gene was significantly higher in isolates of pulmonary tract and burn specimens than isolates from blood. Molecular methods have been reported to be superior to the phenotypic methods for identification of *P. aeruginosa* by designing a multiplex PCR assay based on oprL and oprI genes for molecular detection of *P. aeruginosa*. Determination of different virulence genes of *P. aeruginosa* isolates suggests that they are associated with different levels of intrinsic virulence and pathogenicity. Significant correlations between some virulence genes and source of infections indicates implementation of infection control measures will help in controlling the dissemination of virulence genes among *P. aeruginosa* isolates.

**Keywords:** *Pseudomonas aeruginosa*; Polymerase chain reaction

**Introduction**

*Pseudomonas aeruginosa* is an opportunistic pathogen capable of infecting virtually all tissues, can infect immunocompromised individuals and responsible for hospital acquired infections [1]. Burn patients, mechanically ventilated patients, and cystic fibrosis (CF) patients are particularly susceptible to *P. aeruginosa* infections. It is a major cause of morbidity and mortality in patients with cystic fibrosis [2]. *P. aeruginosa* infections in hospitals mainly affect the patients in intensive care units and those having catheterization, burn, and/or chronic illnesses [3].

*Pseudomonas aeruginosa* possesses a variety of virulence factors that may contribute to its pathogenicity. *P. aeruginosa* also has a large number of virulence factors such as exotoxin A, exoenzyme S, nan1 and Las genes [1]. The outer membrane proteins of *P. aeruginosa* OprP and OprL play important roles in the interaction of the bacterium with the environment as well as the inherent resistance of *P. aeruginosa* to antibiotics where the consequence of the presence of these specific outer membrane proteins that have been implicated in efflux transport systems that affect cell permeability [4]. As these proteins are found only in this organism, they could be a reliable factor for rapid identification of *P. aeruginosa* in clinical samples [5].

*Pseudomonas aeruginosa* can cause pulmonary damage by different mechanisms. Exoenzyme S, encoded by the exoS gene, is an ADP ribosyltransferase that is secreted by a type-III secretion system directly into the cytosol of epithelial cells [6]. Exotoxin A, encoded by the toxA gene, inhibits protein biosynthesis. LasBelastase, a zinc metalloprotease encoded by the LasB gene, has an elastolytic activity on lung tissue [7]. In addition, the phospholipids contained in pulmonary surfactants may be hydrolysed by two phospholipases Cencoded by PLC-H and PLC-N, respectively [8]. The gene called nan1 encodes a sialidase that is responsible for adherence to the respiratory tract [9]. An extracellular neuraminidase is thought to play an important role in implantation of the bacterium but the genetic basis of this process is still unknown [10].

Although conventional microbiological methods for identifying *P. aeruginosa* from clinical and environmental samples are reliable, they require several days to be completed. Rapid detection of isolates causing hospital infections is very important for consequent treatment decision of patients. PCR has the potential for identifying microbial species rapidly by amplification of sequences unique to a particular organism [11].

In this study, our aim was to evaluate oprL, oprI as reliable factors for rapid identification of *P. aeruginosa* isolated from pulmonary tract, blood and burn samples based on PCR amplification of I lipoprotein (oprI) for detection of genus and I lipoprotein (oprL) for detection of species of this organism and to detect toxA, exoS, nan1 Land Las genes by PCR in different isolates of *P. aeruginosa* in order to find out any relation between these virulence factors and special manifestation of...
P. aeruginosa infections, we detected virulence factors among these isolates by using PCR.

Patients and Methods

A total number of 30 clinical isolates of P. aeruginosa were obtained from different samples from the Department of Clinical microbiology Ain shams University hospitals in the period between October 2014 and February 2015. Ten P. aeruginosa isolates were burn infections, 10 isolates from pulmonary tract and 10 isolates from blood infections. Twelve of the isolates were isolated from community acquired infections and eighteen isolates were obtained from Hospital acquired infections. The isolates were identified based on the colony morphology on Nutrient agar, blood agar and MacConkey agar and were confirmed using IMViC biochemical tests.

Detection of Virulence Genes by PCR

Genomic DNA was prepared from overnight cultures grown on Tryptose Soy broth, 10 ml of broth was centrifuged, 360 µl ATL±50 µl protease was added and incubated at 4 h at 55°C. 400 µl AL was added for 10 min at 55°C were added, then 400 µl ethanol were added; 700 µl were transferred to spin column, centrifuged at 8000 rpm/1 min. The rest of the sample was transferred to spin column and centrifuged. Then 700 µl of wash 1 buffer was added centrifuged. Transfer to new wash tube and 700 µl wash 2 was added. Spin column transferred to new wash tube, centrifuged at 13000/3 min, then transfer to elution tubes and add 100 µl preheated elution Buffer (70°C). Incubate 3 minutes at room temperature; centrifuge 7000 rpm/1 min [10].

PCR amplification was carried out using thermal cycler (BioRad, USA) with specific primers for oprI, oprL, toxA, exoS, nan1 and lasB.

PCR was carried out in 50 µl volume reaction mixtures containing 1 µl of each primer, 10 µl of crude template DNA and 25 µl Qagen master mix. The annealing temperature was 55°C for oprI, oprL, toxA and 58°C for exoS, nan1 and lasB. PCR products were separated by gel electrophoresis on 2% agarose gel containing 0.5 µg/ml ethidium bromide.

Statistical Analysis

Data has been collected and entered to the computer using SPSS (Statistical Package for Social Science) program for statistical analysis, (version 17; Inc., Chicago. IL). Descriptive statistics has been done, where qualitative data has been expressed as frequency and percent. Chi square test has been used to detect significance of the results; P value was calculated at significant level (0.10). Bar charts have been plotted.

Results

The oprI and oprL genes were detected in all of 30 P. aeruginosa isolates collected. However, presence of tox A gene in clinical samples was different. According to (Table 1) the presence of tox A gene in isolates from burn and pulmonary tract was significantly higher than that from blood (P<0.001). Our results showed that all tested isolates harbored lasB gene. However, difference between exoS prevalence in isolates from pulmonary tract and burn isolates was statistically significant higher than that from blood (P<0.1). The nan1 gene, other virulence factor studied in this research, was found in 4 (40%) of 10 isolates from pulmonary tract, 4 (40%) of 10 from burn however it wasn’t found in isolates from blood infections. The prevalence of nan1 gene was significantly higher in isolates of pulmonary tract and burn specimens than isolates from blood (P<0.1).

<table>
<thead>
<tr>
<th>Amplified gene</th>
<th>Specific Primer</th>
<th>Amplified region</th>
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<tbody>
<tr>
<td>oprI</td>
<td>PS1, 5'-ATG AAC AAC GTT CTG AAA TTC TCT GCT-3'</td>
<td>250</td>
</tr>
<tr>
<td>oprL</td>
<td>PAL1, 5'-ATG GAA ATG CTG AAA TTC GGC-3'</td>
<td>500</td>
</tr>
<tr>
<td>lasB</td>
<td>lasB lasf 5' GGA ATG AAT ACT TAT TTT GAT 3'</td>
<td>300</td>
</tr>
<tr>
<td>toxA</td>
<td>toxA toxf 5' GAT AAC CAG CTC AGC CAC AT 3'</td>
<td>352</td>
</tr>
<tr>
<td>exoS</td>
<td>exoS exof 5' CTT GAA GGG ACT CGA CAA GGC 3'</td>
<td>504</td>
</tr>
<tr>
<td>nan1</td>
<td>nan1 nan f 5' AGG ATG AAT ACT TAT TTT GAT 3'</td>
<td>1316</td>
</tr>
</tbody>
</table>

Twelve (40%) of the isolates were isolated from community acquired infections (CAI) and 18 (60%) isolates were obtained from hospital acquired infections (HAI). The isolates were then divided mainly into four genetic groups depending on the presence of virulence genes and the relation between these groups and the type of infections (Tables 2 and 3) (Figures 1 and 2). This study shows that the presence of two or three virulence genes is significantly higher among nosocomial infections than community acquired infections (P<0.001). However, most of the isolates from CAI do not contain any virulence genes or may harbor only one virulence gene.

Discussion

Identification of P. aeruginosa has traditionally relied on phenotypic methods. This still is the most accurate standard when dealing with typical isolates of P. aeruginosa. In cystic fibrosis (CF) patients, P. aeruginosa isolates display unusual phenotypic reactions. Moreover, biochemical testing takes long time to perform and requires extensive hands-on work by the technologist, both for setup and for ongoing evaluation. Molecular methods have been reported to be superior to the phenotypic methods for identification of P. aeruginosa [12]. By designing a multiplex PCR assay based on oprI and oprL genes for molecular detection of P. aeruginosa showed that the specificity and sensitivity of the PCR assay were 74 and 100%, respectively. Lavener [13] also noted that all of P. aeruginosa strains contained the oprI and oprL genes (sensitivity=100%, specificity=80%). Similarly in this study, all of the 30 isolates were remarkably positive for both oprI and oprL genes.

Pathogenicity of P. aeruginosa is clearly multifactorial. LasB is one of the most important proteases of P. aeruginosa [14]. In this study all isolates examined harbored lasB gene. This finding is in agreement with previous reports [10]. Mutation of lasB gene reduces markedly P. aeruginosa invasion. Prevalence of the lasB gene in all the environmental and clinical isolates implies the importance of LasB factor to survival of P. aeruginosa in various settings [15]. P. aeruginosa isolates generally express cytotoxicity or invasion phenotypes which is correlated with presence of exoU (encoding exotoxin U) or exoS (encoding exotoxin S) respectively. In our study difference between exoS and tox prevalence in the isolates from pulmonary tract, burn and blood infections was significant (P<0.1) (Table 1). About the nan1 gene, the other virulence factor studied in this research, we found that the prevalence of nan1 was significantly higher in isolates from pulmonary tract and burn than isolates from blood (P<0.1). These results correlate with those studied by Nikbin [10] and Lanotte [11]. nan1 gene has probably a role in CF
pulmonary disease evolution as previously described Lanotte [11]. The low prevalence of this factor among isolates from blood infections may show that the role of this gene in the blood infections is less important than burn and pulmonary tract infections. The differences in the distributions of virulence factor genes in the populations strengthen the probability that some \textit{P. aeruginosa} strains are better adapted to the specific conditions found in specific infectious sites, and thus virulence genes expression differs according to site and severity of infection [10].

This study also showed that \textit{P. aeruginosa} isolates are more virulent in HAIs than that in CAIs. Most of the isolates that were isolated from HAIs contain either three or two virulent genes while most of the isolates that were isolated from CAIs do not contain any virulent gene. These results correlate with those proved by Bradbury [16], who proved that \textit{P. aeruginosa} isolated from nosocomial infections were found to have an increased prevalence of virulence genes. Most of the HAIs isolates were collected from Intensive care units (ICU) and the ICU environments are subject to more stringent disinfection protocols than other environments within the hospital, which may facilitate and promote the acquisition of virulent genes by horizontal transfer between nosocomial strains of \textit{P. aeruginosa}. It may be speculated that acquisition of other resistance mechanisms which may assist survival in the ICU environment might also positively modify the virulent genes acquisition rate [16].

In conclusion, it seems that simultaneous use of \textit{oprI}, \textit{oprL}, and \textit{LasB} genes provides more confident detection of \textit{P. aeruginosa} by PCR. Determination of different virulence genes of \textit{P. aeruginosa} isolates suggests that they are associated with different levels of intrinsic virulence and pathogenicity. This may have different consequence on the outcome of infections. Significant correlations between some virulence genes and source of infections obtained in this research indicate implementation of infection control measures will help in controlling the dissemination of virulence genes among \textit{P. aeruginosa} isolates.

References


