Antimicrobial Resistance Pattern and Presence of Beta-Lactamase Genes in Pseudomonas aeruginosa Strains Isolated from Hospitalized Patients, Babol-Iran

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ABSTRACT

Background: Pseudomonas aeruginosa has recently emerged as one of the main causes of nosocomial infections in the intensive care unit due to vast antibiotic resistance potency. The aim of this study was to investigate the antimicrobial resistance pattern and evaluation of the frequency of beta-lactamase genes in Pseudomonas aeruginosa strains.

Methods: Generally, 42 P. aeruginosa strains were collected from different clinical specimens. The phenotypic and genotypic tests such as disc diffusion, Modified Hodge test and molecular detection of blaIMP, blaIMP1, blaVIM2, and blaKPC genes were performed.

Results: According to the results, by disk diffusion method 33 (78.58%) to Cefotaxime, 31 (73.8%) of strains were resistant to Ceftriaxone, and 31 (73.8%) to Co-trimoxazole, while the lowest resistance was observed in case of Polymyxin B 3 (7.15%). On the other hand 4 (9.52%) of strains were recognized as MHT positive. Moreover, 26 (61.9%) of isolates were detected as multi drug resistance strains. In addition, 2 (4.7%) of isolates harbored blaIMP, 2 (4.7%) blaIMP1 and 10 (23.8%) blaVIM2 genes, whereas the blaKPC gene was not reported.

Conclusion: According to the results, the prevalence of beta-lactamase genes and antibiotic resistance pattern in patients with high levels of hospitalization is essential. Therefore, it is necessary to identify the strains containing β-lactamase genes for better control and treatment.

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Introduction

*Pseudomonas aeruginosa* is a Gram-negative obligate aerobic, spore-free and motile bacilli that can be found in water, soil and the human natural flora (1). This microorganism is regarded as one of the human opportunistic pathogens, which may lead to widespread diseases such as septicemia, pneumonia, wound, respiratory and urinary tract infections, also responsible for approximately 10% of hospital infections, moreover is a major contributor to chronic pulmonary infections with cystic fibrosis patients (2, 3).

The presence of efflux pumps, reduced permeability of the outer membrane and secretion of the β-lactamase enzyme are the main prominent features of this bacteria for the inherent resistance to many antibiotics, including third-generation cephalosporins, imipenem, and aztreonam (4). According to the Ambler's classification, the beta-lactamases are divided into four categories (A to D), in which types A, C and D are serine β-lactamase, while type B is metallo-beta-lactamase (MBL), which are located within the integron and have the ability to integrate into the plasmid or chromosome, so can transfer to other bacteria family, including Enterobacteriaceae sp. It should be noted that MBL have zinc binding motifs around the active site and these active sites are inhibited by the ethylene diamine tetraacetic acid (EDTA), but, clavulanate/tazobactam. The MBL enzymes (Type B) are subdivided into B1-B3 that according to molecular structure the subgroup B1 is classified into four categories: *blaIMP*, *blaVIM*, *blaGIM* and *blaSPM* (5-7). For the first time the *blaIMP*1 enzyme was identified in *Serratia marcescens* in Japan, and *blaVIM*2 was reported in Italy (8). Class A of bacterial β-lactamases which are widely identified in Enterobacteriaceae family are resistant to cephalosporins and inhibited by clavulanic acid and tazobactam. The *blaPER*, *blaGES*, *blaVEBs*, *blaTEMs*, *blaSHVs* and *blaCTX-Ms* are the most known common extended-spectrum beta-lactamases (ESBLs). However *blaKPC* with an intrinsic carbapenemases activity is classified in class A (9).

Carbapenems (imipenem and meropenem) are the most important antimicrobial antibiotics used to treat *P. aeruginosa* strains (10). In the past two decades antibiotic resistance is a critical clinical problem and a serious threat to national health. Infections caused by antibiotic-resistant microorganisms lead to prolonged hospitalization and an increase in mortality rates and higher medical costs compared with antibiotic susceptible microorganisms. Most of the studies that researchers have done on this bacterium are about aspects of drug resistance, which is an important treatment factor to limit the hospital infections.

Regarding the importance of *P. aeruginosa* infections and the frequency of multidrug resistance strains (MDR), the phenotypic and genotypic identification is concerned; so, the aim of this study was to investigate the antibiotic resistance patterns and beta lactamase genes such as *bla-IMP1*, *blaIMP*, *blaKPC* and *bla-VIM2* in *P. aeruginosa* strains isolated from hospitalized patients.

Materials and Methods

Sampling and isolation of bacteria

In this descriptive cross-sectional study, in the period from November 2016 to August 2017, a total of 42 *Pseudomonas aeruginosa* strains were collected from ICU ward of educational hospitals in Babol city. Thereafter, identification and confirmation of strains were performed by using microbiological tests and finally stored at -20 °C on BHI broth containing 15% glycerol.

Antimicrobial Resistance testing

To determine the multidrug resistance phenotype, a disk diffusion method (Kirby-Bauer) was used according to Clinical and Laboratory Standards Institute 2016 by following antibiotics.
Modified Hodge Test (MHT)

At first a bacterial suspension equivalent to a 0.5 McFarland standard of *E. coli* ATCC 25922 was inoculated on Muller-Hinton agar medium. The Ertapenem (10µg) antibiotic disc was placed in the center of the culture medium. The test organism, positive and negative controls were cultured in a straight line from the disc edge to the edge of the plate and incubated for 24 hours at 37 °C. A cloverleaf-like indentation of the *E. coli* ATCC 25922 growing along the test organism growth streak within the disc diffusion zone was considered as a positive MHT.

Genotypic tests

In order to perform the molecular reaction test, the bacterial genomic DNA was first extracted by a unique commercial kit (Yektatajhiz, Iran), and then the PCR test was conducted by specific primers which were listed in (Table 1). Each PCR reaction was carried out in a final volume of 25µl using a Mastermix prepared by the Gene Fanavaran (Tehran, Iran). Finally, 1.5% agarose gel electrophoresis was used for identification of DNA fragments. The PCR program is shown in (Table 2).

Result

During 10 months of study a total of 42 *P. aeruginosa* strains were collected from ICU ward of educational hospitals in Babol city. Out of 42 *P. aeruginosa* strains, %62 and %38 were isolated from male and female respectively. The specimens included urine (n=17, 40/47%), blood (n= 11, 26/19%), tracheal aspirate (n=9, 21/42%), wound (n=3, 7/1%), catheter (n=1, 2/38%) and peritoneal fluid (n=1, 2/38%). In accordance to disk diffusion method the most resistance was achieved in Cefotaxime 33 (78.58%), Ceftriaxone 31 (73.8%), and Co-Trimoxazole 31 (73.8%), respectively. In addition the lowest resistance was belonged to Polymyxin B 3 (7.15%) (Figure 1).

Also, 26 (61/9%) of isolates were distinguished as MDR strains. On the other hand 4) 9.52% of isolates were recognized as MHT positive. Among 42 isolates, 10 (23.8%) harbored *blaVIM2*, 2 (4.7%) *blaIMP* and 2 (4.7%) *blaIMP1* due to our results none of the strains were positive for *blaKPC* gene (Figure 2).

Discussion

Carbapenems are one of the best options for treating infections caused by gram-negative bacteria resistant to multiple drugs. In the present study, a high resistance to all available antimicrobials was observed, which is regarded as a serious threat to limit the treatment options in our hospitals. This can be explained by the increasing use of antimicrobial agents over the past decade that has been led to using the selective antibiotics in *P. aeruginosa*. The most resistance genes are located in transportable elements such as plasmids and integrons, thus can easily be transmitted between different strains of a bacterium and even different bacteria. Due to genetic continuity the resistance to the other family of antibiotics including Aminoglycosides, Sulfonamides, and Fluoroquinolones can be occurred.

In a conducted research by Mirbagheri et al. (48.5%) were resistant to imipenem, (100%) to Tobramycin, (62.6%) to ceftazidime, (88.4%) to Meropenem (64.4%) to ceftriaxone and (54.3%) to gentamicin. The frequency of *blaVIM1* and *blaVIM2* genes were (58.7%) and (3.17%) among imipenem-resistant isolates (16). However, in the present study, the highest resistance to cefotaxime (78.58%) and the lowest resistance to Meropenem.
Antimicrobial Resistance Pattern and Presence ofblaIMP, blaIMP1, blaVIM2, and blaKPC.

Table 1.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5 to 3)</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMP1-F</td>
<td>TGAGCAAGTTAATGCTATTC</td>
<td>74 (12)</td>
<td></td>
</tr>
<tr>
<td>IMP1-R</td>
<td>TTAGTGCTGGTTTGGATG</td>
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<td></td>
</tr>
<tr>
<td>VIM2-F</td>
<td>CAGATTGGCGATGTTTGGG</td>
<td>52 (13)</td>
<td></td>
</tr>
<tr>
<td>VIM2-R</td>
<td>AGGTGGGCGCTACACGGCAG</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>IMP-F</td>
<td>GGAATAAGATGCTATATTCTC</td>
<td>18 (14)</td>
<td></td>
</tr>
<tr>
<td>IMP-R</td>
<td>CCAAACCACATAGTTAATCT</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>KPC-F</td>
<td>GTATCGCGCTATGTCCTGCC</td>
<td>63 (15)</td>
<td></td>
</tr>
<tr>
<td>KPC-R</td>
<td>GGTGCGTTTCTACGATTGC</td>
<td>6</td>
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</tr>
</tbody>
</table>

Various statistics have been reported on Pseudomonas aeruginosa resistance to carbapenems in Iran. Since some isolates were resistant to imipenem and meropenem in this study, the isolates studied in this study Belong to the same strain. Generally, in Iran resistance to Carbapenems is lower than other antibiotics; So that the effectiveness of these antibiotics could be promising in the treatment of Pseudomonas aeruginosa infection (17).

Improper use of antibiotics in medicine has led to a rise in resistant strains. This resistance induces changes in the receptor or the location of the antibiotic activity by induction of inactive antibiotics or mutation in the genes encoding the membrane proteins. In a study by Zafer et al., from 122 P. aeruginosa isolates, the following resistance was obtained: Imipenem (39.3%), ceftazidime (60.6%), gentamicin (50%) and ciprofloxacin (43.4%), also (27%) of strains were MBL producer, in which the prevalence of blaVIM2 and blaIMP1 gene were (58.3%) and (2.1%) respectively (18). The results of these studies are similar to those of the present study; therefore, antibiotic resistance seems to be Pseudomonas aeruginosa can vary from city to city and from hospital to hospital. Rahimzadeh et al., examined 51 strains of Pseudomonas aeruginosa that 63% of the samples had the blaIMP gene (19), which is not readily available from the research findings. In Aghamiri et al. study, the frequency of blaIMP and blaVIM genes was reported %9, %33 respectively (20).

Figure 1. Antibiotic Resistance pattern in P. aeruginosa isolates. Imipenem 10µg (IMI), Meropenem 10µg (MRP), Ceftazidime 30µg (CAZ), Cefotaxime 30µg (CTX), Polymyxin B 300µg (PB), Tobramycin 10µg (TOB), Levofoxacin 5 µg (LVOF), Ceftriaxone 30 µg (CTR), Fosfomycin 200 µg (FO), Co-trimoxazole 25 µg (COT).

Figure 2. A: Lanes 1, 2: positive strains for blaVIM2 gene; Lane 3: DNA Size Marker (100bp); Lane 4: Positive control; Lane 5: negative control. B: Lanes 1: DNA Size Marker (100bp); Lane 2: Positive strain for blaIMP1; Lane 3: Positive control; Lane 4: negative control. C: Lane 1: DNA Size Marker (100bp); Lane 2 & 3: Negative Strains; Lane 4: Negative control; Lane 5: Positive strain.
Table 2. The PCR program for amplification of blaIMP1, blaVIM2, blaIMP and blaKPC genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial Denaturation</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final Extension</th>
</tr>
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<tbody>
<tr>
<td>blaIMP1</td>
<td>94°C for 4min</td>
<td>94°C for 40Sec</td>
<td>56°C for 40 Sec</td>
<td>72°C for 45 Sec</td>
<td>72°C for 4min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35 cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaVIM2</td>
<td>94°C for 4min</td>
<td>94°C for 40Sec</td>
<td>54°C for 40 Sec</td>
<td>72°C for 45 Sec</td>
<td>72°C for 4min</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>35 cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaIMP</td>
<td>94°C for 4min</td>
<td>94°C for 40Sec</td>
<td>52°C for 40 Sec</td>
<td>72°C for 45 Sec</td>
<td>72°C for 4min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35 cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>blaKPC</td>
<td>94°C for 4min</td>
<td>94°C for 40Sec</td>
<td>56°C for 40 Sec</td>
<td>72°C for 45 Sec</td>
<td>72°C for 4min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35 cycles</td>
<td></td>
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</tbody>
</table>

Conclusion

As a conclusion, rapid identification and monitoring the epidemiological outbreak of the strains which producing these enzymes is essential.

Acknowledgment

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Conflict of interest

None declared conflict of interest

References


