Pseudomonas Aeruginosa - Characterizing A Multidrug Resistant Pathogen Isolated From Clinical Specimens

Deepak Kumar, Illiyas Maqbool Malla, Sharmila P, Nandhini C, Sivasubramani K

Abstract: Antimicrobial resistance is a threat to public health that requires our immediate attention. Pseudomonas aeruginosa displays an extraordinary resistance many traditional antibiotics which are presently used to treat bacterial infections. P. aeruginosa causes serious nosocomial infections with high morbidity and mortality. In the present study, we investigated 70 clinical isolates of P. aeruginosa, isolated from clinical samples of patients suffering from bacterial infections. The antibiotic susceptibility patterns of the isolates were determined by disc diffusion method. The antimicrobial susceptibility pattern of the P. aeruginosa isolates revealed that most of them were found to be resistant to Piperacillin (75.14%), Tetracycline (67.14%), Erythromycin (94.85%) and a complete resistance was observed with Ciprofloxacin (100%). Regarding the antibiotic sensitivity, 94.85% of them were found to be sensitive to both Vancomycin and Gentamycin followed by Meropenem (87.14%) and Cefepime (54.29%). MAR (multiple antibiotic resistance) index of the isolates were ranged from 0.1-0.8. 40% of the isolates showed a MAR index of 0.5 followed by 27.14% (0.6), 12.86% (0.3), 11.43% (0.4) and 8.57% (0.8). The biofilm formation assay showed that all the tested isolates were able to biofilm. From the results, it was observed that the clinical isolates of P. aeruginosa were resistant to most of the traditionally using antibiotics tested.

Key words: Pseudomonas aeruginosa, Antimicrobial susceptibility, Multiple antibiotic resistance (MAR), Biofilm formation, Bacterial wound infection.

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans (1). This bacterium has been found occupying in diverse habitats like soil, plants surface, coastal marine habitats, and the surface of human and animal skin (2,3). The P. aeruginosa displays very high resistance to antibiotics (intrinsic and acquired), which make it hard and at times even impossible to manage the infection (4). The clinical strains of P. aeruginosa is reported to show resistance against all clinically available antibiotics (4,5). There have been many reports that the prevalence of infections by multidrug-1 (MDR) bacterial strains is increasing fast across the globe (6-9). The National Nosocomial Infections Surveillance System (NNIS) reports put P. aeruginosa at the second place in causing nosocomial pneumonia (17% of cases), urinary tract infection (UTI) and surgical site infection (11% of cases), and the fifth most common organism isolated from all sites of nosocomial infection (9% of cases)(10). The reason has been accredited to the multidrug-resistance (MDR) to several antibiotics which have been acquired by this bacterium with time(11,12). This makes P. aeruginosa bacterial infections difficult to treat and hence there is a high risk of mortality associated with infections by P. aeruginosa(4). This pathogen has an exceptional capacity to develop resistance to antibiotics by the selection for genomic mutations and by the exchange of transferable resistance determinants (5). The genome size of P. aeruginosa varies greatly, ranging from 5.5 to 7 Mbp (13,14). Such variation arises due to the presence of a large accessory genome. Accessory genomes are strain can occupy up to 20% of the whole genome (15).

Accessory genomes are important for carrying virulence and acquired antibiotic resistance genes. The lateral transfer of those genes between strains contributes to the development of MDR virulent strains (16). In addition, mutational changes in chromosomal genes can also contribute to virulence and antibiotic resistance (16,17). The aim of the present study was to characterize the antimicrobial susceptibility and multiple antibiotics resistant pattern of the clinical strains of P. aeruginosa isolated from the clinical specimens of patients suffering from bacterial infections, against a broad range of antibiotics.

MATERIALS AND METHODS

Collection of bacterial isolates Seventy clinical isolates of Pseudomonas aeruginosa isolated from the patients suffering from bacterial infections and maintained in the Department of Microbiology laboratory, Rajah Muthiah Medical College and Hospital (RMMC), ChidambaramTamil Nadu were procured and used in the present study. Biochemical identification of the isolatesIdentity of the strains was further confirmed using standard biochemical methods (21, 22) Antibiotic susceptibility testing The bacterial isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method (23). Mueller Hinton agar (MHA) plates were spread with the overnight cultures of each isolates. The standard antibiotic discs such as Vancomycin, (10 μg/disc), Gentamycin (30 μg/disc), Cefepime (50μg/disc), Meropenem (10 μg/disc) Ciprofloxacin (5 μg/disc), Tetracycline (30 μg/disc), Erythromycin (15 μg/disc) and Piperacillin (100 μg/disc) (Himedia, India) were aseptically placed at equidistance on the plates and allowed to stand for 1 h. Then the plates were incubated at 37 °C for 24 h. After incubation, the antibiotic sensitivity pattern of each isolate was determined according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) and Performance Standards for Antimicrobial Susceptibility Testing (20). Based on the results the isolates were divided.

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Table 1: Antibiotic sensitive pattern of isolated P. aeruginosa

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<tr>
<td></td>
<td>Green: Sensitive</td>
<td>Red: Resistant</td>
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Into two groups based on the zone of inhibition; Sensitive and resistant. Determination of multiple antibiotic resistance (MAR) index of each isolate was determined according to the method of Krumperman, 1983 (24), using the formula:

\[
MAR = \frac{a}{b}
\]

Where ‘a’ represents the number of antibiotics to which the test isolates depicted resistance and ‘b’ represents the total
number of antibiotics to which the test isolate has been evaluated for susceptibility. Biofilm assay The biofilm formation assay for each isolated strain was performed according to the method described by O'Toole and Kolter (25) with some minor modifications. Overnight cultures of each isolated strain were diluted to 1:100 ratios in fresh LB medium. Then 125 μl of each culture was dispensed into sterile polypropylene tubes and were incubated at 37 °C for 72 h. After incubation, 100 μl of 0.25% crystal violet (CV) was added to each tube and were incubated at 37°C for 30 min. After incubation, the strains were discarded and rinsed 2 to 5 times in standing water and allowed to dry. The stained biofilm was solubilized by adding 200 μl of 95% ethanol for 10 min. and the optical density was read at 600 nm (OD600) using spectrophotometer.

RESULTS

Collection and identification 70 clinical isolates of Pseudomonas aeruginosa collected were revived and further confirmed their identity using standard biochemical methods. Antibiotic susceptibility testing Among the 8 different antibiotics tested, the isolates of P. aeruginosa showed higher resistance to Ciprofloxacin, Erythromycin and Piperacillin. Some antibiotics showed higher sensitivity to Gentamycin, Vancomycin, Meropenem and Cefepime. Antibiotic sensitivity pattern of the isolates was displayed in Table 1. The isolates were resistant to majority of the antibiotics. Multiple antibiotic resistance (MAR) MAR (Multiple antibiotic resistance) index revealed 66 isolates higher resistant Erythromycin (94.85%), Piperacillin (75.14%), Tetracycline (67.14%) and lowest resistant Gentamycin and Vancomycin (05. 14%). isolates with MAR index the higher was 0.5(40%) and low MAR index value 0.1,0.2,0.7,0.9 and 1. (Table 2and 3). The MAR values ranged from 0.3 to 0.8.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total number of isolates (n=70)</th>
<th>MDR isolates (n=53)</th>
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<tbody>
<tr>
<td></td>
<td>Resistant (%)</td>
<td>Sensitive (%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>53(75.14%)</td>
<td>17(24.29%)</td>
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<tr>
<td>Cefepime</td>
<td>32(45.71%)</td>
<td>38(54.29%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>70(100%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>66(94.85%)</td>
<td>4(05.14%)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>4(05.14%)</td>
<td>66(94.85%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>47(67.14%)</td>
<td>23(32.86%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4(05.71%)</td>
<td>66(94.85%)</td>
</tr>
<tr>
<td>Meropenam</td>
<td>9(12.86%)</td>
<td>61(87.14%)</td>
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<table>
<thead>
<tr>
<th>MAR Index</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1</th>
</tr>
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<tbody>
<tr>
<td>Number/ (%)</td>
<td>0(0.00%)</td>
<td>0(0.00%)</td>
<td>94(12.86%)</td>
<td>87(12.42%)</td>
<td>26(38.00%)</td>
<td>19(27.14%)</td>
<td>0(0.00%)</td>
<td>6(8.57%)</td>
<td>0(0.00%)</td>
<td>0(0.00%)</td>
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Measurement of biofilm formation
Results of the biofilm assays of each strain of P. aeruginosa revealed that all the strains were able to form biofilms. Out of 70 isolates, 9 strains of P. aeruginosa p01,02,11,26,42,47,56,57,58 (Table 4). were able to produce high level of biofilm.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Isolates</th>
<th>OD value at 600nm</th>
<th>S. No.</th>
<th>Isolates</th>
<th>OD value at 600nm</th>
<th>S. No.</th>
<th>Isolates</th>
<th>OD value at 600nm</th>
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<tr>
<td>1</td>
<td>p.a p01</td>
<td>0.87</td>
<td>25</td>
<td>p.a p25</td>
<td>0.106</td>
<td>49</td>
<td>p.a p49</td>
<td>0.119</td>
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<tr>
<td>2</td>
<td>p.a p02</td>
<td>0.85</td>
<td>26</td>
<td>p.a p26</td>
<td>0.09</td>
<td>50</td>
<td>p.a p50</td>
<td>0.128</td>
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<tr>
<td>3</td>
<td>p.a p03</td>
<td>0.16</td>
<td>27</td>
<td>p.a p27</td>
<td>0.131</td>
<td>51</td>
<td>p.a p51</td>
<td>0.109</td>
</tr>
<tr>
<td>4</td>
<td>p.a p04</td>
<td>0.24</td>
<td>28</td>
<td>p.a p28</td>
<td>0.148</td>
<td>52</td>
<td>p.a p52</td>
<td>0.116</td>
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<tr>
<td>5</td>
<td>p.a p05</td>
<td>0.131</td>
<td>29</td>
<td>p.a p29</td>
<td>0.132</td>
<td>53</td>
<td>p.a p53</td>
<td>0.122</td>
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DISCUSSION
Among infections caused by Gram-negative rods, Pseudomonas aeruginosa has a leading role (2), especially in critically ill and immunocompromised patients. Antimicrobial resistance has led to a serious restriction in treatment options for P. aeruginosa infections, which has become a critical and deadly issue causing a total of 51,000 healthcare infections in the USA per year (3–6). Despite this problem, physicians mainly rely on retrospective non-randomized controlled studies to derive conclusions about the optimal therapeutic management of these infections. 70 clinical isolates of Pseudomonas aeruginosa were procured from the Department of Microbiology (RMMCH), these isolates were confirmed by their colony, morphology, Grams staining and Biochemical tests. Prolonged exposure to antibiotics in recurrent infections and the use of broad-spectrum antibiotics in polymicrobial infections were believed to lead the selection of microorganism. Different studies on P. aeruginosa infections have shown that ICU stay is a major risk factor (26–28). Studies on fluoroquinolone-1 P. aeruginosa (FQ-RPa) infections have shown that ICU stay is an important risk factor (26, 31, 32). This condition highlights the importance of antibiotic use and its efficacy in resistance development. We evaluated the antibiotic sensitivity in all the isolated strains of P. aeruginosa against different antibiotics. A moderate pattern of resistance was observed in all the isolated strains of P. aeruginosa against all the antibiotic treatments while the higher pattern of resistance was observed towards a few antibiotics.
antibiotics such as piperacillin and erythromycin. This reveals that the resistance potential of P. aeruginosa against antibiotics is elevating significantly. However, further studies are required to support this statement. Multiple antibiotic resistant (MAR) in bacteria the overall antibiotic sensitivity pattern depicted the P. aeruginosa in higher resistant to the common antibiotics. The resistant rate of P. aeruginosa to various antibiotics was Erythromycin (94.85%), Piperacillin (75.14%), Tetracycline (67.14%) and showed the lowest resistant Gentamycin and Vancomycin (24). Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections. More recently it has been noted that bacterial biofilms may impair cutaneous wound healing and reduce topical antibacterial efficiency in healing or treating infected skin wounds. It has been shown that biofilms are present on the removed tissue of 80% of patients undergoing surgery for chronic sinusitis. P. aeruginosa represents a common organism which is involved in different types of biofilm-associated infections. Examples of such infections include chronic wounds, chronic otitis media, chronic prostatitis, and chronic lung infections in cystic fibrosis (CF) patients. About 80% of CF patients have a chronic lung infection, caused mainly by P. aeruginosa growing in a non-surface attached biofilms surround by PMN (37). The infection remains present despite aggressive antibiotic therapy and is a common cause of death in CF patients due to constant inflammatory damage to the lungs. In patients with CF, one therapy for treating early biofilm development is to employ DNase to structurally weaken the biofilm. The biofilm formation analysis showed that the majority of the isolated strains of P. aeruginosa were able to form biofilms except for some few strains. The strains designated as P. aeruginosa p01, 02, 11, 26, 42, 47, 56, 57, 58 were able to form biofilms. This leads to the fact that the majority of the isolated strains of P. aeruginosa strains isolated could be associated with wound infections. The effective treatment of infections caused by P. aeruginosa includes prevention when possible and source control measures as necessary and prompt administration of appropriate antimicrobial agents. If antimicrobial susceptibilities are known, de-escalation should be pursued in patients especially with an appropriate clinical response. Hand hygiene and barrier precautions are important to keep the spread of infection in ICUs. Therefore, surveillance is important in providing useful information for physicians in choosing empirical antibiotics.

CONCLUSION

According to the findings of the present study, antibiotic resistance was common among Pseudomonas aeruginosa isolates. Production of biofilm was prevalent in Pseudomonas aeruginosa isolates. Biofilm might be an important player contributing to the multiple drug resistance of the pathogenic isolates.

REFERENCES

aeruginosa Genomic Structure and Diversity. Front Microbiol 2, 150.


