Antimicrobial susceptibility pattern and multidrug resistance index in Pseudomonas aeruginosa among clinical isolates in Denizli, Turkey

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Abstract

Background: Pseudomonas aeruginosa is an important hospital infection agent causing morbidity and mortality with the ability to gain resistance to many antimicrobials. The objective of this study was to determine the sensitivity profiles of nosocomial P. aeruginosa isolates in Denizli, Turkey.

Methods: A total 120 P. aeruginosa strains which were isolated from specimens sent to the microbiology laboratory between January 2015 and December 2015 were investigated. Antimicrobial resistance was determined by agar disc diffusion method using Mueller-Hinton agar according to Clinical and Laboratory Standards Institute recommendations.

Results: With respect to sensitivity pattern, the most sensitive antimicrobials were Amikacin, colistin, tobramycin, netilmicin and gentamicin and the resistance rates were detected as 97%, 96%, 92%, 90%, 83%, respectively over 120 P. aeruginosa strains. The sensitivity rates for the other antimicrobials were 56% for Piperacillin and 54% for Tazobactam. P. aeruginosa strains 62 (52%) isolates showed multiple antimicrobial resistance to 13 antimicrobials

Conclusion: To prevent the spread of the resistant bacteria, it is critically important to have strict antimicrobial policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented. In the meantime, it is desirable that the antimicrobial susceptibility pattern of bacterial pathogens like P. aeruginosa in specialized clinical units to be continuously monitored and the results readily made available to clinicians so as to minimize the development of resistance.

Keywords: Pseudomonas aeruginosa, antimicrobial resistance, clinical isolates, Turkey

Introduction

Known for many years to be a cause of serious wound and surgical infections, but often regarded as a secondary or opportunistic invader rather than a cause of primary infection in healthy tissues, Pseudomonas aeruginosa has now clearly emerged as a major nosocomial pathogen in immunocompromised and debilitated patients, as well as in cystic fibrosis patients (Pier & Ramphal, 2005). P. aeruginosa develops resistance to many antimicrobials and sometimes the sensitivity status can change during treatment. In particular, development of resistance is observed with the use of specific antimicrobial and resistant strains can be transmitted from patient to patient (Aloush et al., 2006). Multiple antimicrobial resistance (MAR) problems arise because of the combination of resistance against various antimicrobials used in therapy and cross resistance development between antimicrobials.

P. aeruginosa shows intrinsic and acquired resistance to many structurally unrelated antimicrobials, and previous exposure to antimicrobials often leads to multidrug-resistant P. aeruginosa strains (Mouton et al., 1993; Ciofu et al., 1994). When P. aeruginosa strains are resistant to antimicrobials, they increase the length of hospital stay and the cost of treatment (Kang et al., 2005). Increasing resistance through the use of false antibacterial agents presents serious problems in the treatment of infections (Mittal et al., 2009). Antimicrobial susceptibility data of P. aeruginosa is limited in Turkey (Berktaş et al., 2011 Er et al., 2015). Because of these facts, it is of crucial importance to isolate and identify the offending strain in order for appropriate antimicrobial

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therapy to be initiated. The objective of this study was therefore, to determine the characteristics and patterns of antimicrobial resistance among isolates of \textit{P. aeruginosa} recovered from clinical specimens in Denizli, Turkey.

\textbf{Materials and Methods}

\textit{Culture and Identification}
This study was carried out in the Department of Microbiology, Denizli State Hospital, a centrally located medical centre in the Denizli city, between January 2015 and December 2015. Samples were collected from the hospitalized patients in different clinic parts of the hospital. Samples were taken from various sources like urine, broncho-alveolar lavage and tracheal aspirates.

The samples were streaked on nutrient agar plates and the plates were incubated at 37°C for 24 hours as described by Cheesborough (1985). Then the characteristic suspected single colonies were subjected to Gram's staining and then sub-cultured in MacConkey agar and blood agar. The pure isolates of \textit{Pseudomonas aeruginosa} were transferred to 1% nutrient agar slant and stored in the refrigerator at 4°C. \textit{P. aeruginosa} was identified by biochemical test (sugar fermentation test) and biochemical tests were performed following the methods described in MacFadden (2000). Motility test of the isolated \textit{P. aeruginosa} was performed following the method described by Cheesbrough (1985).

\textit{Microbiological analyses and antimicrobial susceptibility testing}
\textit{P. aeruginosa} was confirmed by the Vitek2 automated microbiology system (bioMérieux, Marcy l'Étoile, France). \textit{P. aeruginosa} (ATCC 27853) was used as the quality control strain. The antimicrobials used were selected according to the 2004 National Committee for Clinical Laboratory Standard (NCCLS) guidelines: amikacin, colistin, tobramycin, netilmicin, gentamicin, aztreonam, cefepime, ceftazidime, levofloxacin, ciprofloxacin, imipenem, meropenem, tigecycline, piperacillin, piperacillin/tazobactam, tetracycline and sulfamethazol/trimetroprim.

\textit{Antibiogram pattern of \textit{P. aeruginosa}}
Antimicrobial resistance was determined by an agar disc diffusion test (Bauer et al., 1966) using Mueller-Hinton agar (Difco) according to Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2005). Seventeen different antimicrobials were used. For antimicrobial resistance determination, the isolates were grown in Luria-Bertani (LB) broth until the turbidity equal to the 0.5 McFarland standard (approximately 10^8 cfu/ml). Cultures were swabbed on to the Mueller–Hinton agar and all isolates were tested against. Amikacin (AN, 30µg/ml), Colistin (CS, 10µg/ml), Tobramycin (TM 10µg/ml), Netilmisin (NET, 10µg/ml), Gentamicin (GEN, 10µg/ml), Aztreonam (ATM, 30µg/ml), Cefepime (FEP, 30µg/ml), Ceftazidime (CAZ, 10µg/ml), Levofloxacin (LEV, 5µg/ml), Ciprofloxacin (CIP, 5µg/ml), Imipenem (IPM,10µg/ml), Meropenem (MEM, 10µg/ml), Tigecycline (TGC,15µg/ml), Piperacillin (PIP,30µg/ml), Tazobactam/ piperacillin (TZP,30µg/ml), Tetracycline (TE, 30µg/ml), Sulfamethoxol/trimetroprim (SXT, 25µg/ml). The isolates grown in inoculation were evaluated as resistant and the others were evaluated as susceptible. The antibiotic discs were dispensed sufficiently separated from each other so as to avoid overlapping of inhibition zones. The plates were incubated at 37°C, and the diameters of the inhibition zones were measured after 18 hr. All susceptibility tests were carried out in duplicate and were repeated twice if discordant results had been obtained.

\textit{Multiple Antimicrobial Resistance Index}
For all isolates, we calculated the MAR index values (a/b, where “a” represents the number of antimicrobials the isolate was resistant to; and “b” represents the total number of antimicrobials the isolate tested against). A MAR index value ≥ 0.2 is observed when isolates are exposed to high risk sources of human or animal contamination, where antimicrobials use is common; in contrast a
MAR index value \( <or = 0.2 \) observed when antimicrobials are seldom or never used (Krumperman, 1985; Matyar et al., 2008).

**Results**

A total of 120 isolates of *P. aeruginosa* were collected from January 2015 to December 2015 from different patients. With respect to sensitivity pattern, the most sensitive antimicrobials were Amikacin, colistin, tobramisin, netilmicin, gentamycin and the resistance rates were detected as 97%, 96%, 92%, 90%, and 83%, respectively. For the other antimicrobials, the sensitivity rates of *P. aeruginosa* were in the following order: Piperacillin and Tazobactam were recorded 56% and 54% respectively.

**Table 1. Antimicrobial susceptibility pattern of Pseudomonas isolated from clinical samples**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Sensitivity</th>
<th>Resistance</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>116(97%)</td>
<td>4(3%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>115 (96%)</td>
<td>5 (4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>110 (92%)</td>
<td>3 (3%)</td>
<td>7 (5%)</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>108 (90%)</td>
<td>7 (6%)</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>99 (83%)</td>
<td>12 (10%)</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>87 (73%)</td>
<td>27 (23%)</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>87 (73%)</td>
<td>11 (9%)</td>
<td>22 (18%)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>85 (71%)</td>
<td>26 (22%)</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>74 (62%)</td>
<td>44 (37%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>73 (61%)</td>
<td>40 (33%)</td>
<td>7 (6%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>73 (61%)</td>
<td>47 (39%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>69 (57.5%)</td>
<td>29 (24.2%)</td>
<td>26 (22%)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>70 (58%)</td>
<td>49 (41%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>67 (56%)</td>
<td>33 (28%)</td>
<td>20 (17%)</td>
</tr>
<tr>
<td>Tazobactam/Piperacillin</td>
<td>65 (54.2%)</td>
<td>29 (24.2%)</td>
<td>26 (22%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>53 (44.2%)</td>
<td>67 (56%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Sulfamethaxol/Trimethoprim</td>
<td>8 (7%)</td>
<td>112 (93%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Out of the 120 *P. aeruginosa* strains 62 (52%) isolates showed Multiple Antibiotic Resistance (MAR) four to thirteen antimicrobials. The results were given Table 2. The MAR indices ranged from 0.06 to 0.76 isl.

**Table 2. Number of clinical samples and Multiple Antibiotic Resistance Index 120 Pseudomonas aeruginosa strains**

<table>
<thead>
<tr>
<th>Clinical Samples</th>
<th>No. of isolates</th>
<th>Multiple Antimicrobial Resistance Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>38</td>
<td>0.06 (10 isl), 0.12 (5 isl), 0.18 (4 isl), 0.24 (6 isl), 0.29 (4 isl), 0.35 (2 isl), 0.41 (1 isl), 0.49 (1 isl), 0.53 (1 isl), 0.58 (1 isl), 0.65 (1 isl), 0.76 (1 isl), 0.70 (1 isl)</td>
</tr>
<tr>
<td>Blood</td>
<td>22</td>
<td>0.06 (5 isl), 0.12 (2 isl), 0.18 (2 isl), 0.24 (3 isl), 0.29 (1 isl), 0.35 (1 isl), 0.44 (2 isl), 0.53 (1 isl), 0.57 (1 isl)</td>
</tr>
<tr>
<td>Abscess</td>
<td>14</td>
<td>0.06 (1 isl), 0.12 (1 isl), 0.18 (5 isl), 0.24 (4 isl), 0.29 (10 isl), 0.65 (1 isl)</td>
</tr>
<tr>
<td>Tracheal aspirate</td>
<td>37</td>
<td>0.06 (3 isl), 0.12 (2 isl), 0.18 (4 isl), 0.24 (6 isl), 0.29 (2 isl), 0.35 (4 isl), 0.41 (1 isl), 0.47 (5 isl), 0.59 (4 isl), 0.65 (3 isl)</td>
</tr>
<tr>
<td>Ear</td>
<td>2</td>
<td>0.12 (1 isl), 0.06 (1 isl)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>2</td>
<td>0.06 (1 isl), 0.18 (1 isl)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>1</td>
<td>0.65 (1 isl)</td>
</tr>
<tr>
<td>Mucus</td>
<td>4</td>
<td>0.06 (1 isl), 0.35 (1 isl), 0.47 (1 isl), 0.23 (1 isl)</td>
</tr>
</tbody>
</table>

**Discussion**
Our results were similar to those of Jamasbi (2008) who reported sensitivity to Amikacin of 97%. However other studies have reported lower rate of sensitivity (Sharma et al., 2010; Picao et al. 2008; Behera et al., 2008; Hocquet et al., 2007). An increased sensitivity of P. aeruginosa to Colistin was also seen in this study. Our results were similar to Kumar et al (2014). Tobramycin has a narrow spectrum of activity, but it is often used to eliminate P. aeruginosa in patients with cystic fibrosis (Hamed & Deponnett, 2017). This aminoglycoside antimicrobial is commonly used to treat different Gram-negative bacteria (Bullitta et al., 2015) and has been reported to have good clinical outcome (Gonzalez & Spencer, 1998). Sensitivity to Tobramycin was seen in 92% isolates in our study, while relatively lower rates of sensitivity have been observed in other studies elsewhere (Obritisch et al., 2004; Javiya et al. 2008; Franco et al., 2010). Sensitivity rate to aminoglycosides (gentamicin, netilmicin, amikacin) in our study was high. Gentamicin has been used with excellent results in the treatment of sepsis due to Pseudomonas spp., in burn patients (Stone, 1966). Strateva et al. (2007) in Bulgaria reported higher resistance to P. Aeruginosa to aminoglycosides while Fady et al., (2005) Nigeria reported relatively lower resistance levels.

Sensitivity rate of Cefazolin was showed in 73%. Some researchers have reported Cefazolin sensitivity rate to P. aeruginosa in clinical samples (Gultekin et al., 2004; Durmaz-Çetin et al., 2004; Ersoz et al., 2004; Eksi et al., 2007; Kurtoglu et al., 2008; Gayyurhan et al., 2008; Tuncoglu et al., 2009). Cefepime is one of the few antimicrobials described to have constant antipseudomonal activity over the years, although publications on cefepime resistance are growing in number in recent years (Eksi et al., 2007; Gayyurhan et al., 2008; Pakoz et al., 2011; Ece et al., 2014; Kotwal et al., 2014). Our results were similar to Eksi et al (2007) who also reported sensitivity to Cefepime was 74.5%.

Some researchers have reported ceftazidime sensitivity rate to P. aeruginosa in clinical samples (Yapar et al., 2000; Ayyildiz et al., 2000; Demirci et al., 2001; Cesur et al., 2002; Al-Jasser & Elkhizzi., 2004; Gultekin et al., 2004; Durmaz-Çetin et al., 2004; Ersoz et al., 2004; Ciftci et al., 2005; Yücel et al., 2006; Eksi et al., 2007; Gayyurhan et al., 2008; Kurtoglu et al., 2008; Afifi et al., 2013). Levofloxacin sensitivity rate to P. aeruginosa in clinical samples have been reported in a number of studies (Pakoz et al., 2011; Sen et al., 2014). Among the quinolones, Levofloxacin, a broad spectrum antimicrobial quinolones, is found to be effective against a variety of the clinical isolates, especially Enterococcus spp. and Pseudomonas aeruginosa (Fuchs et al., 1996; Hans et al., 1999).

Only 69 (57.5%) isolates were susceptible to meropenem. Meropenem sensitivity rate to P. aeruginosa in clinical samples has been reported by other authors (Fidan et al., 2005; Gales et al., 2006; Gayyurhan et al., 2008; Somilky et al., 2012; Afifi et al., 2013; Ece et al., 2014; Sen et al., 2014; Yayan et al., 2015). We found that 61% isolates were sensitive to Ciprofloxacin in our study, similar to other studies (Sharma et al., 2010; Javiya et al., 2008; Gokale & Metgud, 2012). However, lower sensitivity rates have been reported by Franco et al. (2010) and Prakash & Saxen (2013). Carbapenems are the drugs of choice for many infections caused by gram positive and gram negative bacteria (Nicolaou 2008; Shah, 2008). Sensitivity to Imipenem was observed in 61% of isolates in our study. Much lower rates have been observed by others (Franco et al., 2010; Picao et al., 2008; Behera et al., 2008) while higher rate of sensitivity have been reported by Javiya et al. (2008) and by Hocquet et al. (2007).

Our rate of tigecycline sensitivity was 58%. Similar sensitivity pattern has been reported elsewhere (Somilky et al., 2012; Chaudhary et al., 2013). Sensitivity rate to P. aeruginosa to pipercillin found in our study are similar to others (Andrade et al., 2003; Gultekin et al., 2004; Al-Tawfig, 2007; Berktas et al., 2011; Yayan et al., 2015). The Piperacillin/Tazobactam combination was effective in only about half of the isolates which is comparable to that of Javiya et al. (2008), while higher sensitivity was reported by Hocquet et al. (2007). The sensitivity pattern of tetracycline and trimethoprim observed in our study has been reported by other researchers elsewhere (Eksi et al., 2007; Gayyurhan et al., 2008; Ullah et al., 2009; Rıfatıoglu et al., 2009; Sen et al., 2014; Toroglu et al., 2013; Sen et al., 2014). Tetracycline is a bacteriostatic antimicrobial and used to select mutants of multidrug resistance (Alonso et al., 1999).
The MAR indices give an indirect suggestion of the probable source(s) of the organism. The MAR indices in this work were greater than 0.20, this confirms the report of Olayinka et al. (2004) that the MAR index greater than 0.20 indicates that the organisms must have been originated from an environment where antimicrobials are often used (Olayinka et al., 2004). Thus, the result of the MAR index in this work can be interpreted that these pathogens might have been originated from where these antimicrobials are used. The multidrug resistance of P. aeruginosa from the hospital was 11.10% which confirms the report of Hota et al. (2009) that outbreaks of multidrug-resistant P. aeruginosa colonization or infection can occur in urology wards, a burn unit, haematology/oncology units, and adult and neonatal critical care units and that various medical devices and environmental reservoirs can be implicated in the outbreaks of the pathogen.

To prevent the spread of the resistant bacteria, it is critically important to have strict antimicrobial policies while surveillance programmes for multidrug resistant organisms and infection control procedures need to be implemented. In the meantime, it is desirable that the antimicrobial susceptibility pattern of bacterial pathogens like P. aeruginosa in specialized clinical units to be continuously monitored and the results readily made available to clinicians so as to minimize the resistance.

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