Antimicrobial susceptibility pattern and multidrug resistance ındex 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, tobramisin, 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 Piperacilin 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 *P. aeruginosa* recovered from clinical specimens in Denizli, Turkey.

Materials and Methods

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 *Pseudomonas aeruginosa* were transferred to 1% nutrient agar slant and stored in the refrigerator at 4°C. *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 *P. aeruginosa* was performed following the method described by Cheesbrough (1985).

Microbiological analyses and antimicrobial susceptibility testing

P. aeruginosa was confirmed by the Vitek2 automated microbiology system (bioMérieux, Marcy l'Etoile, France). *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.

Antibiogram pattern of 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), Sulfamethaxol/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.

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: Piperacilin and Tazobactam were recorded 56% and 54% respectively.

Table1. Antimicrobial susceptibility pattern of *Pseudomonas* isolated from clinical samples

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

Out of the 120 P. *aeruginosa* strains 62 (52%) isolates showed Multiple Antibiotic Resistance (MAR) four to thirteen antimicrobials. The results were given Table2. 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

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

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 (Bulitta *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, amikac) 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 Fadeyi *et al.,* (2005) Nigeria reported relatively lower resistance levels.

Sensitivity rate of Aztreonam was showed in 73%. Some researchers have reported Aztreonam 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; Ayyıldiz *et al.*, 2000; Demirci *et al.,*2001; Cesur *et al.,*2002; Al-Jasser & Elkhizzi., 2004; Gultekin *et al.*, 2004; Durmaz- Cetin *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; Somily *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 (Nicolau 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 (Somily *et al.*, 2012; Chaudhary *et al.*, 2013). Sensitivity rate to *P. aeruginosa* to piperacillin 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; Rifaioglu *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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