

Beta-lactamases production and antimicrobial resistance ratio of *Pseudomonas aeruginosa* from hospitalized patients in Kahramanmaras, Turkey

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Abstract

Sixteen isolates of *P. aeruginosa* were collected from different hospitals in Kahramanmaras among 2006-2007 and tested for the level of resistance to the widely used antipseudomonal antibiotics and used in local medicinal and veterinary practice. The aim of this study was to determine the antibiotic resistance to *P. aeruginosa* strains isolated in Microbiology Laboratory of different hospitals in Kahramanmaras between 2006-2007. These strains were mostly isolated from urine and few from tracheolaryngeal aspirate, tracheal secretion, mucus, bronchoalveolar lavage. The antibiotic resistance rates were as follows: Penicillin (PEN) 100%, Amoxicillin (AMO) 94%, Cefazolin (CEF) 87.5%, Cefoxitin (CEFX) 81%, Nitrofrantoin (NIT) 75%, Chloramphenicol (CHL) 62.5 %, Tetracycline (TET) 56%, Ceftriaxone (CEFT) 44%, Ofloxacin (OFL) and Gentamycin (GEN) 37.5%, Meropenem (MER) and Streptomycin (STR) 31%. Among 16 isolates of *P.aeruginosa* from wounds showed 8 (50%) β -lactamase activity, whereas 8 isolates of *P.aeruginosa* from urine showed no β -lactamase activity. All *P. aeruginosa* strains 16 (100%) isolates showed multiple antibiotic resistance towards three to eleven antibiotics.

Key words

P. aeruginosa, Antibiotic resistance, Beta-lactamase activity

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen. It is the leading cause of nosocomial infections related to intensive care unit (ICU), particularly ventilator-associated pneumonia (Pollack, 2000; Brazas and Hancock, 2005). Of particular concern is the limited number of effective anti-pseudomonal agents including β -lactams, aminoglycosides and fluoroquinolones used in therapeutic practice due to the multiplicity of mechanisms of resistance of *P.aeruginosa* (Pellegrino *et al.*, 2002; Poole, 2005). Antibiotics like imipenems and meropenem are usually active against multi-drug resistant isolates *P.aeruginosa*, however, resistance to these compounds has also become a growing therapeutic problem (Livermore, 2002). In recent years, a notable increase in the prevalence and multi-drug resistant (MDR) of *P.aeruginosa* reported in critically ill hospitalized

patients has been noted with subsequent high morbidity and mortality, both locally and worldwide (Aloush *et al.*, 2006; Karlowsky *et al.*, 2003; Flam *et al.*, 2004; Obritsch *et al.*, 2004; Nourer *et al.*, 2005).

In view of the above, the present study was conducted to determine the status of antimicrobial resistance, underlying conditions, and determination of *P.aeruginosa* isolates with β -lactamase from patients.

Materials and Methods

Isolation of bacterial strains and identification: 16 isolates were collected from hospitalized patients in Kahramanmaras among 2006-2007 and tested for antipseudomonal antibiotics. Ethical approval is not necessary for this paper. Isolates were considered to be presumptive *Pseudomonas*

spp. if they were Gram-negative rods that *Pseudomonas* spp., was isolated by incubated at 42, 37, and 20°C on *Pseudomonas* Selective Medium (Oxoid CM 457). Confirmation of isolates was performed by using classic biochemical tests like casein hydrolysis, pigment production on milk-agar with cetrimide (Brown and Foster, 1970; Toroglu et al., 2005), oxidase and catalase reaction, oxidative-fermentation of glucose test, citrate utilizing capability and lipase activity (Mac Faddin, 2000; Matyar et al., 2009; Gilligan, 1995; Toroglu et al., 2005).

Antibiotic resistance activity: Antibiotic 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 (2005). Twelve different antibiotics were used. For antibiotic resistance determination, the isolates were grown in Luria-Bertani (LB) broth until the turbidity was equal to the 0.5 Mc Farland standard (approximately 10^8 cfu ml⁻¹). Cultures were swabbed on to the Mueller-Hinton agar and all isolates were tested against Meropenem (MER, 10 µg ml⁻¹), Amoxicillin (AMO, 20 µg ml⁻¹), Penicillin (PEN, 10 µg ml⁻¹), Nitrofrantoin (NIT, 300 µg ml⁻¹), Cefazolin (CEF, 30 µg ml⁻¹), Cefoxitin (CEFX, 30 µg ml⁻¹), Ceftriaxone (CEFT, 30 µg ml⁻¹), Gentamycin (GEN, 10 µg ml⁻¹), Tetracycline (TET, 30 µg ml⁻¹), Streptomycine (STR, 10 µg ml⁻¹), Chloramphenicol (CHL, 30 µg ml⁻¹), Ofloxacin (OFL, 5 µg ml⁻¹). The isolates those 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. Reference strain of *P. aeruginosa* ATCC 27853 was used as control organism for verification of the antibacterial effect of the discs on Mueller-Hinton agar plates.

β-lactamase production: The production of β-lactamase was detected with the iodometric slide test (MacFaddin, 2000; Toroglu and Toroglu, 2009). Firstly, iodine solution was added to penicillin solution. A heavy suspension of the organism to be tested emulsified in a drop of freshly prepared penicillin - iodine solution as flamed side of a glass slide followed by addition of starch solution. An indication of β-lactamase production was clearing of solution, clearing of purple color to white within 5 min. But the whole mixture does not have to be clear; clearing of definite clumps or areas was sufficient to denote a positive result. All the bacterial isolates were tested for the production of β-lactamases.

Multiple antibiotic resistance (MAR) index: For all isolates, we calculated the MAR index values (a/b, where a represents the number of antibiotics the isolate was resistant to, b

represents the total number of antibiotics the isolate tested against). A MAR index value ≥ 0.2 was observed when isolates were exposed to high risk sources of human or animal contamination, where antibiotics use was common; in contrast a MAR index value ≤ 0.2 was observed when antibiotics were seldom or never used (Toroglu and Keskin, 2011; Matyar et al., 2008; Krumperman, 1985)

Results and Discussion

The majority of patients with *P.aeruginosa* bacteremia had an underlying immunosuppressed state or some device present that predisposed them to the development of infection, this being similar to findings in other published reports (Aliaga et al., 2002; Pollack, 2000). *P.aeruginosa* commonly cause bronchopulmonary infections and less frequently urinary tract infections, infections of surgical wounds and bacteremia (Pollack, 2000). And also *P.aeruginosa* was the third most common pathogen among bloodstream isolates in the most recent SENTRY study (Diekema et al., 1999). The patients were mainly distributed among intensive care units, surgical and hematological wards, whereas a predominance of patients from the general surgical and transplant services were found (Weisstein et al., 1997). Some researchers reported that *P.aeruginosa* strains were important pathogens among urine, wound, blood, tracheolaringeal aspirate, tracheal secretion, mucus and bronchoalveolar lavage (Al-Jasser and Elkhizi., 2004; Gul et al., 2004; Eksi et al., 2007)

The antibiotic resistance rates were as follows: Penicillin 100%, Amoxicillin 94%, Cefazolin 87.5%, Cefoxitin 81%, Nitrofrantoin 75%, Chloramphenicol 62.5 %, Tetracycline 56%, Ceftriaxone 44%, Gentamycin and Ofloxacin 37.5%, Meropenem and Streptomycine 31% (Table 1, 2).

Penicillins are bactericidal antibiotics which inhibits bacterial cell wall synthesis (Trevor et al., 2001). These bacteria offer resistance to penicillins by production of lactamases and by permeability barrier of the cell surface (Suginaka et al., 1975).

Matyar et al. (2009) found that cefazolin, cefoxitin and nitrofurantoin were resistance to *Pseudomonas*. Several studies have reported increased resistance of *P.aeruginosa* isolates to Cephalosporins (Singh et al., 2003; Khorasani et al., 2008). But third generation antibiotics Ceftriaxone showed 44 % resistance. Cephalosporins have been used in infections caused by *P.aeruginosa* (Cavallo et al., 2000; Gales et al., 2001), also show good activity against *P.aeruginosa* (Tsuji et al., 2003; Takeda et al., 2007).

Aminoglycosides have good activity against clinically important Gram-negative bacilli (Gonzalez and Spencer, 1998). Gentamisin has been used with excellent

Table 1 : Antibiotic resistance pattern of *Pseudomonas aeruginosa*

Antibiotics	Inhibition (mm)	Resistance	Inhibition zone (mm)	Intermediate	Inhibition zone (mm)	Sensitive
Mer	0-10	5 (31%)	14	1 (6%)	21-40	10 (62.5%)
Amx	0-10	15 (94%)	-	0 (0%)	22	1 (6%)
Pen	0-14	16 (100%)	-	0 (0%)	-	0 (0%)
Nit	0-11	12 (75%)	-	0 (0%)	17-26	4 (25%)
Cef	0	14 (87.5%)	-	0 (0%)	19-29	2 (12.5%)
Cefx	0-8	13 (81%)	-	0 (0%)	22-36	3 (19%)
Ceft	0-10	7 (44%)	15-17	5 (31%)	23-26	4 (25%)
Gent	0-10	6 (37.5%)	13	2 (12.5%)	16-26	8 (50%)
Tet	0-14	9 (56%)	16-18	5 (31%)	19-25	2 (12.5%)
Str	0-7	5 (31%)	12-14	3 (19%)	16-27	8 (50%)
Chl	0-12	10 (62.5%)	14	3 (19%)	25-30	3 (19%)
OfI	0-11	6 (37.5%)	-	0 (0%)	23-37	10 (62.5%)

Table 2 : Prevalence of the antibiotic resistance and β -lactamase production among 16 clinical *Pseudomonas aeruginosa* isolates

No. of patient	Data on resistance										
1. <i>P.aeruginosa</i>	Amo	Pen	Tet								
2. <i>P.aeruginosa</i>	Mer	Pen	Tet	OfI							
3. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx						
4. <i>P.aeruginosa</i>	Amo	Pen	Cef	Ceft	Gen	Str					
5. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Chl					
6. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Gen	Chl				
7. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Tet	Chl				
8. <i>P.aeruginosa</i>	Mer	Amo	Pen	Nit	Cef	Cefx	Ceft	OfI			
9. <i>P.aeruginosa</i>	Mer	Amo	Pen	Cef	Cefx	Tet	Str	OfI			
10. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Ceft	Tet	Chl			
11. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Ceft	Tet	Chl			
12. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Ceft	Tet	Chl			
13. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Ceft	Gen	Tet	Chl		
14. <i>P.aeruginosa</i>	Amo	Pen	Nit	Cef	Cefx	Gen	Tet	Str	Chl	OfI	
15. <i>P.aeruginosa</i>	Mer	Amo	Pen	Nit	Cef	Cefx	Gen	Str	Chl	OfI	
16. <i>P.aeruginosa</i>	Mer	Amo	Pen	Nit	Cef	Cefx	Ceft	Gen	Str	Chl	OfI

Table 3 : Multiple antibiotic resistance (MAR) index and β -lactamase production among 16 clinical *P. aeruginosa* isolates

Source of isolates	Total isolates	MAR index	β -lactamase
Urine	4	0.25, 0.33, 0.83, 0.91	All (-)
Wound	5	0.41, 0.58(2), 0.66, 0.75	3(+) 2(-)
Mucus	1	0.66	1(+)
Blood	2	0.66(2)	1(+) 1(-)
Tracheolaringeal aspirate	2	0.5, 0.66	1(+) 1(-)
Tracheal secretion	1	0.5	1(+)
Bronchoalveolar lavage	1	0.83	1(+)
Total	16		

+ = Present; - = Absent

results in the treatment of sepsis due to *Pseudomonas* spp., in burn patients (Stone, 1966). Resistance to gentamycin was recorded in 37.5 % isolates. Strateva *et al.* (2007) from Bulgaria reported 79.7 % resistance to *P.aeruginosa*, but among these group, Streptomycin showed 31% resistance. Our results were similar to previous report in which 40.2% resistant strain were reported from Iorin, Nigeria (Fadeyi *et al.*, 2005).

Carbapenems, mainly meropenem, have been extensively used for the treatment of infections caused by multi resistant *P.aeruginosa* strains. Recent data suggest that almost 15 % of *P.aeruginosa* isolates were resistant to meropenem (Gales *et al.*, 2006). Resistance to meropenem was showed in 31 % . Amongst the *P.aeruginosa* isolates in our study. Matyar *et al.* (2009) reported that meropenem resistance rate was the lowest among the carbapenem antibiotics in their study.

Fluoroquinolones are antibiotics that are very effective against many Gram-negative microorganisms, including *P.aeruginosa*. However, resistance to these antibiotics has been reported in recent years as well. The resistance rate was in ofloxacin with 37.5%. Similarly, some researchers reported ofloxacin resistance rate from 31% to 62.5 % in different studies (Swiatlo *et al.*, 2000; Prosser and Beskid,1995).

Tetracycline is a bacteriostatic antibiotic and used to select mutants of multidrug resistance (Alonso *et al.*, 1999). *P.aeruginosa* is resistant to tetracycline due to low permeability of the outer membrane of the bacteria. In the present study it showed 56% resistance to tetracycline.

The most frequently encountered clinical resistance was through β -lactamase enzymes. This resistance to commonly used agents in *Pseudomonas* infection, which reduced the effectiveness of β -lactam antibiotics leads to widespread use for this reason, depending on which of these enzymes in routine laboratory determination of resistance before treatment is important (Normdan and Guibert,1998). As a result of this, we compared our results both of them. Some researchers detected metallo β -lactamase production rate from 5% to 62% in *P. aeruginosa* strains (Fidan *et al.*, 2005, Magalhaes *et al.*, 2005; Toraman *et al.*, 2005; Gayyurhan *et al.*, 2008). And few researchers detected induced β -lactamase activity production rate from 52.9% to 92% in *P.aeruginosa* (Eksi *et al.*, 2007; Öngüt *et al.*, 2006).

In the present study, among the 16 isolates of *P. aeruginosa* 8(50%) showed β -lactamase activity especially isolated from wound (Table 3). While 8 (50%) isolates of *P.aeruginosa* showed no β -lactamase activity, especially isolated from urine.

In present study, the MAR index values ranged from 0.25 to 0.91 in urine samples and 0.41 to 0.75 in wound samples. Both mucus and blood samples showed 0.66 MAR index value. While the MAR index values ranged from 0.5 to 0.66 among tracheolaringeal aspirate, it showed 0.5 in tracheal secretion, The MAR index value of bronchoalveolar lavage was 0.83. MAR index higher or equal to 0.2 has been said to be an indication of isolates originating from an environment where antibiotics were often used (Krumperman, 1985). All *P.aeruginosa* strains 16 (100%) isolates showed multiple antibiotic resistance three to eleven antibiotics. Our results were similar to Blasco *et al.* (2008) who reported that 100% of *P.aeruginosa* isolates from water reservoirs and cooling system presented multiple antibiotic resistance.

It is concluded that Meropenem and Streptomycin might be suggested for treatment of infections caused by *P.aeruginosa*. Penicillin, Amoxicillin and Gentamycin were non advisable antibiotics for *P.aeruginosa* infections according to the MAR index value. It emphasizes the importance of a conservative approach to antibiotic therapy and continued antimicrobial susceptibility testing surveillance programs to curtail the problem of antibiotic resistance.

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