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Research

Susceptibility patterns and cross resistances of antibiotics against *Pseudomonas aeruginosa* in a teaching hospital of Turkey

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Abstract

Background: *Pseudomonas aeruginosa* is the third most common pathogen responsible for nosocomial infections and the prevalence of multiple resistant isolates has been increasing. Ninety-nine clinical isolates were studied in order to assess the current levels of susceptibility and cross-resistances of widely used antipseudomonal antibiotics against *P. aeruginosa* and to determine some resistance mechanisms by phenotypic methods.

Methods: MICs of isolates for nine antipseudomonal antibiotics were determined by the E test method.

Results: Thirty-six percent of isolates were resistant to more than one group of antibiotics. The rates of susceptible isolates were ciprofloxacin 75%, amikacin 73%, ceftazidime 65%, meropenem 63%, imipenem 63%, piperacillin/tazobactam 60%, cefoperazone/sulbactam 59%, cefepime 54% and tobramycin 44%. The majority of carbapenem resistant isolates were susceptible to ciprofloxacin and amikacin.

Conclusion: Ciprofloxacin seems to be the most active agent against *P. aeruginosa* followed by amikacin in our unit. The usefulness of combinations of these antibiotics and β-lactams should be tested in treating multi-drug resistant *P. aeruginosa*.

Background

Pseudomonas aeruginosa is an important bacterial pathogen most frequently responsible for nosocomial infections and infections in immunocompromised patients. It accounts for 21% of the bacterial isolates from the surgical intensive care unit and for 14% of the bacterial isolates from febrile neutropenic patients in our unit, being the third most common isolate from both groups [1]. The prevalence of multiple resistant *P. aeruginosa* isolates has been increasing, so this study was undertaken in order to assess the current level of susceptibility and cross-resistances of widely used antipseudomonal antibiotics against *P. aeruginosa* in our hospital and an attempt was made to determine possible resistance mechanisms by phenotypic methods.

Methods

Bacterial strains

Ninety-nine consecutive, nonduplicate *P. aeruginosa* isolates were collected between June 2000 and May 2001 from various clinical materials at our hospital, which is a 700-beds teaching hospital in Istanbul, Turkey. Repeated

isolates from the same patient were excluded. All isolates were identified by a positive reaction to oxidase and production of pyocyanin. Of these isolates, 25 were from surgical wound or abscess, 25 from urine, 13 from blood, 12 from transtracheal aspirates, 9 from pleural fluid, 9 from cerebrospinal fluid, 5 from ear and 1 from peritoneal fluid. Forty-five % of them were isolated on the surgical intensive care unit.

Susceptibility tests

MICs for imipenem, meropenem, cefepime, ceftazidime, cefoperazone/sulbactam, piperacillin/tazobactam, ciprofloxacin, tobramycin, amikacin were determined by the E test method (AB Biodisk, Sweden). After overnight incubation, MIC breakpoints were interpreted according to the NCCLS guidelines [2]. MIC breakpoints of cefoperazone/sulbactam were interpreted according to the firm's recommendations. *P. aeruginosa* ATCC 27853 was used as reference strain.

Detection of group 1 inducible β-lactamases

The prevalence of inducible β-lactamases was investigated by disk approximation test method [3]. Ceftazidime (30 μg) disk was placed 20 mm. (centre to centre) from the imipenem (10 μg) disk on Mueller-Hinton agar plate inoculated with the test organism. After overnight incubation, distinct flattening of the inhibitory zone around the ceftazidime disk on the side nearest to the imipenem disk was regarded as the presence of inducible β-lactamase.

Detection of extended-spectrum β-lactamase (ESBL) activity

ESBL activity was investigated by double disk synergy method [4]. Ceftazidime (30 μg), cefepime (30 μg), aztreonem (30 μg) and cefotaxime (30 μg) disks (Oxoid) were placed 25 mm. (centre to centre) from the amoxicillin/clavulanic acid (20/10 μg) disk on Mueller-Hinton agar plate inoculated with the test organism. After overnight incubation, enhancement of the inhibition zone around one of these disks toward the clavulanate-containing disk indicated the presence of ESBLs.

Detection of metallo-β-lactamase activity

The presence of metallo-β-lactamase was investigated by the modified Hodge test [5]. An imipenem disk (10 μg) was placed at the centre of the Mueller-Hinton agar plate inoculated with an overnight culture suspension of imipenem-sensitive *E. coli*, which was adjusted to one-tenth turbidity of the McFarland no.0.5 tube. Imipenem-resistant isolates from the overnight culture plates were streaked heavily from the edge of the disk to the periphery of the plate. The presence of a distorted inhibition zone after overnight incubation was interpreted as the presence of metallo-β-lactamase.

Statistical analysis

Susceptibility data were compared by using a chi-square test. Correspondence analysis were performed by Cohen's kappa measurement.

Results

Table 1 shows the minimum inhibitory concentrations (MICs) of the tested antibiotics. Ciprofloxacin was the most in vitro active antibacterial agent followed by amikacin and ceftazidime ($p < 0.05$). Thirty-six % of isolates were resistant to more than one group of antibiotics. There were cross-resistances between antibiotics (Table 2). About all of meropenem resistant isolates were also resistant to imipenem ($\kappa = 0.93$, $p < 0.001$) and 75% of them were resistant to piperacillin/tazobactam ($\kappa = 0.61$, $p < 0.001$). Seventy-five % of carbapenem resistant isolates were susceptible to ciprofloxacin and amikacin. An important part (50–86%) of ceftazidime resistant isolates were also resistant to other β-lactams, especially cefepime ($\kappa = 0.68$, $p < 0.001$). Thirty-five -65% of ciprofloxacin resistant isolates were also resistant to β-lactams and 65% of them were susceptible to amikacin.

Table 1: Comparative MIC values of antibiotics tested for P.aeruginosa isolates.

MIC (μg/mL)	IP	MP	PM	TZ	CFS	PTc	Cl	TM	AK
MIC_{50}	1,5	0,75	8	2	12/6	8/4	0,25	16	8
MIC_{90}	>32	>32	>256	>256	>256/128	>256/4	>32	>256	48
R	**35**	**36**	**28**	**22**	**32**	**36**	**20**	**51**	**20**
I	**1**	**0**	**17**	**12**	**8**	**3**	**4**	**4**	**6**
S	**63**	**63**	**54**	**65**	**59**	**60**	**75**	**44**	**73**

IP: imipenem, MP: meropenem, PM: cefepime, TZ: ceftazidime, CFS: cefoperazone/sulbactam, PTc: piperacillin/tazobactam, Cl: ciprofloxacin, TM: tobramycin, AK: amikacin, R: resistant, I: intermediate, S: susceptible.

Table 2: Cross resistances of P.aeruginosa isolates.

Isolates resistant		number of isolates resistant to								
To	n	**IP**	**MP**	**PM**	**TZ**	**CFS**	**PTc**	**CI**	**TM**	**AK**
IP	35		34 (97)	17 (49)	11 (31)	22 (63)	25 (71)	8 (23)	30 (86)	9 (26)
MP	36	34 (94)		18 (50)	12 (33)	23 (64)	27 (75)	9 (25)	32 (89)	10 (28)
PM	28	17 (61)	18 (64)		19 (68)	18 (64)	19 (68)	13 (46)	23 (82)	13 (46)
TZ	22	11 (50)	12 (55)	19 (86)		13 (59)	15 (68)	11 (50)	16 (73)	8 (36)
CFS	32	22 (69)	23 (72)	18 (56)	13 (41)		22 (69)	9 (28)	30 (94)	11 (34)
PTc	36	25 (69)	27 (75)	19 (53)	15 (42)	22 (61)		7 (19)	29 (81)	13 (36)
CI	20	8 (40)	9 (45)	13 (65)	11 (55)	9 (45)	7 (35)		17 (85)	7 (35)
TM	51	30 (59)	32 (63)	23 (45)	16 (31)	30 (59)	29 (57)	17 (33)		18 (35)
AK	20	9 (45)	10 (50)	13 (65)	8 (40)	11 (55)	13 (65)	7 (35)	18 (90)	

IP: imipenem, MP: meropenem, PM: cefepime, TZ: ceftazidime, CFS: cefoperazone/sulbactam, PTc: piperacillin/tazobactam, CI: ciprofloxacin, TM: tobramycin, AK: amikacin. *The numbers in parentheses denote percent of isolates

Inducible β-lactamases were detected in 53% of isolates. Forty-eight (80%) of 65 isolates non-resistant to ceftazidime were positive for inducible beta-lactamase. ESBLs were detected in only 4 isolates by DDS method. Metallo-β-lactamases were detected in none of the isolates.

Discussion

P. aeruginosa is inherently resistant to many antimicrobial agents mainly due to the synergy between multi-drug efflux systems or a type 1 AmpC β-lactamase and low outer membrane permeability [6–8]. Inducible AmpC β-lactamase was detected in 53% of our isolates. The presence of inducible β-lactamase in 80% of isolates susceptible to ceftazidime suggests that resistance may emerge during treatment via selection of derepressed mutants from inducible populations. Resistance can also arise by the acquisition of plasmids encoding β-lactamases. The rate of strains with acquired resistance to ceftazidime has been estimated to range from 10% to 40% [9]. Our rate of ceftazidime resistance was 22%. In addition, various extended spectrum β-lactamases have been found in *P. aeruginosa*[9]. In our study, ESBLs were detected in only 4 isolates by DDS method. However, clinical laboratory detection may be difficult due to a combination of resistance mechanisms. The presence of AmpC system and class D ESBLs confer resistance to β-lactamase inhibitors, so synergy can not be detected.

The only β-lactam active against derepressed mutants and ESBLs are carbapenems but resistance to carbapenems has been increasing. The development of antibiotic resistance during the treatment is more frequent in imipenem than in ciprofloxacin and ceftazidime [7,10]. Our results demonstrate that carbapenems are second in affectivity after ceftazidime among other β-lactams with the resistance rate of 36%. Although still rare, isolates with metallo-β-lactamases have been reported from around the world [8,11]. In this study, we used the modified Hodge test, sensitivity of which was 100%, to screen metallo-β-lactamase-producing isolates and found no producers [5].

One third of our isolates were multiple resistant. There were cross-resistances between amikacin and/or ciprofloxacin and/or β-lactams. These data indicate that a high number of isolates probably have resistance due to impermeability or multi-drug efflux or a combination of multiple unrelated resistance mechanisms. Ciprofloxacin showed the highest in vitro antibacterial activity followed by amikacin in our centre. The major resistance mechanisms to quinolones are mutations in the target genes, which confer resistance only to quinolones, and mutations in regulatory genes for drug efflux pumps. The latter one, called Mar (multiple antibiotic resistance) mutation, results in cross-resistance to chemically unrelated antibiotics [6,8,12].

Although comparison between studies is difficult since the patient populations of the centres and the methods of studying differ, interestingly, we found a higher level of resistance to the β-lactams and a lower level of resistance to ciprofloxacin in contrast to other surveys and our 1995–1999 survey [13–16]. The incidence of resistance is dependent on the patterns of antibiotic usage. The relationship between the emergence of resistance of group 1 β-lactamase-producing organisms and the prior use of extended-spectrum cephalosporins is clearly proven [17]. The reduction of ceftazidime resistance had been observed after restricted use of it [18]. Our high level of sus-

ceptibility to ceftazidime and the low level of susceptibility to cefepime may reflect the increased use of cefepime and the decreased use of ceftazidime in recent years in our unit.

Conclusions

Resistance of *P. aeruginosa* to β-lactams seems common in our centre. About two-thirds of isolates were resistant to one or more antibiotics tested, with ciprofloxacin showing the highest in vitro antibacterial activity. Our findings suggest that ciprofloxacin may be of significant value for the treatment of severe infections caused by *P. aeruginosa* and may be more useful than β-lactams for combined treatment with amikacin. However, in order to confirm this suggestion antibiotic combinations should be tested in vitro and in vivo. In addition, these isolates from only one centre may be predominantly clonal, so isolates from different centres should be tested.

Authors' contributions

S Gençer planned and carried out the design of the study, the laboratory studies and coordinated all procedures; Ö Ak, N Benzonana, A Bat rel participated in the laboratory studies; S Özer participated in the design and coordination of the study.

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