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Antibiotic resistance in *Pseudomonas aeruginosa*: an Italian survey

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In order to assess the current level of resistance to widely used antipseudomonal antibiotics in clinical isolates of *Pseudomonas aeruginosa*, a national survey was undertaken. Fifteen hospitals throughout Italy participated in the study. The University of Catania tested the antibiotic susceptibility of 1005 consecutive clinically significant *P. aeruginosa* collected from March to June 1995. Lack of susceptibility, according to NCCLS breakpoints, was at the following rates: meropenem, 9.1%; imipenem, 19.3%; ceftazidime, 13.4%; carbenicillin, 27.3%; piperacillin, 12%; ticarcillin/clavulanic acid, 22.8%; amikacin, 10.6%; and ciprofloxacin, 31.9%. About half of the isolates (44.4%) were not susceptible to at least one of the antibiotics tested.

Introduction

Pseudomonas aeruginosa is an important nosocomial pathogen, causing high mortality in susceptible patients. In 1994, *P. aeruginosa* isolation accounted for 13.9% of the 25,266 consecutive aerobic bacteria isolates and for 20.9% of the 16,863 Gram-negative bacteria isolates examined in an Italian survey of clinically significant isolates from in- and out-patients (S. Stefani, unpublished observation). *P. aeruginosa* resistance can arise by various mechanisms including mutational derepression of the *amp*C chromosomal β-lactamases,[1] acquisition of plasmid- or transposon-mediated β-lactamases,[2] reduced uptake of aminoglycosides across the outer and cytoplasmatic membranes or production of enzymes,[3] modification of DNA gyrase (in the case of quinolone resistance),[4] and loss of D2 porin (resulting in imipenem resistance).[5] Multi-drug resistance has also been associated with multi-drug efflux.[6]

In order to assess the current level of susceptibility to the most common antibiotics with antipseudomonal activity against *P. aeruginosa*, an Italian survey was undertaken.

Materials and methods

Bacterial strains

Fifteen microbiology laboratories throughout Italy participated in this survey. From March to June 1995 each laboratory was asked to collect up to 200 consecutive, clinically significant *P. aeruginosa* isolates from in-patients. Repeat isolates or isolates from out-patients were excluded. The strains were sent to the University of Catania for re-examination and antibiotic susceptibility testing.

Identification of bacteria

All the isolates were re-examined by the University of Catania for a positive reaction to oxidase and production of pyocyanin on pseudomonas P agar (Difco, Milan, Italy). Strains giving positive reactions in both tests were accepted as *P. aeruginosa* and were not identified further. Oxidase-positive but pyocyanin-negative strains were identified by the API 20NE system (BioMérieux, Marcy L'Etoile, France). All other isolates were discarded.

Susceptibility tests

MICs were determined by dilution on IsoSensitest agar (Unipath, Milan, Italy). The following antibiotics were tested: meropenem (Zeneca, Milan, Italy), ticarcillin/clavulanate (SmithKline Beecham, Milan, Italy), piperacillin (Lederle, Catania, Italy) and amikacin, imipenem, carbenicillin, ceftazidime and ciprofloxacin (all from commercial sources). The inocula (10^4 cfu/spot) were plated using a multipoint inoculator (Denley). The MIC was defined as the lowest antibiotic concentration that inhibited visible growth. *P. aeruginosa* ATCC 27853 and *P. aeruginosa* NCTC 10662 were used as reference strains.

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Breakpoints used for all antibiotics tested were according to NCCLS criteria.[7]

Data analysis

The results were processed using Microsoft Access 2.0 and Excel 5.0 programs run on an IBM-compatible computer. Susceptibility data were compared using a chi-squared test.

Results and discussion

In total, 1153 *P. aeruginosa* isolates were sent to the University of Catania; 794 isolates were found to be oxidase-positive and pyocyanin producers and so were confirmed as *P. aeruginosa*. The identity of 211 strains (oxidase-positive and pyocyanin-negative) was confirmed using API 20NE. The remaining strains were discarded. Of the 1005 confirmed *P. aeruginosa* isolates, 35.5% were from the respiratory tract, 22.7% from urine, 19.5% from skin and soft tissue, 3.9% from blood, 2.8% from the ear and 11.4% from other sites; data were not available for 4.2%.

One-hundred and eighty-seven *P. aeruginosa* were isolated from patients in intensive care units (ICUs); the remainder were from other units.

The MICs of the tested antibiotics for *P. aeruginosa* isolates are summarized in Table I. Only 559 *P. aeruginosa* isolates (55.6%) were fully susceptible. Comparison of our data with those of another recent UK survey[8] shows the high incidence in Italy of resistant *P. aeruginosa* strains for all the antibiotics tested.

Meropenem was the most active compound followed by amikacin ($MICs_{90}$ = 4 mg/L and 32 mg/L, respectively). Resistance to meropenem was rarer than that to other antibiotics tested with <4% of isolates being resistant and 5% with intermediate susceptibility. Meropenem was four times more active than imipenem (MIC_{90} = 4 mg/L and 16 mg/L respectively). However, the activity of meropenem against these isolates was moderate because the MICs were higher than those of the fully susceptible strains. The difference in MICs of imipenem and meropenem for the imipenem-resistant strains has been attributed to the poorer β-lactamase-inducing ability of meropenem, improved β-lactamase stability, high affinity for penicillin binding proteins 2 and 3 or the possibility that meropenem diffuses through the outer membrane by non-specific and D2 imipenem-specific pathways.[9] One of these mechanisms could increase the steady-state periplasmic concentration of meropenem and thus reduce its MIC.

Table II shows the activity of all antibiotics tested against isolates resistant to one drug. The majority of meropenem-resistant *P. aeruginosa* were resistant to imipenem but not *vice versa*, as almost half of imipenem-resistant strains were susceptible to meropenem; moreover, the strains resistant to meropenem were also resistant to carbenicillin and ciprofloxacin, probably indicating an intrinsic mechanism of resistance, efflux or overproduction of outer membrane protein M(OprM).[10] Piperacillin-resistant strains were cross-resistant to carbenicillin and ticarcillin/clavulanate, which is a pattern typical of plasmid-encoded β-lactamases. Almost all the strains resistant to amikacin were resistant to ciprofloxacin. Cross-resistance between β-lactam and/or amikacin and/or ciprofloxacin was quite common, probably determined by impermeability or by multi-drug efflux.[6] Studies of resistance mechanisms in resistant isolates will be reported separately.

The incidence of resistance amongst isolates from different centres was compared. In general it was equally distributed between the centres, but in this context the incidence of resistance was dependent on the origin of the strains, probably reflecting the patterns of antibiotic usage for prophylaxis and therapy in the different hospital units. This was particularly evident for ciprofloxacin resistance, with one centre showing a low level of resistance (7.3%) and another a high level (59.4%). The majority of strains tested at the former centre came from paediatrics, while those from the latter were from a haematology unit, and the differing levels of resistance found are in accordance with the low and high use of this drug in these units respectively. Resistance to all the antibiotics tested was commonest amongst isolates from patients in ICUs than amongst those from other units. This probably reflects the higher use of antibiotics in ICUs. Interestingly, we found a high level of resistance to imipenem (40.6%) and ciprofloxacin (38.2%) and a low level of resistance to piperacillin, meropenem and amikacin (data not shown).

In conclusion, resistance of *P. aeruginosa* to penicillins, ceftazidime, ciprofloxacin and amikacin seems common in Italy. About half the isolates were resistant to one or more antibiotics tested, with meropenem showing the highest antibacterial activity and ciprofloxacin the lowest. The patterns of susceptibility were equally distributed in Italy. Resistance to all the antimicrobials was more common in ICUs than in other hospital units. Overall, our findings indicate that meropenem, which has been available in Italy since the end of 1994, retains a high level of activity against clinical isolates of *P. aeruginosa* and suggest that it has potential as an antipseudomonal agent.

References

1. Curtis, N. A. C., Eisenstadt, R. L., Rudd, C. & White. A. J. (1986). Inducible type-I β-lactamase of Gram-negative bacteria and resistance to β-lactam antibiotics. *Journal of Antimicrobial Chemotherapy* **17**, 51–61.

2. Jacoby, G. A. & Sutton, L. (1979). Activity of β-lactam antibiotics against *Pseudomonas aeruginosa* isolates carrying R-plasmids determining different β-lactamases. *Antimicrobial Agents and Chemotherapy* **16**, 243–5.

Table I. Distribution of MICs (mg/L) of antibiotics tested for 1005 isolates of *P. aeruginosa*

MIC (mg/L)	Meropenem	Imipenem	Ceftazidime	Carbenicillin	Piperacillin	Ticarcillin/ clavulanate	Amikacin	Ciprofloxacin
≤0.03	1							
0.03	9							8
0.06	56							20
0.12	127						3	146
0.25	170	18	2		7	8	7	213
0.5	199	133	51	9	12	13	28	188
1	128	369	293	14	32	7	77	109
2	117	209	266	9	160	4	238	37
4	107	82	153	8	259	19	297	25
8	38	88	106	9	156	43	161	30
16	45	82	37	16	94	207	87	49
32	8	16	26	145	86	290	36	124
64		8	24	331	78	185	31	43
128			27	190	47	116	29	13
256			20	130	31	73	9	
512				72	39	40	2	
>1024				72	4			
MIC_{50}	0.5	1	2	64	8	32	4	0.5
MIC_{90}	4	16	16	512	128	256	32	32
% Sensitivity	90.9	80.7	86.7	72.7	88.0	77.2	89.4	68.1

Solid lines indicate breakpoints for fully susceptible strains; dashed lines indicate breakpoints for resistant strains; values between the lines are intermediate.

Table II. Cross-resistance of *P. aeruginosa* isolates

Isolates resistant to	n^a	% Susceptible to other antibiotics							
		meropenem	imipenem	carbenicillin	ceftazidime	piperacillin	ticarcillin/clavulanate	amikacin	ciprofloxacin
Meropenem	91	–	9.0	20.5	54.4	71.4	33.0	75.1	23.2
Imipenem	194	47.4	–	46.0	65.0	74.7	55.6	61.8	25.7
Carbenicillin	274	65.3	54.8	–	45.9	50.7	14.6	74.3	35.4
Ceftazidime	134	62.0	49.2	19.4	–	66.4	32.0	65.6	35.8
Piperacillin	121	65.3	59.5	16.5	62.8	–	22.3	71.9	23.9
Ticarcillin/clavulanate	229	67.2	62.4	7.8	60.2	59.0	–	75.1	36.2
Amikacin	107	78.0	53.8	56.0	66.5	79.0	64.8	–	5.5
Ciprofloxacin	319	75.9	59.3	46.7	74.5	72.3	56.5	69.5	–

[a]Insusceptible strains; some strains were resistant to more than one drug.

3. Shannon, K. & Phillips, I. (1982). Mechanisms of resistance to aminoglycosides in clinical isolates. *Journal of Antimicrobial Chemotherapy* **9**, 91–102.

4. Iyobe, S., Hirai, K. & Hashimoto, H. (1991). Drug resistance in *Pseudomonas aeruginosa* with special reference to new quinolones. In *Pseudomonas aeruginosa in Human Disease* (Homma, J. Y., Tanimoto, H., Holder, I. A., Hoiby, N. & Doring, G., Eds), pp. 209–14. Karger, Basle.

5. Buscher, K. H., Cullmann, W., Dick, W. & Opferkuch. W. (1987). Imipenem resistance in *Pseudomonas aeruginosa* resulting from diminished expression of an outer membrane protein. *Antimicrobial Agents and Chemotherapy* **31**, 703–8.

6. Li, X.-Z., Ma, D., Livermore, D. M. & Nikaido, H. (1994). Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: active efflux as a contributing factor to β-lactam resistance. *Antimicrobial Agents and Chemotherapy* **38**, 1742–52.

7. National Committee for Clinical Laboratory Standards. (1995). *Standard Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria which Grow Aerobically; Approved Standard M7-A2.* NCCLS, Villanova, PA.

8. Chen, H. Y., Yuan, M., Ibrahim-Elmagboul, I. B. & Livermore, D. M. (1995). National survey of susceptibility to antimicrobials amongst clinical isolates of *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy* **35**, 521–34.

9. Fung-Tomc, J. C., Huczko, E., Banville, J., Menard, M., Kolek, B., Gradelski, E. *et al.* (1995). Structure–activity relationships of carbapenems that determine their dependence on porin protein D2 for activity against *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* **39**, 394–9.

10. Sumita, Y. & Fukasawa, M. (1996). Meropenem resistance in *Pseudomonas aeruginosa*. *Chemotherapy* **42**, 47–56.

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