IN VITRO ACTIVITY OF AMINOGLYCOSIDES, UREIDOPENICILLINS AND CEPHALOSPORINS AGAINST *P. AERUGINOSA* ISOLATED IN KUALA LUMPUR

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SUMMARY

170 clinical isolates of Pseudomonas aeruginosa *were tested for* in vitro *susceptibility to gentamicin, amikacin, tobramycin, netilmicin, kanamycin, streptomycin, cefotaxime, ceftriaxone, cefoperazone, ceftazidime, moxalactam, azlocillin, piperacillin and ticarcillin.*

Against 93 gentamicin-sensitive strains, the most active antibiotics were in descending order, ceftazidime, tobramycin, gentamicin, amikacin, and the ureidopenicillins.

Against 77 gentamicin-resistant strains, only ceftazidime, amikacin and moxalactam had mode minimum inhibitory concentrations within achievable peak serum levels after standard therapeutic dosage.

There was no correlation between cephalosporin resistance and aminoglycoside resistance except for cefoperazone, which, together with the ureidopenicillins and ticarcillin, showed marked decrease in activity against gentamicin-resistant strains.

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INTRODUCTION

Pseudomonas aeruginosa is currently one of the most important opportunistic pathogens causing potentially fatal infections in hospitalized patients. Infections by this organism are notoriously difficult to treat largely because of the organism's innate resistance to many antibiotics,[1] its increasing resistance to currently available anti-pseudomonal drugs[2,3] and the vulnerability of the patients whose defences are often compromised.[4]

For the past two decades, the aminoglycoside drugs have been the mainstay of antipseudomonal therapy. The first highly active aminoglycoside, gentamicin, was introduced in 1963.[5] Since then newer derivatives like tobramycin, amikacin, sisomicin and netilmicin have been promoted either because they had higher *in vitro*[6,7] activity against pseudomonas or because they were active against some gentamicin-resistant strains.[8,9]

The first antipseudomonal penicillin, carbenicillin, was introduced in 1969.[10] The successful development of this drug led to the appearance of

ticarcillin, an analogue of carbenicillin with improved antipseudomonas activity[11] and more recently, to a number of new ureido derivatives of penicillin such as piperacillin, azlocillin and mezlocillin, all of which showed significant activity against *Pseudomonas aeruginosa.*[12,13] With the advent of modern cephalosporins, more β-lactam antibiotics became available for the effective treatment of pseudomonas infections. These include cefsulodin which is exclusively effective against *Pseudomonas aeruginosa,*[14] broad-spectrum cephalosporins like cefotaxime, cefoperazone, ceftriaxone, ceftazidime and moxalactam, which is an oxa-β-lactam.[15] The activities of some of these new cephalosporins have been found to rival those of the more active aminoglycosides.[16]

In the present study, the *in vitro* activities of six aminoglycosides, three penicillins, four cephalosporins and an oxa-β-lactam were compared against 170 clinical isolates of *Pseudomonas aeruginosa* in the University Hospital.

MATERIALS AND METHODS

Organisms

170 isolates of *Pseudomonas aeruginosa* were randomly chosen from a bank of pseudomonas isolates obtained from clinical specimens processed in the Department of Medical Microbiology, University Hospital, Kuala Lumpur between December 1981 and December 1982. Most of the isolates were from urine, tracheal secretions and various wound swabs. The organisms were identified by standard laboratory methods and kept on nutrient agar slopes or freeze-dried soon after isolation.

Antibiotics

Antibiotic powders were obtained from Sigma (gentamicin, streptomycin, amikacin), Eli Lilly (tobramycin, moxalactam), Schering (netilmicin), Meiji Seika (kanamycin), Beecham (ticarcillin), Glaxo (ceftazidime), Roche (ceftriaxone), Hoechst (cefotaxime), Pfizer (cefoperazone), Bayer (azlocillin) and Lederle (piperacillin). Stock solutions at 10,000 mg/l were kept at −20°C. When required for the preparation of antibiotic agar plates, working solutions were made in sterile distilled water. All antibiotic agar plates were kept at 4°C and used within two weeks of preparation.

Minimum Inhibitory Concentrations (MICs)

MICs were determined using the agar plate dilution method. Two-fold antibiotic dilutions were made in Mueller-Hinton agar (Difco Laboratories). Strains of *Pseudomonas aeruginosa* were grown on nutrient agar overnight and suspended in nutrient broth to a turbidity equivalent to 0.5 McFarland standard barium sulphate solution, using a Junior Coleman spectrophotometer. The suspensions were further diluted 1 in 100 and inoculated onto antibiotic plates by a Denley Multipoint inoculator delivering approximately 10^3 bacteria per "spot". The plates were then incubated overnight at 36°C. The lowest antibiotic concentration that permitted no growth of the organism was considered the MIC. *Escherichia coli* NCTC 10418 was used as the control organism.

RESULTS

Table I shows the percentage susceptibilities of the 170 clinical isolates of *Pseudomonas aeruginosa* to the 14 antibiotics tested. It can be seen that ceftazidime and amikacin were the most predictably active, inhibiting respectively 98.8 and 97.7% of the isolates. The penicillins and moxalactam came next (70.6 – 77.7% inhibition) followed by the third group of newer aminoglycosides and cephalosporins, which were active against 38.2 – 54.7% of the isolates. The two older aminoglycosides, streptomycin and kanamycin, inhibited less than 10% of the isolates tested.

Considering the activity of aminoglycosides on gentamicin-sensitive strains (Fig. 1), tobramycin, gentamicin and amikacin showed the highest activity with MIC_{90s} of 4.0, 4.9 and 5.9 mg/l respectively. Netilmicin with a MIC_{90} of 14.5 mg/l was almost three times less active than gentamicin. Kanamycin and streptomycin showed

TABLE I

SUSCEPTIBILITIES OF 170 CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* TO 14 ANTIBIOTICS

Antibiotics	No. susceptible*	(%)
Ceftazidime	168	(98.9)
Amikacin	166	(97.7)
Piperacillin	132	(77.7)
Azlocillin	132	(77.7)
Ticarcillin	126	(74.1)
Moxalactam	120	(70.6)
Gentamicin	93	(54.7)
Tobramycin	91	(53.5)
Cefoperazone**	88	(51.8)
Ceftriaxone	81	(47.7)
Cefotaxime	68	(40.0)
Netilmicin	65	(38.2)
Streptomycin	15	(8.8)
Kanamycin	3	(1.8)

*Isolates were considered susceptible if MIC was $\leqslant$ 8 mg/l of gentamicin, tobramycin and netilmicin; $\leqslant$ 16 mg/l of amikacin, ceftazidime, cefotaxime, cefoperazone, cetriaxone and moxalactam; $\leqslant$ 32 mg/l of kanamycin and streptomycin; $\leqslant$ 128 mg/l of ticarcillin, azlocillin and piperacillin.

**If moderately susceptible strains with MICs of 32 and 64 mg/l were included the total no. susceptible would be 152 or 89.4%.

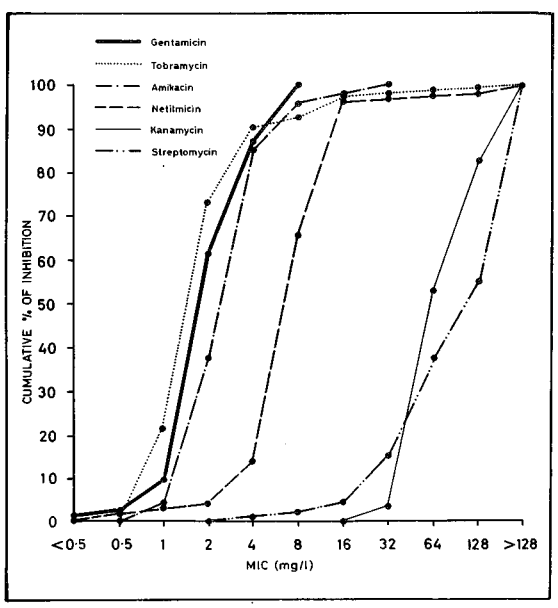

Fig. 1 Inhibitory activity of six aminoglycosides against 93 gentamicin-sensitive clinical isolates of *Pseudomonas aeruginosa*.

little activity with MIC_{90s} well above 128 mg/l. The percentage of gentamicin-sensitive isolates which were resistant to amikacin, tobramycin and netilmicin were 2.5%, 7.5% and 34.5% respectively.

Against gentamicin-resistant isolates (Fig. 2), amikacin (MIC_{90} 7.9) was the only effective aminoglycoside. 97.5% of these isolates were sensitive to amikacin. There was almost complete cross-resistance among gentamicin, tobramycin and netilmicin with only 6.5% of the gentamicin-resistant isolates being sensitive to netilmicin.

Of the β-lactam antibiotics (Fig. 3), ceftazidime showed the greatest degree and predicability of activity. Its MIC_{90} (3.7 mg/l) was almost nine times lower than that of the next most active agent moxalactam (MIC_{90} 29.2 mg/l). The other B-lactams had rather similar MIC_{50s} ranging from 9 mg/l (azlocillin) to 24 mg/l (ticarcillin) but a wider range of MIC_{90s} from 29 mg/l (moxalactam) to $>$ 128 mg/l (ticarcillin).

There was no significant difference in the activities of moxalactam, ceftazidime, cefotaxime

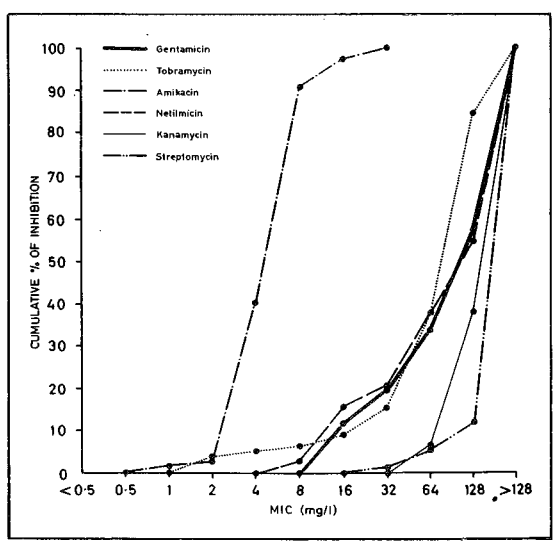

Fig. 2 Inhibitory activity of six aminoglycosides against 77 gentamicin-resistant clinical isolates of *Pseudomonas aeruginosa*.

and ceftriaxone against gentamicin-sensitive and gentamicin-resistant isolates. However cefoperazone, the ureidopenicillins and ticarcillin were much less active against gentamicin-resistant isolates (Table II)

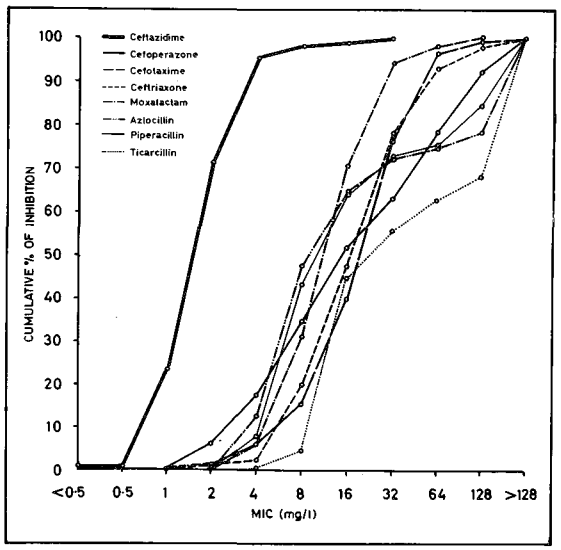

Fig. 3 Activity of 8 β-lactam antibiotics against 170 strains of *P. aeruginosa*.

TABLE II

INVITRO* ACTIVITIES OF AMINOGLYCOSIDES AND β-LACTAMS AGAINST GENTAMICIN-SENSITIVE AND RESISTANT ISOLATES OF *PSEUDOMONAS AERUGINOSA

Antibiotics	Mode MICs (mg/l)	
	gentamicin-sensitive (93 isolates)	gentamicin-resistant (77 isolates)
Gentamicin	2	> 128
Tobramycin	2	> 128
Amikacin	4	8
Netilmicin	8	> 128
Kanamycin	128	> 128
Streptomycin	>128	> 128
Ceftazidime	2	4
Cefoperazone	16	128
Moxalactam	16	16
Ceftriaxone	16	32
Cefotaxime	32	32
Azlocillin	8	> 128
Piperacillin	8	> 128
Ticarcillin	16	> 128

DISCUSSION

In the University Hospital, Kuala Lumpur, gentamicin is the most often used antipseudomonal antibiotic. In addition it is used as first line empirical treatment for severe gram-negative sepsis. In 1983, 23.4% of all pseudomonas isolated in the hospital and 52.3% of all pseudomonas isolated in the surgical wards were gentamicin-resistant. Thus cross-infection with gentamicin-resistant *Pseudomonas aeruginosa* has occurred extensively in our hospital.

Bacterial resistance to aminoglycosides may be due to a variety of mechanisms including membrane impermeability to the drugs and the presence of plasmids that code for aminoglycoside inactivating enzymes.[17] While permeability resistance would affect the entry of aminoglycosides into bacterial cells, inactivating enzymes, specifically acetylate amino groups or adenylate or phosphorylate the hydroxyl groups on individual aminoglycoside molecules. There are many acetylating, adenylating and phosphorylating enzymes known to modify currently used aminoglycosides. Two of the acetylating enzymes, AAC (6')-II which is exclusively present among *Pseudomonas aeruginosa* strains, and AAC(3)-III which is mainly present among *Pseudomonas aeruginosa* inactivate kanamycin, gentamicin, tobramycin and netilmicin but not amikacin.[18] The results of our study showed that one or both of these enzymes might have been responsible for the aminoglycoside resistance among the *Pseudomonas aeruginosa* strains circulating in our hospital.

Resistance to β-lactam antibiotics among the *Pseudomonas aeruginosa* is again mainly determined by cell membrane impermeability and enzymic destruction of these antibiotics by β-lactamases. Virtually all isolates of *Pseudomonas aeruginosa* produce an inducible, chromosomally-determined β-lactamase which is mainly a cephalosporinase. Some strains, in addition, produce plasmid-mediated β-lactamases which can destroy either cephalosporins or penicillins.[17] Livermore, Williams and Williams[15] described a PSE1 and PSE4 β-lactamase which was active against carbeni-

cillin, cefoperazone and cefsulodin and a TEM2 enzyme which was active against carbenicillin and cefoperazone. All three β-lactamases were inactive against moxalactam, ceftazidime and ceftriaxone. Of the eight β-lactam antibiotics we studied, cefoperazone alone of the third generation cephalosporins, the ureidopenicillins and ticarcillin showed decreased activities against the gentamicin-resistant isolates. This suggested the possibility that our pseudomonas strains which carried plasmids determining aminoglycoside-inactivating enzymes also carried plasmids mediating the production of β-lactamases which can inactivate cefoperazone, the ureidopenicillins and ticarcillin.

For the therapy of pseudomonas infections it appeared from our results that the two older aminoglycosides, kanamycin and streptomycin were totally ineffective. For gentamicin-sensitive strains, the aminoglycosides on the whole had better activity, weight for weight than the cephalosporins and penicillins. However, the low toxicity of the β-lactam antibiotics should make them preferable to the more toxic aminoglycosides in the treatment of patients with impaired renal function. For gentamicin-resistant strains, ceftazidime and amikacin appeared to be the most dependable. Moxalactam had a MIC which is readily attainable in the serum but the use of this drug has been curtailed by the report of coagulopathies following its use.[19]

Our finding that ceftazidime was superior to most other cephalosporins against pseudomonas was in agreement with the results of other workers.[15,20] In addition, of the 170 isolates tested, only two were found to be resistant with MICs of 32 mg/l. This drug has been said to possess one of the widest usable antimicrobial spectrum among the β-lactam antibiotics[16] and has been used successfully as a single agent in the treatment of severe *Pseudomonas aeruginosa* infections.[21]

Amikacin has many advantages over gentamicin including having higher and more predictable serum levels[22] and being least affected by aminoglycoside-inactivating enzymes.[18] Many hospitals have been keeping amikacin in reserve for the therapy of gentamicin-resistant strains in order to prevent the emergence of amikacin-resistance. This practice no longer seems appropriate in the light of recent findings that amikacin resistance existed even in hospitals where the drug has never been used and that no increase in amikacin-resistance has been observed in the same hospitals following unrestricted use of the drug.[23,24] This can be due to the fact that the acetylating enzymes which inactivate amikacin also inactivate all other currently used aminoglycosides.

Hence the use of drugs like gentamicin, tobramycin and kanamycin would also select out amikacin-resistant strains and the only way to reduce the risk of increasing amikacin resistance appears to be the suspension of the use of all aminoglycosides. However aminoglycosides are indispensable at the moment and because of the high incidence of gentamicin-resistant organisms in our hospital, it might be more appropriate to use amikacin instead of gentamicin as first line therapy for severe gram-negative pseudomonas infections.

ACKNOWLEDGEMENT

The authors are grateful to Professor Alan Percival of the Department of Bacteriology and Virology, University of Manchester, United Kingdom for the help and advice given in the preparation of this manuscript; to Mrs S.T. Soo and Encik Abdul Aziz for technical assistance.

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