SUSCEPTIBILITY OF 57 STRAINS *PS. PSEUDOMALLEI* TO SOME NEW B-LACTAMS AND OTHER ANTIBIOTICS.

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SUMMARY

Fifty seven strains of *Pseudomonas pseudomallei* were tested for *in vitro* susceptibility to 15 antimicrobial agents.

Amongst the generally recommended antibiotics for therapy of melioidosis, only 86%, 84% and 58% of the strains were found to be sensitive to trimethoprim-sulphamethoxazole, chloramphenicol and tetracycline respectively. Of the newer B-lactams, in descending order of activity were, ceftazidime, ceftriaxone, cefotaxime, cefoperazone and cefuroxime. But on a weight for weight basis, ceftazidime was the most active agent and as such, may be considered in the therapy of acute septicaemic melioidosis.[5]

INTRODUCTION

Melioidosis, a glanders-like disease of man, has been reported to be endemic in South East Asian countries such as Thailand, Hong Kong,[1] Singapore[2] and Malaysia[3] as well as in Queensland, Australia.[4] The traditional chemotherapeutic agents for the treatment of the septicaemic as well as the chronic forms of the disease have been cholramphenicol and tetracycline in large doses for prolonged periods of time. This is because the causative organism, *Pseudomonas pseudomallei*, is intrinsically resistant to the penicillins as well as the aminoglycosides.

Recently, we have encountered strains of *Ps. pseudomallei* resistant to chloramphenicol, tetracycline or to both. Therefore, it has become necessary to look for alternative chemotherapeutic agents. Preliminary reports from Thailand and Hong Kong have indicated the use of a third generation cephalosporin – ceftazidime, in the successful treatment of acute pulmonary melioidosis.[5]

We have recorded since 1977, about 50 cases of human melioidosis as well as 10 in animals. There are but few reports of *in vitro* studies of antibiotic susceptibility of *Ps. pseudomallei*. Because of the therapeutic dilemma facing physicians who must treat such patients, it was considered appropriate to examine the *in vitro* susceptibilities of these strains to the newer cephalosporins, monobactams and quinolones as well as the traditionally used drugs.

MATERIALS AND METHODS

Forty seven clinical isolates of *Ps. pseudomallei* and 10 strains from animals (six guinea-pigs, three monkeys, one goat) isolated in Malaysia from 1978 to 1986 were used in this study. Of the 47 human isolates, 27 were from blood cultures, 13 from pus and seven from urines. Isolates were identified by colonial morphology, gram stained appearance and biochemically. Confirmation was by slide agglutina-

tion with specific antiserum. These strains were kept on nutrient agar slopes or freeze-dried soon after isolation. A number of the earlier isolates were sent for identification and confirmation to the Computer Trials Laboratory, National Collection of Type Cultures, Colindale, London.

Most of the antibiotics selected for the study were obtained as laboratory standard powders of known potency from the manufacturers. The remainder was pure preparations intended for clinical use. Stock solutions were made and stored at −70°C.

Minimal inhibitory concentrations (MICs) were determined by the agar dilution method using unsupplemented Mueller-Hinton agar (Difco, USA), containing two-fold dilutions of the antibiotics. Iso-Sensitest agar (Oxoid, England) was used for testing trimethoprim-sulphamethoxazole. Overnight cultures of the individual strains were suspended in Mueller-Hinton broth (MHB) and adjusted turbidimetrically using a Junior Coleman spectrophotometer, to approximately 10^8 organisms/ml. The suspensions were further diluted 1:100 in MHB just prior to inoculation onto antibiotic-containing plates and antibiotic-free control plates. A Denley multipoint inoculator was used to deliver approximately $10^3 - 10^4$ organisms per "spot". The plates were incubated overnight at 37°C. The MIC was defined as the lowest concentration of antibiotic completely inhibiting the visual growth of the organism.

Standard reference strains of *E. coli* (NCTC 10418) and Oxford Staphylococcus (NCTC 6571) were included in all individual runs.

RESULTS

Fifty seven strains of *Ps. Pseudomallei* were tested against 15 antimicrobial agents. Table 1 shows the MIC at which 90% of the strains were inhibited (MIC_{90}), the range of MIC values for each antibiotic and the percentage susceptibilities of the strains.

The earlier B-lactam antibiotics had very poor activity against *Ps. pseudomallei* : all the strains had MICs of more than 16 mg/L to ampicillin. Against carbenicillin, the first penicillin to have anti-pseudomonal activity, only two strains of *Ps. pseudomallei* had MICs of $\leqslant 128$ mg/L, while with ticarcillin, 12 strains had MICs of $\leqslant 128$ mg/L.

The aminoglycoside gentamicin exhibited no activity against *Ps. pseudomallei*, for all strains tested had MICs well above eight mg/L. As for kanamycin, only nine strains were found to be sensitive with MICs $\leqslant 16$ mg/L.

Amongst the drugs that have been traditionally used in the treatment of melioidosis, 58% of the strains had MICs $\leqslant 8$ mg/L to tetracycline, 84% with MICs $\leqslant 16$ mg/L to chloramphenicol and 86% had MICs $\leqslant 32$ mg/L to trimethoprim-sulphamethoxazole. One isolate was resistant to all three agents (MICs of 16 mg/L to tetracycline, >128 mg/L to cholramphenicol and >32 mg/L to trimethoprim-sulphamethoxazole). In addition, five other isolates were resistant to both tetracycline (MICs >8 mg/L) and chloramphenicol (MICs >16 mg/L) and two isolates were found to be resistant to trimethoprim-sulphamethoxazole (MICs >32 mg/L) and chloramphenicol (MICs >16 mg/L).

Three of the newer B-lactam antibiotics showed very good *in vitro* activity; the $MICs_{90}$ of ceftazidime, cefotriaxone and cefotaxime were 2, 4 and 8 mg/L respectively and on a weight for weight basis, ceftazidime was the most active agent. Cefoperazone had moderate activity; 36 strains (63%) had MICs <16 mg/L, while cefuroxime showed poor *in vitro* activity, all the strains had MICs $\geqslant 16$ mg/L. Aztreonam, a monobactam with activity against aerobic gram-negative bacilli, had only marginal activity

Table 1. *In vitro activities of 15 antibiotics against 57 strains of Ps. pseudomallei*

Antibiotic	MIC(mg/l) MIC_{90}	Range	% susceptible
Traditional drugs used in treatment:			
Chloramphenicol(⩽16)*	32	8–>128	84
Tetracycline (⩽8)	16	8– 128	58
Trimethoprim-sulphamethoxazole (⩽32)	>32	4– >32	86
B-lactams:			
Ceftazidime (⩽8)	2	1– 4	100
Ceftriaxone (⩽8)	4	2– 8	100
Cefotaxime (⩽16)	8	4– 16	100
Cefoperazone (⩽16)	32	16– 64	63
Cefuroxime (⩽8)	32	16– 64	0
Ticarcillin (⩽128)	256	32–>256	21
Carbenicillin (⩽128)	>128	128–>128	4
Ampicillin (⩽16)	128	32–>128	0
Aminoglycosides:			
Kanamycin (⩽16)	32	16– 64	16
Gentamicin (⩽8)	64	16–>128	0
Quinolone:			
Pefloxacin (⩽1)	16	4– 64	0
Monobactam:			
Aztreonam (⩽16)	32	4– 64	14

*( ) *Denotes breakpoint MICs.*

against *Ps. pseudomallei* ; only eight strains (14%) had MICs ⩽16 mg/l. Similary pefloxacin, a quinolone derivative, showed poor *in vitro* activity (MIC_{90} 16 mg/L).

DISCUSSION

Melioidosis remains a problem in three aspects – clinical diagnosis, laboratory confirmation and management. Therapeutic problems are related to the organism as well as the host. The disease affects mainly immunocompromised hosts and the organism has a propensity to cause chronic infections or remain latent for prolonged periods of time.[6] In addition, this organism is not susceptible to commonly used anti-pseudomonal drugs.

The traditional drugs used to treat both the acute septicaemic and chronic forms of the disease have been tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole.[7,4] However, these drugs are bacteriostatic in their action and in addition, there have been reports of the emergence of resistance to these three drugs.[5] Twenty-four of our strains had MICs >8 mg/L to tetracycline, nine with MICs >16 mg/L to chloramphenicol and eight strains with MICs>32mg/L to trimethoprim-sulphamethoxazole. Furthermore, there was one strain that was resistant to all three drugs. Hence, there was an urgent need to look for alternative drugs such as the newer B-lactams and quinolones which have shown activity against other pseudomonads, with the view of using them in the management of acute septicaemic melioidosis which is a life threatening illness.

A recent paper[1] reported the good *in vitro* activities of ceftazidime, piperacillin and carumonam and comparable activity with ceftriaxone, RO 15–8074 and cefotaxime, while tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole showed only moderate activity. Our findings are similar to theirs and in decreasing order of activity of the newer B-lactams were ceftazidime > ceftriaxone > cefotaxime > cefoperazone > cefuroxime. For further clinical evaluation, we would recommend only the first three drugs. So *et al*[5] reported the successful treatment of pulmonary melioidosis with ceftazidime and we also had good results of treatment with ceftazidime in a patient with acute septicaemic illness where the organism was found to be resistant to tetracycline, chloramphenicol and trimethoprim-sulphamethoxazole.

Aztreonam, a monobactam, was only marginally better than cefuroxime and should not be considered in the therapy of melioidosis. This is also true of the quinolone derivative, pefloxacin. Chau *et al*[1] also reported similar findings with ciprofloxacin and ofloxacin.

In conclusion, the use of B-lactams such as ceftazidime, ceftriaxone and cefotaxime may be considered in the therapy of acute septicaemic melioidosis followed by tetracycline and chloramphenicol for a more prolonged period of time in view of the chronicity of the disease.

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