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Antimicrobial resistance in *Burkholderia pseudomallei*

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Abstract

Four strains of *Burkholderia pseudomallei* were used to determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time-kill curves with 13 single antimicrobial agents: ceftazidime, piperacillin, imipenem, amoxicillin/clavulanic acid, doxycycline, cotrimoxazole, kanamycin, rifampicin, ciprofloxacin, trovafloxacin, clarithromycin, azithromycin and meropenem. The time-kill studies were also performed with 33 pairs of combinations of the above antimicrobial agents: 15 combinations which would be expected to be used for acute therapy and 18 combinations for maintenance therapy. The results show that the single and combination antimicrobial agents with bactericidal effects against the four strains of *B. pseudomallei* which should be used for clinical trials in acute melioidosis are: imipenem, meropenem, and imipenem + azithromycin. The combination antimicrobial agents which should be further studied for the ability to eliminate biofilm and intracellular killing effect are ciprofloxacin + clarithromycin, ciprofloxacin + azithromycin, and imipenem + azithromycin.

Keywords: *Burkholderia pseudomallei*; Antimicrobial resistance; Macrolides

1. Introduction

Relapse in melioidosis treatment with various antimicrobial agents including ceftazidime and amoxicillin/clavulanic acid have been reported (Ashdown, 1988; Chaowagul et al., 1993). The explanation for this is the ability of *Burkholderia pseudomallei* to survive within phagocytic cells (Pruksachartvuthi et al., 1990; Vorachit et al., 1995a), produce glycocalyx, and form microcolonies in infected tissue (Vorachit et al., 1995b). Since β-lactams do not penetrate intracellular sites and kill non-multiplying bacteria, therapy with β-lactams may not prevent future relapse of melioidosis. β-Lactams with good in vitro activity also fail in the treatment of intracellular infection such as typhoid and Legionnaire's disease (McEniry et al., 1988). The biofilm of a strain susceptible to ceftazidime and cotrimoxazole showed high resistance to ceftazidime and cotrimoxazole (Vorachit et al., 1993) explaining the relapse of melioidosis treated with those antimicrobial agents. Moreover, the time-kill studies showed that ceftazidime, ciprofloxacin and cotrimoxazole were not bactericidal for susceptible strains of *B. pseudomallei* (Sookpranee et al.,

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1991). Therefore, the ideal antimicrobial agents for melioidosis therapy should have bactericidal effect, should be able to penetrate phagocytic cells, and eliminate or inhibit the production of glycocalyx.

2. Materials and methods

2.1. Bacteria

Four strains of *B. pseudomallei* isolated from melioidosis patients provided by N.J. White (Faculty of Tropical Medicine, Mahidol University) were tested. The criteria for the selected strains were: ceftazidime susceptible (CTZ-S), ceftazidime resistant (CTZ-R), cotrimoxazole susceptible (SXT-S), and cotrimoxazole resistant (SXT-R).

2.2. Antimicrobial agents

Determinations of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were done on 13 antimicrobial agents: ceftazidime (CTZ), piperacillin (PIP), imipenem (IPM), amoxicillin/clavulanic acid (AUG), doxycycline (DOXY), cotrimoxazole (SXT), kanamycin (K), rifampicin (RIF), ciprofloxacin (CIP), trovafloxacin (TROVA), clarithromycin (CLA), azithromycin (AZI) and meropenem (MERO). Thirty-three pair combinations of the above antimicrobial agents were tested in the time-kill assay.

2.3. Methods

MIC and MBC were determined using the broth dilution (macrodilution) method as described by the National Committee for Clinical Laboratory Standards (NCCLS M7-A4) (Jorgensen et al., 1997). Time-kill assay (Knapp and Moody, 1992) was performed in duplicate for measuring the rate of bacterial killing, and the synergistic and antagonistic effect of single and combined antimicrobial agents. The antimicrobial concentration used was $1 \times$ MIC, $2 \times$ MIC or $4 \times$ MIC. Most concentrations chosen represented levels reached in blood, unless the MIC was higher than the blood level. Colonies were counted at time intervals 0, 2, 4, 6, and 24 h, in duplicate.

2.4. Interpretation

Bactericidal activity was recorded if bactericidal activity decreased by $3 \log_{10}$ CFU/ml or more compared with the growth control.

3. Results

With the tube dilution technique, the MBC results showed bactericidal activity of AUG, IPM and MERO against the four strains of *B. pseudomallei*. CTZ and PIP showed the bactericidal effect for strain CTZ-S and SXT-R (Table 1).

Bactericidal effects were observed for MERO, IPM and IPM + AZI against the CTZ-S, CTZ-R and SXT-R strains. MERO and IPM + AZI were effective against the SXT-S strain (Fig. 1). Bactericidal effects were also observed for CIP + CLA and CIP + AZI against CTZ-S strain; for CIP + CLA against the SXT-S strain; and for CIP, CIP + CLA and CIP + AZI against SXT-R strain. No bactericidal effect were observed on the CTZ-R strain (Fig. 2).

An antagonistic effect was observed when testing AUG + SXT with CTZ-R and SXT-R strains, SXT + CIP with CTZ-S strain, AUG + AZI with SXT-S strain, AUG + RIF with CTZ-S and SXT-R strains and MERO + AZI with CTZ-S and SXT-S strains (Table 2). Some combination drugs showed decreased activity when compared with the most effective drug alone, such as the combination of SXT and DOXY or CTZ which are commonly used for the treatment of melioidosis (Table 2).

4. Discussion

The MIC result alone is not enough to indicate the effectiveness of antimicrobial agents against *B. pseudomallei*. Although the MICs of DOXY, SXT and PIP are less than the break points, those antimicrobial agents did not have a complete

bactericidal effect as shown in the time-kill studies. The resistance of strain SXT-S to AUG may be explained by the insensitivity of the β-lactamase enzyme to clavulanic acid, but the mechanism of CTZ resistance in strain CTZ-R could be the change in β-lactamase which caused hydrolysis of ceftazidime (Godfrey et al., 1991). Kanamycin does not show a bactericidal effect against *B. pseudomallei* but in combination with CTZ or PIP, a bactericidal effect against strain CTZ-S, CTZ-R, SXT-S and SXT-R was seen.

Although the 14,15-membered macrolides, CLA and AZI, did not have inhibitory or bactericidal effect against these four strains of *B. pseudo-*

Table 1
Minimum inhibitory concentration and minimum bactericidal concentration of four strains of *B. pseudomallei*

Antimicrobial agents	CTZ-S		CTZ-R		SXT-S		SXT-R	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Amoxicillin/clavulanic acid	4.0	4.0	4.0	4.0	128	>256	4.0	8.0
Ceftazidime	4.0	4.0	>256	>256	8.0	>256	4.0	4.0
Piperacillin	2.0	4.0	16	>256	16	>256	2.0	4.0
Imipenem	0.25	0.5	0.125	8.0	0.5	1.0	0.25	0.5
Meropenem	1.0	2.0	2.0	4.0	1.0	2.0	0.5	2.0
Azithromycin	32	>256	32	>256	32	>256	16	>256
Clarithromycin	64	>256	64	>256	32	>256	32	>256
Trovafloxacin	2.0	128	4.0	32	1.0	8.0	4.0	16.0
Ciprofloxacin	4.0	128	2.0	64	1.0	8.0	2.0	128
Rifampicin	8.0	>256	32	>256	16	>256	8.0	>256
Cotrimoxazole	32	>256	16	>256	16	256	64	>256
Doxycycline	2.0	>256	1.0	>256	1.0	8.0	1.0	>256
Kanamycin	32	64	16	128	32	32	16	64

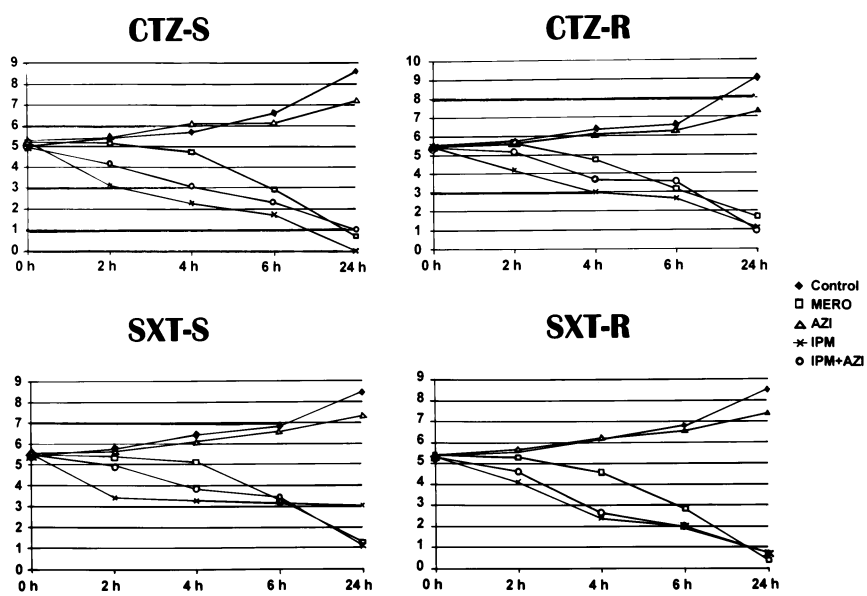

Fig. 1. Time-kill curves against MERO, AZI, IPM and IPM + AZI. This figure shows the bactericidal effect of MERO, IPM and IPM + AZI against the CTZ-R and SXT-R strains; MERO and IPM + AZI against the SXT-S strain.

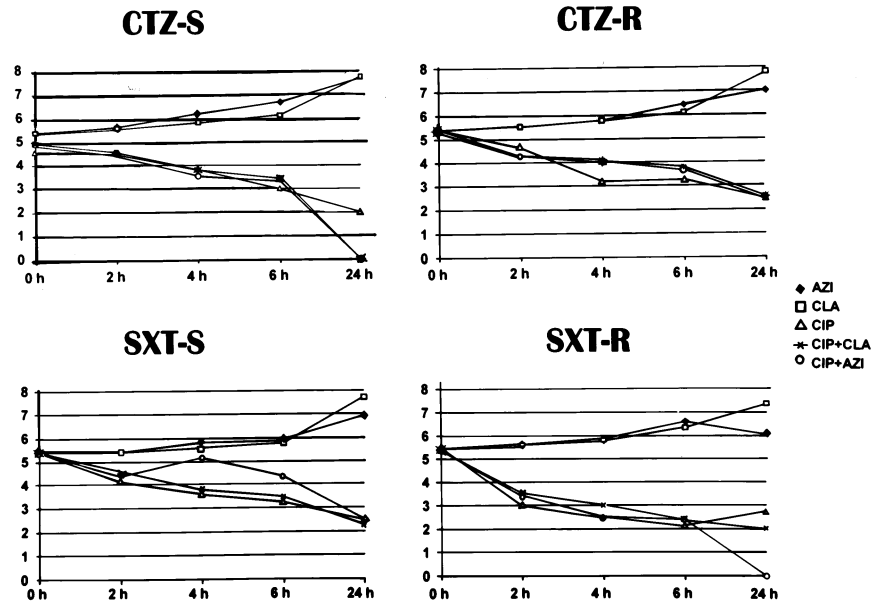

Fig. 2. Time-kill curves against AZI, CLA, CIP, CIP + CLA and CIP + AZI. This figure shows the bactericidal effect of CIP + CLA and CIP + AZI against CTZ-S strain; CIP + CLA against the SXT-S strain; CIP + CLA and CIP + AZI against SXT-R strain.

mallei, it has been reported that the combined use of CIP + CLA or AZI has a bactericidal effect on *P. aeruginosa* (Kobayashi et al., 1993). Both CLA and AZI inhibited alginate production of biofilm producing bacteria or mucoid-type bacteria (Kobayashi, 1995; Yasuda et al., 1993) and AZI at sub MIC level suppressed the synthesis of elastase, protease, lecithinase and DNAse by *P. aeruginosa* as it did with erythromycin, CLA and roxithromycin (Mizukane et al., 1994). Veringa et al. (1989) reported that glycocalyx production was inhibited in *P. aeruginosa* upon contact with clindamycin and consequently the bacteria were easily eliminated by the phagocytic activity of PMNs. As shown in our previous study (Vorachit et al., 1995b), *B. pseudomallei* is a biofilm producing bacteria either in vitro or in vivo. Therefore, the combination of CIP + CLA or CIP + AZI should be further investigated for the ability to eliminate glycocalyx as well as intracellular killing effect. From this in vitro study, the carbapenem antibiotics; IPM and MERO performed better against *B. pseudomallei* than other agents. The combination of IPM + AZI may be another good alternative drug for the acute therapy which was expected to eliminate all bacteria without forming the biofilm.

5. Conclusions

Antimicrobial agents with bactericidal effect on the four strains of *B. pseudomallei* are: IPM, and MERO for single agent treatment; kanamycin combined with CTZ, PIP, MERO or IPM and IPM combined with AZI, SXT or CIP for acute therapy; and ciprofloxacin combined with AZI or CLA for maintenance therapy. The combination of quinolone plus macrolides may be a good alternative for the maintenance treatment of melioidosis because ciprofloxacin can penetrate phagocytic cells and the macrolide can reduce or inhibit the production of glycocalyx. However, this would require clinical studies to test the relevance of our in vitro data.

Table 2
Time-killing assays: maximum $\log_{10}$ reduction of the most active agents from the combination inoculum at 24 h[a]

Antimicrobial agents	CTZ-S	CTZ-R	SXT-S	SXT-R
Ceftazidime + cotrimoxazole	−1.0937	0.3553	1.1627	−0.7269
Ceftazidime + kanamycin	1.4072	0.4929	0.3010	1.1760
Ceftazidime + ciprofloxacin	−0.3187	0.0260	1.8819	−1.9311
Piperacillin + ciprofloxacin	−1.0280	2.9183	1.7191	−1.6020
Piperacillin + cotrimoxazole	−1.9387	1.1512	0.6559	−2.0150
Piperacillin + kanamycin	−0.5228	5.9547	3.7160	1.6282
Imipenem + cotrimoxazole	−0.5740	−0.0669	0.1480	−0.4771
Imipenem + kanamycin	−0.5740	1.0669	2.2552	0
Imipenem + ciprofloxacin	−0.3979	0.2555	0.5149	−0.4771
Amoxicillin/clavulanic acid + ciprofloxacin	0	−0.6989	2.2138	−2.0669
Amoxicillin/clavulanic acid + cotrimoxazole	0	−2.6872	−0.5081	−3.3351
Amoxicillin/clavulanic acid + kanamycin	0	0.4771	3.5563	0.4771
Cotrimoxazole + doxycycline	−0.3010	−0.9156	−0.0871	0.0644
Cotrimoxazole + clarithromycin	1.0078	−0.0086	−0.3610	1.2430
Cotrimoxazole + rifampicin	0.0920	−0.6283	−0.8239	1.4559
Cotrimoxazole + ciprofloxacin	−2.5963	−1.1512	0.0791	1.3679
Cotrimoxazole + azithromycin	−0.3162	0.1938	−0.2218	0.4618
Doxycycline + ciprofloxacin	0.6020	−0.8908	−0.9178	−1.6020
Doxycycline + azithromycin	0.1047	−0.1245	0.4393	0.8525
Doxycycline + clarithromycin	1.3771	0.3919	−0.1047	0.8578
Doxycycline + rifampicin	0.9286	0.5354	0.0806	0.0763
Ciprofloxacin + clarithromycin	2.0	0.0511	0.0271	−1.3010
Ciprofloxacin + azithromycin	2.0	−0.1127	−0.0289	0.6989
Amoxicillin/clavulanic acid + clarithromycin	−3.748	−1.7206	−3.0234	−2.9637
Amoxicillin/clavulanic acid + azithromycin	−1.7403	−0.2216	−2.2825	−0.9637
Amoxicillin/clavulanic acid + rifampicin	−2.1760	−1.7611	−1.6989	−2.7688
Rifampicin + clarithromycin	2.6590	−0.5180	1.3245	1.968
Rifampicin + azithromycin	−0.4134	1.2418	1.1826	0.8266
Trovafloxacin + clarithromycin	1.0413	−0.5929	−0.0084	0.7917
Trovafloxacin + azithromycin	0.3424	−1.0969	−1.0312	0.0347
Meropenem + kanamycin	0.6989	1.6989	1.2430	0.3979
Meropenem + azithromycin	−4.1875	−0.0791	−2.3367	−1.3979
Imipenem + azitromycin	1.0	0.1139	1.903	0

[a] Antagonism, ≤ -2; synergism, ≥ 2.

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