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In Vitro Susceptibilities of *Pseudomonas pseudomallei* to 27 Antimicrobial Agents

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Clinical isolates of *Pseudomonas pseudomallei* isolated in Thailand from 1981 to 1989 were tested for their in vitro susceptibilities to 27 antimicrobial agents, including older and newer quinolones, broad-spectrum cephems, carbapenems, monobactams, penicillins, tetracyclines, chloramphenicol, rifamycin, sulfamethoxazole, trimethoprim, and fosfomycin. Tosufloxacin, meropenem, CS-533, and minocycline were active against *P. pseudomallei* at levels comparable to or even greater than those of antimicrobial agents tested in previous studies, such as ciprofloxacin, ceftazidime, imipenem, carumonam, and piperacillin. Drug-resistant *P. pseudomallei* was found in only 1% of the isolates. The drug-resistant *P. pseudomallei* isolates displayed a unique pattern of susceptibility to the above-listed drugs.

Pseudomonas pseudomallei is the causative agent of melioidosis, which was first described in 1912 in Myanmar (11). The organism can be found in water and wet soil, with seasonal variations in its incidence in tropical, endemic areas, especially Southeast Asia and northern Australia (1, 11, 13, 18). Case reports in those areas have shown both an increasing incidence and a changing spectrum of infection (3, 18). *P. pseudomallei* infects the human body via several routes, such as respiratory and cutaneous (18). On the basis of the clinical manifestations, the infection is classified into three main categories: asymptomatic, localized, and septicemic (3, 18). The last form causes the highest mortality. *P. pseudomallei* may cause an intracellular infection (18). Although patients with melioidosis have been treated with antimicrobial agents, treatment is still a great problem, with a high mortality in the case of the septicemic form. Moreover, drug (chloramphenicol)-resistant isolates of *P. pseudomallei* have been noted since 1988 (7).

In this study, we investigated the in vitro susceptibility of *P. pseudomallei* isolated in Thailand to various antimicrobial agents. The agents included those which have recently been recommended for the treatment of infections caused by *P. aeruginosa*.

All the *P. pseudomallei* strains used were clinical isolates. Of those, 27 strains were isolated in Ubon-Rajathanee, Thailand, in 1989, and the remaining 70 strains were collected in the Department of Medical Sciences, Soi Bamrasnaradura Hospital, Nonthaburi, Thailand, from 1981 to 1989. They were isolated from patients with skin or soft tissue infections (pus), with respiratory tract infections (throat swab and sputum), with septicemia (blood), with splenic abscesses (pus), or with urinary tract infections (urine).

The antimicrobial agents were kindly provided by their manufacturers. Norfloxacin, enoxacin, ofloxacin, ciprofloxacin, lomefloxacin (SC-47111; NY-198 [21]), fleroxacin (Ro 23-6240; AM833 [5]), temafloxacin (TA167 [10]), and tosufloxacin (T-3262 [9]) were categorized as a newer class of quinolones (6, 22) as opposed to an older class of agents, such as that represented by nalidixic acid. Antimicrobial agents used as broad-spectrum cephems were cefuzonam, cefotaxime, ceftazidime, cefepime (14), and E1040 (19). Those used as carbapenems were imipenem, CS-533 (RS-533 [17]), and meropenem (SM-7338 [8]). Monobactam antimicrobial agents used were aztreonam and carumonam. Piperacillin was used as one of the penicillins which have been used for the treatment of *P. aeruginosa* infections. Tetracyclines used were tetracycline and minocycline; the latter possesses a more extensive ability to penetrate membranes. Sulfamethoxazole and trimethoprim were used either alone or in combination at a ratio of 5:1 instead of the conventional ratio of 19:1, on the basis of pharmacokinetic data showing that the two drugs are absorbed to different degrees within the human body (18a). Chloramphenicol, rifampin, and fosfomycin were also used. The quinolones were initially dissolved in 0.1 N NaOH. Rifampin, sulfamethoxazole, trimethoprim, and chloramphenicol were initially dissolved in methanol. The other compounds were dissolved in distilled water.

Susceptibility testing of bacterial strains was done by the agar dilution method with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) by the standard procedure (12). The test bacteria grown overnight at 37°C in L broth (15) were diluted to approximately 10^6 CFU/ml. Aliquots of the bacterial suspensions (27 samples per plate) were inoculated on the surfaces of antimicrobial agent-containing agar plates with a multiloop inoculator (Microplanter, type MIT-P; Sakuma Seisakusho Co., Ltd., Tokyo, Japan). The final inoculum size was approximately 10^4 CFU of bacteria per spot, and incubation was done for 20 h at 37°C. The MIC was defined as the lowest concentration of an antimicrobial agent that allowed no visible growth of bacteria, fewer than five very tiny colonies, or a barely visible haze in the area of the spot on the agar plate (12). *Escherichia coli* NIHJ and *Staphylococcus aureus* 209P were used as reference strains for quality control (12).

The MICs of each antimicrobial agent against *P. pseudomallei* are summarized in Table 1. Several antimicrobial agents in the groups of newer quinolones, broad-spectrum cephems, carbapenems, monobactams, penicillins, and

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TABLE 1. MICs of 27 antimicrobial agents for *P. pseudomallei* clinical isolates

Antimicrobial agent	MIC (μg/ml)[a]		
	50%	90%	Range
Quinolones			
Nalidixic acid	25	50	3.13–≥200
Norfloxacin	12.5	12.5	1.56–50
Enoxacin	6.25	6.25	3.13–25
Lomefloxacin	6.25	6.25	3.13–25
Ofloxacin	6.25	6.25	0.78–12.5
Fleroxacin	3.13	6.25	1.56–12.5
Temafloxacin	3.13	6.25	0.78–12.5
Ciprofloxacin	3.13	3.13	0.78–6.25
Tosufloxacin	1.56	3.13	0.39–6.25
Broad-spectrum cephems			
E1040	25	50	6.25–≥100
Cefepime	12.5	12.5	3.13–50
Cefuzonam	3.13	6.25	1.56–25
Cefotaxime	3.13	3.13	0.78–12.5
Ceftazidime	0.78	1.56	0.39–3.13
Carbapenems			
CS-533	1.56	1.56	0.78–3.13
Meropenem	0.78	0.78	0.39–3.13
Imipenem	0.39	0.78	0.2–1.56
Monobactams			
Aztreonam	25	25	6.25–50
Carumonam	3.13	3.13	0.78–12.5
Penicillin: piperacillin	1.56	1.56	0.39–3.13
Tetracyclines			
Tetracycline	6.25	12.5	0.78–12.5
Minocycline	1.56	3.13	0.78–3.13
Chloramphenicol	12.5	25	6.25–≥200
Rifamycin: rifampin	25	25	3.13–25
Others			
Sulfamethoxazole	50	50	12.5–100
Trimethoprim	25	25	1.56–50
Sulfamethoxazole-trimethoprim[b]	12.5	12.5	0.78–25
Fosfomycin	≥200	≥200	≥200

[a] 50% and 90%, MIC for 50 and 90% of isolates tested, respectively. All drugs were tested against all 97 isolates, except for fosfomycin, which was tested against the 27 Ubon-Rajathanee isolates. The final concentrations of antimicrobial agents tested were from 100 to 0.1 μg/ml.

[b] Ratio (sulfamethoxazole/trimethoprim), 5:1. A 2:1 ratio gave a similar result.

tetracyclines showed good activity against *P. pseudomallei*. Those which exhibited an MIC for 90% of *P. pseudomallei* isolates of ≤3.13 μg/ml were tosufloxacin, ciprofloxacin, ceftazidime, cefotaxime, imipenem, meropenem, CS-533, carumonam, piperacillin, and minocycline. The carbapenems showed the greatest activity. In marked contrast, chloramphenicol, rifampin, sulfamethoxazole, trimethoprim (or the combination of sulfamethoxazole and trimethoprim), and fosfomycin showed much less activity against *P. pseudomallei*.

Of the 97 *P. pseudomallei* strains tested, 1 strain (isolated in 1988 and designated 266/31) was highly resistant to chloramphenicol. Of the *P. pseudomallei* strains tested, strain 266/31 displayed the lowest susceptibility to any of the quinolone antimicrobial agents; the MICs of nalidixic acid, norfloxacin, enoxacin, lomefloxacin, fleroxacin, and temafloxacin for strain 266/31 were at least twofold higher than those for the remaining strains. Moreover, strain 266/31 displayed, among the *P. pseudomallei* strains tested, the highest susceptibility to any of the β-lactam antimicrobial agents (broad-spectrum cephems, carbapenems, monobactams, and penicillins) and to rifampin; the MIC of E1040, cefotaxime, or rifampin for strain 266/31 was at least fourfold lower than those for the remaining strains.

Several rough-type variants of *P. pseudomallei* showed twofold to fourfold lower susceptibility to the quinolones or β-lactam antimicrobial agents than did their parent strains.

No significant differences were observed between clinical isolates in 1989 and before, between clinical isolates from patients with different symptoms, or between clinical and environmental (soil) isolates (data not shown).

The present study confirmed previous reports on the in vitro susceptibility to some β-lactam or quinolone antimicrobial agents of *P. pseudomallei* isolated in Thailand, Hong Kong, Vietnam, and Australia (2, 4, 16, 20). We showed here that some additional antimicrobial agents have good in vitro activity against *P. pseudomallei* and that the incidence of drug-resistant isolates is still extremely low with *P. pseudomallei*. The possibility exists that tosufloxacin, ciprofloxacin, and minocycline may also be active against intracellular infections caused by *P. pseudomallei*.

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