INTRODUCTION

Pseudomonas aeruginosa is a major opportunistic bacterial pathogen of nosocomial infections. The multiresistant strains play an important role in the colonization or infection of chronically hospitalized patients. Treatment is difficult and mortality significant. Beta-lactam antibiotics (ceftazidime), aminoglycosides (gentamicin, amikacin), fluoroquinolone (ciprofloxacin) and carbapenem (imipenem) have been mainstays for the treatment of P. aeruginosa infection. It is well known that the intensive use of antimicrobial agents inevitably leads to the appearance of organisms resistant to the drugs (Chen et al, 1995; Oie et al, 1999; Cavallo et al, 2000; Premru and Zupanc, 2002). Currently the treatment for P. aeruginosa infections is based on combinations of various antimicrobial agents because a single antimicrobial agent is often ineffective (Hilf et al, 1989; Korvick and Yu, 1991). The most widely documented synergy is to be found when beta-lactams are combined with aminoglycosides. The effectiveness of these combinations is limited due to the increasing resistance of P. aeruginosa to beta-lactams, and toxicity associated with aminoglycoside therapy (Hallander et al, 1982). For this reason, there is a continuous search for alternative combinations.

Fosfomycin, a bactericidal antibiotic, interferes with cell wall synthesis in both gram-positive and gram-negative bacteria by inhibiting the initial step, which involves phosphoenolpyruvate synthetase (Kahan et al, 1974). A number of studies have shown that fosfomycin can act synergistically with beta-lactams, which inhibit the last step of bacterial cell wall synthesis, and with aminoglycosides which inhibit bacterial protein synthesis (Olay et al, 1978; Takahashi and Kanno, 1984; Chin et al, 1986; Hayami et al, 1999; Okazaki et al, 2002). In this study, we evaluated the synergistic effects of fosfomycin in combination with ceftazidime, gentamicin and imipenem and the synergistic effects of ceftazidime in combination with gentamicin against ceftazidime-resistant P. aeruginosa strains isolated from hospitalized patients by using the checkerboard agar titration method.

MATERIALS AND METHODS

Bacteria

Fifty P. aeruginosa isolates used in this study were isolated from hospitalized patients at Songklanagarind Hospital, Prince of Songkla University, Songkhla, Thailand. Routine isolation,
identification and susceptibility testing methods were performed at the Microbiology Laboratory of the Department of Pathology, Prince of Songkla University, Songkhla, Thailand between September 1997 and May 2003.

Antimicrobial agents

Standard laboratory powders of antimicrobial agents were supplied from Thai Meji Pharmaceutical (fosfomycin), Siam Pharmaceutical (ceftazidime), and Merck Sharp & Dohme, (imipenem). Standard powder of gentamicin was from Sigma Chemical and ceftazidime disks from Oxoid.

Media

Mueller-Hinton agar and Mueller-Hinton broth were used.

Susceptibility test, MIC determinations and FIC testing

The 50 P. aeruginosa isolates were shown to be ceftazidime-resistant strains by an agar disk diffusion susceptibility test (NCCLS, 1999).

The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined using a two-fold agar dilution method (NCCLS, 1999). The range of antimicrobial concentrations was 0.125-1,024 mg/l. Isolates with MIC ≤ 1,024 mg/l were used for the synergy studies.

The synergic effects of the antimicrobial combinations against selected isolates were evaluated using the checkerboard method. The ratio of antimicrobial agents was 1:1 and the concentration range was 0.125-1,024 mg/l. Fractional inhibitory concentrations (FICs) were calculated as the MIC of drug A and B in the combination divided by the MIC of drug A or B alone and the FIC index was obtained by adding the FICs. Synergism, addition, indifference and antagonism is defined as FIC index of ≤ 0.5, > 0.5 to 1, > 1 to 2, and > 2, respectively (Hayami et al, 1999).

RESULTS

The MIC, MIC₅₀ and MIC₉₀ values of the tested antimicrobial agents are shown in Table 1. On the basis of the NCCLS performance standards (NCCLS, 1999), all 50 isolates were resistant to ceftazidime, having MICs ranging from 64 to > 1,024 mg/l, with 23 isolates (46%) showing MICs greater than 1,024 mg/l. Almost all of the isolates were resistant to imipenem (98%) and gentamicin (100%). The MICs of fosfomycin ranged from 8 to > 1,024 mg/l. Thirteen (26%) and 21 (42%) isolates had MICs of gentamicin and fosfomycin over 1,024 mg/l. MIC, MIC₅₀ and MIC₉₀ for the combination of antimicrobial agents are shown in Table 2. The MIC₅₀ levels of the combined antimicrobial agents were two to sixteen times lower than those of a single antimicrobial agent. The interactions between two combined antimicrobial agents, determined by checkerboard agar dilution, are shown in Table 3. The combination of fosfomycin with imipenem exerted a synergistic effect against 11 isolates (38%), and 4 isolates (14%) showed an additive effect. Ceftazidime with gentamicin showed synergism with 7 isolates (39%) and additive effect with 8 isolates (44%). Synergistic and additive effects were noted less often in other combina-
Antimicrobial combinations against P. aeruginosa

Tions. Antagonistic effects were noted when fosfomycin was combined with imipenem (7%), ceftazidime (22%) and gentamicin (27%). No antagonistic effect was noted in ceftazidime and gentamicin combination.

DISCUSSION

The incidence of multi-drug resistant P. aeruginosa strains isolated in hospitals continues to increase. This study was carried out in order to improve the efficacy of treatment. Judging from the MIC₅₀ findings, combined antimicrobial agents showed higher activity than a single antimicrobial agent. Chin et al (1986) reported that the combination of fosfomycin with imipenem was synergistic against 45% of 49 P. aeruginosa strains tested comparable to our finding of 38%. Chin et al (1986) and Hayami et al (1999) reported that the synergistic activity of fosfomycin and ceftazidime combination against 62 and 26 P. aeruginosa strains tested was 31% and 27%, respectively slightly higher than our finding of 11%. The majority of the isolates used in this study was extremely resistant to the testing agents, according to the very high MIC₅₀ and MIC₉₀ findings. Unfortunately in the majority of isolates, the MICs of the combined antimicrobial agents were higher than the plasma concentrations of both drugs that can be achieved and much higher than the MIC breakpoint, on the basis of the NCCLS performance standards (NCCLS, 1999). Nevertheless, the results in this study suggest that the combination of fosfomycin with imipenem and ceftazidime with gentamicin might be alternatives for the treatment of serious infections due to P. aeruginosa. However, further studies of other antimicrobial combinations need to be done to combat infections due to increasingly drug resistant organisms.

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