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In Vitro Activities of Antimicrobial Agents, Alone and in Combination, Against *Acinetobacter baumannii* Isolated from Blood

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In vitro *activities of 15 antimicrobial agents against 90 strains of* Acinetobacter baumannii *isolated from blood cultures from hospitalized patients were determined using the agar dilution method. Imipenem, ofloxacin, and ciprofloxacin had the best antimicrobial activity with minimum inhibitory concentrations (MIC_{50}s) of 0.25 μg/ml and MIC_{90}s of 0.5–1 μg/ml. β-lactam antibiotics other than imipenem had poor activity, with MIC_{50}s ranging from 8 to 64 μg/ml and MIC_{90}s from 32 to ≥256 μg/ml. The checkerboard titration method was used to study the effects of combination of two antimicrobial agents. Combinations of ceftazidime, aztreonam, imipenem, or ciprofloxacin with amikacin showed either synergistic effects or partial synergistic effects for 40.9%–86.4% of 22 tested strains. The best* in vitro *activity was observed with the combination of imipenem and amikacin. No antagonistic effects were observed with the combination of imipenem and amikacin. Synergistic effects were confirmed by time-kill curve studies. In conclusion, imipenem, ofloxacin, and ciprofloxacin were the three most active agents against human blood isolates of* A. baumannii. *The combination of a β-lactam or ciprofloxacin with amikacin was synergistic for some of the isolates.*

INTRODUCTION

Acinetobacter baumannii, previously named *Acinetobacter calcoaceticus* var. *anitratus,* is a nonfermentative, Gram-negative coccobacillus with low virulence. It is widely present in nature (Bauman, 1968; Rosenthal, 1974) and recognized as a frequent commensal of the skin, respiratory, and genital tracts (Rosenthal, 1974; Al-Khoja and Darrell, 1979). This organism is capable of producing a wide spectrum of infections, including pneumonia, tracheobronchitis, endocarditis, septicemia, meningitis, and intraabdominal, as well as infections of the genitourinary tracts, soft tissues, and surgical wounds (Glew et al., 1977). Although *A. baumannii* was well accepted as an occasional pathogen, until recently most isolates were considered to represent either colonization or environmental contaminants (Gardner et al., 1970; Glew et al., 1977; French et al., 1980). However over the past decade, the incidence of nosocomial infections due to *Acinetobacter* has increased (Ramphal and Kluge, 1979; Retailliau et al., 1979; Bergogne-Berezin and Joly-Guillou, 1985; Bergogne-Berezin et al., 1987).

Antibiotic resistance is a major problem for patients infected with all *Acinetobacter* species, especially those with *A. baumannii* (Castle et al., 1978; Ramphal and Kluge, 1979; Devaud et al., 1982; Tjernberg, 1990; Seifert et al., 1993). This resistance affects the selection of appropriate antibiotics for treating such patients. However, a limited amount

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of data have been published on the in vitro susceptibility of clinical isolates of *A. baumannii*, and even fewer data are available concerning the efficacy of antibiotic combinations against this problem pathogen (Glew et al., 1977).

Acinetobacter calcoaceticus was found to account for 4.8% of the total nosocomial infections during the period 1981–1989 at the National Taiwan University Hospital, both a primary- and tertiary-care medical center that had a 1200-bed capacity before 1991 and had grown to 1500 beds by 1992. Furthermore, *A. calcoaceticus* caused 8.3% of all cases of nosocomial bacteremia during this same time (Chang et al., 1990). Most of these isolates were *A. baumannii* (Chen et al., 1991). To determine the susceptibility of *A. baumannii* isolates causing bacteremia and their combination effect against this organism, bacteria isolated from blood cultures of patients hospitalized in the National Taiwan University Hospital were collected to check their in vitro susceptibility to various antimicrobial agents, alone and in combination.

MATERIALS AND METHODS

Bacterial Isolates

In this study we included 90 strains of *A. baumannii* isolated from blood cultures taken from patients at National Taiwan University Hospital during the period 1988–1991. No duplicate isolates from the same patient were included. During the study period, the BACTEC blood culture system (BACTEC 660; Johnson Laboratories, Towson, MD, USA) was used. All strains were identified by both the Vitek system (Vitek-AMS 120; bioMerieux Vitek, Hazelwood, MO, USA) and conventional laboratory methods (Pickett et al., 1991).

Antimicrobial Agents

The 15 antimicrobial agents used in this study were supplied by their manufacturers as laboratory standard powders, including ticarcillin from Smith-Kline Beecham (U.K.), piperacillin and minocycline from Cyanamid (New York), cefotaxime from Hoechst (Germany), cefoperazone from Pfizer (New York), ceftazidime from Glaxo (U.K.), ceftriaxone from Hoffman-La Roche (Switzerland), aztreonam and amikacin from Bristol-Meyers Squibb (New York), imipenem from Merck Sharp and Dohm (New Jersey), gentamicin from Schering-Plough (New Jersey), tobramycin from Eli Lilly (Indiana), ciprofloxacin from Bayer (Germany), ofloxacin from Daiichi Pharmaceutical (Japan), and norfloxacin from Kyorin Pharmaceutical (Japan). All of them were prepared according to the manufacturers' recommendation.

Antimicrobial Susceptibility Testing

The agar dilution method, as described by the National Committee for Clinical Laboratory Standards (NCCLS, 1993), was used to check the minimum inhibitory concentration (MIC) of the tested antimicrobial agents against each isolate. We used Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) incorporated with one of the antimicrobial agents ranging from 0.03 to 128 μg/ml. Inocula of 10^4 organisms were applied using a Steers replicator. The agar plates were examined after incubation for 18 h at 35°C. The MIC was defined as the lowest concentration of antibiotic resulting in complete inhibition of visible growth on agar. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as internal controls. The break points for a reading judged as to be susceptible were based on the standards of the NCCLS (1994).

Effects of a Combination of Two Antimicrobial Agents

The antibacterial effects of a combination of two antimicrobial agents were assessed by the checkerboard titration method (Eliopoulos and Moellering, 1991). Serial dilutions of two different antimicrobial agents were mixed in cation-supplemented Mueller-Hinton broth (Difco Laboratories). Inocula were prepared from colonies grown on sheep blood agar after overnight culture. The final bacterial concentration after inoculation was 5×10^5 colony forming units cfu/ml. After 24 h of incubation at 35°C, the MIC was determined to be the minimal concentration at which there was no visible growth. The fractional inhibitory concentration (FIC) was calculated for each combination that inhibited growth using the following formula: FIC = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone) (Eliopoulos and Moellering, 1991). The minimum FIC of the calculated FICs was defined to be the FIC index. Synergism was defined as an FIC index of ≤ 0.5; antagonism was defined as an FIC index of ≥ 2. An FIC index between 0.5 and 1.0 was regarded as partially synergistic. An FIC index of 1.0 was regarded as additive.

We randomly selected 22 strains to examine the efficacy of antibiotic combinations. Combinations of antimicrobial agents selected for testing included ceftazidime with amikacin, aztreonam with amikacin, imipenem with amikacin, and ciprofloxacin with amikacin.

For those strains that showed a synergistic effect, the time-kill curve study was also used to assess the combination bactericidal effect. We used cation-supplemented Mueller-Hinton broth incorporated

with either a single antimicrobial agent or a combination of two drugs. The concentration of each antimicrobial agent in media was set at a level equal to the MIC of the tested strain. Inocula of 10^6 cfu/ml of bacteria harvested from colonies grown overnight were used in these experiments. Tubes were incubated at 35°C and continuously agitated. A small volume of the medium was removed to count the viable bacteria at the following times: prior to shaking and at 1, 3, 5, and 24 h after starting incubation. If the final concentration of viable bacteria growing on media containing a combination of two antimicrobial agents was 100-fold less than the concentration of bacteria growing on media containing a single antimicrobial agent, synergism was considered to be present. If the final concentration of viable bacteria in media containing two antimicrobial agents was >100-fold higher than the concentration of bacteria growing on media containing a single antimicrobial agent, the result was regarded to be antagonism (Eliopoulos and Moellering, 1991).

RESULTS

Antimicrobial Susceptibility to a Single Agent

Table 1 shows the range of the MIC results observed, as well as the MIC_{50}, MIC_{90}, and percentage of susceptible strains among the 90 isolates. β-lactam antibiotics other than imipenem showed poor activity against *A. baumannii*. The MIC_{50}s for most β-lactam agents were ≥8 μg/ml; the MIC_{90}s for the same agents were ≥32 μg/ml. The percentages of strains susceptible to β-lactams other than imipenem ranged from 4.4% to 57.8%. Imipenem had excellent activity against *A. baumannii*, with an MIC_{50} of 0.25 μg/ml and MIC_{90} of 1 μg/ml. A total of 98% of the *A. baumannii* strains were susceptible to imipenem.

Of three tested aminoglycosides, gentamicin was less active than tobramycin and amikacin. The MIC_{50} and MIC_{90} of gentamicin were 16 and ≥256 μg/ml, respectively. Slightly less than one half (45.5%) of the strains were susceptible to gentamicin; 74.5% and 86.6% of the strains were susceptible to tobramycin and amikacin, respectively.

Fluoroquinolones, especially ofloxacin and ciprofloxacin, demonstrated good activity against *A. baumannii*. The MIC_{50} and MIC_{90} of both agents were 0.25 and 0.5 μg/ml, respectively. All strains were susceptible to ofloxacin; 97.8% were susceptible to ciprofloxacin. Minocycline showed good activity against most of the strains, but some strains were resistant. The MIC_{50} was 0.125 μg/ml and the MIC_{90} was 128 μg/ml.

TABLE 1 In vitro Activity of 15 Antimicrobial Agents Against 90 Strains of *Acinetobacter baumannii*

	Minimum Inhibitory Concentration (MIC) (μg/ml)			
Agent	Range	MIC_{50}	MIC_{90}	Susceptible %
Ticarcillin	0.5–≥256	32	64	45.5
Piperacillin	0.5–≥256	32	64	44.3
Cefotaxime	0.5–≥256	16	32	17.7
Cefoperazone	2–≥256	16	≥256	54.5
Ceftazidime	0.5–≥256	8	32	57.8
Ceftriaxone	1–≥256	32	128	10.0
Aztreonam	8–≥256	64	128	4.4
Imipenem	≤0.03–32	0.25	1	97.8
Gentamicin	0.125–≥256	16	≥256	45.5
Tobramycin	0.25–≥256	1	64	74.5
Amikacin	0.5–≥256	2	32	86.6
Norfloxacin	0.125–64	2	8	80.0
Ofloxacin	0.06–2	0.25	0.5	100.0
Ciprofloxacin	0.03–2	0.25	0.5	97.8
Minocycline	≤0.03–≥256	0.125	128	87.7

Effects of a Combination of Two Antimicrobial Agents

Results of the antibacterial effects of four different combinations against 22 strains tested by the checkerboard titration method are shown in Table 2. No matter the combination, synergistic and partial synergistic effects could be demonstrated in some of the tested strains. The percentage of strains showing synergistic or partial synergistic effects ranged from 40.9 to 86.4%. However, some other strains showed an additive or antagonist effect. Of the four combinations of antimicrobial agents tested, imipenem in combination with amikacin demonstrated the best results. Synergism, partial synergism, and additive effects were found in 36.4, 50, and 13.6%, respectively, of the tested strains, and no antagonistic effect was found in any of the 22 tested strains.

For those strains showing a synergistic effect, the

TABLE 2 Effect of a Combination of Antimicrobial Agents Against 22 Strains of *Acinetobacter baumannii* (%)[a]

Effect	CAZ + AMK	AZT + AMK	IPM + AMK	CPFX + AMK
Synergism	18.2	22.7	36.4	9.1
Partial synergism	22.7	40.9	50.0	40.9
Additive	31.8	22.7	13.6	31.8
Antagonism	27.3	13.6	0	18.2

[a]CAZ, Ceftazidime; AZT, aztreonam; IPM, imipenem; CPFX, ciprofloxacin; AMK, amikacin.

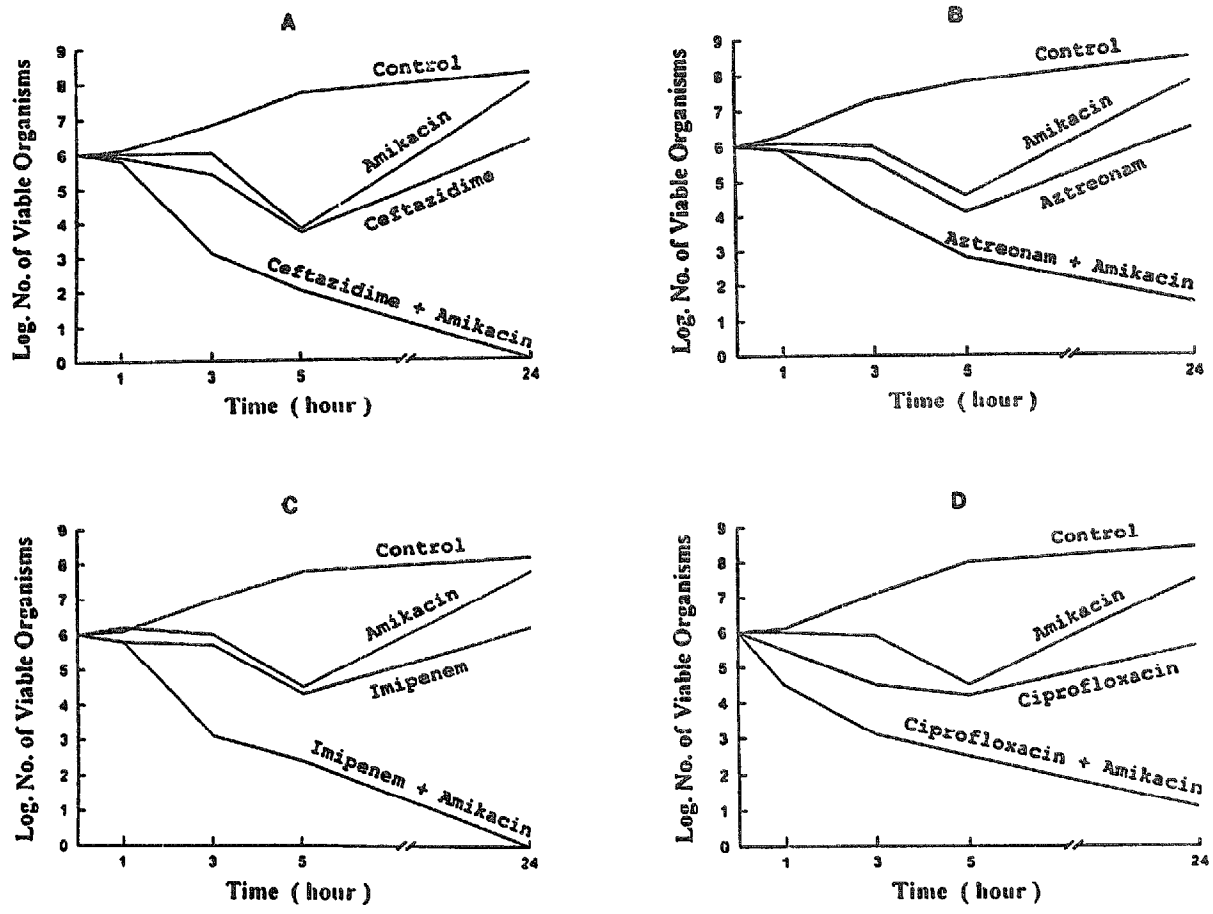

FIGURE 1 Time-kill curves of ceftazidime, aztreonam, imipenem, ciprofloxacin, and amikacin against one strain of *Acinetobacter baumannii* (strain no. 90-6335). (A) Ceftazidime and amikacin, (B) aztreonam and amikacin, (C) imipenem and amikacin, and (D) ciprofloxacin and amikacin. The concentration of each antimicrobial agent was equal to the minimum inhibitory concentrations of the individual agent against the tested strain.

time-kill curve study also demonstrated a synergistic bactericidal effect. Figure 1 shows the results of one of the tested strains (strain no. 90-6335).

DISCUSSION

Although a small number of community-acquired *Acinetobacter* infections have been recognized (Goodhart et al., 1977; Rudin et al., 1979), *A. baumannii* is generally regarded as a nosocomial pathogen (Ramphal and Kluge, 1979; Retailliau et al., 1979; Bergogne-Berezin and Joly-Guillou, 1985; Bergogne-Berezin et al., 1987). Several nosocomial outbreaks due to *Acinetobacter* have been reported (Castle et al., 1978; French et al., 1980; Holton, 1982; Hartstein et al., 1988). A nationwide study in the United States found the *Acinetobacter* species to be responsible for 3.11 nosocomial infections per 10,000 patients discharged (Retailliau et al., 1979). In Taiwan, the incidence of nosocomial *Acinetobacter* infections is even higher. In a previous study at the National Taiwan University Hospital, the rate of nosocomial infection of *A. calcoaceticus* was found to be approximately nine times that reported by Retailliau et al. (27.6/10,000 discharges) during the period 1981–1989 (Chang et al., 1990). Similar to others (Ramphal and Kluge, 1979; Bergogne-Berezin et al., 1987), we noted that the incidence of nosocomial infection with *A. baumannii* increased from 21/10,000 discharges in 1981 to 33/10,000 discharges in 1989. The increase in the rate of nosocomial bacteremia due to *Acinetobacter* was even more remarkable (3/10,000 discharges in 1981 to 11/10,000 discharges in 1989) (Chang et al., 1990).

Patients who acquire *Acinetobacter* infection usually have a history of malignancy, burns, immunosuppression, and/or major surgery (Glew et al., 1977; Bergogne-Berezin et al., 1987). A previous study also showed that patients usually had these predisposing factors before they developed *Acinetobacter* bacteremia (Chen et al., 1991). Mortality rates for patients with *Acinetobacter* bacteremia may be as high as 46% (Chen et al., 1991). Thus, the selection of appropriate antibiotics—probably a combination—for such a rapidly increasing pathogen becomes even more important.

This study found that resistance to various antimicrobial agents, especially β-lactam antibiotics,

was common in strains isolated from patients with bloodstream infections at our hospital. The prevalence of resistance to β-lactam antibiotics was generally higher at our hospital than that found in previous studies from the United States and Europe (Garcia et al., 1983; Stiver et al., 1986; Rolston and Bodey, 1986; Traub and Spohr, 1989; Tjernberg, 1990; Seifert et al., 1993). Like other investigators, we found that imipenem was active against *A. baumannii* (Garcia et al., 1983; Stiver et al., 1986; Rolston and Bodey, 1986; Traub and Spohr, 1989; Tjernberg, 1990; Seifert et al., 1993). However, we noted that the MICs of our strains were higher than those found in other studies. With a few exceptions (Garcia et al., 1983; Urban et al., 1993), isolates described in previous studies were usually susceptible to imipenem. However, we found 2.2% of our *A. baumannii* strains to have an MIC > 4 μg/ml.

Aminoglycosides resistance of our strains was also common, a finding similar to previous studies (Stiver et al., 1986; Rolston and Bodey, 1986; Traub and Spohr, 1989; Tjernberg, 1990; Seifert et al., 1993). As for susceptibility to fluoroquinolones, our strains were highly susceptible to ciprofloxacin. This result is similar to reports from the United States in 1986 (Rolston and Bodey, 1986), but different from reports from Germany in 1989 and 1993 (Traub and Spohr, 1989; Seifert et al., 1993). Ciprofloxacin was released early in Germany, and widespread use followed quickly. In contrast, ciprofloxacin was not approved by the US Food and Drug Administration until 1987 and was not introduced into clinical use in Taiwan until 1991. This fact may explain why strains from Germany had higher MICs and a lower percentage of susceptibility than strains from Taiwan, the United States, and other European countries (Tjernberg, 1990).

Older agents such as ampicillin, carbenicillin, and first- and second-generation cephalosporins were used to treat *Acinetobacter* infections during the 1970s. Most of these agents are now inactive against this bacterium (Traub and Spohr, 1989; Tjernberg, 1990; Seifert et al., 1993). However, doxycycline still has activity against *A. baumannii* (Traub and Spohr, 1989). We found that a related antibiotic, minocycline, was also active against most isolates. However, our strains had a higher MIC_{90} than those strains reported by Traub and Spohr.

Because β-lactam antibiotics are easily available over the counter and have been widely used in Taiwan, it is not surprising that resistance to β-lactam antibiotics is very common in various bacteria isolated from patients in Taiwan, such as *E. coli*, *Enterobacter* spp., *P. aeruginosa*, and *Haemophilus influenzae* (Chang et al., 1994). The resistance rates of these species are in general much higher than those of similar species isolated from patients in most western countries. Resistance to β-lactam antibiotics of *Acinetobacter* has been shown to be caused by β-lactamase production (Morohoshi and Saito, 1977; Joly-Guillou et al., 1988), and β-lactamase production is inducible by β-lactam antibiotics (Morohoshi and Saito, 1977). Although we did not attempt to detect β-lactamase production in this study, our previous work has shown that 40%–100% of 13 tested bacterial species obtained from Taiwanese elaborated β-lactamase (Chang et al., 1991). Thus, it is reasonable to speculate that β-lactamase production induced by the widespread use of β-lactam antibiotics in Taiwan is responsible for the high resistance rates of *Acinetobacter* to β-lactams that we observed.

A β-lactam antibiotic in combination with an aminoglycoside has been demonstrated to be synergistic, both in vitro and in vivo, to many bacteria, including *P. aeruginosa*, another commonly seen pathogenic nonfermenter. Using a combination treatment for *Acinetobacter* infections is common practice as well. However, until now only one study has demonstrated that a combination of carbenicillin with an aminoglycoside was synergistic to some *Acinetobacter* isolates (Glew et al., 1977). This study showed that a combination of ceftazidime, aztreonam, imipenem, or ciprofloxacin with amikacin was synergistic for some strains, but could also be antagonistic or additive for other strains. There was no correlation between susceptibility to individual antibiotics of the tested strains and the results of the combination effect. Combinations could be synergistic for both resistant and susceptible strains. It could also be antagonistic, no matter whether the strain was susceptible or resistant to the antibiotics to be combined. Therefore, whether it is advisable to use combination therapy in patients with *Acinetobacter* infection probably needs to be evaluated individually. However, our results need to be examined further by in vivo studies before a firm conclusion can be made.

In summary, isolates of *A. baumannii* from patients with bacteremia in Taiwan were generally resistant to β-lactam antibiotics. Many strains were also resistant to aminoglycosides; a small percentage was resistant to imipenem and ciprofloxacin. However, despite such resistance, combination therapy using a β lactam antibiotic or ciprofloxacin with amikacin may still be synergistic or partially synergistic in some isolates, and imipenem in combination with amikacin was the best choice of combination treatment for *Acinetobacter* infections among the four combinations tested.

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