NETILMICIN SYNERGY WITH CARBENICILLIN OR CEFAMANDOLE AGAINST *SERRATIA*

D. J. FLOURNOY

The Microbiology Section of the Laboratory Service, Veterans Administration Hospital, Oklahoma City, Oklahoma, U.S.A.

(Received for publication September 21, 1977)

Twenty clinical isolates of *Serratia* sp. were tested against netilmicin, gentamicin, carbenicillin and cefamandole alone (broth and agar dilution) and in combination (agar dilution). Broth and agar dilution minimal inhibitory concentrations agreed to within a two-fold dilution in 96% of the tests. Overall, 95% of the isolates were susceptible to netilmicin regardless of susceptibility to gentamicin or carbenicillin. Netilmicin-carbenicillin synergy was seen in 55% of the strains and netilmicin-cefamandole in 70%. These results indicate that combinations of netilmicin with carbenicillin or cefamandole may be clinically useful.

Netilmicin (Schering 20569) is a derivative of sisomicin[1] which is produced by *Micromonospora inyoensis*[2]. Netilmicin has received recent attention in the form of studies dealing with its: pharmacology[3,4], serum assay[5] and activity against various microorganisms[6~18]. Several reports have noted synergism between netilmicin and carbenicillin[8,15] and between netilmicin and penicillin[17]. This study compares the effects of netilmicin or gentamicin in combination with carbenicillin or cefamandole against twenty strains of *Serratia* sp.

Materials and Methods

Antimicrobials: Schering Corporation supplied netilmicin and gentamicin powder. Cefamandole powder was a gift from Eli Lilly and Company. Carbenicillin was manufactured by Beecham Laboratories.

Organisms: Twenty clinical isolates of *Serratia* sp. were chosen for the study. Their sources and numbers were: urine (10), respiratory (7), blood (2) and wound (1) cultures performed at the Oklahoma City Veterans Administration Hospital (14), Oklahoma Childrens Memorial Hospital (4) and University Hospital (2). Ten strains were susceptible to 1.25 μg/ml of gentamicin or less and ten were resistant. Isolates designated 3D2 and 3F2 were *Serratia liquifaciens* while the remaining were *Serratia marcescens*. All identifications were confirmed by conventional methods.

Susceptibility test methods: Minimal inhibitory concentrations (MICs) of individual antimicrobials, were determined by a broth microdilution method and by agar dilution. The broth method utilized MUELLER-HINTON broth (Difco, Ca^{++} 7 mg/liter, Mg^{++} 2.9 mg/liter) as the diluent. The final volume in each microtiter plate well was 0.1 ml. A standard two-fold agar dilution method employed MUELLER-HINTON agar (Difco, Ca^{++} 63 mg/liter, Mg^{++} 18 mg/liter). A Steers replicator was used to inoculate the plates.

Inocula for both methods consisted of a 1: 100 dilution, in MUELLER-HINTON broth, of a bacterial suspension which had been adjusted to equal the turbidity of a 0.5 MACFARLAND standard. All Petri and microtiter plates were incubated for 16~18 hours at 35°C after inoculation. Two-fold dilutions were used throughout the study. MICs were repeated on several occasions. The MIC was taken as

This investigation was supported by a grant from Schering Corporation.

Please address requests for reprints to D. J. FLOURNOY, Veterans Administration Hospital (113), 921 NE 13th St., Oklahoma City, Oklahoma 73104, U.S.A.

the highest dilution of antimicrobial in which no visible growth appeared.

Synergy testing: Agar dilution was the method used to test combinations of antibiotics. Checkerboard patterns were used and included antibiotic levels which could be useful chemotherapeutically as well as levels which could not. Synergy was defined as a reduction of the MIC values for both antibiotics by at least four-fold. Partial synergy was noted when a four-fold reduction in the MIC of one compound was accompanied by a less than four-fold reduction in the MIC of the other compound. No reduction of the MIC in either compound was termed indifference. Antagonism occured whenever the MIC of an antibiotic combination was greater than the MIC of the most active single antibiotic.

Results

MICs for individual antibiotics (netilmicin, gentamicin, carbenicillin and cefamandole) were done by the broth and agar dilution methods. Both methods yielded results which agreed within a two-fold dilution with the exception of cefamandole against isolates 3E1, 3F1 and 3B2. Broth dilution MICs were more than a two-fold dilution higher than agar dilution for all isolates. This difference was reproducible on numerous occasions.

Table 1 notes the results of agar dilution MICs using netilmicin, carbenicillin and cefamandole alone and in combination. Synergism was observed between netilmicin and carbenicillin in 11 of 20 (55%) isolates tested and between netilmicin and cefamandole in 14 (70%) of the isolates.

Gentamicin, carbenicillin and cefamandole were tested alone and together as seen in Table 2. One isolate (5%) showed synergism between gentamicin and carbenicillin and 5 (25%) showed synergism between gentamicin and cefamandole.

Discussion

Several investigators have studied the synergistic effects of netilmicin. SMITH *et al.* demonstrated

Table 1. MICs of netilmicin (N), carbenicillin (CB) and cefamandole (CM) alone and in combination

Isolate	MIC (μg/ml)				
	CB	CB–N (Effect)	N	N–CM (Effect)	CM
3A1	125,000	0.23~0.62 (PS)	1.25	0.31~320 (S)	1,280
3B1	125,000	0.23~0.62 (I)	0.62	0.31~320 (PS)	1,280
3C1	62,500	31,250~0.07 (PS)	0.62	0.15~20 (S)	80
3D1	125,000	31,250~0.07 (S)	2.5	0.31~10 (S)	40
3E1	62,500	7,812~0.07 (S)	2.5	0.15~10 (S)	80
3F1	62,500	31,250~0.07 (PS)	0.62	0.31~2.5 (PS)	40
3G1	125,000	0.47~0.31 (PS)	0.62	0.31~1.25 (PS)	320
3H1	125,000	31,250~0.31 (S)	1.25	0.31~10 (S)	40
3A2	62,500	15,625~0.07 (S)	0.62	0.31~5 (PS)	80
3B2	125,000	7,812~0.15 (S)	10	5~2.5 (PS)	10
3C2	7.8	1.9~0.15 (S)	0.62	0.15~1.25 (S)	5
3D2	2,000	2,000~0.07 (I)	1.25	0.31~1.25 (S)	5
3E2	7.8	1.9~0.15 (S)	1.25	0.31~1.25 (S)	10
3F2	7.8	1.9~0.15 (S)	5	0.31~2.5 (S)	20
3G2	3.9	0.47~0.15 (S)	1.25	0.31~2.5 (S)	20
3H2	7.8	1.9~0.07 (S)	1.25	0.31~2.5 (PS)	5
3A3	15.6	15.6~0.15 (I)	5	0.31~5 (S)	320
3B3	3.9	1.9~0.07 (PS)	1.25	0.15~2.5 (S)	40
3C3	3.9	1.9~0.15 (PS)	0.62	0.15~1.25 (S)	10
3D3	1.9	0.47~0.15 (S)	0.62	0.15~1.25 (S)	20

S (synergy), PS (partial synergy), I (indifference)

Table 2. MICs of gentamicin (GM), carbenicillin (CB) and cefamandole (CM) alone and in combination

Isolate	MIC (μg/ml)				
	CB	CB–GM (Effect)	GM	GM–CM (Effect)	CM
3A1	125,000	62.5～160 (I)	160	160～0.62 (I)	1,280
3B1	125,000	62.5～160 (I)	160	80～320 (PS)	1,280
3C1	62,500	62.5～40 (I)	40	20～40 (PS)	80
3D1	125,000	62.5～80 (I)	80	40～10 (PS)	40
3E1	62,500	62.5～40 (PS)	80	20～10 (S)	80
3F1	62,500	62.5～40 (I)	40	20～40 (I)	40
3G1	125,000	62.5～40 (I)	40	40～10 (I)	320
3H1	125,000	62.5～80 (I)	80	20～40 (I)	40
3A2	62,500	62.5～40 (I)	40	20～10 (S)	80
3B2	125,000	62.5～20 (PS)	40	2.5～5 (PS)	10
3C2	7.8	3.9～0.07 (PS)	0.31	0.15～2.5 (PS)	5
3D2	2,000	2,000～0.15 (I)	0.31	0.15～5 (I)	5
3E2	7.8	1.9～0.15 (PS)	0.31	0.07～10 (I)	10
3F2	7.8	1.9～0.07 (S)	0.31	0.15～1.25 (PS)	20
3G2	3.9	0.95～0.15 (PS)	0.31	0.07～10 (PS)	20
3H2	7.8	3.9～0.15 (PS)	0.31	0.07～5 (I)	5
3A3	15.6	7.8～0.15 (PS)	1.25	0.31～10 (S)	320
3B3	3.9	3.9～0.15 (I)	0.62	0.07～10 (S)	40
3C3	3.9	1.9～0.07 (PS)	0.62	0.07～5 (PS)	10
3D3	1.9	1.9～0.07 (I)	0.62	0.07～5 (S)	20

S (synergy), PS (partial synergy), I (indifference)

synergy between netilmicin and penicillin against *Streptococcus faecalis* and *Streptococcus faecium*[17]. FU and NEU noted[8] netilmicin-carbenicillin synergy against 21 of 46 *Pseudomonas* sp. isolates. True synergy was lacking in the majority of cases when netilmicin was combined with cefazolin, chloramphenicol or clindamycin against *Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa* strains. Netilmicin-carbenicillin synergistic activity was also absent against two *Serratia* sp. isolates. The results here showed that netilmicin acted synergistically with a new cephalosporin, cefamandole, against 14 of 20 *Serratia* sp. strains. In addition, netilmicin-carbenicillin synergy was demonstrated.

POGWIZD and LERNER[15] found that gentamicin and netilmicin produced opposite effects regarding susceptibility. Their isolates were: (1) highly gentamicin-carbenicillin resistant, netilmicin susceptible; (2) gentamicin-carbenicillin susceptible, netilmicin resistant or (3) moderately gentamicin resistant, carbenicillin susceptible, netilmicin resistant. The present study showed that all of the isolates, except one, were susceptible to netilmicin irregardless of gentamicin susceptibility. The only netilmicin-resistant strain was also resistant to gentamicin and carbenicillin. POGWIZD and LERNER also reported synergy for netilmicin-carbenicillin and gentamicin-carbenicillin against most of the *Serratia* sp. isolates they tested. Carbenicillin and gentamicin or netilmicin was not active synergistically against isolates highly resistant ($\geq$8,000 μg/ml) to carbenicillin. This study found synergy between carbenicillin and netilmicin or gentamicin in isolates which were highly ($\geq$8,000 μg/ml) resistant to carbenicillin, but the synergistic levels were not clinically attainable.

It thus appears that netilmicin is very active against the *Serratia* sp. strains at this institution. Combinations of netilmicin with carbenicillin or cefamandole may also be clinically useful in the future.

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