

THE COMPARATIVE SYNERGISTIC ACTIVITY OF AMIKACIN,
GENTAMICIN, NETILMICIN AND AZLOCILLIN, MEZLOCILLIN,
CARBENICILLIN AND TICARCILLIN AGAINST
SERRATIA MARCESCENS

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The synergistic activities of netilmicin, gentamicin and amikacin combined with carbenicillin, ticarcillin, azlocillin and mezlocillin were investigated against 32 *Serratia marcescens* isolates. Synergy could be demonstrated by killing curve technique, isobologram plots as well as susceptibility data with any of the aminoglycosides and penicillins combinations. No antagonism was shown with any of the combinations. The majority of the isolates were resistant to the aminoglycosides and penicillins. Combination of either ureido-penicillin or carbenicillin with gentamicin or netilmicin did not reduce the MIC values to levels achievable in serum but did reduce the MIC levels of both agents to those achievable in urine. The combination of ureido-penicillins or carbenicillin and ticarcillin with amikacin reduced the inhibitory levels of all isolates to levels achievable in both serum and urine.

Infections caused by *Serratia marcescens* have become an increasingly severe problem in hospitals^{1,9,12,13}. In our own institution the majority of *Serratia* infections are nosocomial in origin and involve the urinary tract while sepsis or respiratory infections are rare. Antibiotic therapy of serious infections produced by *Serratia* has been difficult because of the development of multiply drug-resistant strains¹⁰. The effectiveness of combinations of antibiotics particularly gentamicin-carbenicillin against *Serratia* have been reported both *in vitro* studies and in treatment of selected infections^{2,10,11,14}. The availability of the new aminoglycosides amikacin⁸) and netilmicin^{3,4}) and the two new broad-spectrum semisynthetic ureido-penicillins, azlocillin and mezlocillin (NEU, submitted for publication), prompted us to investigate the comparative activities of these aminoglycoside-penicillin combinations against *Serratia marcescens*.

Materials and Methods

Netilmicin and gentamicin were supplied by Schering Corporation, and azlocillin and mezlocillin by Delbay. Carbenicillin and amikacin were gifts from Beecham Laboratories and Bristol Laboratories respectively. Fresh dilutions of all antibiotics were prepared by a two-fold dilution method with sterile medium or distilled water. Isolates of *Serratia marcescens* were obtained from patients hospitalized at the Columbia-Presbyterian Medical Center and from neighboring hospitals. The isolates were selected to be different strains on the basis of antibiograms, R-factor enzymes and bacteriocin typing.

Susceptibility and Synergy Test Methods: The minimal inhibitory concentration (MIC) was determined by the agar-dilution method using MUELLER-HINTON agar (BBL) and by a broth-dilution method with MUELLER-HINTON broth (BBL) as described previously^{3,4}.

Synergy was tested by the agar-dilution method using MUELLER-HINTON agar (BBL). Two-fold

serial dilutions of both compounds were mixed and incorporated in the agar which was used as soon as it had hardened. A dilution of an overnight culture containing 10^5 CFU was applied to the surface of the agar containing the various concentrations of drug combinations by means of a replicator as described previously³⁾.

Synergy was defined according to the following criteria: (i) a reduction of the MIC values of both antibiotics by four-fold or more; (ii) any definite concavity of the isobologram obtained by graphing the data; (iii) a decrease of 1 log or more in viable cell count occurring at the end of 4 hours with the antibiotic combination compared with that obtained with the most active single antibiotic. Partial synergy was defined as a four-fold or greater reduction in MIC values of one compound but less than four-fold reduction in the MIC value of the second compound. Indifference was either no reduction in MIC values or only two-fold. Antagonism was defined as four-fold increase in MIC of either compound³⁾.

Results

We decided to compare the synergistic activity of amikacin, gentamicin and netilmicin combined with azlocillin since work in our laboratory (NEU and FU, submitted) had indicated that azlocillin because of its antipseudomonas activity might show the greatest synergy against strains of *Serratia marcescens*. Table 1 compares the percent of strains which showed synergy when the three aminoglycosides were combined with azlocillin against strains most of which are multiply resistant. In terms of overall synergy there is no statistical difference in the three groups since the synergy raised only

from 47% to 59%. However, in all instances there is greater synergy shown with the resistant strains whether with amikacin which is the most active agent or with netilmicin the least active.

A more direct comparison of the three agents is shown in Fig. 1. In the absence of penicillin, amikacin inhibited 50% of strains at a concentration of $6.3 \mu\text{g/ml}$. In the presence of azlocillin from 3.1 to $50 \mu\text{g/ml}$ there was no increase in the number of strains inhibited, but at concentrations of $100 \sim 200 \mu\text{g/ml}$ $60 \sim 70\%$ were inhibited at $6.3 \mu\text{g/ml}$ and more importantly at $25 \mu\text{g/ml}$ of amikacin all strains were inhibited in the presence of $100 \mu\text{g/ml}$ of azlocillin.

Fig. 1. Cumulative number of strains of *Serratia* inhibited by amikacin in the presence of increasing concentrations of azlocillin.

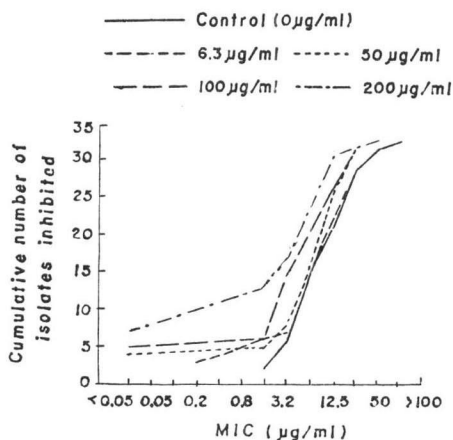


Table 1. Comparative synergistic activity† of aminoglycosides and azlocillin against *Serratia marcescens*

Antibiotic combination	No. tested	Sensitivity to aminoglycoside*	No. isolates	Synergy No.	Synergy %	Overall synergy %
Amikacin	32	R	11	8	73%	50
Azlocillin		S	21	8	38%	
Gentamicin	32	R	21	12	57%	47
Azlocillin		S	11	3	27%	
Netilmicin	32	R	24	15	63%	59
Azlocillin		S	8	4	50%	

† Synergy was tested using agar dilution method in MHA.

* R = MIC $\geq 12.5 \mu\text{g/ml}$ for gentamicin and netilmicin and for amikacin $\geq 25 \mu\text{g/ml}$.

Table 2. Synergy† of amikacin and carbenicillin or ticarcillin against *Serratia marcescens*

Antibiotic	No. tested	Sensitivity to penicillin*	No. isolates	No. showing synergy	Total percent synergy
Carbenicillin	32	R S	26 6	6 5	45
Ticarcillin	32	R S	26 6	8 5	39

† Synergy was tested using agar dilution method in MHA.

* R = MIC \geq 200 μ g/ml. S = MIC \leq 100 μ g/ml.

Combination of amikacin and carbenicillin was synergistic against 45% of isolates and the amikacin-ticarcillin combination was synergistic for 39% of isolates (Table 2). For example, an isolate with an amikacin MIC of 100 μ g/ml had an amikacin MIC of 12.5 μ g/ml in the presence of 100 μ g/ml carbenicillin or ticarcillin and an isolate with an amikacin MIC of 12.5 μ g/ml had an amikacin MIC of 3.1 μ g/ml in the presence of 100 μ g/ml of either penicillin. In this particular study, 16% of the isolates were resistant to amikacin MIC $>$ 25 μ g/ml but all were synergistically inhibited when amikacin was combined with carbenicillin or ticarcillin.

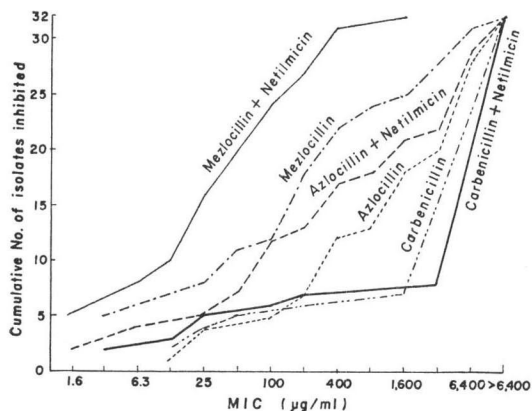
In comparison gentamicin did not show a significant increase in the percent of strains which were inhibited at 6.3 μ g/ml when azlocillin was added at concentrations up to 200 μ g/ml. Netilmicin which had very poor activity against these strains, inhibiting only 25% alone, inhibited 50% in the presence of 200 μ g/ml of azlocillin. At concentrations of 25 μ g/ml both gentamicin and netilmicin in the presence of 200 μ g/ml of azlocillin inhibited only 70~80% of the isolates.

Since netilmicin has excellent activity against many gram-negative organisms except *Serratia*⁴³, we compared the effect of the addition of three penicillins to netilmicin. Table 3 demonstrates that the greatest synergy is again seen with resistant strains. Mezlocillin and azlocillin show comparable overall synergy with the greatest percent synergy shown by mezlocillin and netilmicin. If one compares the agents at their respective MIC values, at a netilmicin MIC of 6.3 μ g/ml, 17 of 32 strains were inhibited by azlocillin-netilmicin, 18 of 32 by mezlocillin-netilmicin and only 10 of 32 by carbenicillin-netilmicin. At a concentration of 50 μ g/ml of netilmicin all strains were inhibited if azlocillin was present at one-fourth of its MIC value, and 94% with mezlocillin, but only 66% with carbenicillin. Fig. 2 shows the data when plotted from the aspect of the penicillins. Even at concentrations of 200 μ g/ml of carbenicillin only 22% of isolates were inhibited in the presence of one-fourth the netilmicin MIC, whereas in the presence of 200 μ g/ml of azlocillin 56% were inhibited and 84% were inhibited with mezlocillin when netilmicin was added at one-fourth its MIC value, a value which is attainable in both serum and urine.

Table 3. Comparative synergy of penicillins combined with netilmicin against *Serratia marcescens*

Penicillin added	No. tested	Sensitivity to aminoglycoside	No. isolates	Showing synergy	Total synergy %
Azlocillin	32	R S	24 8	15 (63%) 4 (50%)	59
Carbenicillin	32	R S	24 8	2 (8%) 2 (25%)	12
Mezlocillin	32	R S	24 8	19 (79%) 0 (0%)	59

Fig. 2. Comparative activities of carbenicillin, azlocillin and mezlocillin when combined with netilmicin at the concentration of one-fourth of its MIC values.



Interestingly it is not possible to generalize which isolates will show synergy with a particular combination. For example, Fig. 3 demonstrates that strain 3035 which is resistant to gentamicin and netilmicin and sensitive to amikacin is synergistically inhibited by the addition of azlocillin to the gentamicin and netilmicin, but no synergy is seen with amikacin. In contrast strain 2953 is synergistically inhibited by amikacin-azlocillin, but not by gentamicin-azlocillin and netilmicin-azlocillin.

Fig. 4 shows that the combination of netilmicin and mezlocillin is synergistic when examined by the killing curve technique. There is a two log reduction in colony forming units in four hours and an eight log reduction in 24 hours. The combined concentrations of netilmicin was $6.3 \mu\text{g/ml}$ and of mezlocillin $800 \mu\text{g/ml}$ both of which would be achieved in urine; whereas, the individual MIC values of both $25 \mu\text{g/ml}$ and $3,200 \mu\text{g/ml}$ would rarely be achieved. Similar effects were seen with amikacin and gentamicin when combined with any of the three penicillins.

Fig. 3. Isobolograms showing the activities of amikacin, gentamicin and netilmicin combined with azlocillin against *Serratia marcescens* 2953, and against *Serratia marcescens* 3035.

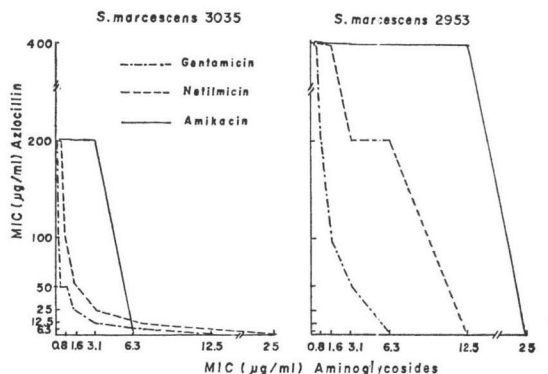
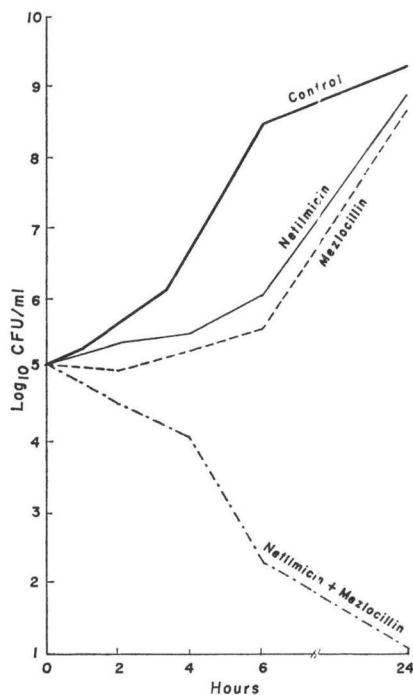


Fig. 4. Bactericidal activity showing synergy against *Serratia marcescens* 3035.

The concentration of netilmicin was $6.3 \mu\text{g/ml}$ and that of mezlocillin was $800 \mu\text{g/ml}$; both are one-fourth of MIC values.



Discussion

As previously noted the majority of our nosocomial infections produced by *Serratia marcescens* are those involving the urinary tract and less than 5% *Serratia* infections are septicemias. Thus we

were concerned to see if the combination of a penicillin and an aminoglycoside would inhibit strains that are usually resistant to the aminoglycoside or the penicillin when used alone. We were particularly concerned about the activity of netilmicin against *Serratia* since work from our group^{3,4)} had shown that netilmicin although active against many gentamicin-resistant *Escherichia coli*, *Citrobacter* and *Klebsiella* was not active against gentamicin-resistant *Serratia*. Furthermore, 34% of the isolates of *Serratia* in our institution had amikacin MIC values above 12.5 µg/ml. From these studies it is clear that at the concentrations which could be achieved in blood, addition of carbenicillin, mezlocillin or azlocillin would not reduce the MIC of gentamicin or netilmicin to a level that was clinically meaningful for the majority of these *Serratia* isolates. In contrast addition of azlocillin, carbenicillin, mezlocillin or ticarcillin to amikacin would reduce the MIC of all isolates to a level that was therapeutically achievable.

In the presence of concentrations of azlocillin or mezlocillin at 400~1,200 µg/ml, which can be achieved in urine, the gentamicin and netilmicin MIC values of strains of *Serratia* that were in excess of 50 µg/ml were reduced to levels of 6.3~25 µg/ml in the majority of instances and the amikacin MIC values were all 12.5 µg/ml or less. Carbenicillin, however, when combined with netilmicin or gentamicin did not lower the MIC of the aminoglycoside to levels that could be achieved in urine.

Although the addition of azlocillin or mezlocillin to netilmicin could increase the percent of strains inhibited at achievable urinary concentrates from 59 to 75%, the same concentration of azlocillin inhibited all isolates when combined with amikacin at concentrations achievable in serum for both agents.

These observations suggest that the combined use of an aminoglycoside and one of these newer ureido-penicillins could be useful in the treatment of *Serratia* urinary infections which are resistant to both the aminoglycosides and semi-synthetic penicillins. The studies do suggest, however, that the use of carbenicillin and the aminoglycosides is less likely to result in success than when one of the ureido-penicillins is used. The killing curve data further suggests that the action of the two agents is bactericidal and not merely inhibitory. These data with *Serratia* are in agreement with our earlier studies with *Pseudomonas* in which we observed that the synergistic effect of antibiotics is most likely to be clinically meaningful with the most active compounds³⁾. Since we are already seeing resistance to amikacin develop, use of combination agents may be necessary to control nosocomial infections due to *Serratia*.

References

- 1) BURTON, D. S. & D. A. NAGEL: *Serratia marcescens* infections in orthopaedic surgery. Clin. Ortho. 89: 145~149, 1972
- 2) FARHOUDI, H. O.; T. BANKS & N. ALI: A case report of *Serratia* endocarditis with review of literature. Med. Ann. Dist. Columb. 43: 406~410, 1974
- 3) FU, K. P. & H. C. NEU: *In vitro* synergistic effect of netilmicin, a new aminoglycoside antibiotic. Antimicrob. Agents & Chemoth. 10: 511~518, 1976
- 4) FU, K. P. & H. C. NEU: *In vitro* study of netilmicin (Sch 20569) compared with other aminoglycosides. Antimicrob. Agents & Chemoth. 10: 526~534, 1976
- 5) MAKI, D. G.; C. G. HENNEKENS, C. W. PHILIPS, W. V. SHAW & J. V. BENNETT: Nosocomial urinary tract infection with *Serratia marcescens*: An epidemiologic study. J. Infect. Dis. 128: 579~587, 1973
- 6) MILLS, J. & D. DREW: *Serratia marcescens* endocarditis: a regional illness associated with intravenous drug abuse. Ann. Int. Med. 84: 29~35, 1976
- 7) MYEROWITZ, R. L.; A. A. MEDEIROS & T. F. O'BRIEN: Recent experience with bacilleemia due to gram-negative organisms. J. Infect. Dis. 124: 239~246, 1971
- 8) PRICE, K. E.; D. R. CHISHOLM, M. MISIEK, F. LEITNER & Y. H. TSAI: Microbiological evaluation of BB-K8, a new semisynthetic aminoglycoside. J. Antibiotics 25: 709~731, 1972
- 9) SCHABERG, D. R.; R. H. ALFORD, R. ANDERSON, J. J. FARMER, III, M. ANN MELLEY & W. SCHAFFNER: An outbreak of nosocomial infection due to multiply resistant *Serratia marcescens*: Evidence of inter-hospital spread. J. Infect. Dis. 134: 181~188, 1976
- 10) THOMAS, F. E., Jr.; J. M. LEONARD & R. H. ALFORD: Sulfamethoxazole-trimethoprim-polymyxin

- therapy of serious multiply drug-resistant *Serratia* infections. *Antimicrob. Agents & Chemother.* 9: 201~207, 1976
- 11) TRAUB, W. H. & I. KLEBER: *In vitro* additive effect of polymyxin B and rifampin against *Serratia marcescens*. *Antimicrob. Agents & Chemother.* 7: 874~876, 1975
 - 12) VERBIST, L.; J. SPAEPEN & J. VANDEPITTE: *In vitro* sensitivity of hospital strains of *Serratia marcescens* to chemotherapeutic agents. *Chemotherapy* 22: 43~54, 1976
 - 13) WEBB, S. F. & A. VALL-SPINOSA: Outbreak of *Serratia marcescens* associated with the flexible fiber bronchoscope. *Chest* 68: 703~708, 1975
 - 14) WEINSTEIN, R. J.; L. S. YOUNG & W. L. HEWITT: Comparison of methods for assessing *in vitro* antibiotic synergism against *Pseudomonas* and *Serratia*. *J. Lab. Clin. Med.* 86: 853~862, 1975