In Vitro Comparison of Netilmicin, a Semisynthetic Derivative of Sisomicin, and Four Other Aminoglycoside Antibiotics

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Received for publication 13 February 1976

One hundred isolates of Pseudomonas and Enterobacteriaceae, of which 85 were chosen because of their resistance to gentamicin or amikacin, were tested for susceptibility to netilmicin (SCH 20569), a new semisynthetic derivative of sisomicin, and to four other aminoglycosides. Tests were performed in Mueller-Hinton agar and, with 43 of these isolates, also in Mueller-Hinton broth. Most isolates of Escherichia coli, Klebsiella, Enterobacter, Citrobacter, and Serratia that were gentamicin resistant proved to be susceptible to netilmicin and amikacin. Tests of representative isolates of this group showed that they owed their resistance to the production of aminoglycoside adenylating enzymes. Four isolates of Serratia, detected by their resistance to amikacin, were also highly resistant to netilmicin but were susceptible to gentamicin. These isolates produced aminoglycoside-acetylating enzymes. Gentamicin-resistant Proteus and Providencia were, in general, highly resistant to netilmicin but were susceptible to amikacin. These isolates also produced aminoglycoside-acetylating enzymes. Most gentamicin-resistant strains of Pseudomonas were resistant to netilmicin, either by enzymatic aminoglycoside modification or by other undefined mechanisms. Thus, like amikacin, netilmicin extends the aminoglycoside susceptibility pattern of Enterobacteriaceae to include gentamicin-resistant isolates that produce aminoglycoside adenylating enzymes. It is ineffective against strains, some of them susceptible to amikacin, gentamicin, or tobramycin, that produce aminoglycoside-acetylating enzymes.

The purpose of this study was to compare the susceptibility of clinical isolates of Pseudomonas and Enterobacteriaceae to netilmicin (SCH 20569), a new semisynthetic derivative of sisomicin developed by the Schering Corp. Netilmicin is O-2,6-diamino-2,3,4,6-tetraoxy-α-D-glycerohex-4-enopyranosyl-(1 → 4)-O-[3-deoxy-4-C-methyl-3-(methylamino)-β-L-arabinopyranosyl-(1 → 6)]-2-deoxy-1-N-ethyl-p-d-glucosamine. It is being prepared for clinical testing in humans (J. A. Waitz, personal communication, Schering Corp., Bloomfield, N.J.). For comparison, we also determined the susceptibility of our bacteria to gentamicin, tobramycin, sisomicin, and amikacin.

MATERIALS AND METHODS

Organisms. Ninety-three of the one hundred strains selected for study were isolated in our diagnostic microbiology laboratory between January 1971 and September 1974. Eighty-five were resistant either to gentamicin or amikacin by repeated standard Kirby-Bauer disk tests. The 100 strains included 36 isolates of Pseudomonas aeruginosa, 10 other Pseudomonas species (5 P. maltophilia, 2 P. cepacia, 1 P. acidovorans, 1 P. putida, and 1 unidentified species), 18 Escherichia coli, 9 Klebsiella pneumoniae, 6 Enterobacter (7 E. cloacae, 2 E. liquefaciens), 8 Serratia marcescens, 4 Providencia (not specified), 2 Proteus mirabilis, 1 P. vulgaris, 1 P. rettgeri, and 2 Citrobacter freundii. Included among these strains were P. aeruginosa GN315 and A20897, and Pseudomonas species strain A20621, obtained from K. E. Price, Bristol Laboratories. These strains produced the aminoglycoside acetyltransferase AAC(6')-4 (for nomenclature see section on enzymatic assays) (4, 11). Also included were E. coli K-12 carrying the R factor R5, which produces a similar enzyme, and E. coli K-12 carrying the R factor JB66, which produces aminoglycoside adenylyltransferase AAD(2'). Both strains were obtained from J. Davies, University of Wisconsin (2, 3). Our isolates, P. aeruginosa strain POW and C. freundii strain WIL (previously erroneously identified as E. coli), were earlier shown to produce gentamicin adenylyltransferases determined by R factors (5, 8, 9). Two P. aeruginosa strains Pre and Kru, were clinical isolates obtained from S. Lerner, University of Chicago.

Antibiotics. The aminoglycosides used in this study were laboratory standard preparations. Gen-
tamicin (a mixture of approximately equal parts gentamicin C1, C9, and C12), sisomicin, and netil-
micin were supplied by J. A. Waitz of the Schering Corp.; tobramycin was supplied by R. S. Griffith, Eli
Lilly & Co.; amikacin (BB-K8), a semisynthetic
kanamycin derivative, was obtained from G. E.
Wright, Bristol Laboratories.

Quantitative determination of aminoglycoside
susceptibility. The standard assay for microbial in-
hibition was performed with each of the five amin-
glycosides by an agar dilution technique. For this
purpose serial twofold dilutions of the aminoglyco-
sides in duplicate Mueller-Hinton agar (BBL) plates
were inoculated with $10^{-2}$ and $10^{-3}$ dilutions of an
overnight growth at 37 C in Mueller-Hinton broth
(Difco) (approximately 10$^4$ and 10$^5$ organisms) by
means of a Steers-Foltz apparatus. The minimal
inhibitory concentration (MIC) was defined as the
lowest concentration of antibiotic that prevented
any visible growth of the organisms after overnight
incubation at 37 C. The MIC values would have
been unchanged in 97% of the tests performed if the
MIC endpoint had been defined as a 90% reduction
in number of colonies formed. Tests performed with
inocula of 10$^4$ and 10$^5$ organisms showed the same
MIC of netilmicin, or one differing by only one
dilution, with 85 of the 100 strains examined.

Twenty-five isolates of P. aeruginosa, one P. puti-
tida, and seventeen isolates of Enterobacteriaceae
representative of the different susceptibility pat-
terns shown in the agar dilution tests were assayed
with gentamicin and netilmicin for MIC and for
minimal bactericidal concentration (MBC) by a
broth dilution procedure. For this purpose, serial
twofold dilutions of the aminoglycosides in duplicate
tubes containing 1 ml of Mueller-Hinton broth were
inoculated with 0.1 ml of a 10$^{-3}$ dilution of an over-
night growth (approximately 10$^5$ organisms). The
MIC was defined as the lowest concentration of anti-
biotic in those tubes showing no turbidity after 18
h of incubation at 37 C. MIC was also determined
after 42 h. After 18 h of incubation, the contents of
nonturbid tubes were subcultured with a 0.01-ml loop
into antibiotic-free Mueller-Hinton agar. The
MBC was defined as the lowest concentration of
antibiotic that permitted the growth of 10 or fewer
colonies.

Single lots of Mueller-Hinton broth and agar were
used for these studies. Samples of each were anal-
yzed for total Mg$^{2+}$ and Ca$^{2+}$ concentrations with
an atomic absorption spectrophotometer by S. Natel-
son, Department of Biochemistry, Michael Reese
Hospital. The agar contained (per liter) 60 mg of
Ca$^{2+}$ and 18 mg of Mg$^{2+}$, whereas the broth con-
tained 7 and 3 mg/liter, respectively.

To compare the effects of the different antibiotics
in tests of each type (i.e., agar MIC, broth MIC or
MBC), the results of each test were recorded sepa-
rateldehyde if the organisms were inhibited
in an MIC test or killed in an MBC test by 5 pg or
less of gentamicin, tobramycin, sisomicin, or netil-
micin per ml and by 10 pg or less of amikacin per ml.
Strains that required 20 pg or more of the former
antibiotics per ml or 40 pg of amikacin per ml for
inhibition or killing were considered resistant. Or-
ganisms requiring 10 pg of one of the former agents
per ml or 20 pg of amikacin per ml for inhibition or
killing were considered intermediate. These values
were selected on the basis of the therapeutic drug
concentrations attained in serum by treatment with
genitamicin, tobramycin, sisomicin, and amikacin.
The resistant, susceptible, and intermediate values
selected for netilmicin were the same as those for
genitamicin and sisomicin, in view of the close chemi-
cal relation between these agents.

Enzymatic assays. From prior unpublished stud-
ies on 32 of the gentamicin- or amikacin-resistant
strains used in this study, information was availa-
ble on results of aminoglycoside resistance tech-
niques by sonic extracts of the bacteria against amin-
glycosides selected from the following: gentamicin
C1, C10, and C12, gentamicin A, sisomicin, tobram-
ycin, amikacin, kanamycin A, kanamycin B, neomy-
cin B, and paromomycin. The assays were per-
formed according to published methods (1, 2, 6, 9).
Bacteria whose extracts gave at least five times the
counts per minute of a similarly processed extract of
a comparable antibiotic-susceptible strain were
considered to be resistant to aminoglycoside antibi-
tics.

The enzymatic nomenclature was taken from the
Commission on Chemoresistance, International So-
ciety of Chemotherapy (S. Mitsuhashi, personal com-
munication, Gunma Univ., Maebashi City, Japan).
The enzymes carried by our isolates had substrate
specificities similar to or the same as the following:
AAD(2'), aminoglycoside 2'-nucleotidyltransferase
(gentamicin adenylyltransferase); AAC(6')-4, amin-
glycoside 8'-acetyltransferase-4 (8'-N-gentamicin
acyltransferase or kanamycin acetyltransferase);
AAC(3'), aminoglycoside 3'-acetyltransferase (3'
-gentamicin acetyltransferase I); AAC(2')-2, amin-
glycoside 2'-acetyltransferase-2 (2'-N-gentamicin
acytetransferase II).

RESULTS

Enterobacteriaceae: MICs and MBCs. Amino-
glycoside susceptibility patterns were similar
among the 36 isolates of E. coli, K. pneumo-
\textit{niae}, and Enterobacter studied by the agar di-
lution method (Fig. 1). In general, the majority of
the gentamicin-resistant isolates were suscep-
tible to netilmicin and amikacin. They were
resistant to tobramycin and were resistant or
intermediate to sisomicin. Only 8% of the iso-
lates were resistant to netilmicin, and 14% were
resistant to amikacin. Fig. 2 shows that 24 of
the 36 isolates of Enterobacteriaceae were
resistant to gentamicin but susceptible to netil-
micin, whereas only one isolate was resistant
to netilmicin but susceptible to gentamicin.
Five isolates that were either intermediate or
resistant to gentamicin had similar MICs with
netilmicin. The susceptibility of two genta-
micin-resistant \textit{C. freundii} isolates, not shown in
Fig. 1, resembled the pattern predominant in this
group of bacteria: these isolates were suscep-
tible to netilmicin and amikacin.

Two patterns of susceptibility to aminoglyco-

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Minimal Inhibitory Concentration (μg/ml)

Fig. 1. MICs of aminoglycosides tested with Enterobacteriaceae by the agar dilution method using as an inoculum a 10⁻³ dilution of an overnight growth. Antibiotics tested were gentamicin (●), amikacin (○), sisomicin (□), tobramycin (■), and netilmicin (▲).

Fig. 2. Comparative MICs of gentamicin and netilmicin for E. Coli, Klebsiella-Enterobacter, and Proteus-Providencia organisms. Note that the scale is logarithmic. The boundary between the two shaded lines indicates results that differ by twofold. The boundary between the shaded and the unshaded zones indicates results that differ by fourfold.

Fig. 3. Comparative MICs of gentamicin and netilmicin for isolates of P. aeruginosa and S. marcescens tested by the agar dilution method. Note the logarithmic scales. For significance of shaded zones, see Fig. 2.

Sides were found by agar dilution tests among isolates of Serratia marcescens (Fig. 3). Three isolates that were resistant to gentamicin were susceptible to netilmicin. Four isolates that were susceptible to gentamicin were resistant to netilmicin. One isolate was nearly equally susceptible to both agents. Seven of the eight isolates were resistant or intermediate to tobramycin and sisomicin (Fig. 1). The gentamicin-resistant isolates were susceptible to amikacin, whereas the four gentamicin-susceptible isolates that were resistant to netilmicin were also resistant to amikacin.

Most of the Proteus and Providencia isolates scored by the agar dilution method were resistant to gentamicin, netilmicin, tobramycin, and sisomicin (MIC <10 μg/ml), but for the most
part were susceptible or intermediate to amikacin (MIC ≤ 20 µg/ml) (Fig. 1). One *P. mirabilis* isolate was equally susceptible to all the agents tested.

Thirteen isolates of the *E. coli*, *Klebsiella*, *Enterobacter* group, two of the *Proteus* isolates, and two of the *Providencia* isolates were tested by the tube dilution method. In 88% of the tests, MICs after 18 h of incubation at 37 °C were identical to or only one dilution different from the agar dilution MIC (Fig. 4). There was 88 and 86% agreement (within one dilution) between 18-h and 42-h MICs and MBCs, respectively.

*P. aeruginosa*: MICs and MBCs. Nearly all of the gentamicin-resistant isolates were resistant or intermediate to netilmicin in agar dilution MIC tests (Fig. 3). Fifty percent of the isolates gave either identical or twofold different MICs with gentamicin and netilmicin whereas 22% gave fourfold different results. Nine (25%) isolates had greater than fourfold higher MICs with gentamicin, whereas only one (3%) had a greater than fourfold higher MIC with netilmicin. Amikacin was the most effective agent tested: only 55% were resistant or intermediate (Fig. 5). The superiority of amikacin was enhanced if we compared the frequency of resistant isolates alone. Eight percent were resistant (MIC ≥ 40 µg/ml) to amikacin, compared with 81% to gentamicin, 92% to netilmicin, 72% to sisomicin, and 50% to tobramycin (MIC ≥ 20 µg/ml).

The MICs for the 25 isolates of *P. aeruginosa* tested by the broth dilution method with gentamicin and netilmicin were generally lower than those obtained by the agar dilution method and enhanced the differences in MICs between those two agents (Fig. 4). Forty-eight percent of the isolates tested were scored as resistant to gentamicin, whereas only 8% were scored as resistant to netilmicin (Fig. 5). For isolates in which we obtained definite end points (i.e., < 80 and ≥ 0.6 µg/ml), the mean ratio of the agar dilution MIC to the broth dilution MIC was 6.

Judged by the MBC test, most of these 25 isolates of *P. aeruginosa* would be classified as resistant both to gentamicin and netilmicin (Table 1). The MBCs were four- to eightfold higher than the tube dilution MICs. They were, however, comparable to the MIC values obtained by agar dilution. Table 1 also shows that MIC values obtained by the tube dilution test were similar after 18 or 42 h of incubation.

*Pseudomonas* species: MICs and MBCs. With 9 of the 10 isolates selected for study, the agar dilution MICs of all five aminoglycosides were equal to or greater than 80 µg/ml. With one isolate of *P. putida*, MICs were 10, 20, and 40 µg of amikacin, tobramycin, and netilmicin per ml, respectively. By the broth dilution method, the MICs of gentamicin and netilmicin were 40 and < 0.6 µg/ml, respectively, for this isolate. The MBC for this isolate was 10 µg of netilmicin per ml.

Tests with organisms capable of modifying aminoglycosides. From prior unpublished work, supplemented by some determinations on netilmicin, we had data on the aminoglycoside-adenylating and -acetylating activity of 32 strains from the group submitted for susceptibility determinations. The strains had been selected for assay of these enzymatic activities as part of an investigation of mechanisms of gentamicin resistance. The available data gave evidence of a correlation between the enzymatic activities of the isolates examined and the results of the susceptibility tests.

The 10 *Enterobacteriaceae* isolates that were resistant or intermediate to gentamicin and produced the enzyme AAD(2') were susceptible to netilmicin and amikacin (Table 2). The four gentamicin-resistant isolates of *P. aeruginosa* that also produce this enzyme had netilmicin MICs that were only 10 to 20 µg/ml (resistant or intermediate) (Table 2) and, in addition, by the broth dilution tests had MICs of < 0.6 to 1.2 µg/ml (susceptible). All but one of the 14 adenylating isolates were resistant or intermediate to tobramycin and sisomicin. The ade-
nylating isolates included 7 of the 24 Entero-
bacteriaceae that were resistant to gentamicin-
and susceptible to netilmicin in Fig. 2 and 4 of
the 9 Pseudomonas isolates in Fig. 3 that had
greater than fourfold higher MICs with genta-
icin than with netilmicin. The remaining 17
Enterobacteriaceae and 5 Pseudomonas iso-
lates in these two groups were not assayed, but
we think it likely that they also produced AAD(2').
Other adenylylating isolates include C. freundii strain WIL and two of the three
Serratia shown in Fig. 3 that were resistant to
gentamicin and susceptible to netilmicin.
Fifteen of the 32 assayed isolates produced
enzymes with substrate specificities resembling
those of three classes of aminoglycoside acetyl-
transferases (4), namely AAC(6’)-4, AAC(3),
and AAC(2’) (6, 14) (Table 2). All of these 15
isolates, which included P. aeruginosa, S. mar-
cescens, Proteus, Providencia, and E. coli K-12,
were highly resistant to netilmicin, having MICs of 40 or more µg/ml (Table 2). Eight
isolates that produce acetyllating enzymes re-
presentative of each of the three classes of ami-
oglycoside acetyltransferases acetylated netil-
icin. With two exceptions, the 15 acetyllating
isolates were also resistant to sisomicin and
tobramycin.

In contrast to the nearly uniform resistance
of the acetyllating strains to the aminoglyco-
sides cited above, three Providencia strains and
one Proteus strain were susceptible to amika-
cin, and one Proteus and two Pseudomonas
isolates were intermediate. The remaining
cetyllating acetyllating isolates were resistant to amikacin,
and most of them produced enzymes either
known to be AAC(6’)-4 or closely resembling it.
AAC(6’)-4 is the only enzyme known to acetyl-
ate amikacin. Five isolates that produced this
enzyme were susceptible to gentamicin. They
included four isolates of Serratia that were resi-
stant to netilmicin (Fig. 3) and one strain of
E. coli K-12 carrying R5 (Fig. 2). In addition, P.
aeruginosa GN315, which had a higher MIC to

![Fig. 5. MICs of aminoglycosides for P. aeruginosa isolates. (A) Thirty-six isolates tested by the agar
dilution method; (B) 25 isolates tested by the broth dilution method. For antibiotics tested see Fig. 1.]

| Antibiotic | Determination | Cumulative no. of isolates with MIC or MBC (µg/ml) of: | \( <0.6 \) | 0.6 | 1.2 | 2.5 | 5 | 10 | 20 | 40 | 80 | >80 |
|------------|--------------|-----------------------------------------------------|---|---|---|---|---|---|---|---|---|---|
| Gentamicin | MIC (18 h)   | 3 3 3 12 12 13 13 14 19 25                       | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
|            | MIC (42 h)   | 2 2 4 8 12 12 13 13 14 25                       | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
|            | MBC          | 0 0 0 0 4 9 12 12 12 25                       | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
| Netilmicin | MIC (18 h)   | 4 4 3 19 23 23 23 24 24 25                       | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
|            | MIC (42 h)   | 0 0 0 3 10 19 21 23 23 24 25                       | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |
|            | MBC          | 0 0 0 0 0 0 0 8 17 21 22 25                       | 8 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |

* MIC and MBC were determined by the broth dilution method (see Materials and Methods).
netilmicin than to gentamicin, carried this enzyme (Fig. 3). AAC(6')-4 does not acetylate gentamicin C1, a component of the gentamicin complex. The unimpaired activity of gentamicin C1, together with possible residual antibiotic activity of the acetylated components, may account for the effectiveness of gentamicin against these organisms (4).

Three isolates resistant to netilmicin (one P. aeruginosa strain Kru, one E. coli, and one K. pneumoniae) had previously been found not to acetylate or adenylylate gentamicin. In contrast to the organisms that were resistant by virtue of their enzymatic modification of aminoglycosides, these isolates were uniformly resistant to all five of the aminoglycosides, with MICs of nearly identical magnitude. Resistance in these isolates may be caused by restricted access of these agents to their site of action, as has been demonstrated for streptomycin resistance in some strains of P. aeruginosa (13).

DISCUSSION

Our data suggest that many gentamicin-resistant E. coli, Klebsiella, Enterobacter, Citrobacter, and Serratia were susceptible to netilmicin and to amikacin. Gentamicin-resistant Proteus and Providencia were, in general, resistant to netilmicin but were susceptible to amikacin. Amikacin-resistant Serratia were also resistant to netilmicin but were susceptible to gentamicin.

P. aeruginosa and the other Pseudomonas isolates occupy a special position, because their MICs varied with experimental conditions. A systematic difference between broth and agar dilution MIC has been observed with P. aeruginosa and other aminoglycosides, attributed to the higher concentration of the divalent cations Mg²⁺ and Ca²⁺ in Mueller-Hinton agar than in Mueller-Hinton broth (7, 12). Since the concentration of these cations in agar more closely approximates that of serum, it has been suggested that the data obtained in agar may be of more clinical significance (7, 12).

The foregoing results of susceptibility determinations are in general accord with those of others (J. A. Waitz and G. H. Miller, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 92, 1975; and J. J. Rahal, Jr., M. S. Simberkoff, and K. Kagan, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 93, 1975).

In general, strains harboring a gentamicin adenylating enzyme were resistant to gentamicin but susceptible to netilmicin. In our experience, these enzymes have been found most frequently in E. coli, Klebsiella, Enterobacter, and Citrobacter. Thus, it seems likely that netilmicin may prove to be a therapeutically useful agent against many isolates of these genera. The P. aeruginosa isolates that harbored adenylating enzymes conceivably might respond to therapy with netilmicin, especially if found in the urine, since in 80% of the isolates, MICs did not exceed 20 μg/ml, even by agar dilution.

Preliminary experiments in our laboratory indicate that netilmicin is not refractory to attack by AAD(2'), when tested at saturating

| Enzyme | Organism | No. of isolates | MIC by agar dilution (μg/ml) |
|--------|----------|----------------|-----------------------------|
| AAD(2') | Coliforms⁶ | 8 | Gentamicin: 10-40; Netilmicin: <0.6-2.5; Sisomycin: 5-20; Tobramycin: 10-40; Amikacin: 1.2-10 |
| | P. aeruginosa | 4 | Gentamicin: >80; Netilmicin: 10-20; Tobramycin: 10-80; Amikacin: 5 |
| | S. marcescens | 2 | Gentamicin: 20-40; Netilmicin: <0.6-1.2; Tobramycin: 10-20; Amikacin: 2.5-5 |
| AAC(6')-4 | E. coli K-12-R5 | 1 | Gentamicin: 0.6; Netilmicin: 40; Sisomycin: 5; Tobramycin: 40 |
| | P. aeruginosa strain GN315 | 1 | Gentamicin: 10; Netilmicin: >80; Tobramycin: 80; Amikacin: 40 |
| | S. marcescens | 2 | Gentamicin: >80; Netilmicin: >80; Sisomycin: >80; Tobramycin: >80; Amikacin: 20-80 |
| AAC(3) | P. aeruginosa strain Pre | 1 | Gentamicin: >80; Netilmicin: >80; Tobramycin: 80; Amikacin: 5 |
| AAC(2')-2 | Proteus⁴ | 3 | Gentamicin: 10-40; Netilmicin: 40-80; Tobramycin: 20-40; Amikacin: 10-40 |
| | Providencia | 3 | Gentamicin: 20-40; Netilmicin: 40-80; Tobramycin: 20-20; Amikacin: 2.5-10 |

*Enzymatic nomenclature is given in Materials and Methods.

⁶ Coliforms include: E. coli, Klebsiella, Enterobacter, and Citrobacter species. Proteus include: P. mirabilis, P. vulgaris, and P. rettgeri. All were clinical isolates except for one strain, E. coli K-12 (JR66).

⁴ Extracts from these isolates acetylated netilmicin.

⁴ P. aeruginosa A20897 and Pseudomonas species strain A20521.

⁵ Extracts from one of these isolates acetylated netilmicin; the other isolates were not tested.
concentrations of substrate. A possible remaining mechanism for the susceptibility of adenyllylating bacteria to this antibiotic is a low affinity of netilminic for AAD-2(2") at concentrations comparable to the MIC for susceptible bacteria. Confirming preliminary observations of J. A. Waitz (personal communication), we have found that at least one adenyllylating enzyme, AAD-(2") from E. coli K-12 carrying JR66, adenyllylated netilminic at concentrations of 1 to 5 µg/ml to an appreciably lesser extent than gentamicin C1 or the gentamicin complex. An additional mechanism, as yet untested, is that the adenyllylated netilminic retains antibiotic activity.

A number of varieties of aminoglycoside acetyltransferases with somewhat different substrate specificities are known to exist in bacteria (4). From our observations and those of others, it seems likely that our collection included at least three different varieties of acetyltransferase. The bacteria that harbored any of these enzymes were, in general, highly resistant to netilminic. In contrast to the results with the adenyllyltransferase enzyme, our preliminary experiments indicate that netilminic at concentrations of 1 to 5 µg/ml is acetylated, as well as gentamicin C1, or C1a, by each of the three varieties of acetyltransferases encountered in our collection. Judging by the isolates we have examined, resistance to netilminic owing to acetyltransferase activity is relatively infrequent in Escherichia, Klebsiella, and Enterobacter, but is common in Proteus, Providencia, and Pseudomonas.

Thus, it appears that netilminic, like amikacin, may prove effective against Enterobacteriaceae and possibly some Pseudomonas strains that are resistant to gentamicin owing to aminoglycoside-adenyllylating enzymes. The therapeutic ineffectiveness of netilminic against bacteria possessing aminoglycoside-acetylating enzymes may be mitigated by the susceptibility of some of these bacteria to amikacin and, in a few instances, to gentamicin and tobramycin.

ACKNOWLEDGMENTS

This study was supported by a grant from the Schering Corp., Bloomfield, N.J., and by the Medical Research Institute Council, Michael Reese Hospital and Medical Center.

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