# Netilmicin — A Recent Advance In Aminoglycoside Therapy

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# Netilmicin — A Recent Advance In Aminoglycoside Therapy

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# Introduction

These proceedings are a synthesis of papers presented at a series of medical symposia held in Hong Kong, Singapore, Kuala Lumpur and Penang during March 1982. The discussion session from each meeting is also included.

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# Microbiology of netilmicin

## Introduction

Despite the progress which has been made in the development of new classes of antibiotics, such as the development of the third generation cephalosporins, the aminoglycosides alone or in combination remain the antibiotics of choice for the treatment of severe Gram-negative infections. Aminoglycosides have in the past had three basic problems: they had a limited antibacterial spectrum, a problem of resistance development and serious side-effects such as nephro and ototoxicity.

However, great advances have been made since the development of the first aminoglycoside, streptomycin, in 1943. Streptomycin had all three of these problems: a limited spectrum, rapid development of resistance, and significant ototoxicity. It is therefore used only in very selective cases today. Neomycin came next and later kanamycin, but most important was the development of gentamicin in 1963, because this was the first broad spectrum aminoglycoside which encompassed such important organisms as Pseudomonas. Therefore gentamicin was, and still is, utilized extensively throughout the world and can be considered the standard aminoglycoside against which all new aminoglycosides must be compared. However, in the last few years reports have been coming in from all parts of the globe on the development of resistance to gentamicin. The importance of the resistant strains varies from country to country, from city to city and even from hospital to hospital. In certain areas it is of quite minor significance; in other places, such as Manila, it can be of very great importance.

New aminoglycosides such as gentamicin, tobramycin, amikacin and sisomicin have been developed. However, this paper discusses the most recent and most promising new aminoglycoside, netilmicin.

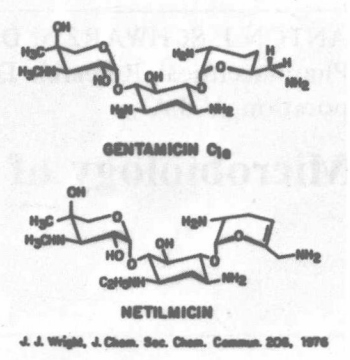


*Fig. 1.* Chemical structure of gentamicin and netilmicin.

## Structure of netilmicin

Figure 1 shows the chemical structure of netilmicin. Netilmicin is a semi-synthetic aminoglycoside. It originates from sisomicin which is isolated from *Micromonospora inyoensis.* The gentamicin $C_{1A}$ structural formula is also shown for comparison. The first difference is a double bond between the 4′ and 5′ position. This double bond differentiates sisomicin from gentamicin $C_{1A}$. Then sisomicin is chemically changed by putting an ethyl group on the number 1 position. This ethyl group plays a very important role in the activity of netilmicin against the gentamicin resistant strains.

## Antibacterial spectrum of netilmicin

Figure 2 shows in vitro results comparing netilmicin with gentamicin. Two different types of data are illustrated. On the left hand side are data obtained in our own laboratories compared with data from the literature. This is to demonstrate that the two sources agree. The upper half of Figure 2 concerns Enterobacter. On the x axis the MICs are listed and on the y axis the cumulative percentage of sensitive strains. Gentamicin is compared with netilmicin and both antibiotics exhibit excellent activity against Enterobacter. Almost 100% of the strains were sensitive to an MIC of approximately 1 $\mu$g/ml. The geometric mean MIC is 0.74 $\mu$g/ml for netilmicin. The lower half of Figure 2 clearly demonstrates that both antibiotics were highly effective against *E. coli;* again netilmicin was as active as gentamicin.

In Figure 3 similar data are shown for Klebsiella, a very important

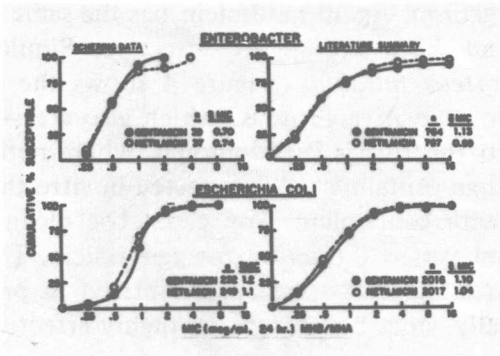


*Fig. 2.* Pattern of sensitivity to gentamicin and netilmicin.

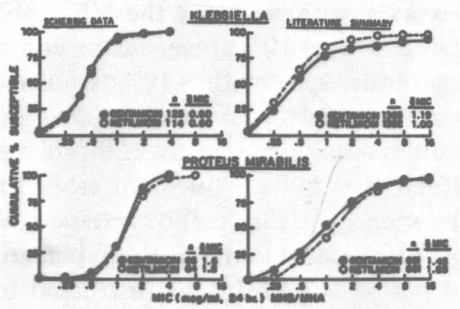


*Fig. 3.* Pattern of sensitivity to gentamicin and netilmicin.

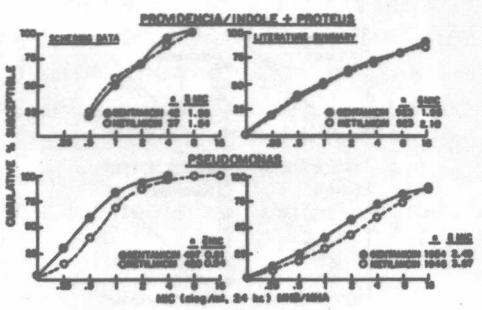


*Fig. 4.* Pattern of sensitivity to gentamicin and netilmicin.

Gram-negative organism. Again netilmicin has the same level of activity as gentamicin and both are highly effective. Similar results were achieved with *Proteus mirabilis*. Figure 4 shows the results for Providencia and Indole positive Proteus, which also are very similar. The only exception to the rule is Pseudomonas, where netilmicin is somewhat less active than gentamicin. When tested in vitro the activity is 1.5 times less than with gentamicin. The geometric mean MIC for netilmicin is 0.9 μg/ml versus 0.6 μg/ml for gentamicin. This difference is real in in vitro testing, but as we have noticed in practice it is not meaningful clinically, since both drugs are highly effective.

## Effect of test conditions on the activity of netilmicin

Figure 5 shows the effect of the inoculum size on the different aminoglycosides. On the x axis we have listed the MICs obtained on Müller-Hinton agar inoculated with ~ $10^6$ organisms as compared to the MICs on Müller-Hinton agar inoculated with ~ $10^4$ organisms. The plots show that the MICs for all four tested aminoglycosides increased only 2-4 fold when the inoculum was increased by 100-fold, representing a very minor inoculum effect. It is quite a different story for the beta lactam antibiotics as can be seen in Figure 6. For instance, with the new third generation cephalosporins, cefotaxime and cefaperazone, the points are significantly spread out and the MICs are increased by as much as 64-fold when the increase in inoculum size is 100-fold.

### *Protein binding*

It is well known that protein binding can be important not only from a testing standpoint but also from the clinical standpoint. It is also well

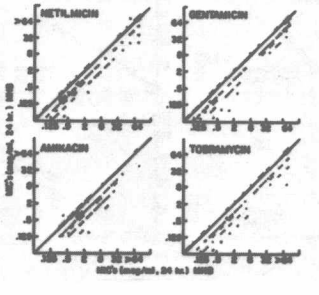


*Fig. 5.* Effect of inoculum size on the in vitro activities of selected aminoglycosides.

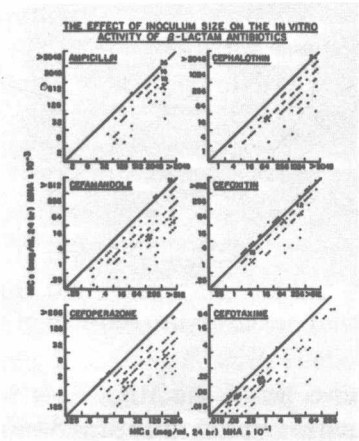


*Fig. 6.* Effect of inoculum size on the in vitro activities of β-lactam antibiotics.

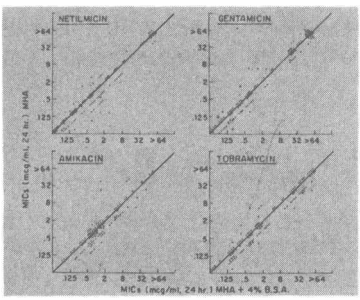


*Fig. 7.* Effect of bovine serum albumin on the in vitro activities of aminoglycosides.

recognized that aminoglycosides generally have a low serum protein binding and this is confirmed in Figure 7. On the x axis are the MICs on Müller-Hinton broth which has been supplemented with 4% bovine serum albumin. This 4% bovine serum albumin yields a similar concentration of protein to human serum. On the y axis are the MICs on the same Müller-Hinton broth without bovine serum albumin. Most of the points are close to the line, meaning that there is little protein binding. This is true for netilmicin, gentamicin, tobramycin and amikacin.

*Effect of pH*

Figure 8 shows the effect of medium pH, which is important from the standpoint of testing but also possibly of importance from the clinical

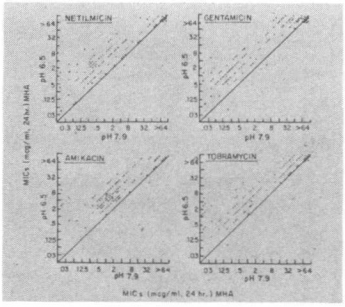


*Fig. 8.* Effect of medium pH on the in vitro activities of aminoglycosides.

aspect. These graphs have listed the MICs on a medium with a pH of 7.9 on the x axis and with an acidic pH of 6.5 only on the y axis. Here the points are not on the line of equality, they are above the line. This shows that in a more acid pH the MICs are increased, indicating that the aminoglycosides in general are less active in a more acid pH. This means that all aminoglycosides tested – netilmicin, amikacin, tobramycin and gentamicin – are four to eight times more active in a slightly alkaline medium.

### *Effect of addition of $Ca^{++}$ and $Mg^{++}$ to the medium*

Figure 9 shows one additional aspect which becomes very important when testing for Pseudomonas sensitivity. With Pseudomonas, the addition of calcium and magnesium ions to the culture medium influences the results. On the x axis is the ratio of the MIC in agar supplemented with calcium and magnesium ions over the MIC of unsupplemented broth versus, on the y axis, the numbers of strains. If calcium and magnesium ions had no effect, this ratio would be 1 and the curve in Figure 9 would be centred over the number 1. However there is actually a shift

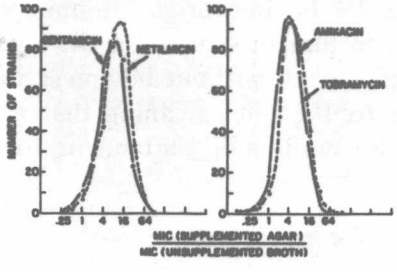


*Fig. 9.* Effect of supplementation of test media with $Ca^{++}$ and $Mg^{++}$ on the susceptibility of Pseudomonas (N = 295).

to the right which means that when the medium is supplemented with calcium and magnesium ions, the MICs are increased roughly eight times. This is not unique for netilmicin; it is also true for gentamicin, amikacin and tobramycin, in fact, for all the aminoglycosides.

*Disc testing*

Figure 10 illustrates the disc diffusion sensitivity test data for netilmicin. On the x axis are the MICs and, on the y axis, the zone size in millimetres. This particular study was done with a variety of non-Pseudomonas organisms including *E. coli*, Klebsiella and Enterobacter. The vertical line is at the 8 μg level which is the breakpoint for netilmicin. Everything below that is considered sensitive. The regression line (diagonal) intercepts the 8 μg line showing that the sensitivity breakpoint for a 30 μg disc, which this figure represents, would be 17 mm in zone diameter. One could also use a 10 μg or a 20 μg disc and the slope of the regression line would be similar, but it would move down somewhat. The zone diameter would be smaller and therefore the test would be less discriminating. In order to have a clearer end point we selected the 30 μg disc for these measurements.

Figure 11 represents similar studies with Pseudomonas. For Pseudomonas results with the 10 μg, 20 μg and 30 μg disc are indicated. The slope is somewhat different from that with the other organisms. The zone sizes are similar in that the zone around the 10 μg disc is smaller than that around the 20 μg, and the zone around the 30 μg is the largest. However, taking the same 8 μg breakpoint and the 30 μg disc, the sensitivity breakpoint in zone diameter is 12 mm. So, to summarize, there are two different sensitivity diameters for the 30 μg disc: one for non-Pseudomonas which is 17 mm and one for Pseudomonas which has a different slope and which is 12 mm.

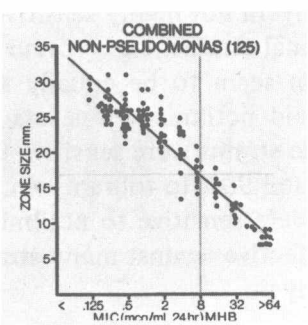


*Fig. 10.* Sensitivity diameter of a 30 μg netilmicin disc for non-Pseudomonas organisms.

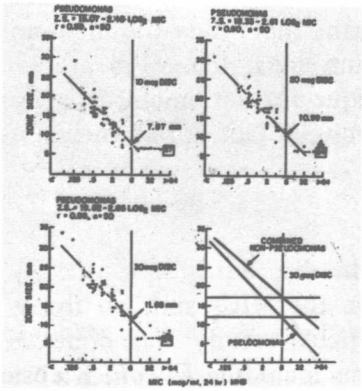

*Fig. 11.* Evaluation of 10, 20 and 30 μg netilmicin discs with Pseudomonas.

## In vitro sensitivity of clinical isolates

Table 1 shows the results of a study in Germany by Professor Braveny, in which isolates from his hospital were tested against netilmicin, gentamicin, tobramycin and amikacin. The breakpoint for netilmicin was 8 μg/ml, for gentamicin and tobramycin 4 μg/ml and for amikacin 16 μg/ml. For *E. Coli,* of the 222 strains isolated from his patients, 99% were sensitive to netilmicin, 96% sensitive to gentamicin and tobramycin, and 100% to amikacin. The picture is somewhat different for Klebsiella, where 96% were sensitive to netilmicin, but only 62% to gentamicin and 69% to tobramycin. This indicates very clearly that a number of strains of Klebsiella were resistant to gentamicin and tobramycin but were fully sensitive to netilmicin and amikacin. In the case of Serratia, 93% of the strains isolated were sensitive to netilmicin but only 36% to gentamicin and 43% to tobramycin. In this particular hospital over 50% of all the Serratia strains were highly resistant to gentamicin and to tobramycin but highly sensitive to netilmicin.

Table 2 shows additional strains, e.g. *Proteus mirabilis,* Indole positive Proteus. All of them seem to be equally sensitive to netilmicin. Even though we have said netilmicin was less active against Pseudomonas in vitro, 96% of the strains were sensitive to netilmicin compared with 89% to gentamicin and 90% to tobramycin. 100% of the staphylococcal strains evaluated were sensitive to netilmicin. These results indicate that netilmicin is effective against many strains which are resistant to gentamicin and tobramycin.

TABLE 1

*Activity of netilmicin and other aminoglycosides**

| | | Geometr. mean MIC | Cumulative: susceptible to indicated concentration (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 20.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 ⩾ 256 |
| *Escherichia coli* (n = 222) | Netilmicin | 0.58 | 1 | 14 | 74 | 94 | 98 | 99 | 99 | | 100 | | |
| | Gentamicin | 0.71 | 1 | 10 | 64 | 90 | 95 | 96 | 98 | 99 | | 100 | |
| | Tobramycin | 0.86 | 1 | 5 | 50 | 83 | 94 | 96 | | 99 | 100 | | |
| | Amikacin | 1.74 | 1 | | 4 | 39 | 82 | 94 | 100 | 100 | | | |
| *Klebsiella spp.* (n = 136) | Netilmicin | 0.73 | 1 | 20 | 75 | 83 | 86 | 87 | 96 | 99 | 100 | | |
| | Gentamicin | 1.70 | 2 | 24 | 55 | 58 | 61 | 62 | 80 | 86 | 96 | 99 | 100 |
| | Tobramycin | 1.50 | 3 | 14 | 47 | 60 | | 69 | 90 | 99 | 100 | | |
| | Amikacin | 1.23 | | 1 | 13 | 64 | 95 | 98 | 99 | 100 | | | |
| *Enterobacter spp.* (n = 57) | Netilmicin | 0.37 | 2 | 44 | 97 | 98 | 100 | 100 | 100 | | | | |
| | Gentamicin | 0.68 | 4 | 42 | 75 | 77 | | 80 | 95 | 98 | | 100 | |
| | Tobramycin | 0.84 | | 21 | 74 | 77 | | 80 | 95 | 98 | | 100 | |
| | Amikacin | 1.01 | | 2 | 12 | 83 | 98 | | | 100 | | | |
| *Serratia spp.* (n = 14) | Netilmicin | 2.10 | | | 7 | 36 | 71 | 93 | 93 | | 100 | | |
| | Gentamicin | 6.56 | | | 36 | 36 | 36 | 36 | | 64 | 93 | 100 | |
| | Tobramycin | 8.00 | | | | 14 | 29 | 43 | | 71 | 100 | | |
| | Amikacin | 2.69 | | | | 14 | 57 | 86 | 100 | 100 | | | |

Breakpoints: Netilmicin 8 mcg/ml
Gentamicin 4 mcg/ml
Tobramycin 4 mcg/ml
Amikacin 16 mcg/ml

*Braveny, Institut für Medizinische Mikrobiologie, Universität München

TABLE 2

*Activity of netilmicin and other aminoglycosides**

| | | Geometr. mean MIC | Cumulative: susceptible to indicated concentration (mg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 20.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 ⩾ 256 |
| *Proteus mirabilis* (n = 119) | Netilmicin | 1.45 | 1 | 3 | 13 | 52 | 89 | 96 | 97 | | 100 | | |
| | Gentamicin | 1.23 | | 4 | 27 | 72 | 90 | 94 | | | 97 | 98 | 100 |
| | Tobramycin | 1.14 | | | 27 | 71 | 90 | 98 | | 99 | 100 | | |
| | Amikacin | 3.79 | | 1 | 2 | 7 | 32 | 74 | 93 | 99 | 100 | | |
| *Indole-pos. proteus* (n = 49) | Netilmicin | 0.64 | 4 | 18 | 63 | 86 | 94 | 100 | 100 | | | | |
| | Gentamicin | 0.69 | | 20 | 59 | 80 | 94 | 100 | | | | | |
| | Tobramycin | 0.70 | | 14 | 61 | 78 | 98 | 100 | | | | | |
| | Amikacin | 1.47 | | 4 | 12 | 49 | 82 | 98 | 100 | 100 | | | |
| *Pseudomonas aeruginosa* (n = 228) | Netilmicin | 1.06 | 1 | 9 | 44 | 77 | 87 | 93 | 96 | | 97 | | 1 |
| | Gentamicin | 0.91 | 2 | 25 | 70 | 83 | 86 | 89 | 90 | 91 | | | 94 100 |
| | Tobramycin | 0.51 | 12 | 55 | 83 | 87 | 90 | 90 | 91 | 94 | 96 | 99 | 100 |
| | Amikacin | 1.34 | | 2 | 16 | 66 | 83 | 95 | 99 | 99 | | | 100 |
| *Citrobacter spp.* (n = 20) | Netilmicin | 0.50 | | 35 | 90 | 95 | 95 | 95 | 95 | | 100 | | |
| | Gentamicin | 0.71 | | 20 | 70 | 85 | | 90 | 100 | | | | |
| | Tobramycin | 0.81 | | 15 | 65 | 80 | 85 | 85 | 100 | | | | |
| | Amikacin | 1.00 | | | 15 | 85 | 100 | 100 | 100 | 100 | | | |
| *Staphylococcus aureus* (n = 155) | Netilmicin | 0.16 | 79 | 92 | 95 | 96 | 99 | 100 | 100 | | | | |
| | Gentamicin | 0.24 | 54 | 83 | 92 | 96 | 96 | 96 | | | 97 | 99 | 100 |
| | Tobramycin | 0.22 | 61 | 82 | 93 | 96 | 96 | 96 | | 99 | | | 100 |
| | Amikacin | 0.60 | 28 | 37 | 58 | 73 | 87 | 93 | 99 | 99 | 100 | | |

Breakpoints: Netilmicin 8 mcg/ml
Gentamicin 4 mcg/ml
Tobramycin 4 mcg/ml
Amikacin 16 mcg/ml

*Braveny, Institut für Medizinische Mikrobiologie, Universität München

## Resistance

The different mechanisms of resistance towards aminoglycosides are listed in Table 3. There are three possible mechanisms, the first of which is alteration of the target site. The target site for an aminoglycoside is the 30 s subunit of the ribosome. This site can be changed due to a mutation in the bacterial chromosome. This particular mechanism of resistance does not play an important role clinically at present but it is a mechanism for streptomycin resistance. Change of permeability of the cell membrane is also rare, although it has been described in certain cases for Pseudomonas. When this form of resistance occurs, all aminoglycosides are equally influenced, since aminoglycosides cannot enter the cell and therefore cannot be effective. Sometimes in this situation it may be useful to add a beta lactam antibiotic in order to open up the cell wall and allow the aminoglycoside to reach its target site.

The most important and the most frequent resistance mechanism against aminoglycosides is enzyme-medicated inactivation of aminoglycosides caused by three different types of enzymes. One type of enzyme acetylates the amino groups (AAC); another phosphorylates hydroxyl groups (APH); and the third nucleotidylates or adenylates hydroxyl groups (ANT).

Figure 12 demonstrates the attack points of these enzymes on an aminoglycoside molecule. The structure of the molecule shown, that of kanamycin B, allows the demonstration of all the different attack points of these enzymes. Firstly there is the 2″ hydroxyl group. This is one of the most important sites because the majority of resistant strains will adenylate this hydroxyl group. There are two more hydroxyl groups, 4′ and 3′, which can be adenylated or phosphorylated. There are also three amino groups, 6′, 2′ and 3′, which can be acetylated. Any of these enzymes can therefore inactivate this molecule.

TABLE 3

*Mechanisms of resistance*

| |
|---|
| Alteration in target site |
| Change in permeability of cell membrane |
| Enzyme mediated inactivation by: |
| Acetylation (AAC) |
| Phosphorylation (APH) |
| Nucleotidylation (ANT) |

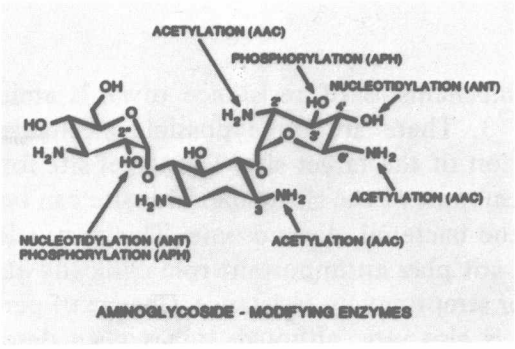


*Fig. 12.* Attack points of enzymes on an aminoglycoside molecule (kanamycin B).

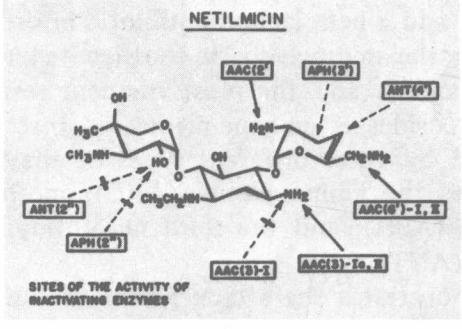


*Fig. 13.* Sites of activity of enzymes on netilmicin.

When the netilmicin structure is examined we can deduce which enzymes can inactivate netilmicin and against which netilmicin will be stable. Figure 13 shows the structure of netilmicin. The broken arrows represent enzymes which cannot attack netilmicin and the solid arrows those which can attack and inactivate netilmicin. Obviously, enzymes cannot attack netilmicin at the 3′ and 4′ position because netilmicin has no hydroxyl groups there. At the 2″ position the hydroxyl group is protected by the ethyl group in the 1 position which denies the enzyme access through stearic hindrance, thus protecting the hydroxyl group against two different types of enzymes. As can be deduced from this diagram, netilmicin should be stable against all adenylating and phosphorylating enzymes. However, netilmicin is vulnerable to the acetylating enzymes at three positions. Most of these acetylating enzymes inactivate netilmicin, so organisms which carry these enzymes will be resistant to netilmicin.

Figure 14 shows the results of testing in vitro strains of gentamicin