NETILMICIN
SUMMARY OF PRECLINICAL MICROBIOLOGICAL STUDIES

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Preliminary microbiological studies with Netilmicin showed it to be a broad-spectrum aminoglycoside antibiotic with activity against many aminoglycoside-resistant strains. Studies carried out with approximately 2,500 clinical isolates demonstrated that Netilmicin was active against aminoglycoside-resistant strains with the following types of resistance patterns: adenylylating strains, ANT (2″) and ANT (4′); phosphorylating strains, APH (3′) and APH (2″) and acetylating strains, AAC (3)-I. Netilmicin was not active against strains containing the acetylating enzymes, AAC (3) (other than type I), AAC (2′) and AAC (6′) nor was it active against permeability resistant mutants. This same pattern of susceptibilities was also observed in experimental mouse infections.

A summary of the results of more than 40 strains, in which the activity of Netilmicin against Gentamicin-resistant strains was measured, is consistent with the above-mentioned activities. That is, Netilmicin was active against 80~90% of Gentamicin-resistant isolates of *Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella* and *Staphylococcus*. These organisms usually owe their Gentamicin-resistance to 2″-inactivating enzymes (ANT(2″), APH(2″)). Gentamicin-resistant isolates of *Providencia* and indole-positive *Proteus* showed lower susceptibilities to Netilmicin consistent with the presence of acetylating enzyme, AAC (2′). The activity of Netilmicin against Gentamicin-resistant *Pseudomonas* was variable, consistent with the presence of a variety of different resistance mechanisms.

Netilmicin's excellent activity against strains containing ANT (2″) enzymes is considered to be of great importance since this type of strain is thought to be responsible for most aminoglycoside resistance in the U. S. A. and Europe.

Introduction

Netilmicin is a novel semisynthetic aminoglycoside antibiotic first synthesized from Sisomicin by J. J. Wright[1]. Netilmicin has broad-spectrum microbiological activity and has been shown to be active against many aminoglycoside-resistant strains[2,3,4,5]. In addition, experimental animal models designed to determine either nephrotoxicity or ototoxicity have suggested that Netilmicin may be less toxic than other aminoglycosides of this family[6]. This paper summarized the early pilot studies[4] which led to the development of Netilmicin and presents the results of recent studies with large numbers of clinical isolates which substantiate the advantages seen in the early studies.

Materials and methods

In vitro Activity : *In vitro* activity was studied using an agar dilution method on Mueller-Hinton agar. An inoculum of approximately 10^3 to 10^4 organisms was delivered onto the agar surface from an overnight culture in Mueller-Hinton broth using an inocula replicating apparatus. The minimal inhibitory concentration (MIC) was defined as the lowest concentration at which less than six discrete colonies were visible. *In vitro* activity was also studied by means of conventional broth dilution tests with Mueller-Hinton broth as the medium. The inoculum used was 0.05 ml of a 1:1000 dilution of an overnight culture; the volume of medium in each tube was 3 ml. The MIC was defined as the lowest concentration that prevented visible growth.

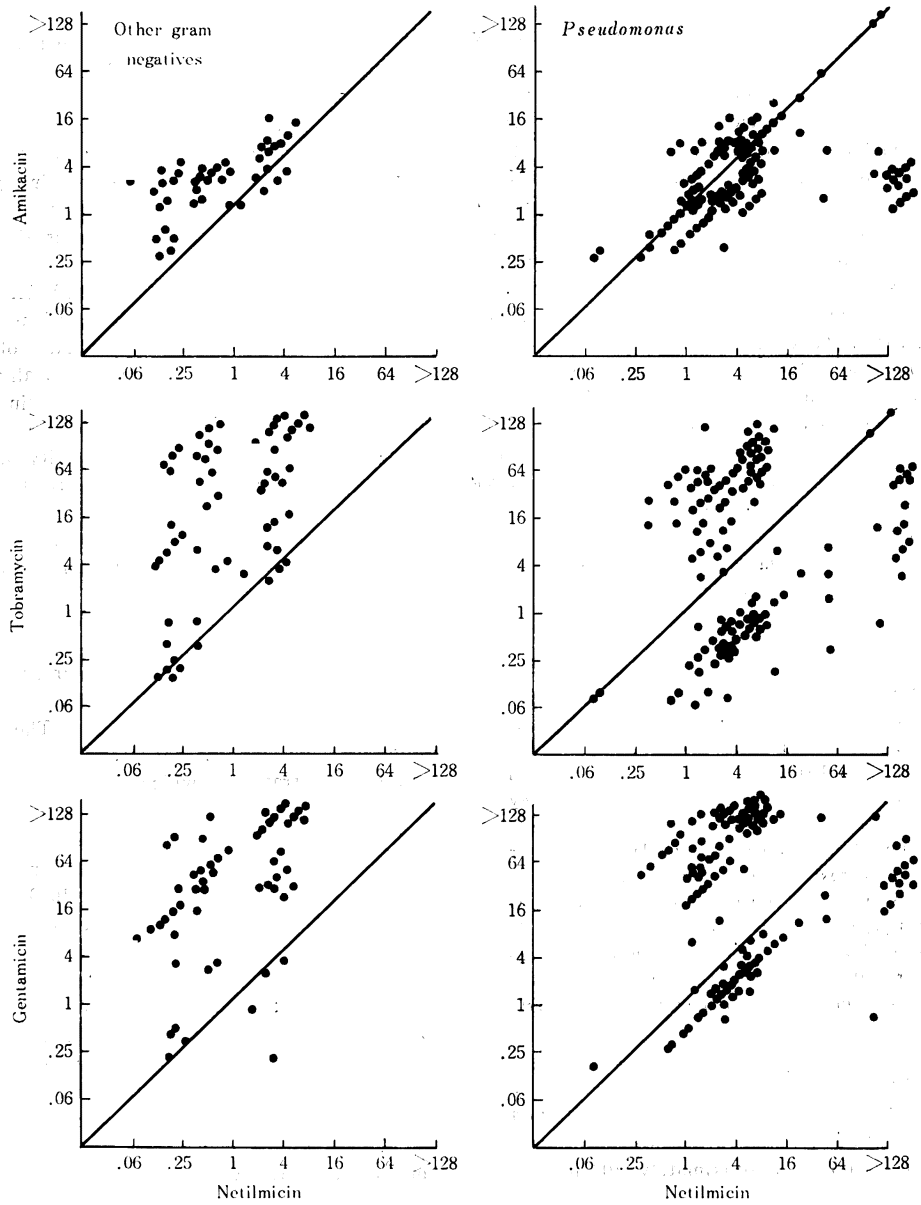
In vivo Tests: Mouse protection tests were performed with groups of ten male CF1 mice weighing approximately 20 g each. Mice were dosed once subcutaneously 1 h after intraperitoneal infection with approximately 10^7 organisms/mouse. Usually, five to seven dose levels were used in each test, and mean protective dose (PD_{50}) values were

calculated by probit procedures. Comparative studies were always carried out simultaneously using the same inoculum. Cats used in the ataxia tests were mongrel males weighing between 2.5 and 4 kg each. The antibiotics were administered subcutaneously. Ataxia studies were similar to those described earlier[7].

Results and discussion

Figure 1 shows data obtained in 1974 and 1975, in which MICs of Netilmicin were compared to those obtained with Gentamicin, Tobramycin and Amikacin[4]. In these and other studies, Netilmicin was shown to have good activity against aminoglycoside-sensitive isolates. Its potency against non-*Pseudomonas* gram-negatives was shown to be similar to that of Gentamicin while it was also found to be about 2-fold less active than Gentamicin against *Pseudomonas*. In addition, most Gentamicin-resistant isolates were found to be susceptible to

Fig. 1 The *in vitro* activity of Netilmicin against aminoglycoside-resistant strains. The MIC's (μg/ml, 24 hr.) Mueller-Hinton agar of Netilmicin are compared to either Gentamicin, Tobramycin or Amikacin.



	GM	TOB	Netilmicin	Sch 21561	Sch 21562	AMK	Sch 21420	SISO	Pentisomicin
R	H	H	Et	Et	Et	HABA	HAPA	H	H
g	38.1	29.8	1.3	3.0	5.3	2.0	1.3	25.3	0.73
i	42	31	1.2	0.7	1.3	0.7	0.7	36	1.2
PD_{50}, mg/kg	≥50	≥50	0.5	0.5	—	2	—	—	—

N = 13

Sch 21561: 2′-N-ethyl-netilmicin
Sch 21562: 6′-N-ethyl-netilmicin
Sch 21420: HAPA Gentamicin B

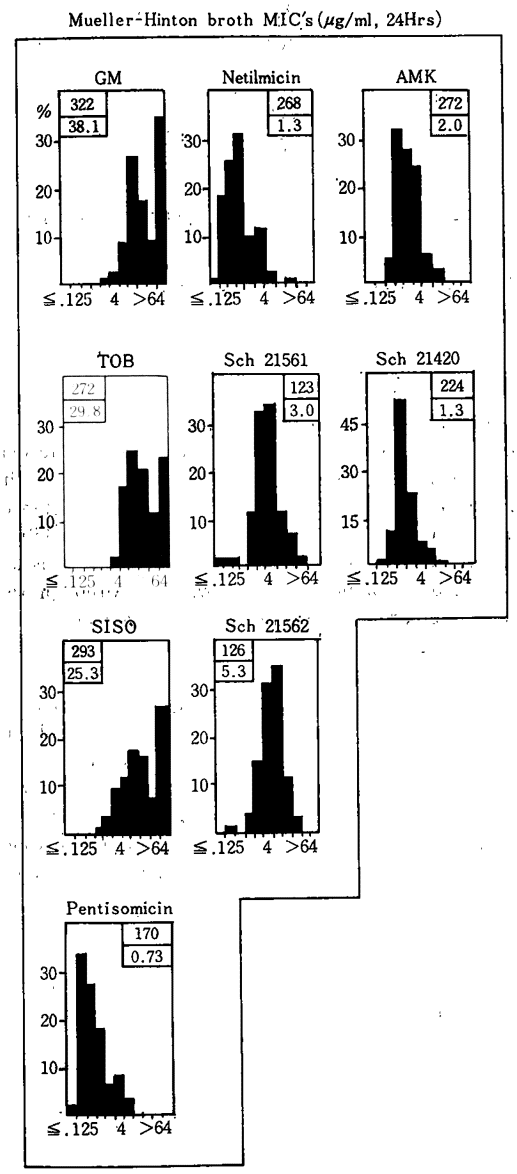
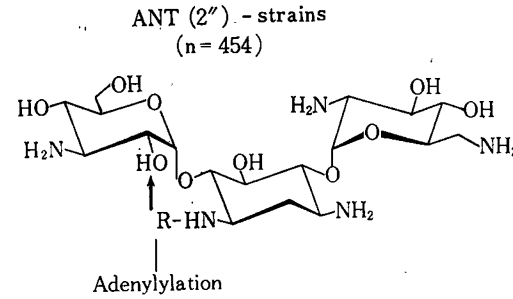


Fig. 2 The activity of nine selected aminoglycosides against strains of bacteria thought to possess the aminoglycoside modifying enzymes, ANT (2″). The histograms show the distribution of MIC's obtained with each aminoglycoside as well as the number of strains tested and the geometric mean MIC (g). The values labelled i represent the average increase in MIC values obtained with these strains compared to normal strains. Average PD_{50} values from 13 experimental mouse infections are given for selected compounds.



Netilmicin. Only a few aminoglycoside-resistant isolates were found to be resistant to Netilmicin. Many of our later studies were directed towards identifying the nature of the aminoglycoside resistance of these isolates.

Results from an early pilot study in cats, Table 1, indicated that Netilmicin might be less ototoxic and nephrotoxic. In this study cats were dosed subcutaneously at 40 mg/kg/day. Gentamicin-treated cats became ataxic after about 14 to 17 days of treatment. Netilmicin-treated cats failed to show ataxia even after 90 days of treatment. There was very little accumulation of Netilmicin in the serum, Table 1, suggesting that nephrotoxicity as well as ototoxicity was low. The results of this study have been substantiated by a large number of investigators using many different experimental animal models[6]. In general, the nephrotoxicity of Netilmicin has been found to be 3 to 4 times less than that of Gentamicin in rats[8,9,10,11], dogs [12,13], rabbits[14] and monkeys[15]. Similarly, the ototoxicity of Netilmicin has been studied in monkeys[15] and guinea pigs[16,17] as well as in the previously mentioned cat experiment. Netilmicin

Table 1 Initial evaluation of the chronic toxicity of Netilmicin

Cat No.	Weight, kg After Day 0	Weight, kg After Day 88	Day of impaired reflexes	Day of ataxia	Day of death	Serum levels 24 hr. after the 90th dose
			Netilmicin, 40 mg/kg/day; s. c.			
8814	5.3	5.7	—	—	92[a]	0.54
8815	4.5	4.7	—	—	92[a]	0.89
8589	4.3	3.8	—	—	89	—
8763	4.1	4.5	—	—	92[a]	1.4
8630	4.2	4.9	—	—	92[a]	0.8
8643	4.8	—	9	—	10[b]	—
8811	4.8	—	13	—	13[b]	—
8793	4.5	—	15	18	18[b]	—
8764	4.2	—	15	18	18[b]	—
8694	4.1	—	11	—	12	—

[a] On Day 92 the study was terminated and the surviving cats were sacrificed in an apparently normal condition.

[b] Four of the gentamicin cats were in a moribund condition and were sacrificed on the day indicated.

has been found to be less ototoxic than either Gentamicin, Tobramycin or Amikacin.

During the last 5 years, we have determined the aminoglycoside susceptibility of about 2,500 clinical isolates in an attempt to determine the most frequent causes of aminoglycoside resistance. These isolates were obtained from 234 different hospitals in the United States, Europe and Latin America. We have classified these strains into 13 different groups, normal strains (1,306 isolates) and 12 different types of resistant isolates. The classification of resistant types was based on the susceptibility of the strains to 9 different aminoglycosides[3]. Netilmicin susceptibilities have been determined for 910 of the 1,255 resistant isolates.

The results of our studies with one type of resistant strain are shown in Figure 2. These strains have a pattern of aminoglycoside susceptibilities consistent with the presence of 2″-adenylylating enzymes. From the graphs of % of strains susceptible vs. MIC shown on the right hand side of the figure, one can see that these strains are resistant to Gentamicin, Tobramycin and Sisomicin. They are sensitive to Netilmicin, two Netilmicin derivatives, Amikacin, Sch 21420 and Pentisomicin. All of the compounds with a 1-N-substituent seem to be active against these strains, and we believe this substituent sterically blocks access of the adenylylating enzymes to the 2″-hydroxyl group[18]. A similar susceptibility pattern was observed in a small number of experimental mouse infections (PD_{50}'s, Figure 2).

Table 2 summarizes some pertinent information about the adenylylating strains in our study. About 36 % of the aminoglycoside-resistant isolates tested have this susceptibility pattern, and we believe it is the most common cause of Gentamicin-resistance in the United States. While this susceptibility pattern has been observed in many different genera, it is most common in *Klebsiella*. Although we have received a large number of *Pseudomonas* isolates with this resistance pattern, 75 % of these were received during 1974 and 1975. During the last three and one-half years, only 26 *Pseudomonas* of this type have been tested. Therefore, we believe the importance of this resistance mechanism is declining in *Pseudomonas* but not in other gram-negatives. Many *Serratia* also have this resistance profile, but here it is usually found in combination with an AAC(6′) enzyme. An additional 113 strains or 9 % of our isolates have this combination so that a total of 45 % of our strains have 2″-adenylylating enzymes.

We have obtained similar information about strains with other resistance patterns. The results are summarized in Figures 3 and 4.

We have found, Figure 3, that Netilmicin was active against strains with resistance patterns corresponding to: 2″-adenylylating strains, 2″-phosphorylating strains, 4′-adenylylating strains, one type of 3-N-acetylating strain, as well as all 3′-phosphorylating strains. Netilmicin was also moderately active against strains with the combination of 2″-adenylylating and 6′-acetylating enzymes depend-

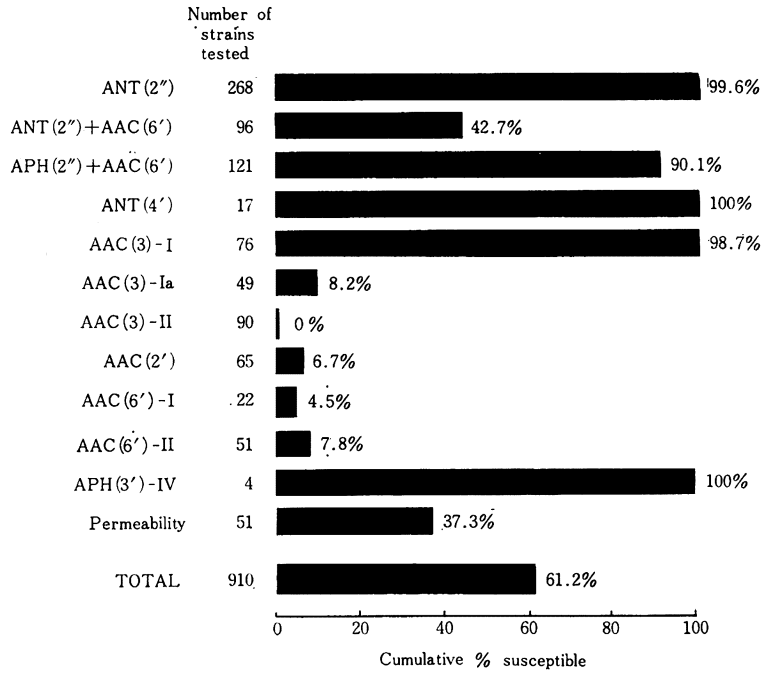
Table 2 "ANT (2″)"-Strains

Total number 454
Number of source 72

% of isolates during		origin	%	Organism	number
1974	76.0	USA	82.6	*Acinetobacter*	1
1975	33.3	CANADA/MEXICO	2.4	*Citrobacter*	7
1976	21.6	S. AMERICA	0.2	*Enterobacter*	23
1977	37.1	EUROPE	14.1	*E. coli*	27
1978	24.3	OTHER	0.6	*Klebsiella*	236
1979	17.7			*P. mirabilis*	7
				P. morganii/P. vulgaris	6
74~79	36.2			*Providencia*	1
				Pseudomonas	109
				Salmonella	1
				Serratia	8
				S. marcescens	28

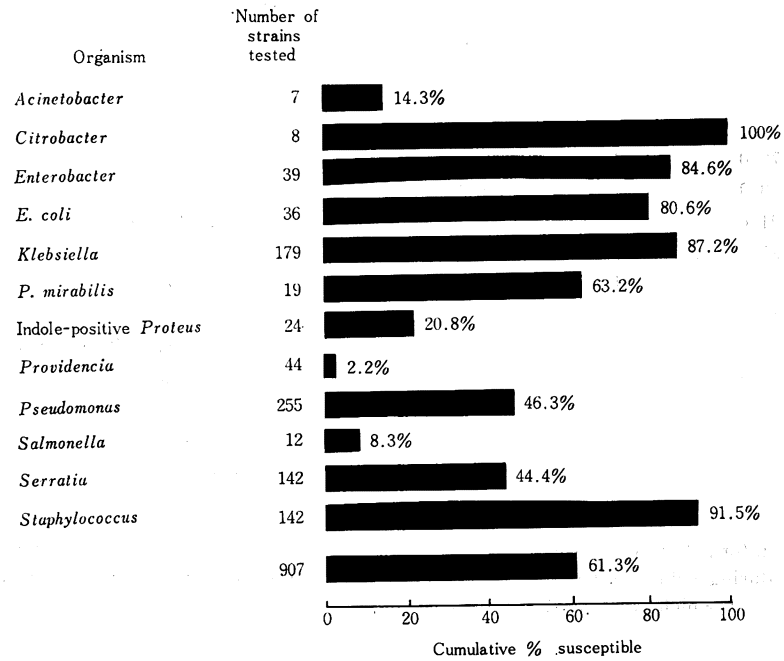
Further information about the 454 ANT (2″) strains which were tested. The percent of isolates during column gives the percentage of aminogycoside-resistant strains tested in a given year which were of this resistance pattern.

Fig. 3 Activity of Netilmicin against aminoglycoside-resistant strains



Summary of the activity of Netilmicin against 910 aminoglycoside-resistant strains. The data has been grouped according to resistance pattern. An MIC value of 8 μg/ml or less has been classified as susceptible in Figures 3, 4, 5 and 6.

Fig. 4 Activity of Netilmicin against aminoglycoside-resistant strains



Summary of the activity of Netilmicin against 907 aminoglycoside-resistant strains. The data have been grouped according to the type of organism studied rather than by resistance patterns (Fig. 3).

ing upon the levels of resistance. Netilmicin had little or no activity against the other types of resistance mechanisms studied. Overall, Netilmicin was active against 61 % of the 910 tested strains.

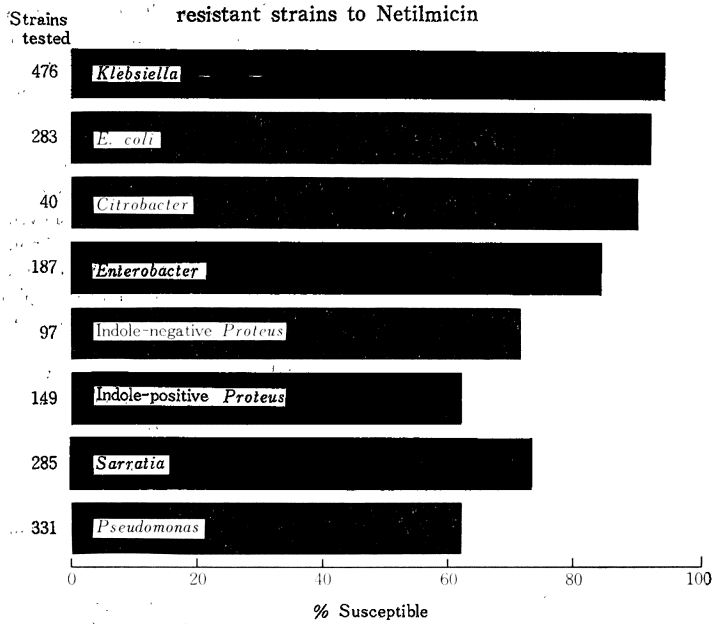
Figure 4 shows the same data arranged by organism rather than by resistance pattern. Because 2″-inactivating enzymes are largely responsible for aminoglycoside resistance in *Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella* and *Staphylococcus*, Netilmicin is active against 80 to 90 % of the resistant isolates encountered in these genera. On the other hand, since 2′-acetylating enzymes are responsible for resistance of *Providencia* and indole-positive *Proteus*, resistant isolates from these genera are usually not susceptible to Netilmicin. *Pseudomonas*, where a variety of resistance mechanisms are encountered, shows variable susceptibility to Netilmicin.

The results of these studies have been substantiated by a large number of investigators. Their findings are summarized in Figures 5 and 6. Figure 5 is a summary of data obtained from approximately 40 different studies carried out in Europe and the United States. Resistant strains of *Klebsiella*, *E. coli*, *Citrobacter*, *Enterobacter*, and indole-negative *Proteus* are usually susceptible to Netilmicin. Where acetylating mechanisms are encountered, indole-positive *Proteus*, *Serratia* and *Pseudomonas*, Netilmicin is less active.

Figure 6 shows that Netilmicin, in addition to being active against aminoglycoside-resistant strains, has good activity against all of the pathogens which are usually treated with an aminoglycoside. This figure summarizes data obtained from more than 100 different investigators around the world. They found that Netilmicin was active against more than 90 % of the tested strains of *Klebsiella*, *E coli*, *Citrobacter*, *Enterobacter*, *Proteus*, *Serratia* and *Staphylococcus* and approximately 85 % of *Pseudomonas*.

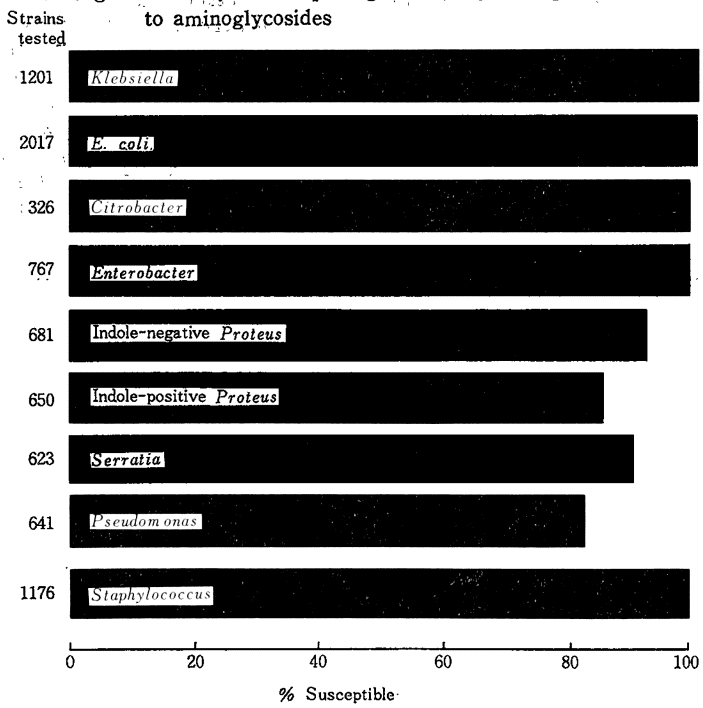
On the basis of our studies and those carried out by other investigators, we believe that Netilmicin is an aminoglycoside antibiotic with activity against many aminoglycoside-resistant strains which also offers the promise of reduced toxicity. Because of this activity and safety profile, we believe Netilmicin will find a role as an aminoglycoside of first-choice for the treatment of life-threatening infections. Because of its activity against both Gentamicin- and Netilmicin-resistant strains, Amikacin will continue to prove useful as a reserve antibiotic for resistant organisms[19,20,21].

Fig. 5 Summary of susceptibility of Gentamicin-resistant strains to Netilmicin



Summary of Netilmicin MIC data submitted to Schering Laboratories by outside investigators. The strains tested were resistant to either Gentamicin, Tobramycin or Amikacin.

Fig. 6 Activity versus pathogens usually susceptible to aminoglycosides



Summary of Netilmicin MIC data against aminoglycoside-susceptible strains submitted to Schering Laboratories by outside investigators.

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Netilmicin 基礎試験成績の要約

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Netilmicinは，広範囲の抗菌スペクトルを有し，他剤耐性株に対しても感受性を示す新しいアミノ配糖体系抗生剤である。約2,500株の臨床分離株を用いた本試験成績では，本剤はアミノ配糖体系抗生剤不活化酵素のうち，adenylylating enzyme の ANT (2″) およびANT (4′)，phosphorylating enzyme の APH (3′) および APH (2″)，ならびに acetylating enzyme の AAC (3)-I をもつ菌株に対して抗菌力を示し，これら以外の AAC (3)，AAC(2′) および AAC (6′) をもつ菌株や permeability resistant mutants に対しては抗菌力を示さなかった。この成績はこれらの耐性株を用いた実験的マウス感染症においても同様であった。

Gentamicin 耐性株についての多数の試験成績においても，上記と同様の結果が得られており，*Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella* および *Staphylococcus* における Gentamicin 耐性株では，本剤はその80～90%に抗菌力を示しているが，これらの耐性株は，通常，Gentamicin の 2″ 位の不活化によるものであり，ANT (2″) や APH (2″) の enzyme を有する。また *Providencia* や indole-positive *Proteus* の耐性株では，acetylating enzyme AAC (2′) を有するために，本剤の抗菌力は弱い。*Pseudomonas* では，種々の耐性機構に応じて本剤の抗菌力に変動が見られている。

欧米でのアミノ配糖体系抗生剤の耐性株は，その大部分が ANT (2″) による不活化であると考えられており，この点において，Netilmicin の抗菌力からみた有用性が高い。