IN VITRO ACTIVITY OF SISOMICIN, AN AMINOGLYCOSIDE ANTIBIOTIC, AGAINST CLINICAL ISOLATES

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Sisomicin, a new aminoglycoside antibiotic which is produced by *Micromonospora myoensis*, was studied against 565 clinical isolates of gram-negative bacilli and gram-positive cocci. With the exception of *Serratia marcescens*, over 90% of isolates of gram-negative bacilli were inhibited by 1.56 μg/ml or less of sisomicin. Sisomicin was slightly more active than gentamicin and tobramycin against isolates of *Escherichia coli*, *Proteus mirabilis* and *Klebsiella* spp. It was substantially more active than butirosin and kanamycin against all gram-negative bacilli. Isolates of gram-negative bacilli which were resistant to gentamicin and tobramycin were also resistant to sisomicin. Most of these isolates were sensitive to amikacin.

Gram-negative bacilli are responsible for an increasing number of serious infectious complications occurring in hospitalized patients.[1] Often these organisms are susceptible to only a few antibiotics. Aminoglycoside antibiotics, which have a broad spectrum of activity against *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, have been useful for the treatment of serious infections.[2] However, nephrotoxicity and auditory toxicity of these drugs limit their therapeutic usage. Recently, a new aminoglycoside, sisomicin was isolated from the fermentation broth of *Micromonospora myoensis*.[3] Its spectrum of antimicrobial activity *in vitro* is similar to gentamicin sulfate. In animal toxicity studies sisomicin was found to have slightly less audiotoxicity than gentamicin. However, the nephrotoxicity of both drugs were similar.[4] The *in vitro* activity of sisomicin was determined against clinical isolates of bacteria and compared with gentamicin, tobramycin, amikacin, butirosin and kanamycin. The activity of sisomicin is similar to that of gentamicin and tobramycin.

Materials and Methods

Susceptibility tests were conducted on 478 clinical isolates of gram-negative bacilli, and 87 clinical isolates of gram-positive cocci, using the dilution technique with an automatic microtiter system.**[5] All organisms were inoculated into MUELLER-HINTON Broth (Difco) and incubated at 37°C for 18 hours. For gram-negative bacilli, a 0.05-ml sample of a 10^{-3} dilution of this broth (approximately 10^5 colony forming units/ml) was used as inoculum for the sensitivity testing. For gram-positive cocci, a 0.05-ml sample of 10^{-2} dilution of this broth (approximately 10^6 colony forming units/ml) was used as inoculum for susceptibility testing.

All gram-negative bacilli used in this study were cultured from blood specimens obtained from patients between 1967 and 1973. The majority of these patients were hospitalized at this

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** Canalco: Autotiter IV Instruction Manual.

institution and had malignant diseases. A total of 100 isolates each of *P. aeruginosa*, *E. coli* and *Klebsiella* spp., 85 isolates of *Proteus* spp., 40 isolates of *Enterobacter* spp., and 53 isolates of *Serratia marcescens* were used.

All gram-positive cocci used in this study were cultured from specimens obtained from hospitalized patients, most of whom did not have cancer. A total of 25 isolates of *Streptococcus pyogenes*, 12 isolates of *Diplococcus pneumoniae*, and 50 isolates of *Staphylococcus aureus* was studied. The susceptibility of isolates of *S. aureus* to penicillin G was determined by means of the broth dilution method. Those isolates inhibited by less than 0.10 μg/ml were considered to be penicillin G susceptible, and those isolates resistant to more than 25 μg/ml were considered to be penicillin G resistant.

Sisomicin and gentamicin used in this study were supplied as powders by Schering Corporation, Bloomfield, New Jersey. Butirosin, tobramycin and amikacin were supplied by Parke, Davis, and Company, Eli Lilly and Company, and Bristol Laboratories, respectively. Two fold serial dilutions of the antibiotics were made with MUELLER-HINTON broth, and the minimum inhibitory concentration (MIC) was determined after incubation at 37°C for 18 hours. Comparison studies were conducted simultaneously with kanamycin, gentamicin, amikacin, butirosin and tobramycin.

Results

The *in vitro* activity of sisomicin against gram-negative bacilli and gram-positive cocci is shown in Fig. 1. All isolates of *Klebsiella* spp. were inhibited at a concentration of 0.39 μg/ml. Over 90% of *E. coli*, *P. aeruginosa*, *Enterobacter* spp., *Proteus* spp. were inhibited at a concentration of 1.56 μg/ml, while only 66% of *S. marcescens* spp. were inhibited at this concentration. All isolates of *Staphylococcus aureus* (penicillin sensitive and penicillin resistant) were inhibited by a concentration of 0.78 μg/ml or less. All of the isolates of *Diplococcus pneumoniae* and 92% of *Streptococcus pyogenes* were inhibited by a concentration of 1.56 μg/ml.

The effect of inoculum size on the MIC of sisomicin was determined against 10 isolates each of *P. aeruginosa*, *Klebsiella* spp., and *E.*

Fig. 1. *In vitro* activity of sisomicin against gram-negative bacilli and gram-positive cocci.
Numbers in parenthesis indicate number of isolates tested.

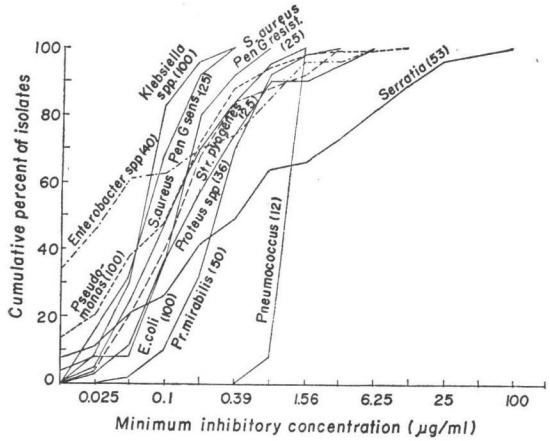
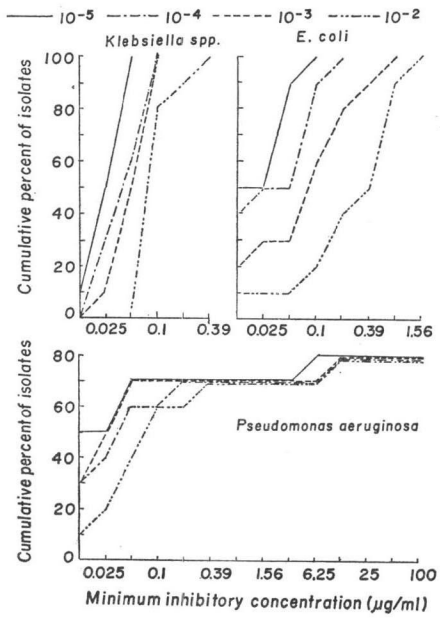


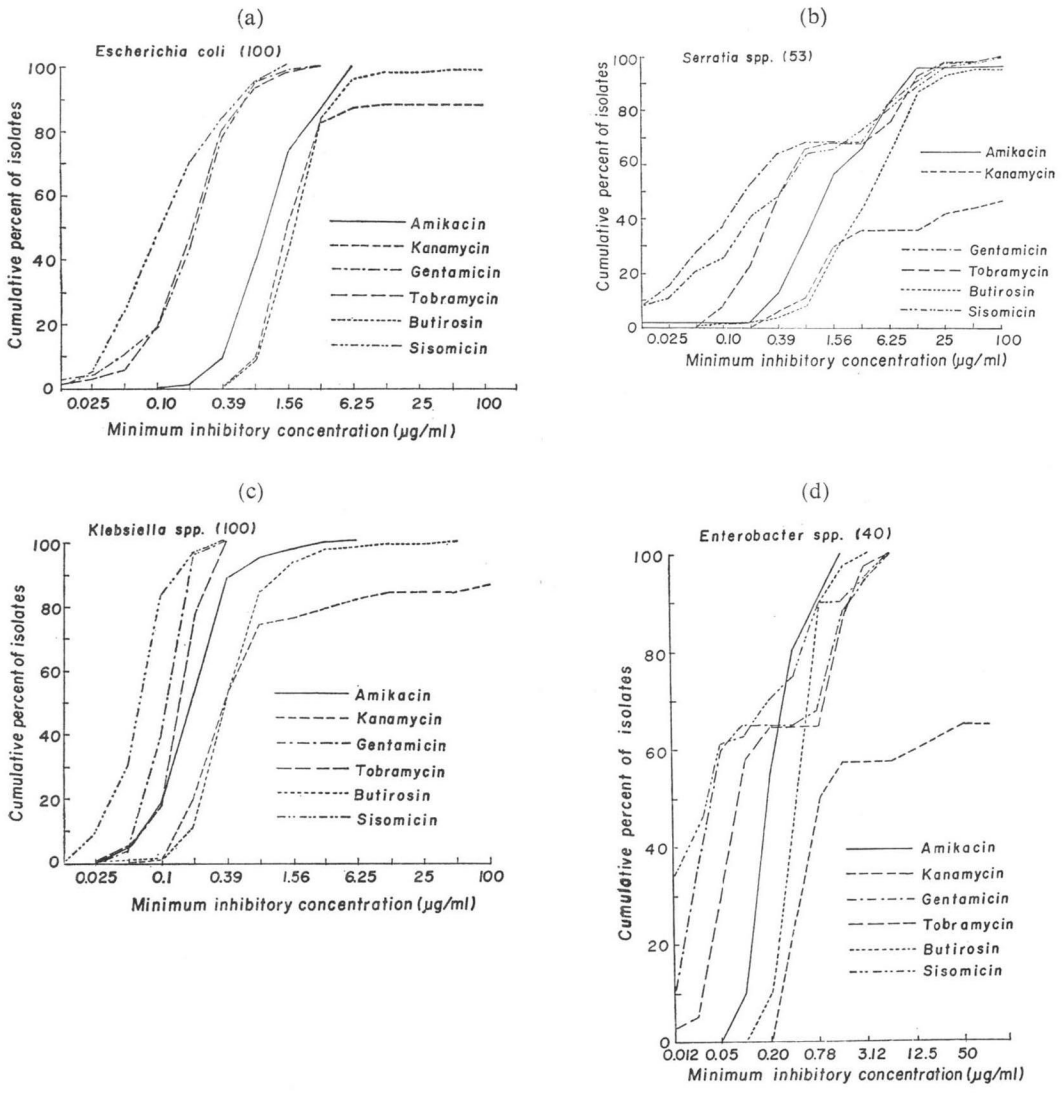
Fig. 2. Effect of inoculum size on the activity of sisomicin.

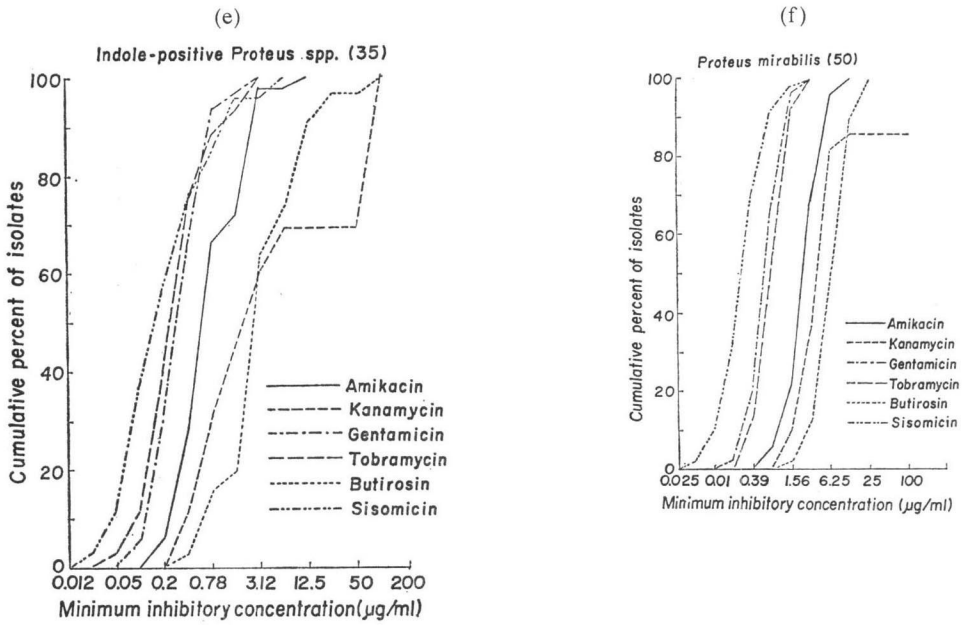


coli (Fig. 2). The inocula used were 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions of an 18 hour broth culture of the test organisms (containing approximately 10^8 colony forming units/ml). The greatest effect of inoculum size on the MIC was observed with *E. coli*.

Fig. 3 compares the *in vitro* activity of sisomicin with that of five other aminoglycoside antibiotics: gentamicin sulfate, kanamycin sulfate, tobramycin, butirosin sulfate, and amikacin, against *Enterobacteriaeceae*. Sisomicin was consistently more active than butirosin sulfate, kanamycin sulfate and amikacin. It was also slightly more active than gentamicin and tobramycin against *E. coli*, *P. mirabilis* and *Klebsiella* spp. The concentration of gentamicin and tobramycin which inhibited all isolates of *E. coli* was 3.12 μg/ml, whereas the concentration of sisomicin was 1.56 μg/ml. All strains of *Klebsiella* spp. were inhibited by sisomicin, gentamicin

Fig. 3. Comparison of activity of sisomicin with gentamicin, tobramycin, amikacin, kanamycin, and butirosin against *Enterobacteriacae*.
Numbers in parentheses indicate number of isolates tested.

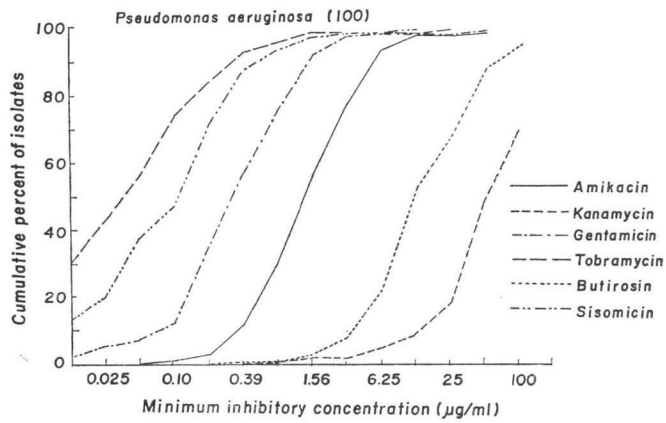




and tobramycin at a concentration of 0.39 μg/ml. At the same concentration kanamycin, butirosin, and amikacin inhibited only 88 %, 53 %, 52 %, respectively, of these isolates. The activity of sisomicin against *Enterobacter* spp. and *S. marcescens* was quite similar to the activity of gentamicin and tobramycin. Sisomicin, gentamicin, and tobramycin inhibited over 90 % of the isolates of indole-positive *Proteus* spp. at a concentration of 1.56 μg/ml. Kanamycin and butirosin were the least active antibiotics against indole-positive *Proteus* spp.

The *in vitro* susceptibility of 100 isolates of *P. aeruginosa* to the six aminoglycoside antibiotics is shown in Fig. 4. At a concentration of 0.39 μg/ml, sisomicin and tobramycin were the most active, inhibiting 88 % and 93 %, respectively. At the same concentration, gentamicin inhibited 57 % and amikacin inhibited only 12 %. At a concentration of 1.56 μg/ml, gentamicin, tobramycin, and sisomicin inhibited over 90 % of the isolates. At this concentration, amikacin inhibited 57 % of isolates, whereas kanamycin and butirosin were least active, and inhibited

Fig. 4. Comparison of activity of sisomicin with tobramycin, gentamicin, amikacin, butirosin and kanamycin against 100 isolates of *Pseudomonas aeruginosa*.



only 5% of isolates.

The effect of media on the activity of aminoglycoside antibiotics was determined against 10 isolates of *E. coli*, *P. aeruginosa*, and *Klebsiella* spp. All of the antibiotics were most active against *E. coli* in MUELLER-HINTON broth (Fig. 5a). At a concentration of 0.39 μg/ml, both sisomicin and gentamicin inhibited all 10 isolates, and tobramycin inhibited 8 of these organisms. In brain heart infusion broth, the order of activity was similar with sisomicin, gentamicin and tobramycin being the most active. In trypticase soy broth, the results also were similar, but sisomicin was slightly more active than gentamicin. Tobramycin was the most active antibiotic against *P. aeruginosa* in all three media tested (Fig. 5b). The activity of sisomicin was similar to that of gentamicin in all 3 media. All six antibiotics were consistently more active against *Klebsiella* spp. in MUELLER-HINTON broth (Fig. 5c). Sisomicin was more active than gentamicin or tobramycin in MUELLER-HINTON broth. However, sisomicin was not as active as gentamicin in trypticase soy broth. The activity of amikacin and butirosin in these media was quite similar. Kanamycin was the least active antibiotic and its activity was greatest in MUELLER-HINTON broth.

Fig. 5a. Effect of media on activity of aminoglycoside antibiotics against 10 isolates of *Escherichia coli*.

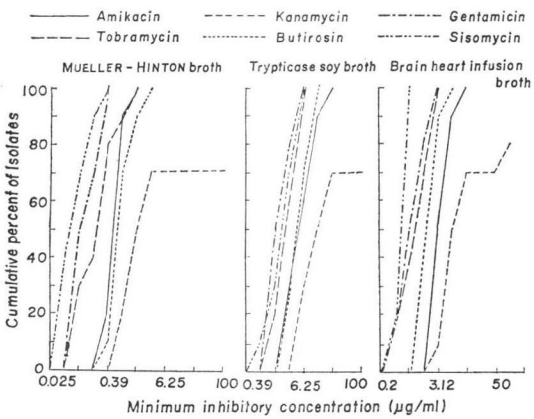


Fig. 5b. Effect of media in activity of aminoglycoside antibiotics against 10 isolates of *Pseudomonas aeruginosa*.

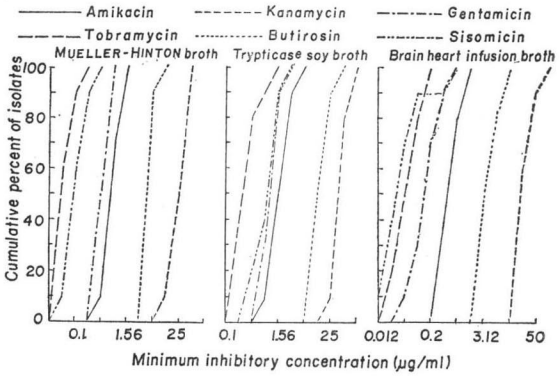


Fig. 5c. Effect of media on the activity of aminoglycoside antibiotics against 10 isolates of *Klebsiella* spp.

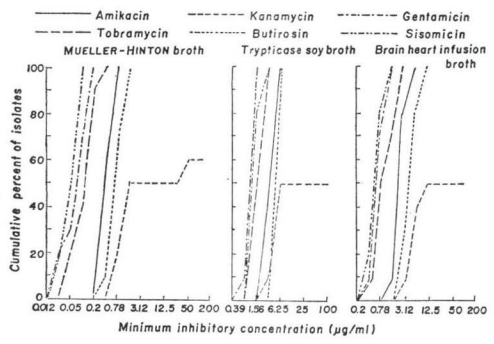
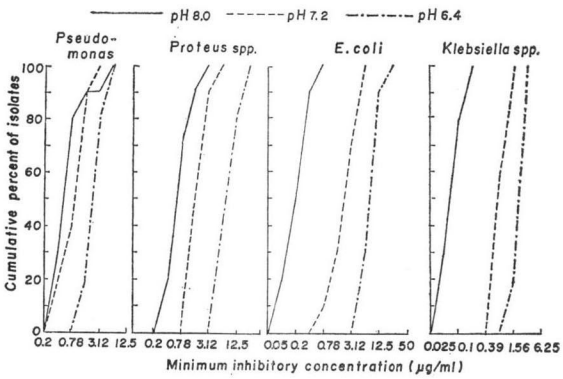


Fig. 6. Effect of pH on the activity of sisomicin in Trypticase soy broth.



The effect of pH on the activity of sisomicin in trypticase soy broth was determined against 10 isolates each of *Pseudomonas aeruginosa*, *Proteus* spp, *E. coli*, and *Klebsiella* spp. (Fig. 6). Sisomicin was most active against all organisms at the most alkaline pH. The greatest effect of pH activity on sisomicin was observed against isolates of *Escherichia coli* and *Klebsiella* spp.

Fourteen isolates of gram-negative bacilli known to be resistant to gentamicin or tobramycin or both were tested for their susceptibility to sisomicin (Table 1). All ten isolates of *S. marcescens* and four isolates of *P. aeruginosa* were also resistant to sisomicin. With exception of *Pseudomonas*, these isolates were most sensitive to amikacin.

Table 1. Activity of sisomicin against organisms resistant to gentamicin or tobramycin

Organisms	Source	Minimal inhibitory concentration (μg/ml)					
		Sisomicin	Butirosin	Kanamycin	Gentamicin	Tobramycin	Amikacin
Pseudomonas aeruginosa	Blood	100	>100	>100	>100	>100	25
	Blood	25	>100	>100	50	50	25
	Blood	12.5	>100	100	50	25	100
	Blood	12.5	>100	>100	50	25	50
Serratia marcescens	Blood	6.25	12.5	>100	12.5	12.5	1.56
	Blood	6.25	3.12	>100	12.5	12.5	1.56
	Blood	6.25	6.25	>100	12.5	12.5	0.78
	Blood	12.5	6.25	>100	12.5	12.5	6.25
	Blood	25	12.5	100	25	25	3.12
	Blood	25	12.5	>100	25	12.5	6.25
	Urine	100	25	>100	100	100	12.5
	Blood	50	25	100	12.5	12.5	6.25
	Blood	12.5	25	>100	12.5	12.5	12.5
	Sputum	12.5	25	12.5	50	>100	>100

Discussion

Sisomicin is an aminoglycoside antibiotic with broad spectrum activity against gram-negative bacilli. The *in vitro* activity of this antibiotic is similar to that of gentamicin and tobramycin. Sisomicin is slightly more active than gentamicin and tobramycin against *E. coli*, *P. mirabilis* and *Klebsiella* spp. It was also more active than gentamicin against *P. aeruginosa*, but less active than tobramycin.

Our results are in general agreement with those of other investigators.[6~9] However, most of our isolates of gram-negative bacilli were somewhat more sensitive to aminoglycoside antibiotics. For example, whereas 100% of our isolates of *E. coli* were inhibited by 1.56 μg/ml sisomicin, only about 60% of the isolates studied by LEVISION and KAYE were inhibited at this concentration. All of our isolates of *Klebsiella* spp. were inhibited by 0.39 μg/ml sisomicin, whereas less than 60% of their isolates were inhibited by 0.8 μg/ml. However, WAITZ *et al.* found that all of their isolates of *S. marcescens* were inhibited by 0.5 μg/ml sisomicin whereas only 72% of our isolates were inhibited by 3.12 μg/ml sisomicin. Our isolates of *P. aeruginosa* were also more sensitive to sisomicin; 92% were inhibited at a concentration of 0.79 μg/ml. WAITZ *et al.* found 71% of their isolates inhibited by 1 μg/ml sisomicin, YOUNG and HEWITT found about 80% inhibited by 2 μg/ml sisomicin and LEVISON and KAYE found only 75% of their isolates inhibited by 3.12 μg/ml sisomicin.

The type of media utilized affects the activity of aminoglycoside antibiotics and may explain some of these differences. WAITZ *et al.* also used MUELLER-HINTON broth, whereas LEVISON

and KAYE used heart infusion broth. In our study, these antibiotics were less active in brain heart infusion broth than in MUELLER-HINTON broth. Other investigators have found differences in the activity of various antibiotics depending on the media utilized.[10] Factors, such as pH and calcium and magnesium concentration of media, affect the activity of aminoglycoside antibiotics.[11] Since these antibiotics are more active in an alkaline pH, it has been suggested that alkalinization of the urine would be advantageous when aminoglycosides are used for the treatment of urinary tract infections.[12]

Most isolates of gram-negative bacilli which are resistant to gentamicin or tobramycin are also resistant to sisomicin. Fourteen of our isolates had an MIC of 12.5 μg/ml or greater with gentamicin and tobramycin. All of these isolates had an MIC of 6.25 μg/ml or greater with sisomicin. CROWE and SANDERS found similar results with 16 isolates of resistant gram-negative bacilli.[8] Only one of these isolates was sensitive to sisomicin. Many isolates of gramnegative bacilli resistant to these other aminoglycoside antibiotics are sensitive to amikacin.[6]

The role of sisomicin in the clinical management of gram-negative bacillary infections has not been determined. Clinical studies are in progress to ascertain its efficacy and toxicity in relationship to the other aminoglycoside antibiotics.

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