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In Vitro Antibacterial Activity of Spectinomycin

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The in vitro inhibitory and bactericidal activities of spectinomycin hydrochloride were tested against a variety of bacteria. The antibiotic was inhibitory at 31.2 μg/ml to most strains of *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Staphylococcus epidermidis*. Concentrations of antibiotic exhibiting bactericidal activity exceeded the inhibitory concentration by at least fourfold. Regression graphs were plotted for results obtained with 30-, 100-, 200-, and 300-μg spectinomycin discs; tentative interpretative standards are proposed.

Spectinomycin hydrochloride, a basic antibiotic prepared from *Streptomyces spectabilis*, has been found to be highly effective in the therapy of gonorrhea (2, 3, 5). Because, with the usually recommended dosages, peak concentrations of this agent in the blood may be about 100 μg/ml (9, 11) and urinary concentrations may attain 1,000 μg/ml, this investigation was undertaken to study the activity of spectinomycin against bacteria other than *Neisseria gonorrhoeae*.

MATERIALS AND METHODS

Spectinomycin (Trobicin) was supplied as a sterile dry powder in 100-mg vials through the courtesy of R. T. Pfeifer (The Upjohn Co., Kalamazoo, Mich.). The antibiotic was dissolved in sterile distilled water.

Minimal inhibitory concentrations (MIC) were determined in Mueller-Hinton agar (MHA) and Trypticase soy agar (TSA), both obtained from BBL, by the agar dilution technique (12). The inoculareplicating device described by Steers and co-workers (10) was used, and the concentrations of spectinomycin tested were 1,000, 500, 250, 125, 62.5, 31.2, 15.6, and 7.8 μg/ml. Inoculum size was adjusted so as to deposit 10^5 colony-forming units (CFU) on the surface of the agar. The MIC represented the lowest concentration at which there was no growth, a fine barely visible haze, or no more than three discrete colonies.

Bactericidal activity of spectinomycin was determined against three different sizes of inocula (10^3, 10^5, and 10^7 CFU/ml) of two strains of each of the following species by use of procedures described by Otto et al. (7): *Escherichia coli*, *Staphylococcus aureus*, and *Proteus mirabilis*. Twofold dilutions of spectinomycin were prepared in tubes containing 0.5 ml of Mueller-Hinton broth (BBL). To each tube was added 0.5 ml of an appropriate dilution of 6-hr broth cultures of the test organisms to provide the desired inoculum size. Colony counts of each of the diluted broth cultures were performed by a pour plate technique. At the end of 18 hr of incubation at 35 C, 0.5 ml of the broth in each tube exhibiting no visible turbidity was pipetted into correspondingly labeled tubes containing 15 ml of molten (50 C) brain-heart infusion agar, and pour plates were prepared. The pour plates were incubated at 35 C for 72 hr, and the concentrations of spectinomycin that resulted in 99.9% kill of the initial inoculum were calculated.

Disc diffusion tests were performed according to the method described by Bauer et al. (1) with the standards proposed in the Federal Register (21CFR, Part 147.2, 36: 6899-6902, 1971). Discs containing 30, 100, 200, and 300 μg of spectinomycin were supplied by The Upjohn Co. and were tested against 120 bacterial isolates. To select the 120 test strains, the MIC against each of 368 clinical isolates of bacteria was determined.

RESULTS AND DISCUSSION

Table 1 shows the MIC of spectinomycin against 368 clinical isolates of bacteria. The majority of strains of *Enterobacteriaceae*, with the exception of *Serratia marcescens* and of species of *Proteus*, were inhibited by 31.2 μg/ml. Most staphylococci and group D streptococci required 62.5 μg/ml for inhibition. Few strains of *Pseudomonas aeruginosa* and *Herellea vaginicola* were inhibited by 62.5 μg/ml, and most required 500 or 1,000 μg/ml for inhibition.

The MIC values obtained in MHA and those obtained with the same strains in TSA are shown in Table 2. The MIC values of approximately 56% of the strains were identical in both media; however, those of 38% were at least twofold greater in TSA than in MHA. Although heavier growth of some strains of *S. epidermidis* or group D streptococci on TSA than on MHA may have accounted for some differences in MIC, this factor alone cannot explain these differences completely. Nearly 35% of strains of *P. aeruginosa* had a higher MIC in TSA than in MHA. These findings are suggestive of those

TABLE 1. *Minimal inhibitory concentrations of spectinomycin against various bacteria*

Organism	Strains (no.)	Cumulative percent inhibited at each concentration (μg/ml)[a]							
		7.8	15.6	31.2	62.5	125	250	500	1,000
Escherichia coli	97	20	87	88	89	89	90	94	
Klebsiella	38	32	87	90	90	92	97		
Enterobacter	20	5	100						
Serratia	5	—	20	60	60	80	100		
Citrobacter diversus	7	—	57	86	100				
Proteus mirabilis	25	—	—	24	96	96	96	96	100
Other *Proteus* spp.	18	—	11	50	67	72			
Pseudomonas aeruginosa	40	—	—	3	8	13	35	85	95
Herellea	16	—	—	6	6	44	69	81	
Staphylococcus aureus	27	—	—	4	93	96			
S. epidermidis	42	—	5	79	83	83	83	83	86
Group D streptococci	33	—	3	21	94	100			

[a] Agar dilution technique with Mueller-Hinton agar.

TABLE 2. *Relationship of minimal inhibitory concentrations (MIC) in Mueller-Hinton agar (MHA) and Trypticase soy agar (TSA)*

Organism	Strains (no.)	MIC range in MHA (μg/ml)	Fold relationship of MIC in TSA			
			−2	1	+2	≧+4
Staphylococcus aureus	17	62.5–125	—	9[a]	8	—
S. epidermidis	26	15.6–62.5	—	3	21	2
Group D streptococci	22	31.2–125	—	2	20	—
Escherichia coli	39	7.8–15.6	—	34	5	—
Klebsiella	14	7.8–250	1	10	3	—
Enterobacter	10	7.8–15.6	—	10	—	—
Serratia	5	15.6–250	2	3	—	—
Proteus mirabilis	11	21.2–62.5	5	6	—	—
Other *Proteus* spp.	10	15.6–62.5	1	8	1	—
Pseudomonas aeruginosa	23	31.2–1,000	1	14	8	—
Herella vaginicola	8	125–1,000	1	5	2	—
Total	185		11	104	68	2

[a] Number of strains.

observed with tetracycline, polymyxin B, and gentamicin, the activities of which are affected by cation content of the media (4, 6, 13–16).

The inhibitory activity of spectinomycin was related to inoculum size. The minimal bactericidal concentration (MBC) of spectinomycin resulting in killing of 99.9% of the original inoculum within 72 hr as determined by plate count was at least four times the MIC and was usually greater (Table 3), thereby characterizing this antibiotic as being bacteriostatic rather than bactericidal (7).

Regression graphs of the MIC against the zone diameters about each of four discs containing 30, 100, 200, and 300 μg of spectinomycin are shown in Fig. 1 through 4.

In general, anticipated concentrations of an antibiotic in the blood should exceed the MIC by three to five times for an organism to be considered susceptible (8). In the case of spectinomycin, therefore, since blood levels in the order of 100 μg/ml are readily attainable, it may be reasonable to propose tentatively that organisms inhibited by 31.2 μg or less/ml be considered susceptible, that organisms inhibited by 62.5 or 125 μg/ml (concentrations equivalent to or slightly less than peak blood concentrations) be placed into the intermediate category, and that organisms for which concentrations in excess of 125 μg/ml are required be considered resistant. Separation of organisms into these three categories can be accomplished most readily by using the 100-μg disc (Table 4). The 100-μg disc achieves somewhat better separation of points on the regression graph than does

TABLE 3. *Bactericidal activity of spectinomycin with various inoculum sizes*

Organism	Inoculum size (CFU/ml)	MIC[a] (μg/ml)	Concn resulting in 99.9% kill (μg/ml)
E. coli U6642	2.8×10^3	7.8	
	2.8×10^5	15.6	250
	2.8×10^7	31.2	
E. coli (ATCC 25922)	3.8×10^3	7.8	
	3.8×10^5	15.6	250
	3.8×10^7	62.5	250
S. aureus G2510	1.3×10^3	31.2	
	1.3×10^5	31.2	125, 250, 500
	1.3×10^7	—[b]	
S. aureus (ATCC 25923)	4.7×10^2	31.2	
	4.7×10^4	31.2	500, 1,000
	4.7×10^6	—[b]	
P. mirabilis U7616	3.2×10^3	31.2	
	3.2×10^5	31.2	
	3.2×10^7	125	
P. mirabilis U7572	3.6×10^3	31.2	
	3.6×10^5	31.2	1,000
	3.6×10^7	125	1,000

[a] Broth dilution technique.
[b] Growth through 1,000 μg/ml.

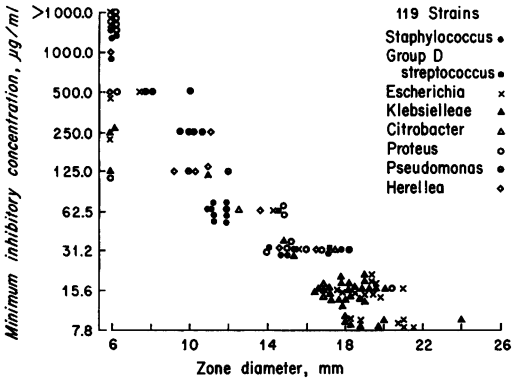

FIG. 1. *Regression graph for 30-μg spectinomycin disc.*

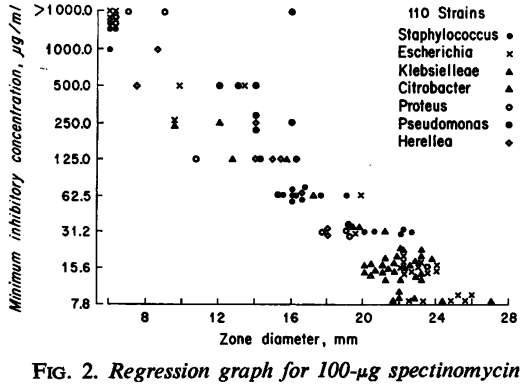

FIG. 2. *Regression graph for 100-μg spectinomycin disc.*

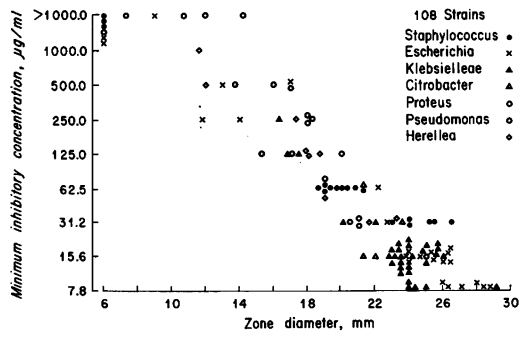

FIG. 3. *Regression graph for 200-μg spectinomycin disc.*

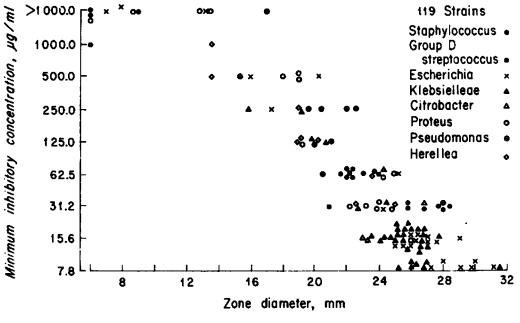

FIG. 4. *Regression graph for 300-μg spectinomycin disc.*

TABLE 4. *Suggested susceptibility status on basis of zone diameters obtained with 100-μg spectinomycin discs*

Zone diam (mm)	Status
≧18	Susceptible
15–17	Intermediate
≦14	Resistant

the 30-μg disc and therefore appears to be preferable for routine use.

Because very high urinary concentrations of spectinomycin are attained, it may be possible that a second disc with a higher antibiotic content would be desirable for testing urinary isolates of bacteria. However, this has not generally been the practice with other antibiotics and would require documentation of the clinical response of bacteriuria due to a variety of organisms for which the MIC of spectinomycin is known.

ACKNOWLEDGMENT

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