

Medium-Dependent Activity of Gentamicin Sulfate Against Enterococci

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Routine disc diffusion susceptibility tests (Bauer-Kirby technique), employing 5% sheep blood-Mueller-Hinton agar and 10- μ g gentamicin sulfate discs, disclosed that a significant number of clinical enterococcal isolates were sensitive to the antibiotic, as also revealed by the agar dilution technique. With few exceptions, the isolates proved resistant to this antibiotic when tested for susceptibility in Brain Heart Infusion and Trypticase soy broth or agar. The addition of 5% sheep blood to Trypticase soy and Brain Heart Infusion agars resulted in markedly enhanced activity of the antibiotic, indicating medium-dependent activity of gentamicin against enterococci. Human serum and urine failed to support optimal growth of enterococci. Thus, it was not possible to correlate the activity of gentamicin in any of the media examined with that in serum or urine.

Routine, standardized disc diffusion susceptibility tests in our clinical microbiology laboratory indicated that 57 of 58 enterococcal isolates were sensitive to gentamicin sulfate, yielding zones of inhibition greater than 13 mm and ranging up to 23 mm in diameter around 10- μ g gentamicin discs in sheep blood-Mueller-Hinton agar (MHA). It was of interest to determine whether this finding was valid for other media as well or whether the activity of gentamicin sulfate was medium-dependent.

MATERIALS AND METHODS

Bacteria. A total of 58 enterococcal isolates from various clinical sources were presumptively identified with the following tests: fermentation of mannitol and arabinose, reduction of methylene blue in milk, liquefaction of gelatin, and type of hemolysis on 5% sheep blood-agar (4-6, 9); specific anti-group D streptococcal hyperimmune serum was not available. The organisms were maintained on sheep blood-agar (4 C). A strain of *Escherichia coli* of known antibiotic sensitivity served for control purposes.

Media. MHA (Difco), tryptic soy broth or agar (TSB, TSA; Difco), and Brain Heart Infusion broth or agar (BHIB, BHIA; Difco) were used for antibiotic susceptibility tests. For comparative purposes, TSA and BHIA were enriched with 5% sheep blood. The pH of all media had been adjusted to 7.2.

Gentamicin sulfate. Nonsterile gentamicin sulfate powder (579 μ g of activity per mg) was a gift from the Schering Corp., Union, N.J. (batch GMC-8-M-65-1), as were Difco and BBL 10- μ g gentamicin discs (batches 505784 and 9AMW20, respectively). Stock solutions of gentamicin were prepared and stored as described previously (7).

Sensitivity tests. The isolates were tested by the disc sensitivity method by the standardized procedure of Bauer et al. (1). Tube and microtiter broth dilution tests were performed as reported previously (7, 8), employing serial twofold dilutions of gentamicin over the range of 200 to 0.2 μ g/ml; the bacterial inoculum was adjusted to yield 1.5×10^6 organisms per ml at zero time. Agar dilution tests, with the same range of final concentrations of gentamicin, were performed with spot-inoculated bacterial inocula consisting of 1.5×10^8 organisms at zero time (7). The minimal inhibitory concentration (MIC) of gentamicin was defined as the lowest concentration of antibiotic that completely inhibited bacterial growth as judged by visual inspection, after incubation for 16 to 18 hr at 35 C. The minimal bactericidal concentration of gentamicin was the lowest concentration of the drug yielding no growth after subculture from clear tubes or wells (one 3-mm loopful to quarter sectors of 5% sheep blood-agar, incubated at 35 C for 24 hr).

RESULTS

The results obtained with the disc diffusion method, employing various media, are listed in Table 1. It was found that the addition of 5% sheep blood to TSA and BHIA (BBHIA) resulted in significantly enhanced activity of gentamicin against enterococci but not against *E. coli*, as judged by the diameters of the resultant zones of inhibition. Interestingly, the enterococcal isolates yielded less susceptible variants within the zones of inhibition on BBHIA but not within those of any of the other media examined. Chocolatization (80 C, 10 min) of MHA plus 5% sheep blood (BMHA) did not influence the

TABLE 1. Zones of inhibition obtained with gentamicin (10 μ g) discs against 58 clinical isolates on various media^a

Organisms	BMHA ^b	Chocolatized BMHA	Autoclaved BMHA	TSA	BTSA	BHIA	BBHIA
Enterococci							
Range.....	11-23	12-21	8-23	6-17	11-20	6-16	10-21
Mean.....	17.3	17.1	13.6	8.8	16.0	8.8	16.5
Control <i>E. coli</i>	22	22	21	22	21	21	21

^a Diameters were measured in millimeters. Discs measured 6 mm in diameter; therefore a zone reading of 6 mm indicates absence of a zone of inhibition.

^b Abbreviations: BMHA, Mueller-Hinton agar plus 5% sheep blood; TSA, Trypticase soy agar; BTSA, TSA plus 5% sheep blood; BHIA, Brain Heart Infusion agar; BBHIA, BHIA plus 5% sheep blood.

activity of the antibiotic, whereas autoclaved BMHA (121 C, 10 min) gave significantly smaller inhibition zones, the diameters of which approached those of plain TSA and BHIA. The addition of 3% hydrogen peroxide to BMHA, chocolated BMHA, and autoclaved BMHA allowed detection of catalase in BMHA only. Thus, although all media supported luxuriant growth of the enterococci, the activity of gentamicin appeared to be medium-dependent. The MIC values of gentamicin against the 58 enterococcal isolates were generally eightfold lower in BMHA (agar dilution method) than in TSB and BHIB (broth dilution technique), in that greater than 90% of the isolates were inhibited by 12 μ g of gentamicin per ml in BMHA as contrasted with 100 μ g of antibiotic per ml required to inhibit a comparable cumulative per cent of strains in TSB and BHIB (Fig. 1). Comparative agar dilution MIC values of gentamicin against a selected number of enterococcal isolates are shown in Table 2; again, gentamicin was much more active in BMHA than in TSA and BHIA.

An attempt was made to determine the activity of gentamicin against several enterococcal isolates in fresh, clean-voided human urine as well as fresh and heat-inactivated human serum. It was noted that normal urine (initial pH 6.0) did not allow optimal growth of these isolates, in contrast to the control strain of *E. coli* which grew well and was inhibited by 1.5 μ g of gentamicin per ml. Fresh serum (pH 7.4) was found to be unsuitable for the propagation of the selected enterococcal isolates. Heat-inactivated serum from individual donors or pooled sera (pH range 8.0 to 7.8) likewise did not support growth of the enterococci; the *E. coli* grew well and required 1.5 μ g of gentamicin per ml for inhibition. When heat-inactivated serum and urine were added to TSB and BHIB to 50%, respectively, isolates 1, 10, 14, 20, and 39 were characterized by suboptimal growth and MIC values ranging from 3 to 6 μ g/

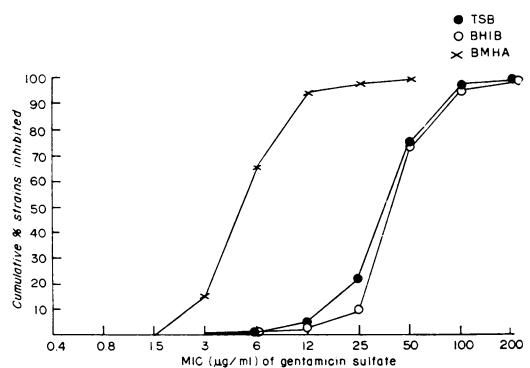


FIG. 1. Medium-dependent activity of gentamicin against 58 enterococcal isolates. Abbreviations: TSB, Trypticase soy broth; BHIB, Brain Heart Infusion broth; BMHA, Mueller-Hinton agar plus 5% sheep blood.

ml; the control *E. coli* was inhibited by 0.8 μ g of gentamicin per ml.

DISCUSSION

The finding that the activity of gentamicin against enterococci is medium-dependent was expected. Several years ago, Bulger (2, 3) stressed the effect of media upon the activity of certain antibiotics; Mueller-Hinton broth was found to compare favorably with human serum. Very recently, Toala and co-workers (5, 6) reported that the hydrogen ion concentration of media significantly influenced the activity of gentamicin, in that acid milieus reduced whereas alkaline media enhanced the activity of the antibiotic. All media used in this study were of identical pH. However, the addition of 5% sheep blood to various solid media drastically enhanced the activity of gentamicin against enterococcal isolates. Of particular interest was the finding that chocolated BMHA yielded inhibition zones comparable to those obtained with BMHA, whereas auto-

TABLE 2. Minimal inhibitory concentrations of gentamicin against selected enterococcal isolates in various media

Isolate no.	Agar dilution technique			Broth dilution technique	
	BMHA ^a	TSA	BHIA	TSB	BHIB
1	6	25	50	50	50
14	3	25	25	50	50
20	6	25	50	50	100
24	6	25	50	50	50
39	6	12	25	50	50
Control <i>E. coli</i>	0.4	0.8	1.5	6	3

^a Abbreviations for BMHA, TSA, and BHIA as in footnote *b*, Table 1. TSB, Trypticase soy broth; BHIB, Brain Heart Infusion broth.

claved BMHA diminished the activity of gentamicin, the zone diameters approaching those obtained with TSA and BHIA. The finding that catalase was detected only in BMHA suggests that a partially heat-labile component(s) of sheep blood (not catalase) was responsible for the enhanced activity of gentamicin in blood-enriched solid media.

The observation that the majority of enterococcal isolates yielded resistant variants within the inhibition zones on BBHIA, but not within those of the other media tested, is inexplicable.

Unfortunately, normal fresh or heat-inactivated human serum and urine did not permit optimal growth of enterococci. This is why no attempt could be made to compute regression lines for gentamicin sulfate against enterococci. As a result of the described findings we decided to exclude gentamicin sulfate from our battery of drug discs routinely used to test for the antibiotic susceptibility of enterococcal isolates.

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