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Medium-Dependent Variation in Bactericidal Activity of Antibiotics Against Susceptible *Staphylococcus aureus*

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Staphylococcus aureus resistant to bactericidal activity of antibiotics caused sepsis in three patients. Bacteriological and clinical responses were not achieved until serum and tissue fluid levels of administered antibiotics exceeded the minimum bactericidal concentration (MBC) of the infecting organism. Fifteen clinical isolates of *S. aureus* were tested in brain heart infusion broth and Mueller-Hinton broth for the MBC of gentamicin, vancomycin, clindamycin, oxacillin, cefazolin, and cephalothin. Results showed significant eightfold or greater broth-dependent differences in the MBC of at least one antibiotic against 87% (13/15) of strains tested. The MBC was unpredictable and varied with the strain, antibiotic, and medium used. No controlled studies are available to indicate the clinical significance of the MBC demonstrated in different media. The necessity for treating serious infection with bactericidal drugs has not yet been established; however, in septicemia such as that caused by bacterial endocarditis, bacteriostatic antibiotics have generally failed to eradicate the infection, whereas bactericidal agents have often been curative. Therefore, in patients unresponsive to usual antistaphylococcal therapy, we suggest that MBC testing be performed in at least two media and that treatment be instituted with antibiotics demonstrating the lowest MBC in all media used.

In vitro antimicrobial susceptibility testing has been considered essential for appropriate antibiotic usage (20). Medium-dependent variation in the minimum inhibitory concentration (MIC) of tetracyclines, aminoglycosides, and polymyxins has been reported (2, 5, 19, 20, 24). Similar medium-dependent differences in minimum bactericidal concentration (MBC) have rarely been described for bacteria against which the tested antibiotics have an equal MIC (16). Refinements in techniques for determining MIC and MBC have included standardization of inoculum size (3) and publication of a standard method for determination of bactericidal activity (1).

Sabath et al. have described clinically significant infections caused by *Staphylococcus aureus* tolerant to the bactericidal action of antimicrobial agents and have demonstrated the potential importance of antibiotic killing activity (21). We now report the MBC of virulent *S. aureus* to be a variable of the liquid medium selected for testing.

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MATERIALS AND METHODS

Patients. The first patient, a 60-year-old male, had septicemia and septic arthritis due to *S. aureus*. Studies performed in brain heart infusion (BHI) broth revealed the MIC of oxacillin to be 0.25 μg/ml and that of cephalothin to be 4.0 μg/ml. Intravenous therapy with 6 g of oxacillin per day for 7 days failed to eradicate the organisms from involved joints in spite of serum and joint oxacillin levels that ranged from 24 to 44 μg/ml. The MBC in BHI broth was 64 μg of oxacillin per ml and 16 μg of cephalothin per ml. Intravenous therapy was therefore changed to cephalothin at 12 g/day, resulting in serum and joint fluid levels ranging from 95 to 200 μg/ml. The infection was controlled, the patient was afebrile, and cultures were sterile 24 h after the beginning of cephalothin therapy.

Patient 2 was a 61-year-old male with endocarditis caused by *S. aureus* with numerous culture-positive septic emboli to skin, pericardium, and synovium. The patient continued to be febrile, and cultures remained positive, in spite of therapy with intravenous nafcillin at 12 g/day for 8 days. The MIC of nafcillin in Mueller-Hinton (MH) broth was 0.25 μg/ml with a MBC of 64 μg/ml. Therapy was changed to cephalothin, vancomycin, and rifampin with respective MICs of 0.25, 0.5, and 0.125 μg/ml and MBCs of 16, 2, and 0.5 μg/ml. Cultures of blood, synovial fluid, and pericardial fluid were sterile, and the patient was afebrile after 3 days on this regimen.

The third patient, a 47-year-old male on hemodialysis, developed endocarditis caused by *S. aureus*. Therapy was begun with intravenous vancomycin; the infecting organism was inhibited by a MIC of 0.5 μg/ml in MH broth and 1.0 μg/ml in BHI broth. Blood cultures remained positive, and the patient continued to be febrile for 6 days while on this therapy, despite a vancomycin serum concentration of 11.3 μg/ml. The MBC of vancomycin was 4 μg/ml in MH broth and 16 μg/ml in BHI broth. Therapy was subsequently changed to intravenous nafcillin at 12 g/day (MIC of 0.125 μg/ml and MBC of 0.5 μg/ml in MH broth; and MIC of 0.125 μg/ml and MBC of 0.5 μg/ml in BHI broth). Cultures were sterile, and the patient became afebrile 36 h after the beginning of intravenous nafcillin therapy.

Methods. Tests for antibiotic MIC and MBC were performed by the broth dilution method of Barry and Sabath (1) in both BHI (pH 7.3) and MH (pH 7.2) broth (Difco Laboratories, Detroit, Mich.). Gentamicin, vancomycin, clindamycin, oxacillin, cefazolin, and cephalothin were tested at concentrations varying by twofold dilutions between 0.125 and 128 μg/ml. Bactericidal activity was defined as the concentration killing at least 99.9% of the initial bacterial inoculum of 10^5 to 10^6 organisms in 24 h. Three determinations were performed on a strain of *Escherichia coli*, and five determinations were performed on a strain of *S. aureus* in BHI, MH, and Trypticase soy broth (Baltimore Biological Laboratories, Cockeysville, Md.) to establish the reproducibility of the method for our laboratory. Fourteen additional strains of *S. aureus* were then individually tested for medium-dependent differences in antibiotic bactericidal activity with BHI and MH broth. Organisms were obtained from blood, bone, or wound cultures from patients with clinical infections.

Susceptibility was defined according to the recommendations of the National Committee for Clinical Laboratory Standards (18). The breakpoint concentrations for susceptibility were less than or equal to 6 μg of gentamicin per ml, 5 μg of vancomycin per ml, 1 μg of clindamycin per ml, 3 μg of penicillinase-resistant penicillins per ml, and 10 μg of cephalosporins per ml. The MBCs for susceptibility were defined as the same antibiotic concentration as the MIC breakpoint suggested by the National Committee for Clinical Laboratory Standards.

Significant medium-dependent variation in the MIC or MBC was defined as an eightfold or greater difference in either the MIC or the MBC when results with BHI and MH broths were compared. Significance was based on 95% confidence limits of the dilution MBC test as performed in our laboratory.

RESULTS

Initial experiments with the single strains of *E. coli* and *S. aureus* demonstrated that 100% (64/64) of repeat MIC determinations were within the mean ±1 dilution of each antibiotic tested. Ninety-five percent (61/64) of the repeat MBC determinations were within the mean ±2 dilutions of each antibiotic tested. The single *E. coli* showed no medium-dependent variation in MIC or MBC.

These experiments with BHI, MH, and Trypticase soy broth, as well as those performed on *S. aureus* strains 1 and 2 (Table 1 and 2), showed no difference between MBCs obtained with BHI and Trypticase soy broth. The Trypticase soy broth gave frequent "skip" zones when clear tubes were subcultured to blood agar plates. Therefore, Trypticase soy broth was eliminated from the experimental design.

The only significant medium-dependent variation in the MIC of antibiotics tested against *S. aureus* was with strain 6 for gentamicin and with strain 11 for vancomycin (Table 1). All the organisms were susceptible to the antibiotics as judged by the MIC. The results of MBC testing are given in Table 2.

DISCUSSION

The importance of antibacterial killing activity in serious infection has been postulated in the past by numerous authors using the serum inhibitory titer of Schlichter and MacLean (22) and the serum bactericidal titer described by Fisher (4, 6–9, 11–15, 21–23). Our cases and other earlier reports demonstrate that the MBC of an antibiotic can be important in the therapeutic result (10, 17, 21). Medium differences in the MIC of aminoglycosides and polymyxins based on variations in calcium and magnesium content have been reviewed (20). Inadequate killing of staphylococci based on a decreased amount of autolytic enzyme activity has been proposed as an explanation for staphylococcal tolerance to the bactericidal action of antibiotics (21). Our results indicate an inconsistent pattern of disparity between MICs and MBCs with different media and would seem to indicate that multiple factors influence in vitro bactericidal activity of antibiotics.

Eighty-seven percent (13/15) of staphylococcal strains tested showed an eightfold or greater difference in MBC when tests in MH and BHI broth were compared. Additionally, 60% (9/15) of the strains demonstrated tolerance (low MIC with high MBC) as defined by Sabath et al. (21) to vancomycin, oxacillin, cephalothin, or cefazolin in at least one of the two broths tested. Taken together, all 15 strains showed either medium-dependent variation in MBC or were tolerant to the bactericidal action of antibiotics in one or both media.

The large differences between the MIC and MBC noted for certain strains of staphylococci on selected media coupled with the apparent importance of antibacterial killing activity in

TABLE 1. *Comparison of MICs of antibiotic against S. aureus in MH and BHI broth*

Strain	Gentamicin (μg/ml)		Vancomycin (μg/ml)		Clindamycin (μg/ml)		Oxacillin (μg/ml)		Cefazolin (μg/ml)		Cephalothin (μg/ml)	
	MH	BHI	MH	BHI	MH	BHI	MH	BHI	MH	BHI	MH	BHI
1	<0.125	0.5	NT[a]	NT	NT	NT	<0.125	<0.125	0.5	0.5	NT	NT
2	0.25	0.25	NT	NT	NT	NT	<0.125	<0.125	0.5	0.5	NT	NT
3	NT	NT	NT	NT	NT	NT	<0.125	<0.125	NT	NT	0.125	0.125
4	0.25	0.5	NT	NT	0.125	0.125	<0.125	<0.125	0.25	0.25	NT	NT
5	0.25	0.25	0.5	0.5	0.125	0.125	<0.125	<0.125	0.125	0.25	NT	NT
6	0.125	1	0.5	2	0.125	0.125	<0.125	<0.125	0.125	0.25	0.25	0.125
7	0.125	0.5	1	1	0.125	0.125	0.25	0.25	0.5	0.5	0.25	0.25
8	0.125	0.5	1	1	0.125	0.125	<0.125	<0.125	0.125	0.125	0.125	0.125
9	0.125	0.125	0.25	1	0.125	0.125	<0.125	<0.125	0.125	0.125	0.125	0.125
10	0.125	0.125	0.5	1	0.125	0.125	<0.125	<0.125	0.5	0.25	0.125	0.125
11	0.125	0.5	0.25	2	0.125	0.125	<0.125	<0.125	0.5	0.25	0.125	0.125
12	0.125	0.5	0.5	2	0.125	0.125	0.25	<0.125	1	0.5	0.25	0.25
13	0.125	0.25	0.5	1	0.125	0.125	<0.125	<0.125	0.125	0.125	0.125	0.125
14	0.25	1	NT	NT	NT	NT	<0.125	<0.125	0.25	0.125	NT	NT
15	NT	NT	0.5	1	NT	NT	0.125	0.125	0.5	0.125	0.25	0.125

[a] NT, Not tested.

TABLE 2. *Comparison of MBCs of antibiotics against S. aureus in MH and BHI broth*

Strain	Gentamicin (μg/ml)		Vanomycin (μg/ml)		Clindamycin (μg/ml)		Oxacillin (μg/ml)		Cefazolin (μg/ml)		Cephalothin (μg/ml)	
	MH	BHI	MH	BHI	MH	BHI	MH	BHI	MH	BHI	MH	BHI
1	4	0.5	NT[a]	NT	NT	NT	0.5	0.125	1	2	NT	NT
2	1	4	NT	NT	NT	NT	32	32	64	64	NT	NT
3	NT	NT	NT	NT	NT	NT	32	32	NT	NT	32	8
4	4	4	NT	NT	>128	>128	0.5	0.125	2	0.25	NT	NT
5	4	2	0.5	8	8	8	0.25	32	0.25	32	NT	NT
6	0.25	4	0.5	2	0.25	0.25	0.25	0.25	0.125	0.25	0.25	2
7	1	4	32	32	4	8	0.5	8	16	4	8	4
8	0.25	2	16	32	16	1	4	16	64	0.25	32	32
9	0.125	2	128	128	4	4	32	8	16	8	8	8
10	0.125	1	8	1	8	1	0.5	0.125	8	0.5	0.5	0.25
11	0.25	1	2	16	4	8	0.25	0.125	0.5	32	0.25	64
12	0.5	8	64	128	32	16	64	64	128	128	64	128
13	0.25	4	2	8	16	1	0.125	0.125	0.25	0.25	0.125	2
14	1	8	NT	NT	NT	NT	0.5	32	0.5	16	NT	NT
15	NT	NT	4	16	NT	NT	0.5	0.5	4	0.5	2	0.5

[a] NT, Not tested.

serious infection make the determination of MBC in a single broth difficult to interpret. Antibiotic MIC has been shown to correlate best with MBC when the test is performed in MH broth (18). No previous study has conclusively shown whether the low or high MBC seen with different media is clinically important. One reason for the variable clinical response to treatment of patients with severe staphylococcal infections may be the selection of media that indicate a low MBC for the antibiotic, whereas the in vivo response is more consistent with the antibiotic having a high MBC.

Our three cases of staphylococcal sepsis failed to demonstrate a clinical or bacteriological response during 6 to 8 days when treatment consisted of antimicrobials that were found to have a high MBC for the infecting organism in either MH or BHI broth. When therapy was changed to antibiotics demonstrating a MBC in broth media that could be exceeded in the serum and tissue fluid, clinical and bacteriological response was evident within 1 to 3 days. Serious staphylococcal infection can have a variable response to therapy. These three cases suggest that the selection of an antibiotic with a high MBC as determined in either MH or BHI broth can lead to failure to eradicate the infecting organism. Therefore, until further clinical correlation is available to indicate which medium is appropriate, we would recommend determination of the MBC in at least two different broth media and selection for therapy of the antibiotic appearing to have the lowest MBC in all media tested.

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