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Pitfalls in Identification of Methicillin-Resistant *Staphylococcus aureus*

J. ANDRÉ MARCOUX AND JOHN A. WASHINGTON, II

Mayo Clinic and Mayo Foundation, Section of Microbiology, and Mayo Graduate School of Medicine, University of Minnesota, Rochester, Minnesota 55901

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Media and techniques for in vitro testing with respect to screening for methicillin-resistant *Staphylococcus aureus* are discussed.

An increasing number of methicillin-resistant strains of *Staphylococcus aureus* are now being identified in laboratories throughout the world. As a rule, these bacteria are also resistant to the related β-lactam semisynthetic penicillins and also to the cephalosporin C derivatives. It has been shown that these pathogens can cause severe and potentially lethal infections in humans (1–4, 6). Such infections must be treated vigorously for a prolonged period with antibiotics such as vancomycin, cephalothin and kanamycin, lincomycin, or gentamicin, most of which are potentially toxic agents. It is imperative for the benefit of the patient that the diagnosis of methicillin resistance be established without doubt.

Use of inactive methicillin discs or mistaking *S. epidermidis* for *S. aureus* probably accounts for some reports of resistant strains of *S. aureus* (4). Another possible source of error is the accidental testing of a mixture of two or more types of bacteria for methicillin susceptibility. Data in the medical literature concerning the influence of various testing media used in the identification of methicillin-resistant *S. aureus* are sparse (5; J. Benner, Clin. Res., p. 119, 1969).

TABLE 1. *Bactericidal activity of methicillin in three broth media*

Staphylococcus aureus strain	Trypticase soy broth			Mueller-Hinton broth			Brain heart infusion broth		
	MIC[a]	MBC[b]		MIC	MBC		MIC	MBC	
		Thio	BAP		Thio	BAP		Thio	BAP
1	3.12	>100	6.25	3.12	6.25	6.25	6.25	>100	6.25
2	3.12	>100	6.25	1.56	3.12	6.25	3.12	>100	6.25
3	3.12	>100	6.25	3.12	6.25	3.12	3.12	>100	6.25
4	3.12	>100	>100	3.12	6.25	6.25	3.12	>100	6.25
5	3.12	>100	6.25	3.12	3.12	3.12	6.25	>100	6.25
6	3.12	>100	>100	1.56	3.12	3.12	3.12	>100	3.12
7	3.12	>100	6.25	3.12	6.25	3.12	6.25	>100	6.25
8	3.12	>100	3.12	3.12	6.25	12.5	3.12	3.12	3.12
9	3.12	>100	3.12	3.12	25	3.12	3.12	>100	3.12
10	3.12	>100	>100	3.12	3.12	3.12	3.12	>100	6.25
11	3.12	>100	3.12	3.12	25	3.12	3.12	>100	3.12
12	3.12	>100	>100	1.56	3.12	3.12	3.12	>100	6.25

[a] Lowest concentration (μg/ml) of methicillin inhibiting visible growth at 18 to 24 hr.
[b] Lowest concentration (μg/ml) producing 99.9% kill after 48 hr of incubation at 37 C. Thio = thioglycolate broth; BAP = blood-agar plate.

Twelve strains of *S. aureus*, so classified on the basis of being coagulase-positive, isolated from clinical material were obtained from the routine antibiotic-sensitivity-testing laboratory. Methicillin susceptibility was measured by the twofold serial dilution technique. An inoculum of 0.5 ml of a 10^{-3} dilution of an overnight broth culture was used. Test strains were incubated overnight, and minimal inhibitory concentration (MIC) determinations were carried out in Trypticase soy broth, Mueller Hinton broth, and brain heart

infusion broth. The MIC was read as that dilution at which there was no visible growth after 24 hr of incubation at 37 C. Subsequently, the minimal bactericidal concentration (MBC) was determined by subculturing 0.05 ml of broth from the clear tubes to blood-agar plates and also to thioglycolate broth. The MBC was read after 48 hr of incubation at 37 C (Table 1).

The MIC values were similar with the three media. No bactericidal activity was noted if subcultures were done in thioglycolate medium when Trypticase soy broth (12 of 12 strains) or brain heart infusion broth (11 of 12 strains) was used to determine the MIC value, but significant bactericidal activity was noted when Mueller-Hinton medium was used to determine the MIC (10 of 12 strains). Significant bactericidal activity was noted if subcultures were done on blood-agar plates after incubation in Trypticase soy broth (8 of 12 strains), but better results were obtained with brain heart infusion (12 of 12 strains) or Mueller-Hinton broth (12 of 12 strains). The best correlation of MBC values was obtained by using either thioglycolate broth or blood-agar plate after incubation in Mueller-Hinton broth.

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