Unusual Susceptibility of Methicillin-Resistant *Staphylococcus aureus* to Erythromycin, Clindamycin, Gentamicin, and Tetracycline at 30°C But Not at 35°C

WILLIAM D. WELCH* AND PAUL M. SOUTHERN

Department of Pathology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Received 20 December 1983/Accepted 14 March 1984

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen in hospital environments, and optimal detection of MRSA requires nonroutine methods in clinical microbiology laboratories. One such method is an incubation temperature of 30°C in contrast to the more commonly used temperature of 35°C. To determine the percentage of MRSA isolates that would be missed if only one temperature were used, we evaluated methicillin resistance and susceptibility of 2,397 *S. aureus* isolates by agar dilution at 30 and 35°C. Of the clinical isolates, 93% showed matching MICs of methicillin at both temperatures. Another 6.8% (162) showed different MICs at 30 and 35°C, with 60 of the 162 isolates (2.5% of all isolates studied) being resistant to methicillin at 30°C but susceptible at 35°C (temperature-discrepant MRSA). MICs of other antimicrobial agents, measured at 35°C, revealed an unusual pattern of susceptibility of these temperature-discrepant MRSA isolates. In contrast to previously reported resistance of MRSA at 35°C to erythromycin, clindamycin, gentamicin, and tetracycline, the temperature-discrepant MRSA isolates were susceptible to these agents. This resistance pattern may be of value in identifying questionable MRSA isolates when only one incubation temperature is used.

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been shown to be responsible for large numbers of infections in several U.S. and European hospitals (4, 9, 11, 14, 15, 17-21). MRSA is a bacterial pathogen of clinical importance because (i) a single strain may be transmitted in the hospital via nurse-patient-physician contact (24), (ii) it may cause life-threatening diseases such as pneumonia and endocarditis, (iii) *S. aureus* is the most frequently isolated organism found in postoperative wound infections (15), (iv) it is almost always resistant to other semisynthetic, penicillinase-resistant penicillins as well as to ampicillin, chloramphenicol, erythromycin, and clindamycin (1, 4, 8, 22), and (v) it requires special laboratory procedures for maximal detection. These special procedures include a 48-h incubation period (5), use of media with increased salt concentrations (3), and incubation of the susceptibility test at reduced temperatures (30 to 31°C) (2, 3). Because a 30°C incubation temperature may affect the interpretation of MICs of drugs against bacteria other than MRSA, Thornsberry et al. (25) recommend screening for MRSA at 35°C. However, in their report (25) comparing 30, 35, and 37°C incubation temperatures, incubation at 30°C continued to be a more sensitive indicator for determining the degree of resistance of *S. aureus* to methicillin (22% more MRSA isolates were found to require MICs of methicillin greater than 50 µg/ml at 30°C than at 35°C).

Since many laboratories continue to use 30 and 35°C incubation temperatures for detection of MRSA, we have examined the frequency of discrepancies of methicillin-resistant and -susceptible *S. aureus* isolates at these two temperatures. Additionally, the antibiograms of the temperature-discrepant *S. aureus* isolates were studied to determine whether this information would be helpful in identifying MRSA at 35°C.

MATERIALS AND METHODS

Organisms. A total of 2,397 clinical isolates of *S. aureus* from Parkland Memorial Hospital, Dallas, Tex., were used in this study. Standard reference strains were *S. aureus* ATCC 25923 and 29213. Isolates identified as *S. aureus* were gram-positive cocci in clusters and were catalase and tube coagulase positive.

Agar dilution assay. The agar dilution method as described by Ericsson and Sherris (12) with a Steers replicator (3) was used. Mueller-Hinton agar (pH 7.2 to 7.4) was prepared and used within 24 h. The inocula for susceptibility studies were prepared by diluting a 2.5- to 3-h Mueller-Hinton broth culture (from four to five isolated colonies) to a density equivalent of 0.5 McFarland standard. Methicillin concentrations tested were 0.5, 2, 8, 16, and 32 µg/ml.

Two identical sets of plates were prepared for each culture, with one incubated at 35°C and one at 30°C. The tests were read after 18 to 24 h of incubation. The MIC was read as that concentration which allowed the growth of no more than one colony. On agar dilution plates without antimicrobial agents added, there was confluent growth on the agar at the application area. The incubators used contained fans to provide constant air circulation and did not have temperature variations of more than 1°C from the selected temperature during the course of the study.

RESULTS

The frequency of resistant (>8 µg/ml) and susceptible (<2 µg/ml) clinical *S. aureus* isolates (2,397) to methicillin at 30 and 35°C is shown in Fig. 1. Of the 162 isolates that showed discrepant MICs at 30 and 35°C, 60 (37%) were resistant at 30°C and susceptible at 35°C (referred to as temperature-discrepant MRSA). All of these discrepant isolates required MICs of 8 and 2 µg of methicillin per ml at 30 and 35°C, respectively. Thus, of all 2,397 *S. aureus* isolates, 60 (2.5%) would not have been detected for resistance if only a 35°C incubation temperature had been used. Conversely, 25 (15%)

* Corresponding author.

of the isolates with discrepant MICs were resistant at 35°C and susceptible at 30°C. Twenty-two of these discrepant isolates required MICs of 2 and 8 µg/ml at 30 and 35°C, respectively. The majority of the discrepancies (72 [44%]) were of resistant isolates, all requiring MICs of >8 µg of methicillin per ml.

The MICs of 11 antimicrobial agents against the 60 temperature-discrepant MRSA isolates are shown in Table 1. Significant numbers of isolates displayed susceptibility to cephalothin (99%), cefamandole (99%), clindamycin (100%), erythromycin (97%), gentamicin (100%), and tetracycline (86%).

DISCUSSION

Detection of methicillin resistance in *S. aureus* is enhanced by a reduced incubation temperature (2, 13), additional length of incubation (48 h) (5), an increase in the salt concentration of the media used (3), and large inoculum size (12). Because these special procedures differ from routine methods used for susceptibility testing, a 35°C incubation temperature only was recommended by Thornsberry et al. (25) for detection of MRSA. Our results showed that 162 of 2,397 isolates (2.5%) would have been identified as methicillin susceptible which actually were methicillin resistant at 30°C, if only a 35°C incubation temperature had been used.

Because MRSA isolates are multiresistant to various antibiotic classes, this resistance pattern has been proposed to be another and perhaps better means of predicting methicillin resistance than methicillin MICs (1). To determine whether a similar pattern of antibiotic resistance was present in our temperature-discrepant MRSA isolates, we measured the MICs of several antimicrobial agents against these strains (Table 1). In contrast to other reports in which MRSA isolates were resistant to erythromycin, clindamycin, gentamicin, and tetracycline (1, 4, 8, 10, 16), we found that greater than 96% of *S. aureus* isolates resistant to methicillin at 30°C but not at 35°C were susceptible to erythromycin, clindamycin, and gentamicin. Of these isolates, 86% were also susceptible to tetracycline. It should be noted that all susceptibility tests with antimicrobial agents other than

TABLE 1. MICs of antimicrobial agents against 60 clinical *S. aureus* isolates resistant to methicillin at 30°C and susceptible to methicillin at 35°C

Antimicrobial agent ^a	% of isolates susceptible at the following MIC (µg/ml):							Not inhibited
	0.125	0.25	0.5	2.0	8.0	16.0	32.0	
Ampicillin	0	0	6	18	27	11	11	24
Cefamandole	0	0	13	86	0	0	0	0
Cefoxitin	0	0	0	15	84	1	0	0
Cephalothin	0	0	93	6	1	0	0	0
Chloramphenicol	0	0	4	0	87	7	2	0
Clindamycin	0	0	100	0	0	0	0	0
Erythromycin	0	0	86	11	2	0	0	1
Gentamicin	0	93	7	0	0	0	0	0
Methicillin (30°C)	0	0	0	0	100	0	0	0
Methicillin	0	0	0	100	0	0	0	0
Penicillin	4	4	8	0	0	0	0	84
Tetracycline	0	0	46	40	0	2	0	11

^a Susceptibility testing was performed at 35°C unless indicated otherwise.

methicillin were performed at 35°C. Thus, our temperature-discrepant MRSA isolates, which were susceptible to cephalothin and cefamandole at 35°C, may actually be resistant to these cephalosporins at 30°C, as reported by Canawati et al. (6, 7).

The unusual pattern of susceptibility to several antimicrobial agents that we have found with the temperature-discrepant MRSA isolates may be of benefit in determining the methicillin susceptibility of questionable *S. aureus* strains. For example, *S. aureus* isolates resistant to methicillin at 31°C but susceptible at 35°C often display susceptible inhibitory zones at 35°C by disk diffusion testing, but they have small colonies within the zone (2). This characteristic appearance, in addition to susceptibilities to erythromycin, clindamycin, gentamicin, and tetracycline at 35°C, may suggest that the isolate in question is actually resistant to methicillin and that additional susceptibility testing at 30°C is warranted.

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FIG. 1. Frequency of methicillin-resistant (≥ 8 µg/ml) and -susceptible (≤ 2 µg/ml) clinical *S. aureus* isolates at 30 and 35°C.

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