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Temperature Effect on the Susceptibility of Methicillin-Resistant *Staphylococcus aureus* to Four Different Cephalosporins

HANNA N. CANAWATI,[1,2] JOYCE L. WITTE,[3] AND FRANCISCO L. SAPICO[1,3]*

University of Southern California School of Medicine, Los Angeles, California 90033,[1] and Department of Pathology[2] and Department of Medicine,[3] Rancho Los Amigos Hospital, Downey, California 90242*

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Forty isolates of methicillin-resistant *Staphylococcus aureus* were tested for in vitro susceptibility to cephalothin, cefamandole, cefotaxime, and moxalactam, using the disk diffusion and microbroth dilution methods at incubation temperatures of 30 and 35°C. Resistance to all four antibiotics was more clearly evident at an incubation temperature of 30°C.

Methicillin-resistant *Staphylococcus aureus* infections are being seen with increasing frequency in the United States (5, 8, 11, 15, 17). The resistance of these microorganisms to the semisynthetic penicillins has posed therapeutic problems, and clinical failures have been reported with earlier cephalosporins despite apparent in vitro susceptibility to these drugs (1, 11, 14).

In a recent communication (7) we reported discordant in vitro disk susceptibility results for the effect of cephalothin on methicillin-resistant *S. aureus* when the incubation temperature was changed from 35 to 30°C. Similar observations had been reported previously with methicillin and other semisynthetic penicillins (2, 6, 16). We also showed in our study that additional isolates of methicillin-resistant *S. aureus* may show resistance to cephalothin by tube dilution susceptibility testing at an incubation temperature of 30°C rather than 35°C.

We studied the antibiotic susceptibility of methicillin-resistant *S. aureus* isolated at our institution to other cephalosporins, including a second-generation cephalosporin (cefamandole) and third-generation cephalosporins (cefotaxime and moxalactam), to determine whether results similar to those of cephalothin can be observed.

Forty clinical isolates of methicillin-resistant *S. aureus*, each from a different patient, were studied. These isolates were recovered from a variety of clinical specimens from January 1978 to April 1979. The isolates were identified by conventional methods (12).

Antibiotic disk susceptibility testing was performed by the method of Bauer and associates (4) and by the agar overlay technique (3). A 30-μg disk was used for each of the four antibiotics studied. One set of plates was incubated at 30°C and the other was incubated at 35°C for 18 to 24 h. The criteria for inhibitor zone diameter standards for cephalothin and cefamandole were obtained from previously published data (13), and those for cefotaxime and moxalactam were obtained from their respective manufacturers. These criteria are listed in Table 1.

We determined minimum inhibitory concentrations (MIC) by a microbroth dilution technique in Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.), using the method of Gavan and Barry (10). One set of tubes was incubated at 30°C and the other was incubated at 35°C for 18 to 24 h. All samples were run in duplicate. A known methicillin-susceptible strain of *S. aureus* (ATCC 25923) and a known methicillin-resistant strain from our culture collection were tested simultaneously each time the test was performed. Isolates with an MIC of 10 μg/ml or less were considered susceptible, and those with an MIC of 20 μg/ml or more were considered resistant.

Table 1 shows the results of the disk diffusion study performed on 40 methicillin-resistant *S. aureus* isolates at incubation temperatures of 30 and 35°C. The results of the Bauer-Kirby and the agar overlay methods were comparable. The agar overlay method, however, gave better-defined zones of inhibition. We therefore elected to use the results obtained by this technique. Many resistant colonies were often seen growing inside cephalothin disk inhibition zones, even in areas immediately adjacent to the disk border, particularly at 30°C. These isolates were interpreted as resistant to cephalothin. This phenomenon was not observed with the other three cephalosporins.

Table 2 shows a summary of the tube dilution susceptibility results of the 40 methicillin-resistant *S. aureus* isolates studied at incubation

TABLE 1. Disk diffusion sensitivity results by the agar overlay method to cefotaxime (CT), moxalactam (MO), cefamandole (CM), and cephalothin (CL) of 40 methicillin-resistant *S. aureus* isolates

Susceptibility	% of all isolates							
	Incubation at 30°C				Incubation at 35°C			
	CT	MO	CM	CL	CT	MO	CM	CL
Susceptible[a]	0	0	37.5	7.5	0	0	55	100
Intermediate[b]	0	0	10	0	7.5	10	40	0
Resistant[c]	100	100	52.5	92.5	2.5	90	5	0

[a] Susceptible = ≥18 mm for cefamandole and cephalothin and ≥23 mm for cefotaxime and moxalactam.

[b] Intermediate = 15 to 17 mm for cefamandole and cephalothin, 19 to 22 mm for cefotaxime, and 15 to 22 mm for moxalactam.

[c] Resistant = ≤14 m for cefamandole, cephalothin, and moxalactam and ≤18 mm for cefotaxime.

temperatures of 30 and 35°C. If an MIC of 160 μg/ml or more is considered indicative of high-degree resistance, then none of the isolates tested were highly resistant at 35°C to cefotaxime, but 72.5% were highly resistant at 30°C. Likewise, 35% were highly resistant to moxalactam at 30°C, compared with 5% at 35°C. Only one isolate (2.5%) was highly resistant to cephalothin at 35°C, compared with 47.5% at 30°C. Cefamandole showed relatively better activity compared with the other three cephalosporins tested. Only one isolate was highly resistant to cefamandole at 35°C, and none were highly resistant at 30°C.

Of the 35 isolates that were phage typed at the Centers for Disease Control in Atlanta, Ga., 28 belonged to phage type 83A, 1 belonged to type 29/52/52A/80, and 6 were nonreactive to the phages used. Although a single phage type was dominant, almost one half of our isolates came from patients who acquired the infection at other institutions before transfer to our hospital, which is a referral facility. Thus, this dominant phage type was not consistently acquired within our hospital by patient-to-patient transfer. The possibility of a single dominant epidemic strain accounting for our isolates, however, cannot be entirely ruled out.

In a previous publication (7), we demonstrated that cephalothin-resistant methicillin-resistant *S. aureus* may be missed by disk diffusion susceptibility testing at the routine incubation temperature of 35°C. This phenomenon is similar to that observed with methicillin (2, 6, 16) and has been attributed to the inhomogeneity of methicillin susceptibility within individual strains.

The present study shows that other cephalosporins, including the more recent ones, may exhibit similar properties. All four cephalosporins tested showed poor in vitro activity against methicillin-resistant *S. aureus*, although cefamandole had slightly better activity. The methicillin-resistant *S. aureus* isolates showed greater resistance to cephalothin, cefamandole, cefotaxime, and moxalactam by disk susceptibility testing at the 30°C incubation temperature. Resistant colonies observed growing inside the cephalothin disk inhibition zones were not seen with the other three cephalosporins.

This study confirms our previous observation that cephalothin tube dilution susceptibility testing is likewise affected by changing the incubation temperature from 35 to 30°C. Greater in vitro resistance was detected at the lower temperature, and the same phenomenon was seen with cefamandole, cefotaxime, and moxalactam. Dyke et al. (9) suggested that, at low temperatures, bacterial cell wall synthesis proceeds at a relatively higher rate than the synthesis of other cell structures, thus accounting for the relatively greater resistance to cell wall inhibitors at lower temperatures.

The clinical failures associated with the use of cephalosporins in methicillin-resistant *S. aureus* infections and the in vitro resistance demonstrated in this study should alert clinicians to the fallibility of cephalosporin disk susceptibility testing of methicillin-resistant *S. aureus* at an incubation temperature of 35°C. Some cephalosporin-resistant strains may be erroneously reported as susceptible by standard techniques.

TABLE 2. MIC of cefotaxime (CT), moxalactam (MO), cefamandole (CM), and cephalothin (CL) against 40 isolates of methicillin-resistant *S. aureus*

Susceptibility	% of all isolates							
	Incubation at 30°C				Incubation at 35°C			
	CT	MO	CM	CL	CT	MO	CM	CL
Susceptible[a]	2.5	0	20	2.5	32.5	5	75	20
Resistant[b]	97.5	100	80	97.5	67.5	95	25	80
Mean MIC ± SE	129 ± 7.9	92 ± 8.4	27.2 ± 3	82.8 ± 10	25.9 ± 4.4	39.8 ± 4.5	14.8 ± 2.3	67.4 ± 10

[a] Susceptible = MIC of 10 μg/ml or less.

[b] Resistant = MIC of 20 μg/ml or more.

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