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Activity of Cephalosporins Against Methicillin-Susceptible and Methicillin-Resistant, Coagulase-Negative Staphylococci: Minimal Effect of Beta-Lactamase

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Eight cephalosporins were tested for their activity against methicillin-susceptible and methicillin-resistant, coagulase-negative staphylococci and for their resistance to β-lactamase from methicillin-resistant, coagulase-negative staphylococci. Susceptibility testing by the agar plate method was evaluated for the effect of inoculum size and duration of incubation. Methicillin-susceptible, coagulase-negative staphylococci were highly susceptible to the cephalosporins, with cephapirin and cephalothin showing the greatest activity, followed by cefazolin and cefamandole. Methicillin-resistant, coagulase-negative staphylococci displayed nearly total cross-resistance to the cephalosporins. Resistance increased with increasing inoculum size. β-Lactamases produced by methicillin-resistant, coagulase-negative staphylococci had a minimal hydrolytic effect on cephalothin, cephapirin, cefazolin, and cefamandole and no measurable effect on cefoxitin. There was no correlation between the anti-staphylococcal activity and resistance to β-lactamases.

Coagulase-negative staphylococci cause not only infections of prosthetic devices (12, 13) but also postoperative wound infections (4), peritonitis related to chronic peritoneal dialysis (8), and vascular and bone infections associated with hemodialysis (2, 20). For over 15 years it has been known that coagulase-negative staphylococci are more resistant to antibiotics than their coagulase-positive counterparts (14). Methicillin resistance, a hallmark of multiple antibiotic resistance for the staphylococcus, remains an oddity in the United States in *Staphylococcus aureus* (15), but from 15 to 40% of all coagulase-negative staphylococci isolated in various clinical microbiology laboratories are methicillin resistant (14, 23, 24). Disk methods of testing coagulase-negative staphylococci probably underestimate the incidence of both methicillin resistance and resistance to cephalosporins (17). Since the cephalosporins have been recommended as the primary alternative agents for treating infections due to methicillin-resistant, coagulase-negative staphylococci (1), we examined the activity of eight currently available cephalosporins against clinical isolates of methicillin-susceptible and methicillin-resistant coagulase-negative staphylococci. We also determined the resistance of five parenteral cephalosporins to β-lactamases produced by methicillin-resistant, coagulase-negative staphylococci.

MATERIALS AND METHODS

Microorganisms. Clinical isolates of coagulase-negative staphylococci were obtained from the Clinical Microbiology Laboratory of the Medical University of South Carolina Hospital. They were identified as coagulase-negative staphylococci by their colony morphology, Gram stain, and ability to produce catalase and to ferment glucose anaerobically (5). They were separated from micrococci by their susceptibility to furazolidone (3). All methicillin-resistant organisms grew on Mueller-Hinton agar (Difco) containing 12.5 μg of methicillin per ml.

Antibiotics. Standard antibiotic powders were gifts from their respective manufacturers: methicillin and cephapirin from Bristol Laboratories, Syracuse, N.Y.; cephalothin, cefazolin, cefamandole, cephaclor, and cefalexin from Eli Lilly Laboratories, Indianapolis, Ind.; cefoxitin from Merck Sharp and Dohme Research Laboratories, West Point, Pa.; and cefuroxime from Glaxo Research Ltd., Greenford, England.

All aqueous solutions of antibiotics were freshly made, and antibiotic-containing agar was prepared less than 24 h before inoculation.

Antimicrobial susceptibility testing. Minimum inhibitory concentrations (MICs) were determined by the agar dilution method of Steers et al. (25). High inocula (10^9 organisms per ml) and low inocula (10^6 organisms per ml) were prepared from overnight cultures in Mueller-Hinton broth and applied to Mueller-Hinton agar plates by a replica-plating apparatus (Melrose Machine Shop, Woodlyn, Pa.) whose plating prong delivers about 0.001 ml to the agar surface. Duration of incubation was for 18 and 48 h at 30°C, and the MIC was defined as the minimum concentration of antibiotic preventing growth of greater than three isolated colonies.

Beta-lactamase activity. Five representative strains which were shown to produce penicillinase by

the rapid slide starch-iodine test of Rosenblatt and Newman (22) were chosen for determination of β-lactamase activity. Whole-cell suspensions for these measurements were prepared as follows. Overnight cultures in Trypticase soy broth (BBL Microbiology Systems) were diluted 1:20 in fresh Trypticase soy broth and shaken at 37°C for 6 h. Methicillin or cefoxitin (0.2 μg/ml) was added for β-lactamase induction, with shaking continued for 2 h. Cells were harvested by centrifugation and resuspended in 0.01 of the original volume of 0.75 mM phosphate buffer, pH 7.0. Bacterial colony-forming units were determined by dilution-spread plating.

Beta-lactamase activity was measured by the alkalimetric titration method (10, 26) with an automatic recording pH-Stat (Metrohm-Herisau Combititrator, Brinkmann Instruments, Westbury, N.Y.). A 25-ml volume of phosphate buffer (0.75 mM) containing 3×10^{-5} mol of antibiotic was brought to pH 7.0 at 37°C. The enzyme reaction was started by the addition of 0.5 ml of the whole-cell suspension, and the rate of β-lactam ring hydrolysis was measured by continuous titration of the penicilloiic acid formed with 0.01 NaOH. Activity was measured as micromoles of substrate hydrolyzed per hour per 10^9 cells and reported as relative to hydrolysis of penicillin, where penicillin G was 100.

RESULTS

Antimicrobial susceptibility. The activities of methicillin and the eight cephalosporins against low and high inocula of methicillin-susceptible, coagulase-negative staphylococci are shown in Table 1. An inoculum size effect was

TABLE 1. *Cephalosporin activity against methicillin-susceptible, coagulase-negative staphylococci*

Antibiotic	No. of strains	Inoculum	Incubation time (h)	Median MIC (μg/ml)	Ranges
Methicillin	28	Low	18	2	0.24–4
			48	4	1–8
	28	High	18	4	2–16
			48	8	2–16
Cephalothin	28	Low	18	0.24	0.03–1
			48	0.24	0.06–1
	28	High	18	0.5	0.12–2
			48	1	0.24–4
Cephapirin	28	Low	18	0.24	0.03–0.5
			48	0.24	0.06–0.5
	28	High	18	0.24	0.6–8
			48	0.5	0.12–16
Cefazolin	28	Low	18	0.5	0.03–1
			48	0.5	0.12–2
	28	High	18	1	0.24–16
			48	2	0.24–31
Cefamandole	28	Low	18	0.5	0.06–1
			48	0.5	0.12–1
	28	High	18	1	0.24–4
			48	1	0.5–8
Cefoxitin	28	Low	18	2	<0.06–8
			48	2	0.12–8
	28	High	18	4	1–8
			48	4	1–8
Cefuroxime	28	Low	18	1	<0.06–4
			48	2	0.24–4
	28	High	18	2	0.24–8
			48	4	0.24–8
Cefaclor	28	Low	18	2	<0.06–4
			48	2	0.5–8
	28	High	18	4	1–63
			48	8	2–>125
Cephalexin	28	Low	18	4	<0.06–31
			48	4	1–31
	28	High	18	8	2–125
			48	16	4–>125

suggested with the two oral cephalosporins. Cephalothin and cephapirin were the most active cephalosporins against the methicillin-susceptible strains, but cefazolin and cefamandole also displayed good activity.

The results for the methicillin-resistant strains were markedly different, as shown in Table 2. Although some resistant strains had an MIC of ≤2 μg/ml for cephalothin, cephapirin, cefazolin, and cefamandole, the median MIC of all cephalosporins at both the low and high inocula was greater than 12 μg/ml, which might be considered beyond the therapeutic range for these compounds. Moreover, for the methicillin-resistant strains, the median MIC at 18 h increased for each cephalosporin by increasing the inoculum. Of the two newer cephalosporins, cefamandole was more active than cefoxitin against both methicillin-susceptible and methicillin-resistant strains.

Beta-lactamase activity. The relative activity of β-lactamase from coagulase-negative staphylococci against five cephalosporins is shown in Table 3. In five strains, using methicillin as the inducer, there was minimal degradation of any cephalosporin. When cefoxitin was used as the inducer, even less β-lactamase activity was demonstrated.

DISCUSSION

In terms of cephalosporin activity against co-

TABLE 2. *Cephalosporin activity against methicillin-resistant, coagulase-negative staphylococci*

Antibiotic	No. of strains	Inoculum	30°C incubation time (h)	Median MIC (μg/ml)	Range (μg/ml)
Methicillin	51	Low	18	31	<1->1,000
			48	250	4->1,000
	52	High	18	500	<4->2,000
			48	2,000	31->2,000
Cephalothin	53	Low	18	16	<0.24-125
			48	31	0.24-125
	54	High	18	31	0.5->125
			48	63	0.5->125
Cephapirin	53	Low	18	16	<0.12-63
			48	16	0.12-125
	54	High	18	31	1->250
			48	31	1-250
Cefazolin	53	Low	18	31	<0.12->150
			48	31	0.5->250
	54	High	18	63	2->250
			48	63	2->250
Cefamandole	53	Low	18	16	<0.12-250
			48	16	0.5-250
	54	High	18	31	1->250
			48	31	2->250
Cefoxitin	30	Low	18	125	<0.02-500
			48	125	8-500
	32	High	18	500	8->500
			48	500	8->500
Cefuroxime	30	Low	18	125	<1-1,000
			48	250	<1-1,000
	32	High	18	1,000	2->1,000
			48	>1,000	16->1,000
Cefaclor	29	Low	18	31	<1-63
			48	63	8-125
	32	High	18	63	8->250
			48	250	63->250
Cephalexin	30	Low	18	250	<1-500
			48	250	8-500
	32	High	18	250	31->500
			48	500	63->500

TABLE 3. *Activity of β-lactamase from coagulase-negative, methicillin-resistant staphylococci against five cephalosporins*

Strain no.	Inducer	Rate of hydrolysis[a]						
		Penicillin G	Methicillin	Cephalothin	Cefazolin	Cephapirin	Cefamandole	Cefoxitin
278	Methicillin	100	0	1.2[a]	N.D.	0.6	1.2	N.D.
283	Methicillin	100	0.2	0.2	1.3	0.1	0.1	<0.1
283	Cefoxitin	100	0	0	1.0	0.6	0	<0.1
288	Methicillin	100	0.1	0.1	1.0	0.4	0.3	<0.1
290	Methicillin	100	0.7	2.0	1.3	0	0	<0.1
293	Methicillin	100	<0.1	0.2	0.4	0.4	0.5	<0.1

[a] Relative rates of hydrolysis (penicillin G = 100) with hydrolysis of penicillin G at a rate ≥12 μmol/mg of bacteria per h. N.D., Not done.

agulase-negative staphylococci, our study agrees with the findings of Laverdiere et al.: methicillin-susceptible, coagulase-negative staphylococci are susceptible to the cephalosporins, whereas methicillin-resistant strains are for the most part resistant to the cephalosporins (17, 18).

Cephapirin and cephalothin were the most active cephalosporins against methicillin-susceptible, coagulase-negative staphylococci, followed by cefazolin and cefamandole, which were followed by cefoxitin and cefuroxime. The oral cephalosporins were the most sensitive to a change in inoculum. In fact, the median MICs for cephalexin and cefaclor at the high inoculum and longer incubation were 8 and 16 μg/ml, respectively. These findings should serve as a cautionary note to clinicians who routinely extend parenteral cephalosporin therapy of methicillin-susceptible, coagulase-negative staphylococcal infections with these oral cephalosporins.

For methicillin-resistant, coagulase-negative staphylococci, no cephalosporin had a median MIC below 12.5 μg/ml even at the lower inoculum. Although there was a wide range of MICs, we found more cross-resistance between methicillin and cephalosporins among methicillin-resistant, coagulase-negative staphylococci than has been reported previously (1). Because of minimal cross-resistance in his study, Archer suggests that cephalosporins would be superior to semisynthetic penicillins for the initial therapy of serious *S. epidermidis* infections (1). Yet in our study, the median MICs of all cephalosporins for methicillin-resistant staphylococci were sufficiently high to serve as a warning to clinicians who might select a cephalosporin on the basis of conventional susceptibility tests to treat a patient infected with a methicillin-resistant, coagulase-negative staphylococcus. Cephalosporin activity should probably not be reported for methicillin-resistant staphylococci unless the strains are subjected to special methods such as increased inoculum size or lowered temperature of incubation which are designed to uncover resistant strains. Several therapeutic experiences would support this contention. Percival failed to eradicate methicillin-resistant, cephalosporin-susceptible *S. albus* from infected peritoneal fluids when cephalothin was administered via the dialysate (21). Hammond and Stiver found that methicillin-resistant *S. epidermidis* causing prosthetic valve endocarditis were not killed in vitro by cephalothin unless gentamicin was added (9). The cephalothin-gentamicin combination produced excellent serum bactericidal activity in vivo.

In the last 5 years there has been renewed interest in extending the species classification of coagulase-negative staphylococci (16). In studying urinary isolates of coagulase-negative staphylococci, we found various patterns of antibiotic susceptibility, depending upon the species (11). Perhaps the discrepancies noted in the present study and other studies regarding the wide range of cephalosporin susceptibility of methicillin-susceptible and particularly methicillin-resistant coagulase-negative staphylococci may be species related. Further studies may clarify whether such variations are indeed species dependent.

We are not aware of any published data on the activity of β-lactamase from coagulase-negative staphylococci against cephalosporins. All five strains tested showed minimal activity against the five parenteral cephalosporins. In general, cefazolin was the most sensitive, whereas no activity was detected against cefoxitin. Farrar and Gramling (6) and Farrar and O'Dell (7) performed similar studies for *S. aureus*. They found a correlation between the sensitivity of cephalosporins to β-lactamase and the effect of inoculum size. We tested fewer strains than they, but nevertheless were unable to find any correlation. Beta-lactamase production in *S. epidermidis*, like that in *S. aureus*, is often plasmid mediated (19). Newer techniques for isolating staphylococcal plasmids which encode different β-lactamases may facilitate the further study of the stability of various cephalosporins to different β-lactamases from coagulase-negative staphylococci.

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