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In Vitro Activities of Ampicillin-Sulbactam and Cefoperazone-Sulbactam against Oxacillin-Susceptible and Oxacillin-Resistant Staphylococci

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Ampicillin-sulbactam and cefoperazone-sulbactam were tested against staphylococci that were collected from 40 different medical centers throughout the United States. Oxacillin-resistant strains were resistant to both drug combinations, but oxacillin-susceptible strains were uniformly susceptible. The latter included strains with borderline susceptibility to oxacillin and methicillin.

In many medical centers within the United States, serious clinical and therapeutic problems have been created by the emergence of staphylococci which are resistant to oxacillin, methicillin, and other penicillinase-resistant penicillins (PRP resistant). Among *Staphylococcus aureus* isolates, such resistance is classically a heteroresistance, since some cells in a broth culture can be resistant while the others may be susceptible. The proportion of resistant cells within a population varies from strain to strain. In vitro tests have been developed to improve the ability to detect heteroresistant populations; e.g., for broth microdilution tests, addition of NaCl to the broth medium has been advocated (1, 18) since that enhances expression of PRP resistance. The question of whether other β-lactams should be tested with added NaCl has not been settled.

Chromosomally mediated intrinsic PRP resistance has been ascribed to the ability to produce a new penicillin-binding protein (PBP 2a) which has low affinity for most β-lactams and is capable of functioning when other binding sites are saturated (4, 6, 7, 15). Strains without such intrinsic resistance may display elevated oxacillin or methicillin MICs. The latter strains may be referred to as having borderline susceptibility, and their decreased susceptibility might be associated with the ability to produce excess amounts of β-lactamase enzymes (13, 16). Strains with borderline susceptibility have been shown to be responsive to therapy with oxacillin, nafcillin, or ampicillin-sulbactam in experimental endocarditis models (5, 17, 19). *S. aureus* isolates with borderline susceptibility should be considered PRP susceptible, although their oxacillin and methicillin MICs are elevated (12). Those elevated MICs can be markedly reduced when a β-lactamase inhibitor is added.

Sulbactam is a β-lactamase inhibitor which has been combined with ampicillin or cefoperazone for therapeutic use (3, 8, 10, 11). In this study, we evaluated the antistaphylococcal activities of both drug combinations. Tests were performed with 97 methicillin-resistant *S. aureus* strains that were initially recovered from clinical material before 1982 (historical strains). More contemporary isolates included 481 staphylococci that were recovered in 1987 and 1988 from blood cultures or significant nosocomial wound infections in 40 different medical centers within the continental United States (9).

Staphylococci with oxacillin MICs of ≤2.0 μg/ml and methicillin MICs of ≤4.0 μg/ml (in broth with 2% NaCl) were categorized as being PRP-susceptible, and all others were considered PRP resistant on the basis of the interpretive criteria recommended by the National Committee for Clinical Laboratory Standards (NCCLS; 14). PRP-susceptible *S. aureus* isolates were further categorized as having borderline susceptibility if the methicillin MIC was 4.0 μg/ml, the oxacillin MIC was ≤2.0 μg/ml, and the cephalothin MIC was ≤2.0 μg/ml (13). The latter strains were all β-lactamase positive and resistant to benzylpenicillin. All staphylococci were tested for β-lactamase activity by using a nitrocefin filter paper spot test, and 92% of the strains were β-lactamase positive.

Broth microdilution susceptibility tests were performed by NCCLS procedures (14). Ampicillin and cefoperazone were both combined with sulbactam at a 2:1 ratio (2, 8, 10, 14), and MICs were expressed as the concentration of active β-lactam in the presence of sulbactam (1 part of sulbactam to 2 parts of ampicillin or cefoperazone). All tests were performed in cation-supplemented Mueller-Hinton broth (50 mg of calcium per liter and 25 mg of magnesium per liter) with and without 2% NaCl added. The inocula were prepared by suspending freshly isolated colonies in a small amount of Mueller-Hinton broth and then adjusting the turbidity to match that of a McFarland 0.5 standard. The inocula contained approximately 5×10^5 CFU/ml, and MICs were recorded after 20 to 24 h of incubation at 35°C in ambient air.

In vitro studies were performed with 578 staphylococci (414 *S. aureus* isolates, 126 *S. epidermidis* isolates, 17 *S. haemolyticus* isolates, 6 *S. warneri* isolates, 4 *S. hominis* isolates, 3 *S. simulans* isolates, 2 *S. auricularis* isolates, 2 *S. capitis* isolates, 1 *S. cohnii* isolate, 1 *S. saprophyticus* isolate, 1 *S. xylosus* isolate, and 1 isolate of an unidentified species). Table 1 presents the results of these studies. Among the PRP-resistant *S. aureus* isolates, the contemporary isolates did not differ from the historical isolates that were selected from our stock culture collection. Both types of PRP-resistant staphylococci were relatively resistant to the two drug combinations, compared with PRP-susceptible strains. Among the PRP-susceptible strains, 72 isolates were categorized as having borderline susceptibility on the basis of the criteria of McDougal and Thornsberry (13). Both types of PRP-susceptible strains were very susceptible to ampicillin-sulbactam and cefoperazone-sulbactam. MICs for PRP-susceptible *S. aureus* strains were increased by addition of

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TABLE 1. In vitro activities of ampicillin-sulbactam and cefoperazone-sulbactam against staphylococci in broth with and without 2% NaCl added

Antimicrobial agent, broth medium, and microorganism[a]	No. of isolates tested	Cumulative % inhibited by a concn (μg/ml)[b] of:						
		≤0.5	1.0	2.0	4.0	8.0	16	32[c]
Ampicillin-sulbactam in CSMHB[d]								
S. aureus								
PRP resistant								
Historical isolates	97			2	7	52	99	
Contemporary isolates	174			1	2	29	70	
Borderline PRP susceptible[e]	72	5	16	61	97	100		
PRP susceptible	71	25	42	92	100			
Coagulase-negative species								
PRP resistant	120		4	13	47	79	88	
PRP susceptible	44	2	39	77	93	100		
Ampicillin-sulbactam in CSMHB–2% NaCl								
S. aureus								
PRP resistant								
Historical isolates	97			3	25	36	100	
Contemporary isolates	174				1	11	75	
Borderline PRP susceptible	72	4	8	33	91	100		
PRP susceptible	71	20	28	74	100			
Coagulase-negative species								
PRP resistant	120			2	25	64	89	
PRP susceptible	44	62	73	84	96	100		
Cefoperazone-sulbactam in CSMHB								
S. aureus								
PRP resistant								
Historical isolates	97			1	4	9	34	56
Contemporary isolates	174					9	38	52
Borderline PRP susceptible	72			25	100			
PRP susceptible	71			25	100			
Coagulase-negative species								
PRP resistant	120			8	63	86	88	89
PRP susceptible	44	2	39	77	93	100		
Cefoperazone-sulbactam in CSMHB–2% NaCl								
S. aureus								
PRP resistant								
Historical isolates	97				2	4	8	12
Contemporary isolates	174					1	11	20
Borderline PRP susceptible	72			5	92	100		
PRP susceptible	71			28	100			
Coagulase-negative species								
PRP resistant	120			8	52	78	87	87
PRP susceptible	44	11	41	77	91	100		

[a] Resistant or susceptible to PRP by NCCLS criteria (14).
[b] Concentration of ampicillin or cefoperazone when combined with sulbactam at a 2:1 ratio.
[c] Ampicillin was not tested at 32 μg/ml.
[d] CSMHB, Cation-supplemented Mueller-Hinton broth.
[e] Borderline PRP susceptible, PRP-susceptible strains by NCCLS criteria (14) categorized as having borderline (seven isolates) or partial borderline susceptibility by the criteria of McDougal and Thornsberry (13).

2% NaCl to the broth medium, but the effect was much greater when PRP-resistant strains were tested (Table 1). Some coagulase-negative staphylococci appear to be inhibited somewhat by added salt, and thus, MICs for PRP-susceptible strains might actually decrease, whereas MICs for PRP-resistant strains increase with the addition of salt.

The NCCLS document for dilution tests (14) defines an MIC breakpoint for susceptibility of ≤8.0 μg/ml for ampicillin-sulbactam, and strains with an MIC of 16 μg/ml are categorized as being moderately susceptible. By those criteria, 29% of the contemporary PRP-resistant *S. aureus* strains were susceptible and another 41% were moderately susceptible. When 2% NaCl was added to the broth medium, only 11% of the PRP-resistant *S. aureus* isolates were susceptible to ampicillin-sulbactam and another 64% were moderately susceptible. MIC breakpoints of ≤2.0 or ≤4.0 μg/ml would separate PRP-susceptible and PRP-resistant strains more effectively.

Cefoperazone alone is expected to be active against PRP-susceptible staphylococci but not against PRP-resistant strains (10, 11). When sulbactam is added to cefoperazone, MICs are lower than those expected for cefoperazone alone. MICs for 9% of the PRP-susceptible staphylococci tested were ≤4.0 μg/ml with or without NaCl, but PRP-resistant staphylococci had much higher cefoperazone-sulbactam MICs. A susceptibility breakpoint of ≤4.0 μg/ml would separate PRP-susceptible strains from PRP-resistant strains better than the current breakpoint of ≤16 μg/ml.

Even with added NaCl, in vitro susceptibility tests did not accurately categorize PRP-resistant strains as resistant to ampicillin-sulbactam or cefoperazone-sulbactam. It seems to be more prudent to test only oxacillin and assume that oxacillin-resistant strains are resistant to the two drug combinations. Oxacillin-susceptible strains, including those with borderline susceptibility, might be assumed to be susceptible to ampicillin-sulbactam and cefoperazone-sulbactam.

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