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Effect of Various Blood Culture Media on Lysostaphin Sensitivity of Staphylococci

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A rapid screening test for *Staphylococcus aureus* that utilizes lysostaphin sensitivity was found to be reliable for organisms cultured in 12 different commercial blood culture media.

In a previous report we described what we believe will be a clinically useful, rapid method for differentiating *Staphylococcus aureus* from non-*S. aureus* staphylococci, based on differential sensitivity to the enzyme lysostaphin (6). This difference appears to be determined by the relative amounts of glycine and serine present in the bridging pentapeptides of the staphylococcal cell wall (1, 2, 7, 8). It has been shown that the amino acid content of the growth medium can change the amino acid content of the staphylococcal peptidoglycan, thereby altering the sensitivity of the organism to lysostaphin (3-5). Since the type of blood culture medium used by clinical microbiology laboratories varies, we evaluated 12 commercially available blood culture media to determine whether the lysostaphin sensitivity test was influenced by the type of medium in which the staphylococci were grown.

The commercially purchased media were inoculated with freshly drawn blood according to manufacturers' recommended procedures. The test organism consisted of 10 strains of *S. aureus* and 10 strains of *Staphylococcus epidermidis* which were randomly selected from among the 168 laboratory strains used during our previous experiments. The media were inoculated with the test organism and then incubated at 37°C for 18 to 24 h. Samples of each bottle were then tested for lysostaphin sensitivity, using the previously described methodology (6). Briefly, this procedure consisted of exposing a 1:10 dilution of the blood culture to 2 μg of lysostaphin per ml and then incubating the specimen on a tilting mixer for 30 min at 37°C. Gram stains prepared pre- and posttreatment were compared, and a 90% or greater reduction in the number of organisms seen was defined as a positive test result. As previously reported (6), with these methods, 98% of 141 strains of *S. aureus* gave a positive result and all 127 non-*S. aureus* staphylococci gave a negative result.

A total of 120 determinations of lysostaphin sensitivity were performed with *S. aureus*, and all 120 tests were positive (Table 1). Of 120 determinations with *S. epidermidis*, 116 gave a negative test. Thus, the assay yielded an overall sensitivity of 100% and a specificity of 96.7%. For Columbia broth, the sensitivity remained 100%, but the specificity dropped to 86.7%.

Our previous report demonstrated that *S. aureus* grown in brain heart infusion (BHI) broth or Vacutainer blood culture bottles containing either tryptic soy broth (TSB) or supplemented peptone broth II could be readily distinguished from non-*S. aureus* staphylococci on the basis of lysostaphin sensitivity. Although cell wall constituents are genotypically determined, alterations in the amino acid content of the growth medium can cause a phenotypic alteration in the peptidoglycan structure; i.e., *S. aureus* grown in a glycine-poor, serine-rich medium will incor-

TABLE 1. *Lysostaphin sensitivity test*[a]

Medium	*S. aureus* (no. positive/no. tested)	*S. epidermidis* (no. positive/no. tested)
TSB (Difco Laboratories)	10/10	0/10
TSB (GIBCO Laboratories)	10/10	0/10
TSB (Pfizer Inc.)	10/10	0/10
BHI broth (Difco)	10/10	0/10
BHI broth (GIBCO)	10/10	0/10
BHI broth (Pfizer)	10/10	0/10
Columbia broth (Difco)	10/10	2/10
Columbia broth (GIBCO)	10/10	2/10
Columbia broth (Pfizer)	10/10	0/10
Bactec no. 6 (Johnston)	10/10	0/10
Bactec no. 7 (Johnston)	10/10	0/10
Trypticase soy broth (BBL Microbiology Systems)	10/10	0/10

[a] Overall sensitivity, 100%; specificity, 96.7%.

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porate serine instead of glycine into the pentapeptide bridge and become more resistant to the action of lysostaphin (4). The converse, using *S. epidermidis*, is also true (4).

Thus, differences in the amino acid content of commercially available blood culture media could limit the usefulness of this rapid screening test for *S. aureus*. For this reason, we studied 12 commercially available blood culture media to determine whether staphylococci cultured in these media would develop a change in lysostaphin sensitivity because of growth factor-induced phenotypic alteration. All *S. aureus* strains demonstrated sensitivity to lysostaphin and gave a positive result, regardless of the culture medium utilized. The four false-positive test results with non-*S. aureus* staphylococci all occurred in Columbia broth. The two strains that gave positive results were the same in each medium. These organisms were verified to be *S. epidermidis* (tube coagulase test negative and anaerobic dextrose test positive), and when subcultured in BHI broth or TSB, the organisms were lysostaphin resistant. These data suggest that these two strains exhibited a growth factor-induced alteration in lysostaphin sensitivity; the presumed mechanism implies that Columbia broth is a relatively glycine-rich, serine-poor medium. As a result, the specificity of the test ranges from 100% for media containing TSB or BHI broth to 86.7% for media containing Columbia broth. We feel that the incidence of growth factor-induced alteration is low and should not be a major deterrent to use of the rapid lysostaphin screening test for *S. aureus*; however, those laboratories using Columbia broth should be aware of the findings described herein.

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