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Detection of Pneumococci in Blood Cultures by Latex Agglutination

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Latex agglutination by use of the Pneumoslide test on clinical blood cultures detected 22 *Streptococcus pneumoniae* strains as the etiological agents in 47 streptococcal septic episodes. The other 25 isolates were identified as viridans streptococci or streptococci of groups A, B, D, or G. The test demonstrated 100% sensitivity, 92% specificity, and predictive values for positive and negative reactions of 91 and 92%, respectively. Two false-positive reactions were caused by strains of viridans streptococci. The two strains continued to give positive reactions when colonies from blood agar plates were tested according to the instructions of the manufacturer. This latex agglutination test is an effective tool for the rapid diagnosis of pneumococci in blood cultures.

Earlier we reported excellent results in detecting beta-hemolytic streptococci in blood cultures with a commercial latex agglutination test kit (K. Browne and K. D. Stottmeier, Abstr. 3rd International Symposium on Rapid Methods and Automation in Microbiology, 1981, 35). Subsequently, the results were confirmed by others (6).

The Neufeld quellung test is the only classical serological test system that allows the rapid identification of pneumococci in blood cultures, provided that pneumococci are seen on the gram-stained smear (5). However, this labor-intensive test depends on the presence of large numbers of intact organisms in specimens or blood cultures, in contrast to the Pneumoslide latex agglutination test (BBL Microbiology Systems, Cockeysville, Md.). We evaluated the specificity and sensitivity of this latex test on simulated and patient blood cultures that were positive by microscopy (×1,000) for gram-positive diplococci or gram-positive cocci in chains.

MATERIALS AND METHODS

To determine the sensitivity and specificity of the Pneumoslide, we selected three clinical isolates of streptococci: one strain of *Streptococcus agalactiae* type III (group B), one strain of *Streptococcus pneumoniae* (type 13), and one unspeciated strain of viridans streptococci with colony morphology similar to that of pneumococci. A single colony of each strain was picked from a sheep blood agar plate and placed in 3 ml of eugonic broth (Scott Laboratories, Fiskeville, R.I.). After overnight incubation in 5% CO_2 at 35°C, 10 serial dilutions in Columbia broth (GIBCO Laboratories, Grand Island, N.Y.) with 10% human blood were made to obtain an inoculum range from 10^0 to 3×10^6 CFU per ml.

After overnight incubation in 5% CO_2 at 35°C, a count of viable units (CFU) was made on sheep blood agar by standard bacteriological methods (4). A 3-ml sample of the overnight broth culture was centrifuged at 1,000 × *g* for 5 min and a Pneumoslide test was performed on the supernatant fluids. The test was repeated after 48 to 72 h of incubation of the broth cultures. According to the manufacturer, specific antibodies (Omniserum) to the 83 pneumococcal capsular polysaccharide antigens are absorbed onto the surface of latex beads. Latex particle aggregation becomes large enough to allow rapid visualization of positive agglutination in the presence of specific pneumococcal antigens. The viability of the organisms was determined after 72 h of incubation by sampling 0.1 ml of the broth cultures on blood agar which was incubated overnight at 35°C in 5% CO_2. All simulated blood culture experiments were performed in a blind fashion, by using coded specimens.

Samples (3 ml each) from routine blood cultures (brain heart infusion [Scott Laboratories] or Columbia broth) were centrifuged at 1,000 × *g*, and the supernatant fluids were tested for latex agglutination reactions, after various periods of incubation, when the bottles appeared visibly positive (hemolysis, media discoloration, and turbidity) and when a Gram stain demonstrated the presence of gram-positive diplococci or gram-positive cocci in chains. In addition, samples of the blood cultures were tested by the Neufeld quellung reaction with the entire battery of pools A through I and 46 type sera (Staten Serum Institute, Copenhagen, Denmark). If the quellung test was negative, the sediment from the centrifuged blood culture sample was used to perform a latex agglutination by Streptex test (Wellcome Diagnostics, Dartford, England) for beta-hemolytic streptococci and group D streptococci (6). Finally, a bile esculin agar plate (Scott Laboratories) was inoculated from the blood culture sediment to identify group D streptococci (5). No biochemical tests were employed to speciate viridans streptococci. They were identified by colony morphology, Gram stain, and the absence of reactivity in all of the above listed tests, as well as resistance to the optochin and bacitracin disk test (0.04 U) (BBL) (6).

RESULTS

Pneumococcal inocula of Columbia broth cultures ranging from 4×10^1 to 7×10^3 CFU per ml yielded strong (4+) agglutination reactions after 18 h of incubation. At that time, the number of CFU in the broth ranged from 8×10^4 to $>10^8$ CFU per ml. A very low inoculum of <1 organism per ml did not result in a positive agglutination after 18 h when there were only 10^3 CFU per ml. However, a 4+ reaction was observed in that bottle after 48 h of incubation. Weak (1+) agglutination reactions were observed after 18 h, when the inoculum exceeded 10^6 organisms per ml. But strong (4+) reactions were obtained when these broths were diluted 1:16 to 1:100 in saline and also after 72 h of incubation when no viable pneumococci were present. We observed no positive agglutination reaction at any time in any blood cultures seeded with various concentrations of *S. agalactiae* type III or the viridans streptococcus.

With both the *S. agalactiae* and the viridans streptococcus, we observed some questionable, i.e., very weak, agglu-

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TABLE 1. Sensitivity and specificity of direct pneumococcal agglutination in clinical blood cultures of 47 septic streptococcal episodes

Streptococci	No. of strains	No. latex positive	No. latex negative
S. pneumoniae types 4, 6, 9, 11, 13, 14, 18, 19, and 22[a]	22	22	0
Viridans[b]	14	2[c]	12
Groups A, B, D, G[b]	11	0	11

[a] Test demonstrated 100% sensitivity.
[b] Test demonstrated 92% specificity.
[c] Strains remained latex positive after retesting of colonies grown on blood agar plates according to the instructions of the manufacturer.

tination reactions after 18 to 48 h of incubation of 10^4 to 10^5 CFU per ml that were not considered positive.

Table 1 depicts results obtained from 47 septic episodes that were culture positive for streptococci by gram-stained smear. Of these, 22 were identified as *S. pneumoniae* by the pneumococcal quellung test (types 4, 6, 9, 11, 13, 14, 18, 19, and 22).

All 22 strains were positive by the Pneumoslide latex agglutination test. Bile solubility and susceptibility to optochin confirmed them as pneumococci. The remaining 25 strains were identified as 14 viridans streptococci and 11 strains of streptococci groups A, B, D, or G. Of the 25 streptococcal strains, 23 were Pneumoslide negative. Two strains of viridans streptococci were Pneumoslide positive. The positive reaction persisted after retesting of colonies of the two strains from blood agar. Colonies of the two strains were negative by quellung test and resistant to optochin and bile.

DISCUSSION

The simulated blood cultures required the presence of at least 10^4 CFU per ml to produce sufficient amounts of capsular polysaccharide to yield a positive latex test. More than 10^9 CFU per ml yielded only weak reactions, until rapidly progressing autolysis of the pneumococci resulted in increasingly stronger agglutination reactions over a period of 72 h after which time the culture was sterile. Initial antigen excess (prozone effect) might explain the phenomenon of weak agglutination reactions in the presence of very large numbers of viable pneumococci, or antigen solubility might be a factor. Detection of pneumococci in blood cultures after often rapid autolysis presents a distinct advantage of the Pneumoslide test over conventional identification tests. Furthermore, this test does not require a battery of expensive antisera and can be performed after minimal training of laboratory personnel in contrast to the Neufeld quellung test which requires considerable technical skill to interpret. However, the quellung test remains an essential epidemiological tool to monitor worldwide distribution of pneumococcal types.

In 2 out of 47 streptococcal episodes, we observed two false-positive reactions by viridans streptococci. Austrian and co-workers (1) "observed frequently that organisms lacking typical pneumococcal morphology and resembling alpha and non-hemolytic streptococci give rise to small but definitely positive capsular precipitin reactions in the presence of omniserum. In 196 cultures of sputum and nasopharyngeal secretions at least one such cross-reaction strain was found." It is apparent that regardless of the serological test, a false-positive reaction with pneumococcal Omniserum can be expected by certain strains of the viridans streptococci.

It is remarkable that streptococci of groups A, B, D, or G, particularly *S. agalactiae* type III, did not cross-react in this test, although it shares antigenic components with pneumococcal type 14 (3).

On the other hand, the Pneumoslide test did identify all pneumococcal types tested, including *S. pneumoniae* type 14 that occurred in three septic episodes. The sensitivity (100%) and specificity (92%) data (2) of the Pneumoslide test on 47 supernatants of streptococcal blood cultures confirm the data of the manufacturer of 98 and 93%, respectively, which apply to tests on pneumococcal colonies from a much larger number of strains.

In conclusion, the latex agglutination BBL Pneumoslide test correctly identified nine different types of pneumococci in 22 septic episodes when gram-positive diplococci were seen on the Gram stain. Positive reactions were observed in simulated blood cultures after pneumococci were no longer viable, having undergone autolysis. False-positive reactions were seen in 2 out of 14 septic episodes with viridans streptococci, but no false-positive reactions were seen in 11 episodes due to group A, B, D, or G streptococci. Predictive values for positive and negative reactions of the test were calculated to be 91 and 92%, respectively (2).

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