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Modification of the Bile Solubility Test for Rapid Identification of *Streptococcus pneumoniae*

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A modified bile solubility test is described which can be used to presumptively identify *Streptococcus pneumoniae* recovered in blood culture.

The rapid identification of microorganisms recovered in blood can provide information about the source of the infectious process and guide the specific therapeutic management of the septic patient. In the present study a modification of the bile solubility test for the rapid presumptive identification of *Streptococcus pneumoniae* recovered in blood cultures was evaluated.

Blood cultures, collected from patients in Barnes Hospital, were analyzed during an 8-month period from November 1977 through June 1978. Blood (10%, vol/vol) was inoculated into one bottle each of tryptic soy broth (TSB; Difco Laboratories) and Thiol broth (Difco), under vacuum with CO_2 and 0.025% sodium polyanetholsulfonate. The routine processing of the blood cultures has been described previously (4).

Gram-stained smears of positive blood cultures were examined for the presence of gram-positive cocci arranged in pairs and chains, consistent with *Streptococcus*. If such organisms were seen, then a second slide was prepared. On one-half of the slide, 1 drop of blood was mixed with 1 drop of 2% sodium deoxycholate. On the other half of the slide, 1 drop of blood was mixed with 1 drop of water, which served as the control. The slide was air-dried at ambient temperature, Gram stained, and examined. The numbers of organisms present in the blood treated with sodium deoxycholate and in the untreated blood were compared. If fewer than one to five cocci per microscopic field were observed in the control, then the test was repeated with the sediment of the blood culture broth centrifuged for 5 min at 2,000 × *g*.

In the second part of the study, laboratory isolates of *S. pneumoniae* and other streptococci were inoculated into 10-day-old, negative blood culture broths. After macroscopic growth of the streptococci was obtained, the cultures were processed as described above.

Catalase-negative, gram-positive cocci were identified with the following tests: *S. pneumoniae*, solubility in 2% sodium deoxycholate and susceptibility to ethylhydrocupreine hydrochloride (optochin); group A streptococci, reactivity with specific fluorescent antiserum; group B streptococci, hydrolysis of hippurate; and group D streptococci, growth in 40% bile and hydrolysis of esculin. During the study streptococci were recovered in blood cultures obtained from 54 patients, and a total of 72 TSB and 77 Thiol broths were analyzed (Table 1). Sodium deoxycholate treatment of TSB and Thiol broths completely solubilized all cells of *S. pneumoniae*, which were recovered from 20 patients. There was a 0 to 25% reduction in the number of other streptococci in the sodium deoxycholate-treated blood compared with the control for 36 of 42 isolates (86%) in TSB and 43 of 52 isolates (83%) in Thiol broth and a 25 to 75% reduction in organisms for six and eight isolates in TSB and Thiol broth, respectively. One isolate of group D enterococcus, recovered in a single Thiol broth, was completely solubilized with the sodium deoxycholate treatment. This was observed when the Thiol broth was retested, but not when the isolate was inoculated into TSB and Thiol broth and retested.

The results with simulated blood cultures are summarized in Table 2. Sodium deoxycholate completely solubilized all *S. pneumoniae*. Treatment of the other streptococci reduced the number of organisms from 0 to 25% for 122 of the 128 isolates (95%) in TSB and 120 of the 128 isolates (94%) in Thiol broth.

The test described herein is a combination of two commonly used laboratory procedures, namely the bile solubility test and the Gram stain test. Neufeld (5) originally reported that *S. pneumoniae* was soluble in bile, and Hawn and Beebe (2) demonstrated that this test could be accurately performed directly on isolated colonies. Bile salts, such as sodium deoxycholate, alter the surface of *S. pneumoniae*, which sub-

TABLE 1. *Effect of sodium deoxycholate treatment on streptococci recovered in blood cultures*

Isolate	Medium	No. of broths tested with a reduction in cell count of:		
		0–25%	25–75%	100%
S. pneumoniae	TSB	0	0	30
	Thiol broth	0	0	25
Streptococcus viridans group	TSB	7	2	0
	Thiol broth	10	1	0
Streptococcus group A	TSB	9	2	0
	Thiol broth	10	1	0
Streptococcus group B	TSB	6	0	0
	Thiol broth	6	1	0
Streptococcus group G	TSB	5	0	0
	Thiol broth	7	0	0
Streptococcus group D	TSB	9	2	0
	Thiol broth	10	5	1

TABLE 2. *Effect of sodium deoxycholate treatment on streptococci artificially inoculated into blood culture broths*

Isolate	Medium	No. of broths tested with a reduction in cell count of:		
		0–25%	25–75%	100%
S. pneumoniae	TSB	0	0	30
	Thiol broth	0	0	30
Streptococcus viridans group	TSB	72	4	0
	Thiol broth	70	6	0
Streptococcus group A	TSB	15	0	0
	Thiol broth	15	0	0
Streptococcus group B	TSB	8	1	0
	Thiol broth	9	0	0
Streptococcus group D	TSB	27	1	0
	Thiol broth	26	2	0

sequently activates the autolytic enzyme, an amidase which splits the muramic acid and alanine bond in the peptidoglycan portion of the cell wall (1). The bile solubility test must be performed with young, viable cells and at a neutral pH, because the bile salts precipitate in acid (3). The single false-positive reaction observed here was with a group D streptococcus recovered in a 5-day-old culture, and the isolate was not solubilized with sodium deoxycholate when retested from freshly inoculated broths.

In the procedure described herein, isolated colonies are not required for the presumptive test, in contrast with the previous studies cited. Wasilauskas and Ellner (6) described a modification of the bile solubility test for the presumptive identification of *S. pneumoniae* from blood cultures. A portion of the blood culture broth was centrifuged, and the pellet of bacteria was suspended in saline and then treated with 2% sodium deoxycholate for 2 h. However, this additional processing of the culture was found to be unnecessary in the present study.

Additional techniques for identifying *S. pneumoniae* include the Neufeld (quellung) capsular precipitin test and susceptibility to optochin. Although the quellung test can be performed directly with the blood culture broth, the anticapsular omniserum is produced only in Denmark and is not commonly used in most microbiology laboratories. The optochin sensitivity test requires isolated colonies of bacteria and therefore is not a rapid diagnostic test.

The test performed as described herein is sensitive and specific, with only one false-positive reaction. For an isolate to be presumptively identified as *S. pneumoniae*, the sodium deoxycholate treatment must completely lyse all cocci. A partial reduction in the number of other streptococci was commonly observed with the sodium deoxycholate treatment. This presumably was due to either a solubilization of older cells or a loss of cells during the staining process. The presumptive identification of streptococci with this modified solubility test must be confirmed by conventional tests.

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