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Detection of Bacteremia with Liquid Media Containing Sodium Polyanetholsulfonate

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Two liquid blood culture media, Tryptic soy broth (TSB) and Thiol broth, containing sodium polyanetholsulfonate were compared in 8,654 cultures. *Pseudomonas* and *Corynebacterium* (including *Propionibacterium*) were isolated significantly more frequently ($P < 0.001$) from TSB than from Thiol. *Escherichia coli*, *Haemophilus*, and Bacteroidaceae were isolated more frequently in TSB; however, the differences were not statistically significant. In no instance was Thiol superior to TSB in detecting bacteremia. In an additional 2,977 cultures, aerobic and anaerobic Vacutainer culture tubes with supplemented peptone broth were inoculated in parallel with TSB and Thiol. Significantly greater rates of detection ($P < 0.01$) in TSB or Thiol were noted with *Pseudomonas*, *E. coli*, *Enterobacter*, viridans, and group A streptococci, Bacteroidaceae, and staphylococci.

In a previous publication (5) from this laboratory comparing Tryptic soy broth (TSB) and Thiol broth, neither containing sodium polyanetholsulfonate (SPS), it was found that isolation rates of *Actinobacillus* and *Pseudomonas* were significantly greater in TSB and that isolation rates of *Streptococcus* and *Corynebacterium* (aerobic and anaerobic) were significantly greater in Thiol. The present report compares these same media, but both with SPS, during a 5.5-month period of study. In addition, the present report includes a limited comparison of TSB and Thiol with SPS (Difco Laboratories) and two Vacutainer culture tubes with supplemented peptone broth (Becton-Dickinson).

MATERIALS AND METHODS

Blood was collected aseptically with a sterile needle and syringe from patients suspected of having bacteremia and was inoculated (10%, vol/vol) into one bottle each of TSB and Thiol broth, both under vacuum and with CO_2. Each bottle contained 100 ml of medium with 0.05% SPS, and neither bottle was vented during incubation. During the studies of the Vacutainer tubes, an additional 4 ml of blood was obtained and divided equally between the two tubes after disinfection of the stopper with povidone-iodine. Two types of venting units for the tubes were supplied by the manufacturer: one to provide an aerobic environment in the tube and the other to maintain an anaerobic environment in the tube by means of an oxygen-impermeable plug that could be displaced if gas pressure within the tube became excessive. Venting units were applied to the tubes within 30 min after they were inoculated with blood.

All units were incubated at 35 C and were inspected daily for 14 days. To ensure prompt recovery of pseudomonads (3), all units without apparent growth were subcultured routinely within 24 h after collection, by sampling with a sterile syringe and needle through the stopper, inoculating chocolate blood agar plates (BioQuest), and incubating in an atmosphere of 10% CO_2 for 48 h. No further subcultures were carried out unless there was visual evidence, or suspicion thereof, of growth. Visible growth was stained and subcultured appropriately for identification and antimicrobial-susceptibility testing of the isolate.

Methods of statistical analysis of the results have been previously reported (5) and are based on those described by Cochran (1).

RESULTS

During the first comparison of TSB and Thiol, 8,654 sets of blood cultures were performed, and 642 isolates were obtained from 611 positive blood cultures (Table 1). Of these isolates, 166 were presumed contaminants on the basis of previously defined arbitrary criteria (5). *Corynebacterium* (including *Propionibacterium*) and *Pseudomonas* were isolated significantly more frequently ($P < 0.001$) from TSB than from Thiol. *Escherichia*, *Haemophilus*, and Bacteroidaceae were isolated more frequently from TSB than from Thiol; however, the differences were not statistically significant ($P < 0.1$). Otherwise, there were no significant

TABLE 1. *Isolates in positive cultures, by medium*

Organism	No. positive in			Total positive[a]	P[b]	Adjusted percent positive[c]
	TSB and Thiol	TSB only	Thiol only			
Bacillus	0	7	7	14	NS	
Clostridium	2	1	1	4	NS	0.8
Corynebacterium	8	70	20	98	<0.001	
Escherichia	87	22	10	119	<0.1	25.1
Salmonella	2	0	0	2	NS	0.4
Citrobacter	2	2	1	5	NS	1.1
Klebsiella	35	12	5	52	NS	10.9
Enterobacter	6	3	2	11	NS	2.3
Proteus	11	3	2	16	NS	3.4
Haemophilus	5	5	0	10	<0.1	2.1
Streptococci						
S. pneumoniae	14	3	3	20	NS	4.2
Group A	6	1	1	8	NS	1.7
Group D	27	5	4	36	NS	7.6
Other groups	0	0	1	1	NS	0.2
Viridans	30	4	5	39	NS	8.2
Acinetobacter	0	4	1	5	NS	1.1
Alcaligenes	1	1	0	2	NS	0.4
Bacteroidaceae	23	10	3	36	<0.1	7.6
Staphylococci						
S. aureus	45	20	11	76	NS	16.0
S. epidermidis	7	19	15	41	NS	
Peptostreptococcus	0	1	0	1	NS	0.2
Peptococcus	0	2	0	2	NS	0.4
Pseudomonas	2	25	0	27	<0.001	5.7
Candida	0	2	0	2	NS	0.4
Torulopsis	0	2	0	2	NS	0.4
CDC group IIIA	1	8	4	13	NS	

[a] Total = 642.
[b] By chi square analysis, for difference between media.
[c] Based on total positive minus 166 presumed contaminants equals 475.

differences in detection rates between these two media. Mean times to detection of positivity in each medium generally were similar (Table 2). The cumulative percentages of cultures positive, by days of incubation, for some of the more commonly encountered genera or groups are shown in Table 3. The presumed contaminants *Bacillus* and *Corynebacterium* (including *Propionibacterium*) had prolonged mean detection times, and fewer than 50% were isolated within the first week of incubation. At least 90% of the isolates listed in Table 3, exclusive of *Staphylococcus epidermidis*, were detected within the first week of incubation. Of interest was the finding that approximately 75% of Bacteroidaceae were detected within the first 3 days of incubation. The number and distribution of patients positive by organism group are listed in Table 4.

During the second phase of this study there were 2,977 blood culture sets (TSB and Thiol bottles and aerobic and anaerobic Vacutainer tubes), among which there were 362 culture sets in which one or more of the four media were positive. For simplicity of presentation, only results pertinent to those groups of organisms in which differences in rates of detection occurred are presented (Table 5). In no instance was either the aerobic or anaerobic Vacutainer tube significantly better than TSB or Thiol in detecting the presence of bacteria. In the cases of *Corynebacterium* (including *Propionibacterium*) and *Pseudomonas*, the statistically significant differences among the four media were entirely attributable to the higher detection rates of those two groups of bacteria in TSB. Significantly greater rates of detection ($P < 0.01$) in TSB or Thiol were noted, however, with *E. coli*, *Enterobacter*, viridans and group A streptococci, Bacteroidaceae, and staphylo-

TABLE 2. *Mean time interval to detection of positivity*

Organism	TSB		Thiol	
	No.	Mean (days)	No.	Mean (days)
Bacillus	7	7.0	7	10.0
Clostridium	3	1.3	3	1.3
Corynebacterium	78	8.6	28	10.1
Escherichia	109	1.8	97	1.7
Salmonella	2	2.0	2	2.0
Citrobacter	4	1.5	3	3.6
Klebsiella	47	2.6	40	2.1
Enterobacter	9	2.2	8	1.3
Proteus	14	1.8	13	2.0
Haemophilus	10	2.9	5	7.2
Streptococci				
S. pneumoniae	17	1.4	17	1.8
Viridans	34	3.0	35	2.8
Group A	7	1.0	7	1.7
Group D	32	1.5	31	1.8
Other groups	0	0	1	1.0
Acinetobacter	4	2.0	1	3.0
Alcaligenes	2	3.5	1	5.0
Bacteroidaceae	33	3.7	26	3.7
Staphylococci				
S. aureus	65	3.9	56	3.5
S. epidermidis	26	3.8	22	6.4
Peptostreptococcus	1	3.0	0	0
Peptococcus	2	5.0	0	0
Pseudomonas	27	3.1	2	2.5
Candida	2	4.5	0	0
Torulopsis	2	12.5	0	0
CDC group IIIA	9	4.0	5	4.8

TABLE 4. *Number and percentage distribution of patients with positive cultures, by organism*

Organism	No.	%	% Adjusted[a]
Bacillus	11	2.9	
Clostridium	3	0.8	1.3
Corynebacterium	88	26.0	
Escherichia	60	17.8	25.9
Salmonella	1	0.3	0.4
Citrobacter	2	0.5	0.9
Klebsiella	21	5.5	9.1
Enterobacter	6	1.6	2.6
Proteus	8	2.1	3.4
Haemophilus	5	1.3	2.2
Streptococci			
S. pneumoniae	11	2.9	4.7
Viridans	17	4.5	7.3
Group A	4	1.1	1.7
Group D	12	3.2	5.2
Other groups	1	0.3	0.4
Acinetobacter	5	1.3	2.2
Alcaligenes	2	0.5	0.9
Bacteroidaceae	17	4.5	7.3
Staphylococci			
S. aureus	33	8.7	14.2
S. epidermidis	38	11.2	
Peptostreptococcus	1	0.3	0.4
Peptococcus	2	0.5	0.9
Pseudomonas	18	4.7	7.8
Candida	2	0.5	0.9
Torulopsis	1	0.3	0.4
CDC group IIIA	11	3.3	

[a] Excluding 148 presumed contaminants.

TABLE 3. *Cumulative percentage positive of some commonly isolated species, by medium*

Organism	TSB						Thiol					
	By day (%)					No. positive	By day (%)					No. positive
	1	2	3	4	7		1	2	3	4	7	
Bacillus			14	43		7					43	7
Clostridium	67	100				3	67	100				3
Corynebacterium				3	41	78	4				21	28
Escherichia	70	88	91	92	96	109	55	93	96		99	97
Klebsiella	43	79	81		96	47	48	88		90	98	40
Enterobacter	67	78			100	9	75	100				8
Proteus	58	86	93		100	14	38	85		92	100	13
Haemophilus		20	90	100		10		20		40	100	5
Streptococci												
S. pneumoniae	59	100				17	29	94	100			17
Viridans	32	59	71	74	97	34	34	69	83		94	35
Group A	29	100				7	67	100				7
Group D	72	97			100	32	61	94	97			31
Bacteroidaceae	6	39	73		94	33	8	50	77		90	26
Staphylococci												
S. aureus	26	65		71	86	65	14	45	70	73	93	56
S. epidermidis	8	31	62	69	96	26	9	18	23	32	68	22
Pseudomonas	4	44	81	93	96	27	50	100				2

TABLE 5. *Comparison of TSB, Thiol broth, and aerobic and anaerobic Vacutainer culture tubes with supplemented peptone broth*

Organism	TSB		Thiol		Supplemented peptone broth in Vacutainer tubes				P^a
					Aerobic		Anaerobic		
	No. positive	Days (mean)	No. positive	Days (mean)	No. positive	Days (mean)	No. positive	Days (mean)	
Corynebacterium[b]	51	8.2	13	12.5	13	8.4	11	7.4	<0.01
Escherichia coli	87	2.0	81	1.8	34	2.1	36	1.8	<0.01
Enterobacter	12	1.0	12	1.9	2	1.0	3	1.3	<0.01
Haemophilus	4	9.8	2	12.0	0		0		[c]
Streptococci									
Viridans	17	1.8	14	2.4	6	1.7	7	1.6	<0.01
Group A	15	1.7	13	2.7	6	1.3	10	1.5	<0.01
Group D	6	1.5	6	1.3	2	2.0	3	1.7	[c]
Alcaligenes	8	2.3	1	2.0	2	5.0	1	3.0	[c]
Bacteroidaceae	24	2.3	26	2.5	9	3.4	8	3.4	<0.01
Staphylococci									
S. aureus	52	2.9	38	4.1	23	3.1	20	3.6	<0.01
S. epidermidis	41	4.5	30	5.4	17	3.2	16	4.2	<0.01
Pseudomonas	28	4.0	3	6.7	12	3.9	7	5.4	<0.01
Candida	6	3.2	2	4.0	2	4.5	1	2.0	[c]

[a] For hypothesis that proportions of positives are the same in all four media.
[b] Includes *Propionibacterium*.
[c] Although $P < 0.05$ in these instances, the sample sizes were too small for determination of significance.

cocci. Greater rates of detection in TSB or Thiol were noted with *Haemophilus*, group D streptococci, *Alcaligenes*, and *Candida*; however, in these instances the sample sizes were too small for valid analysis.

DISCUSSION

At least in regard to the organisms isolated during this study, TSB appears to be superior to Thiol in detecting bacteremia, and the addition of SPS to these two media has eliminated the advantage that Thiol formerly had over TSB with respect to streptococci (and aerobic and anaerobic *Corynebacterium*, for what this is worth). Furthermore, it should be emphasized that the convenience offered by the aerobic and anaerobic Vacutainer culture tubes with supplemented peptone broth is offset by some statistically significant deficiencies in detection rates of certain groups of bacteria. Although this medium (along with supplemented prereduced brain heart infusion-yeast extract broth) has been recommended for blood cultures for anaerobes (2), our data indicate that it missed a substantial number of Bacteroidaceae that were detected concurrently in TSB and Thiol. Previous data (6) from this laboratory have also shown the equivalence of TSB, Thiol, and supplemented and prereduced brain heart infusion-yeast extract broth in recovery of anaerobic bacteria from blood. It seems reasonable to ascribe much of the discrepancy between recovery rates of bacteria in the supplemented Vacutainer culture tubes and in TSB and Thiol broths to the marked difference between volumes of blood used in these two approaches—only 2 ml into each tube versus 10 ml into each bottle. Quantitative studies by Sullivan et al. (4) have shown most bacteremias to be of a fairly low order of magnitude.

It should be stressed that, in addition to composition of media, the variables in blood cultures include volume of blood sampled, atmosphere and duration of incubation, frequency of examination, frequency and types of routine "blind" subcultures, and manner of processing of recognized positive cultures. There is no standardization of any of these aspects of blood culture. Blood culture data, therefore, must be interpreted cautiously, and comparative data certainly must be interpreted in terms of the media and systems being compared.

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