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Controlled Clinical Evaluation of Isolator and ESP Aerobic Blood Culture Systems for Detection of Bloodstream Infections

BARBARA A. KIRKLEY,[1] KIRK A. EASLEY,[2] AND JOHN A. WASHINGTON[1]*

Department of Clinical Pathology[1] and Department of Biostatics and Epidemiology,[2] The Cleveland Clinic Foundation, Cleveland, Ohio

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A controlled clinical evaluation comparing the Isolator system (Wampole Laboratories, Cranbury, N.J.) and the ESP 80A blood culture bottle in the automated ESP system (Difco Laboratories, Detroit, Mich.) was performed with 10,535 blood culture sets from patients with suspected septicemia. Of 1,150 positive cultures, 844 positive cultures from 285 patients with 394 septic episodes fulfilled the study criteria for minimum blood sample requirements in each system and clinical significance of isolates. The Isolator system detected statistically significantly more positive cultures of *Staphylococcus aureus* ($P < 0.001$), *Enterococcus* spp. ($P = 0.007$), *Escherichia coli* ($P = 0.001$), *Alcaligenes xylosoxidans* ($P = 0.02$), *Xanthomonas maltophilia* ($P = 0.01$), *Candida albicans* ($P < 0.001$), and *Candida glabrata* ($P = 0.05$). The Isolator system detected significantly more septic episodes due to *S. aureus* ($P < 0.001$), *X. maltophilia* ($P = 0.02$), and *C. albicans* ($P = 0.004$) than did the ESP 80A bottle; however, the two systems did not otherwise significantly differ in their abilities to detect septic episodes due to other organisms.

The ESP blood culture system (Difco Laboratories, Detroit, Mich.) is a novel system for detecting bacterial growth in broth media. As described by Morello et al. (4), the system uses an electronic sensor to detect pressure changes caused by oxygen consumption and gas production resulting from microbial growth in a closed bottle. The system is automated, provides for aerobic and anaerobic blood culture incubation, and can contain up to 384 bottles. Bottles in the lower shelves for aerobic bottles are agitated, whereas those in the upper shelves for anaerobic bottles are stationary.

The objective of this study was to compare the ESP 80A aerobic bottle with the Isolator. The study compared the recovery of aerobic and facultatively anaerobic bacteria and yeasts from each system inoculated with equivalent volumes of blood at the patient's bedside and incubated under aerobic conditions in the laboratory. Anaerobic cultures were not performed routinely, on the basis of documented background epidemiologic data analysis at this institution over the past 8 years with an aerobic-anaerobic blood culture system, during which time the incidence of septic episodes due to anaerobic bacteria consistently remained well below 1%.

MATERIALS AND METHODS

Blood was collected aseptically with needle and syringe by phlebotomists from patients with suspected sepsis and inoculated at the patient's bedside on an equivalent volume basis into an Isolator tube and an aerobic ESP 80A bottle. During the first half of the study, the Isolator tube was inoculated first, whereas during the second half of the study the ESP bottle was inoculated first. Only those culture sets in which each system contained at least 6 ml of blood were included in the analysis. The volume of blood per container was assessed at the time of specimen accession in the laboratory by comparing the height of the column of blood in the Isolator tube and that of the blood-broth mixture in the bottle with those of volume standards in each system. Care was also taken by the phlebotomists not to fill any ESP bottle with more than 10 ml of blood, since according to the manufacturer, overfilling the bottle would increase the false-positive rate.

The Isolator sediment was inoculated onto blood agar and chocolate agar, which were incubated at 35°C for a full 72 h in an atmosphere of increased CO_2, and onto brain heart infusion blood agar and potato dextrose agar, which were incubated at 30°C for 7 days. Incubation of the blood agar and chocolate agar plates for a minimum of 72 h was done on the basis of prior statistical regression analysis in which clinically significant isolates of aerobic and facultatively anaerobic bacteria were not detected on these media beyond a full 72 h of incubation (our unpublished observations). No anaerobic cultures of the Isolator sediment were performed. Isolators were processed between 7 a.m. and 11 p.m. daily. Plates inoculated with the sediment were examined twice during their first 24 h of incubation and daily thereafter. Isolator tubes received after 11 p.m. were stored at room temperature until the following morning, when they were processed.

Upon receipt in the laboratory, the ESP bottle was processed according to the manufacturer's instructions. Bottles received after 11 p.m. were incubated off site and then delivered to the microbiology laboratory at 7 a.m. for processing. Prior to incubation of the bottle, a special connecting device was placed on the bottle. This connector, which allows the detection of gas consumption and production, automatically vents the bottle. Bottles with attached connectors were accessioned into the ESP system and placed in their designated positions in the lower agitating location of the ESP unit. Bottles were removed when a signal indicating positivity appeared or at the end of 7 days of incubation. Because of staffing limitations, bottles with a positive signal between 4 p.m. and 7 a.m. were not worked up until after 7 a.m. At the end of 7 days of incubation, negative bottles were routinely subcultured. Isolates from positive cultures were identified by standard techniques, and their clinical significance was established according to definitions for bloodstream infections published by the Centers for Disease Control and Prevention (1). Statistical analysis was carried out by methods described by Ilstrup

* Corresponding author. Mailing address: Department of Clinical Pathology, The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44195-5140. Phone: (216) 444-4435. Fax: (216) 445-6984.

TABLE 1. Positive cultures by system

Organism(s)	No. of positive cultures detected				P
	Total	By Isolator only	By ESP only	By both systems	
Staphylococcus aureus	241	109	6	126	<0.001
Staphylococcus epidermidis	129	35	28	66	NS[a]
Staphylococcus spp. (coagulase negative, other)	40	12	12	16	NS
Streptococcus viridans group	7	0	4	3	NS
Streptococcus pneumoniae	6	1	1	4	NS
Enterococcus spp.	51	19	6	26	0.007
Acinetobacter anitratus	15	4	3	8	NS
Alcaligenes xylosoxidans	11	7	0	4	0.02
Citrobacter diversus	7	1	0	6	NS
Enterobacter cloacae	14	4	1	9	NS
Escherichia coli	50	22	6	22	0.001
Klebsiella oxytoca	11	2	2	7	NS
Klebsiella pneumoniae	24	7	5	12	NS
Serratia marcescens	7	2	0	5	NS
Pseudomonas aeruginosa	41	9	11	21	NS
Xanthomonas maltophilia	19	10	1	8	0.01
Candida albicans	127	40	9	78	<0.001
Candida glabrata	44	13	4	27	0.05

[a] NS, not significant.

(2). A new septic episode was defined as the initial isolation of a clinically significant organism, the subsequent isolation of a different clinically significant organism, or the isolation of the same organism after at least a 5-day interval since the previous positive culture with this organism.

RESULTS AND DISCUSSION

Between March and September 1993, a total of 10,535 blood culture sets were collected. A total of 1,150 cultures were positive, of which however, only 844 from 285 patients satisfied the inclusion criteria for analysis (i.e., the appropriate volume of blood per system and clinical significance of the isolate). Table 1 contains the listing of genera and/or species represented by at least six isolates. Statistically significant differences between the two systems were observed with *Staphylococcus aureus*, *Enterococcus* spp., *Escherichia coli*, *Alcaligenes xylosoxidans*, *Xanthomonas maltophilia*, and *Candida albicans*. In each instance, detection was significantly greater in the Isolator system. In Table 2, a variety of species represented by fewer than six isolates are listed. With the exception of anaerobic species which were detected only by the ESP system (Isolator sediment was not cultured anaerobically), the trend towards greater detection by the Isolator system was also noted. There were totals of 28 of these isolates from the Isolator system only, 4 (excluding 7 anaerobes) from the ESP system only, and 27 from both systems. Table 3 lists the number of episodes of bloodstream infections by system. There was a total of 394 septic episodes. Statistically significant differences between systems for *S. aureus*, *C. albicans*, and *X. maltophilia* were noted.

The reason for the major discrepancy between systems with *S. aureus*, regardless of how the data were analyzed, is not entirely clear, but the discrepancy is consistent with our prior observations in comparisons between Isolator and broth-based systems (3, 5). In most instances, ESP 80A failed to detect *S. aureus* when there were only one or two colonies of *S. aureus* on a single plate in the concurrent Isolator sediment culture. Although it is possible that some of these cultures yielding only one or two colonies of *S. aureus* only in the Isolator system represented contaminants, a preliminary result of a recent clinical review in our institution (unpublished observations) has demonstrated that at least 50% of such isolates of *S. aureus*

TABLE 2. Other organisms detected from positive cultures

Organism(s)	No. of positive cultures detected by:		
	Isolator only	ESP only	Both systems
Aerococcus viridans	1		
Corynebacterium spp.	2	1	1
Propionibacterium acnes		3	
Clostridium spp.		1	
Stomatococcus mucilaginosus			1
Streptococcus group B			6
Streptococcus group G			1
Streptococcus bovis	2		2
Citrobacter freundii	1		
Enterobacter aerogenes	5		2
Enterobacter agglomerans		1	
Enterobacter taylorae	1		2
Proteus mirabilis	3		2
Proteus vulgaris			1
Salmonella spp.		1	3
Serratia liquefaciens	1		
Flavobacterium indologenes	1		
Flavomonas oryzihabitans	1		
Pseudomonas putida	1		
Pseudomonas spp.	1		
Bacteroides fragilis		2	
Bacteroides vulgatus		1	
Candida guilliermondii	1		
Candida krusei		1	1
Candida lusitaniae	1		4
Candida parapsilosis	1		
Candida tropicalis	2		
Candida spp.	1		
Cryptococcus neoformans	1		
Rhodotorula glutinis	1		
Rhodotorula rubra			1

TABLE 3. Episodes of bloodstream infections detected by system

Organism(s)	No. of episodes of bloodstream infections detected				P
	Total positive	By Isolator only	By ESP only	By both systems	
Staphylococcus aureus	86	34	1	51	<0.001
Staphylococcus epidermidis	74	17	19	38	NS[a]
Staphylococcus spp. (coagulase negative, other)	34	12	10	12	NS
Streptococcus viridans group	5	0	3	2	NS
Streptococcus pneumoniae	4	1	0	3	NS
Enterococcus spp.	26	9	6	11	NS
Acinetobacter anitratus	7	2	1	4	NS
Alcaligenes xylosoxidans	3	1	0	2	NS
Citrobacter diversus	3	1	0	2	NS
Enterobacter cloacae	9	2	1	6	NS
Escherichia coli	36	13	6	17	NS
Klebsiella oxytoca	4	0	2	2	NS
Klebsiella pneumoniae	8	2	2	4	NS
Serratia marcescens	4	1	0	3	NS
Pseudomonas aeruginosa	21	4	6	11	NS
Xanthomonas maltophilia	11	7	0	4	0.02
Candida albicans	45	18	4	23	0.004
Candida glabratra	14	5	3	6	NS

[a] NS, not significant.

were found clinically significant. It should also be noted that the vast majority of isolates of enterococci, *E. coli*, and *Candida* spp. detected by Isolator only also represented cultures with colonies present on only one of the four agar media inoculated with the Isolator sediment. Whether these "low-order" bacteremias and fungemias would have been detected by the ESP system had an additional 10 ml of blood been obtained with each blood culture set and inoculated into an anaerobic ESP bottle cannot be answered by our study.

There were 175 false-positive ESP bottles in the study, for an overall prevalence of 1.7%. In all of these instances, there were a positive signal from the ESP unit and negative gram-stained smears and subcultures. A strict comparison of this false-positive rate with those published for other automated systems is not possible, since our rate reflects that of a single bottle only. Terminal subcultures of negative ESP bottles yielded 52 isolates, of which 10 were considered clinically insignificant. All but six of the remaining isolates had previously been detected in the Isolator system.

Statistically significant differences in detection times between systems (when both were positive) were limited to *S. aureus* ($P < 0.001$) and *C. albicans* ($P < 0.001$). With *S. aureus*, the mean and median numbers of days were, respectively, 1.2 and 1.0 in the Isolator system and 1.8 and 1.0 in the ESP system. The corresponding numbers of days for *C. albicans* were 1.9 and 2.0 in the Isolator system and 4.0 and 2.0 in the ESP system.

In conclusion, the Isolator system detected significantly more positive cultures than the ESP 80A bottle in the ESP system; however, this difference was limited to *S. aureus*, *X. maltophilia*, and *C. albicans* when the data were analyzed by septic episode.

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