COMPARISON OF A NEW AUTOMATED NONRADIOMETRIC BACTEC SYSTEM AND CONVENTIONAL METHOD FOR PEDIATRIC BLOOD CULTURE

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ABSTRACT

A prospective study of 450 paired blood culture comparing the Bactec NR 730 System and the Conventional Method was done in the Philippine Children's Medical Center among patients diagnosed to have clinical sepsis and treated with antimicrobial therapy. There was a total of 123 clinically significant positive isolates of organism. The Bactec method recovered significantly more organisms than the conventional method (p=0.003). The earliest time of positivity was 18 hours in Bactec versus 72 hours in the conventional medium. The mean positive isolates detected per day was 16.5 in Bactec versus 5.9 in the conventional. The cumulative percentage of positivity with 100% detection was accomplished 3 days earlier in the Bactec system. This study has collaborated earlier reports of the superior advantage of the Bactec system in detection of blood-borne pathogens in infants and children.

Key words: Bactec System
Blood Culture

INTRODUCTION

Sepsis remains to be a major cause of morbidity and mortality in the pediatric age group. Therefore prompt etiologic diagnosis is vital for definitive treatment and survival of septic infants and children.

The method commonly used to detect bacteremia or fungemia is the use of conventional blood culture bottles and visual inspection. Unfortunately, some drawbacks in the blood culture obtained by the conventional method after patients were started on empiric antibiotics prior to obtaining blood samples more often than not fails to yield viable organism. Delay in detection of positive cultures and contamination of culture media are factors likewise observed in some laboratories, which may affect the prompt management of patients with sepsis.

Newer methods need to be utilized for rapid but equally accurate isolation of the blood-borne pathogens that will save labor and favour minimal recovery of extraneous contaminants. Presently, the nonradiometric growth detection system which recognizes bacterial growth in blood culture by detection of carbon dioxide production by the microorganism, is currently in use in most major centers. Some institution here in the Philippines, now utilize this system: the Bactec NR 730, an infrared spectrophotometric method for aerobic, anaerobic, and fungal cultures. Published foreign literature have reported superior evaluation of this method in comparison to the conventional method (1). However, local experience is still limited. This paper aims to evaluate the effectiveness of the Bactec and the conventional system in a local pediatric center.

OBJECTIVES

To compare the effectiveness of the Bactec and the conventional culture method:

1. To identify the microorganisms recovered utilizing both techniques
2. To determine the percentage of clinically significant isolates by comparison, and
3. To determine the speed of recovery of clinically significant isolates using the Bactec and conventional method

METHODOLOGY

Subjects

All pediatric patients admitted at the Philippine Children's Medical Center between June to September 1995, diagnosed to have clinical sepsis (2), started on empiric antimicrobial therapy prior to blood sample collection, were included in the study.

Blood Sampling, Incubation, and Processing of Specimens

The site for venipuncture is cleansed with 70% alcohol followed by povidone-iodine in circular motion and cleansed again with 70% alcohol (3). The pediatric resident and medical technologist wore sterile gloves during extraction to minimize contamination by skin flora. Two ml of blood were drawn from each subject, 1 ml of which was immediately inoculated into a bottle of Bactec Peds

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plus and 1 ml into a bottle with conventional medium for aerobic blood culture. The conventional and Bactec Peds plus bottles were then incubated simultaneously at 37°C, up to 10 days for the conventional and 7 days for Bactec.

Criteria for Positivity

The positive criteria for conventional blood culture is the existence of bacterial colonies on the agar slant or turbidity of the medium. The positive criteria for Bactec system was based on the Growth Value (GV) number. The reading reaching threshold equal to or greater than 20 was considered presumptively positive and a growth value reading less than 20 was considered negative for microorganism. The high Growth Value number (20) was confirmed by gram stain smear and subculture. Identification of the organism and sensitivity test was done using the standard blood culture method.

Bactec Peds plus resin containing medium: was an enriched soybean-case in digest broth with resins for aerobic cultures used with the Bactec NR instrument. It consists of nonionic absorbing and cationic exchange resins. The resin beads in Bactec medium is used for neutralizing the antimicrobial agents and allowing the organism to grow in the blood culture bottle for rapid detection of bacterial infection.

Description of the Bactec NR 730: a blood culture system made use of an infrared spectroscopy to detect carbon dioxide that was produced by the organisms. It consisted of two parts: the first part is incubator and rotary shaker, and the second part is a test module containing the measurement system, power supplies, tray transport and test head assembly that performs the actual blood culture vial testing with the vial test data.

Recording Analysis of Data

The following data were obtained and recorded for each set of positive bottles for both the conventional and Bactec systems - bottle identification, time of sample collection, results and positivity for conventional and Bactec vials, subculture results, organism identification, and use of antimicrobial therapy.

Statistical Analysis

Paired comparison of the two blood culture systems was done using the paired t test.

Definition of Terms

1. Clinical sepsis: Clinical suspicion of infection and evidence of systemic response to infection not attributable to any other disease entities. This systemic response is manifested by two or more of the following conditions as a result of infection:

   Temperature 38°C or 36°C (rectal)
   Heart rate (infants >160 beats/min; children >150/min)
   Resp rate (infants >60/min; children >50/min)
   White blood cell count >12,000 cells/cu ml,
   <4,000 cells/cu ml, or >10% immature (band) forms

2. Time of Positivity: started from the time blood samples were collected in the wards up to the time a positive organism was identified in the gram stain.

3. Criteria for clinically significant isolates included one or more of the following (1,4):
   1. Both bottles of a set showed positive cultures
   2. Same organism grown on other specimens from the same patient (eg. CSF)
   3. Organism identity (the isolated organism is unusual to cause contaminant)
   4. Infectious disease service consulted
   5. Clinical sign and symptom compatible with sepsis

4. Contamination: Bacterial isolates in only one bottle of the set with the other bottle yielding no growth; or isolation of 2 or more organisms in one bottle.

RESULTS

A total of 450 subjects who met the inclusion criteria were included in the study. A total of 450 sets of blood were cultured. There were 123 clinically significant positive cultures. 51 were identified in both media, 23 in the conventional media alone, and 49 in Bactec media alone. All significant microorganisms isolated were similar in both blood culture methods. The two leading microorganisms isolated were members of the Enterobacteriaceae and cougulase-negative staphylococci. In this study, the Bactec system detected significant isolates 81.3% as compared to the conventional method which detected 60.2% only. The Bactec system significantly recovered more organisms in patients on antimicrobial therapy as compared to the conventional method with p value <0.005.

Contaminants were recovered in 25 of 74 (33%) of the positive growth in conventional culture. Those identified were Staphylococcus epidermidis, Staphylococcus aureus, Pseudomonas species, Acinetobacter species, Escherichiae coli, and Candida species. In contrast, Bactec showed no contamination.

Table 1 *Blood culture isolates recovered by the Bactec and conventional system from June to September 1995 at PCMC*

MICROORGANISM	CONVENT	BACTEC	CONVENTIONAL & BACTEC	TOTAL ISOLATES
Aerobic Gram Negative Bacilli				
• Pseudomonas spp.	1	8	15	24
• Acinetobacter spp.	5	9	5	19
• Enterobacter spp.	1	6	8	15
• Salmonella typhi	0	1	3	4
• Klebsiella spp.	0	0	4	4
Escherichiae coli	0	1	2	3
Alkaligenes spp.	0	1	2	3
Citrobacter spp.	0	1	0	1
H. influenzae	0	1	0	1
Aerobic Gram Positive Cocci				
• Coag(-) Staph	11	13	11	35
Coag(+) Staph	5	2	0	7
Strep. pneumoniae	1	3	0	4
Strep. pyogenes	0	0	1	1
Fungi				
Candida spp.	1	3	0	4
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	23	49	51	123

Table 2 and 3 shows the time required for the detection of positivity by the Bactec NR 730 system and conventional method respectively . The earliest time of positivity for clinically significant isolate in Bactec was 18 hours while the conventional method was 72 hours. There were 3 out of 99 (3.0%) organisms detected early by Bactec on the day of receipt of the blood specimen. These were Haemophilus influenzae, Streptococcus pneumoniae and Staphylococcus aureus. However, in the conventional system none was isolated until the third day after blood sampling. More so, 100% of the bacterial isolates were detected on day 5 using Bactec and day 8 with conventional method. The cumulative percentage of the significant isolates detected on subsequent days were compared between the two methods as shown in Figure 1. A 100% detection was accomplished three days earlier in the Bactec system. The mean positivity of isolates per day for Bactec was 16.5 organisms (base=99), while that for the conventional method was 5.9 organisms per day (base=47). This difference was statistically significant with p value <0.001.

DISCUSSION

Antigen detection and DNA probe methods are currently available for rapid detection of microbial agents in other specimens. However, cultures remain to serve as a gold standard for the diagnosis of bacteremia and fungemia.

The conventional broth culture method is the most thoroughly investigated and utilized system. It involves inoculation of the blood specimen to liquid medium in bottles followed by monitoring of growth by manual noninstrumented means such as visual examination for turbidity, hemolysis, gas production or colonies adherent to the vessel walls; microscopic examination of stained broth means; and subculture of broths to agar media. Thus, this is very labor intensive (5).

Combination of the conventional broth culture bottle to an attractable agar medium-coated slide brought about the biphasic agar/broth culture (Septi-Chek) this was introduced as a more convenient alternative to the conventional method especially effective for recovery of fungi.

Several semi-automated and fully automated instruments have become available for early detection of positive isolates. These instruments include the Bactec 460, 660, 730, 860 and 9240 (the latest) series (Becton Dickinson), BactT/Alert (Oragnon-Tehnika), ESP (Difco) and Vital (BioMeriru-Viteh). Aside from detecting carbon dioxide production from microbial metabolism, this measures consumption of atmospheric gases during microbial growth. Methods used include radiometry (CO2 derived from radiolabeled growth substrates), infrared spectroscopy, fluorometry and pH sensors. All documented to detect positive cultures early with up to the minute information on status of all cultures.

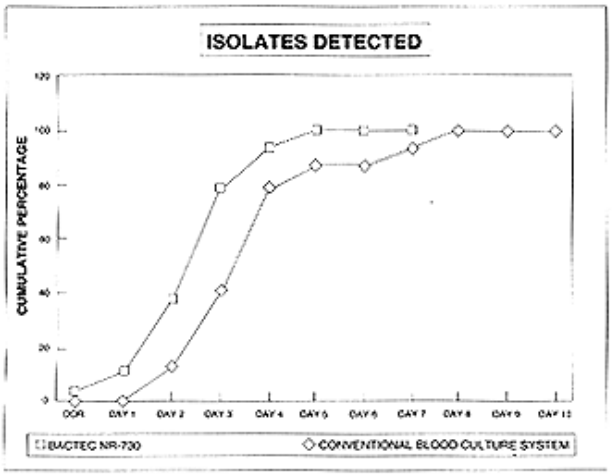
Table 2 *Organisms detected by Bactec NR-450*

	DOR	1	2	3	4	5	6	7	TOTAL POSITIVE
Escherichia coli	1	1	1						3
Enterobacter sp.		5	3	6					14
Pseudomonas sp.			9	9	4	1			23
Acinetobacter sp.		1	3	7	2	1			14
Klebsiella sp.		1	2	1					4
Salmonella typhi					4				4
Alkaligenes sp.			1		2				3
Coag. neg. Staphy			5	15	3	1			24
Coag. pos. Staphy	1		1						2
Strep. pneumoniae			1	1		1			3
Hemophilus influenzae	1								1
Citrobacter			1						1
Fungi (Candida)				2		1			3
Percentage of isolates by day of detection	3.0	8.1	26.3	46.4	15.2	5.1	0	0	---
Cumulative percentage	3.0	11.1	37.4	79.8	93.9	100	100	100	

Table 3 Organisms detected by conventional blood culture system

	DOR	1	2	3	4	5	6	7	8	9	10	TOTAL POSITIVE
Escherichia coli				1	1							2
Enterobacter sp.			2	3	2	1			1			9
Pseudomonas sp.			2	4	5	3			2			16
Acinetobacter sp.			1	5	4							10
Klebsiella sp.			1	2	1							4
Salmonella typhi					3							3
Alkaligenes					2							2
Coag. neg. Staphy			2	4	12	1		3				22
Coag. pos. Staphy				1	2							3
Strep. pneumoniae						1						1
Hemophilus influenzae												0
Citrobacter				1								1
Fungi (Candida)				1								1
Total isolates	0	0	8	22	32	6	0	3	3	0	0	74
Total positive isolates	0	0	6	13	18	4	0	3	3	0	0	47
% of total positive isolates	0	0	12.8	17.7	38.3	8.5	0	6.4	6.4	0	0	
Cumulative %	0	0	12.8	40.4	78.7	87.2	0	93.6	100	100	100	

Fig. 1 Cumulative percentage of positive isolates detected on subsequent days between two blood culture methods



It was neither the Bactec nor the conventional culture system recovered all the clinically significant isolates in our study, the conventional method yielded negative growth in 39.8% and 18.7% in Bactec, as observed by Kumaringhe and co-workers (16.1% vs 19.4%) (6). This can be explained by either no organism grew at all or the causative agents were anaerobic or viral. The most common microorganisms isolated in both methods were members of the Enterobacteriaceae and coagulase-negative staphylococcus, and were also similar to those detected by Kumaringhe. Antibiotics present in the blood that was inoculated in the culture broth could influence the blood culture positivity by inhibiting the growth of the microorganism (7).

In our series, the conventional method had a high rate of contamination which is comparable with the 26.2% contamination rate of Kumaringhe (6). As expected, the use of Bactec NR-730 reduced the frequency of contamination owing to the closed nature of the system. This system also had an improved needle sterilization system that heated the entire length of the needle to minimize contamination (8).

A more obvious advantage of the Bactec system in this study was the earlier detection of positive cultures. In the Bactec system, there were three out of 99 (3.0%) significant positive cultures on the day of receipt of the blood specimen. However, in the conventional system none was isolated until the third day after blood sampling. More so, 100% of the isolates was recovered on day 5 using Bactec but was obtained on day 8 with the conventional system. This was also consistent finding of Kumaringhe demonstrated quicker detection of positive blood cultures with the Bactec. Indeed, for rapid microbial identification and susceptibility tests, this new system has an indisputable advantage for clinicians for prompt and more rationale use of antimicrobials in a septic patient.

CONCLUSION

Bactec NR-730 is proven more advantageous than the conventional culture method detection of clinically significant bloodborne pathogens in septic patients receiving antimicrobial therapy.

RECOMMENDATION

1. The Bactec NR-730 system, an infrared spectroscopic semi-automated instrument with the use of Peds plus broth is recommended in local laboratories owing to its superior advantage of early and accurate identification of positive cultures.

2. A prospective multicenter study with bigger sample size to determine the sensitivity, specificity and predictive value of the Bactec NR system in septic patients is recommended.

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Ang pakikisama at pakikipagkapwa kapag maagang nakagisnan, nakakatulong sa paghubog ng katauhan