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Original Research Article

A Study on the Outcome of Routine Blood Cultures by Conventional Methods in Relation to the Time of Incubation

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Abstract: Presence of microbes in blood i.e. bacteremia carries high risk of morbidity and mortality. Blood cultures form a critical part of evaluation of patients with suspected sepsis. The present study was undertaken to study the effect of duration of incubation for obtaining positive cultures. A total of 220 samples from 107 pediatric patients presenting with suspected bacteraemia were processed aerobically. Cultures were positive in 24.3% of the samples. Most of the positive cultures (76.9%) were obtained after 24 hours of incubation of the broth and no isolates were obtained beyond day 4 of incubation. Incubation beyond four days (unless with specific indication like enteric fever) may be unnecessary for issuing a negative culture report.
Keywords: Blood culture, Time of incubation, bacteremia

INDTRODUCTION

Presence of microbes in blood i.e. bacteremia carries high risk of morbidity and mortality. The detection of microorganisms in a patient's blood has great diagnostic and prognostic significance. Blood culture is the essential investigation for the management of sepsis. Blood cultures provide essential information for the evaluation of a variety of diseases like endocarditis, pneumonia, and pyrexia of unknown origin and particularly, in patients with suspected sepsis. [1]. Many infections in neonatal and pediatric age group can only be established on the basis of etiological agent recovered from blood. One key determinant in the ultimate outcome of patients with sepsis is institution of early and appropriate antimicrobial therapy.

It is not easy to establish a definite diagnosis of sepsis in the neonate. Early clinical signs are mostly non-specific and inconclusive. However, outcome will indeed be jeopardized by delayed initiation of therapeutic action. Therefore, broad-spectrum antimicrobial therapy is started immediately if there is any suspicion of sepsis, while awaiting blood culture results. However, the majority of neonatal blood cultures will remain sterile, implying unnecessary use of antibiotics and presumably triggering multidrug resistance [2], the length of treatment is often based as much on the physician's clinical assessment of the infant's status as on the microbiologic results. Sick newborns may be treated with antibiotics for 5 to 10 days if such therapy is warranted by clinical assessment [2] even if blood cultures remain negative. Some asymptomatic newborns are started on antibiotics because of maternal risk factors. Others receive antibiotics for minor symptoms that resolve quickly. Patients in these groups are often treated until it is clear that blood cultures are not positive. A critical issue for these and other infants is the time at which one can assume that blood cultures negative to date will not subsequently become positive. The literature has not resolved the question of when a blood culture can safely be considered negative. Many laboratories continue to observe culture media for 7 to 10 days [3]. The question arises that at what time of incubation, the routine blood cultures (other than for Salmonellosis or Brucellosis, which would require longer incubation periods), is optimal for recovery of the microbes that may influence the treatment. There are several proposals to arrive at a consensus for the same [4].

Keeping this goal in mind, the study was undertaken to know the percentage of recovery of organisms at various incubation times of the broth by conventional methods of blood cultures.

MATERIALS AND METHODS

A total of 107 paediatric in-patients (less than 18 years of age) including 22 neonates admitted in a tertiary care hospital in Hyderabad were included in the study. Patients presented with prolonged fever or clinical impression of septicaemia. Patients having prolonged fever in the postoperative period, despite antibiotic coverage were also included in the study. Detailed history was taken to identify the possible risk factors. History of antibiotic usage empirically either before or after admission was also obtained.

Blood samples for culture were collected following strict aseptic precautions. If empirical antibiotics were already started, the collection was timed before the next dose of antibiotic was due or about half an hour before the predicted peak of temperature. A second set was also collected in all patients about an hour later from a different venipuncture site. Three sets were collected in cases of suspected or sonographically diagnosed congenital heart disease. About 1 mL of blood in case of neonates and about 5 mL in case of children was collected in each set. Immediately after collection, the blood was inoculated into brain heart infusion (BHI) broth without switching needles. The bottles containing 10 mL of BHI broth were used in case of neonates and 50 mL were used for other children to allow 1:10 dilution. The culture bottles were incubated at 37°C aerobically. After overnight incubation, the samples were subcultered onto blood agar, MacConkey's agar, and chocolate agar. If there was no growth observed on the plates by the next day, subcultures were again repeated from the broth on day 3, day 4 and finally on day 7. If there was any growth, it was identified and antibiotic susceptibility tests were performed according to the standard methods [5-8].

RESULTS AND DISCUSSION

A total of 220 samples from 107 children were processed. Cultures were positive in 26 (24.30%) cases. The culture positivity rate was observed to be highest in neonates (52.63%). Risk factors were identified in 38 (35.51%) of the cases. (Table 1)

Table 1: Prevalence of various microorganisms in blood cultures

Sl No	Bacterial isolate	No of isolates
1	Klebsiella spp.	7 (26.92%)
2	Citrobacter spp	6 (23.01%)
3	Staphylococcus aureus	3 (11.50%)
4	Coagulase negative staphylococci	4 (15.38%)*
5	Acinetobacter spp.	2 (7.69%)
6	Escherichia coli	1 (3.84%)
7	Alkaligenes faecalis	1 (3.84%)
8	Others (viridans streptococci, candida)	2 (7.69%)
	Total	26

*(only 1 confirmed in repeat culture)

Administration of empirical antibiotics was already initiated by the time of collection of sample for culture in 71 (66.35%) of the cases. Of these, only 6 (8.45%) had positive cultures with delayed culture growth. The duration of incubation of the broth (24, 48 and 72 hours and 7 days) after which positive cultures were obtained on plating, was also noted (Table 2).

Table 2: isolates versus duration of incubation of the broth before plating

Sl No	Time of incubation → Isolate	24hrs	48hrs	72hrs	7 days	Total
1	Klebsiella spp.	6	1	-	-	7 (26.92%)
2	Citrobacter spp	5	1	-	-	6 (23.01%)
3	Staphylococcus aureus	3	-	-	-	3 (11.50%)
4	Coagulase negative staphylococci	3	1	-	-	4 (15.38%)*
5	Acinetobacter spp.	1	-	1	-	2 (7.69%)
6	Escherichia coli	0	0	1	-	1 (3.84%)
7	Alkaligenes faecalis	1	-	-	-	1 (3.84%)
8	Others (viridans streptococci, candida)	1	1	-	-	2 (7.69%)
		20 (76.9%)	4 (15.38%)	2 (7.69%)	0	26

None of the cultures were positive beyond 72 hours of incubation of the broth. Almost all the isolates were sensitive to cephalosporins and amikacin, while one isolate of Staphylococcus aureus was resistant to β-lactams.

Septicaemia is a clinical syndrome associated with considerable morbidity and mortality. The timely detection of bacteraemia can have a profound influence on the final clinical outcome. One blood culture set is rarely sufficient to establish or rule out bacteraemia, and multiple cultures could maximize sensitivity. We have processed 220 samples from 107 patients including 22 neonates. In our study, the total number of positive cultures was 26 (24.3%). Neonates are particularly vulnerable to infections because of their weak immunological barrier. A high rate of positive cultures (52.63%) was observed in neonates, Klebsiella spp. being the most common (35%) isolate. A study by Jain et al., also showed similar findings [9].

The duration of incubation of the broth to obtain a positive culture was observed to be more among the samples from the patients who were already on antibiotics by the time of collection. This was observed for one isolate each of Acinetobacter spp. and E. coli. Though this was an interesting finding, the number was too small for a statistical evaluation for its significance. Among the positive cultures, we have observed that 76.92% of cultures were positive by first subculture itself (after 24 hours of incubation of the BHI broth), 15.38% and 7.6% of the cultures were positive by second subculture (after 48 hours) and third subculture (after 72 hours), respectively, while virtually no isolates were obtained later (subculture on day 7). Reller *et al.;* suggested that incubation beyond 7 days is generally unnecessary with relatively few clinically significant isolates detected [10].

But, in a recent study, it has been shown that 99.5% of the isolates were detected by day 4 (after 72 hours of incubation of the BHI broth). The predictive value of blood cultures that were negative at day 4 was similar to that of waiting for seven days of processing before discontinuing therapy [11].

In a study, Blood samples from 1647 suspected neonatal septicaemic cases were cultured over a period of four years. Organisms were identified by conventional methods. Of the 1647 samples cultured, 877 were positive by the end of seven days processing period yielding 781 bacterial isolates and 96 yeasts. Five (0.5%) organisms were recovered on the day the culture was submitted (day 0), and 383 (43.7%) of positive cultures were identified by day 1. [3]. Out of 877, 781(89.1%) isolates were detected as positive by day 2, 858 (97.8%) were detected as positive by day 3, and 873 (99.5%) were detected as positive by day 4. The predictive value of blood cultures that were negative at day 4 was clinically similar to that of waiting for 7 days of processing before discontinuing therapy. No septic infants were missed in the study, since growth is not the only parameter taken into account for the treatment of newborns. The concluded that four days processing period will detect positive blood culture of virtually all important infections and clinical benefit from continuing blood culture processing beyond four days does not justify the time and cost involved.

CONCLUSIONS

In a high burden and resource limited settings like several Government healthcare institutions in our country, four day processing of blood cultures would be sufficient as the number of isolates obtained after four days would be virtually nil and processing beyond four days (unless there is a clear indication like enteric fever) does not justify the time and cost involved in processing the samples. In addition, this approach will save the patient from the cost and risks of prolonged hospital stay or cost and toxicity of "empirical" drugs. In fact, if the microbiology laboratory gives an early blood culture report, it would definitely increase the physician's compliance for which a constant coordination and rapport between the microbiologist and the clinician are extremely essential.

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