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EVALUATION OF SEVEN DAYS OR LESS INCUBATION FOR BLOOD CULTURE AND ITS CLINICAL IMPLICATION

Microbiology

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ABSTRACT

Introduction: Bloodstream infection(BSI) are an important cause of morbidity and mortality. Blood culture is use for timely detection and identification of blood borne infection that is essential for appropriate antimicrobial therapy. The 7days incubation period for blood culture bottle recommended before discarding as negative for conventional method. Most of the times result was not useful to change in antimicrobial therapy based on results. Comparative Study plan to evaluate the result on isolation of organism from blood culture following 3days 5days 7 days incubation protocol.

Method: 470 blood culture collected from all age group patients whomever clinically indicated &, processed as per plan and organism isolated by standard methods .

Results: reveal 19.4 % culture positivity, 98.9 % isolates were detected after following 5days incubation protocol. *Coagulase negative Staphylococci* was most frequent organism.

Conclusion: Study concluded that reporting 5 days incubation of blood culture bottle sufficient to say positive or negative considering its clinical implication except if ask specially for extended incubation.

KEYWORDS

Blood stream infection, blood culture, incubation period

INTRODUCTION

Blood stream infection can have serious immediate consequences including shock, multiple organs failure, Disseminated intravascular coagulation (DIC) and death; toll ranging between 20% to 50%. Hence the timely detection and identification of blood borne pathogens is one of the most important functions of microbiology laboratory. Pathogen could be bacteria viruses fungi &parasites. Bacteremia could be transient continuous or intermittent [1].Blood culture is requested mainly in two situation 1.) When clinical sign &symptoms suggestive of bacteremia or septicemia associated local infection e.g. sepsis in surgical wound, puerperal sepsis, pneumonia, meningitis osteomyelitis or endocarditis.2.) Pyrexia of unknown origin; that could be infective or non infective(malignancy, Autoimmune diseases) [2] Recently many advanced techniques such as nucleic acid probes and PCR have been developed for diagnosis of blood stream infection but blood culture still remains the most reliable method [3].Our hospital is a teaching multispecialty hospital and providing health care to surrounding villages and majority emergency patients are belong to pediatric age group as in this age group symptoms are nonspecific and the outlook is consider to be worst if treatment is delayed or inappropriate antibiotics are given.[4]over that neonates are vulnerable to infection by Nosocomial pathogens[5] hence early diagnosis and treatment required for reducing mortality and hospital stay, cost of treatment[6]. In Developing country including India many hospitals, teaching hospitals are still away from having advanced technology, so we are using conventional method for blood culture as we are not having automated blood culture systems. The conventional method is having its own limitation, prolong time required for incubation, and lead to delay reporting and high degree of contamination and poor sensitivity than automated blood culture systems [7]. However we are following 7 day incubation protocol before giving culture negative, except ask for extended incubation for HACEK group and fastidious organism in case of infective endocarditis[1] and we found not much clinical benefit over shorter incubation in our hospital setting however shorter protocol may not necessarily suited to every laboratory, because the patient population vary. Each laboratory should evaluate the different testing regimens to determine optimum schedule for own setting. Present study were attempted to evaluate the use of 7 days incubation or less with their clinical implication for change in antimicrobial therapy of patient.

METHODS

A total 470 patients were enrolled in study; were from all age group range from newborn to >60 years age, those were suspected case of bacteremia and septicemia from various departments.

Sample collection and Processing:-Age specific volume of blood was collected aseptically from patients;8to 10 ml in adult,5ml for children age 2to 12 years, 2to3ml in infant, toddlers age 1mth to 2years[7],1ml in neonates[8]. Blood inoculated immediately in to the bottle containing brain heart infusion broth in1:5-1:10 dilution [2];so 50ml for adults,30 ml for children[7] and 5ml for neonates [8]and sodium polyanetholesulfonate were added to a concentration .025gm/100ml broth[1]. Blood culture bottles were transported immediately to laboratory. A second sample was obtained from different site on same day after few hrs to rule out skin contaminant, but it was made possible only in 80 cases. Blood culture bottles were inoculated at 37 degree c[1,2]as per standard protocol and subculture were made after 24 hrs, 72hrs (3days), 5days and 7days of incubation on Chocolate agar, 5% sheep blood agar, MacConkey's agar (HIMEDIA)[9] Any growth was try to identify up to species level by colony characteristics and standard biochemical reactions[10]Negative report only given with daily observation of broth for turbidity till 7 days and final subculture report on media after 7th day of incubation. For each isolate recovered, a dialogue with clinician was established for its pathogenic potential and Alteration of antimicrobial therapy. Antimicrobial susceptibility testing performed by the Kirby Bauer Disk diffusion method as per CLSI guideline. Clinical details of patient were recorded as much as possible.

RESULTS

From 470 patients 470 blood samples were processed (additional 80 or 17% paired samples) and19.1% (90/470) found positive for microorganism. Out of all 52.7 % (248/470) were male and 47.3 %(222/470) were female. In our study maximum patients 80.6 % (378/470) were from pediatric age 0-12years and least8 %(38/470) from adult>18 years age. No correlation observed in different occupations. Age wise distribution given in Table-1.

TABLE-1
AGE WISE DISTRIBUTION OF PATIENTS

Age of patients	No of samples	%
Neonates	118	25.1%
1mth-1 year	84	17.87%
1-12 years	176	37.44%
12-18 years	54	11.48%
Adult(>18years)	38	8.0%
Total patients	470	100%

The monomicrobial growth was seen in 96.6 % (87/90) cases and polymicrobial were seen in 3.4 %(3/90) cases. On comparing incubation period required for isolation of organism, it has been found that among all 90 isolates; 21(23.3%)were identified after 24hrs of incubation and 58 (64.4%) after 3 days,10 (11.1%) after 5 days and 1 (1.1%) only isolated after 7 days of incubation of blood culture bottles.

On summation of results 87.7 %(79/90) isolates were identified after 3 days of incubation and 98.9 %(89/90) after 5 days only 1 .1 %(1) after 7 days incubation. It has been observed that out of 5 contaminant; 4 were positive after 7th day and 1 was after 5 days of incubation. they were *Micrococcus(n=2) Gram positive bacilli (n=2), Diphtheroids (n=1).*

Out of 90 isolates 58 (64.4%) were Gram positive bacteria with *Candida sp*. and 32(35.6%) were Gram negative bacteria. Among the 80 (17%) paired samples processed; only 1 isolates was dissimilar it was in combination of *Coagulase negative staphylococci (Cons)* and *Pseudomonas aeruginosa,* instead of single organism. *CoNS* was the most frequent bacteria (33.3%) that is followed by *Staphylococcus aureus* (14.4%), followed by *Klebsiella pneumoniae* (11.1%). Among fungi yeast *Candida sp* identified, two were *Candida albicans*, one was *Candida non albicans*. A summary of length of time required to detect individual microorganism during study, described in TABLE-2.

TABLE –2
DISTRIBUTION OF (90) RECOVERED ISOLATES

Organism	Number of isolates recovered on day					
	1	3	5	7	total	
Cons	6	21	2	1	30	33.3%
Staphylococcus aureus	3	9	1	-	13	14.4%
Enterococcus spp.	1	3	1	-	5	5.5%
Streptococcus pneumoniae	-	2	-	-	2	2.2%
Other streptococci	1	1	-	-	2	2.2%
Neisseria sp	1	2	-	-	3	3.3%
Candida albicans,&other sp	-	2	1	-	3	3.3%
Total gram positive organism	12	40	5	1	58	64.4%
Klebsiella pneumoniae	2	6	2	-	10	11.1%
Pseudomonas aeruginosa	2	3	1		6	6.6%
Escherichia coli	2	3			5	5.5%
Acinetobacter sp.	1	2	1		4	4.4%
Salmonella typhi…..	1	2	1		4	4.4%
Citrobacter sp.	1	2	-		3	3.3%
Total gram negative organism					32	35.6%
Total organism	21	58	10	1	90	100%
Aerobic GPB,Diphtheroid		0/1*		2*/0		
Micrococcus				2*		

Organism*-reported as contaminant

DISCUSSION

Considering the fact that we are providing health care to the nearby village belong to lower socioeconomic status and majority age group of patients were from pediatric age .In the giving circumstances early detection of infection and appropriate treatment can save life and reduce the stay in hospital, ICU care, ultimately relieved from financial burden on family. In our experiences 7 days incubation as recommended was not having much clinical implication for change in antibiotic therapy. Hence present study proceeds.

Present study showed positivity of blood culture was 19.1%(90/470) similar to the study done by,P.V Surase et al 19.88 %[7] and Chokephibulkit, K.et al18 %[11], but less than Ahmad at al 24 %[4] and47% [8] may be because of study involve mainly children and ,neonates, respectively in last two studies, where magnitude of bacteremia and septicemia generally greater than adult. lower culture positivity may be because of ;inadequate volume(each volume increase the recovery by 3%),concurrent administration antibiotic ,fastidious organism ,blood broth ratio, media, temperature use for incubation, [1,7].in our study we found 87.7%% culture found positive after 3rd day and 98.9% after 5 days, nearly similar to the study done by Ahmad et. al recovered>50%after 48hrs[4].and other study detected fastidious organism S*treptococcus pneumoniae* and *Haemophillus influenzae* after 48hrs and 96hrs respectively[7] Das,M. et al reported mean time for detection of fastidious HACEK group of organism by non Automated blood culture was3.4 days.[12]The most common isolate was *CoNS* in present study similar to[7,13,]]it may be because of 89% of population in study belong to pediatrics and it is the frequent cause of bacteremia in this age group[5].Blood culture positivity with Gram positive organism more (64.4%) than gram negative (35.6%) similar to Pal N.et al.62.5%,32.2%[9],54.05and45.9%respectively in other study [13] but other study showed Gram negative bacteria more frequently[7]. The limitation of present study is that paired samples were not able to get in all cases as multiple prick in neonates and kids were not allowed by family members, a well trained phlebotomist were not available in all wards.

CONCLUSIONS

Based on the this survey conclusion was made that 5days of incubation is probably sufficient for aerobic culture for detection of routine bacteria and yeast, as 98.9% isolates were detected after 5days of incubation and antimicrobial therapy was changed, based on positive results but not possible after 7 day incubation, However on 7days patients condition improves and discharged or succumbed with ailment. We caution others to recognize that our result may be influenced by geographic area in which hospital located, patient population we served and antimicrobial ordering pattern of our clinician.

We believe that additional studies should be perform at other institution to determine how widely applicable our results in different geographic area, patient population. In our laboratory we continue to employ 7 days incubation protocol for routine blood culture.

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