Original Article

DOI: 10.7860/JCDR/2022/56676.16759

Assessment of Factors Influencing the Positivity of Blood Culture by BacT/ALERT®3D Microbial Detection System: A Cross-sectional Observational Study

Microbiology Section

RIDDHI PATEL[1], NAIMIKABEN PATEL[2], RUPAL PATEL[3]

ABSTRACT

Introduction: Bloodstream Infections (BSI) are defined as the presence of living microorganisms in the blood. It is a systemic condition that can result in life-threatening sepsis, thus leading to high morbidity and mortality. Blood cultures have become critically important. Positive blood culture results can help a clinician's early diagnosis and start empirical antimicrobial at the correct time. Today many laboratories use modern, automated, continuous-monitoring blood culture systems for the detection of bacterial growth for blood culture. At our hospital, blood culture is done by using automated detection in BacT/Alert instrument (Biomerieux, France).

Aim: To determine the effect of number of blood cultures and volume of blood on positivity rates, contamination rate in blood cultures, and rate of false-positive blood cultures.

Materials and Methods: The present study was a cross-sectional observational study conducted from 1st May 2019 to 31st July 2019. Blood culture requests of all patients were included in the study. All blood culture bottles were processed as per standard laboratory protocols. The effect of number of blood cultures and amount of blood volume on positivity rate, contamination rate, and false-positive blood cultures were studied in detail. The patient's details and microbiological result parameters were extracted from Laboratory Information System (LIS). All the data was analysed in Microsoft Excel 2010.

Results: A total of 761 blood culture bottles were received at the Microbiology laboratory from 604 patients. Maximum (30%) blood cultures were received from 0-10 years of age group. A total of 31% (236/761) of blood cultures were positive. The true pathogen positivity rate was 41.1% and the contamination rate was 58.9%. Single (74.4%) blood culture requests were more than two (25.3%) or three (0.3%) blood cultures. True pathogens were isolated in 9% (41/449) of single blood cultures and in 18% (56/306) of two blood cultures. Overall, 42% of blood cultures had adequate volume and 58% of blood cultures had inadequate volume. However, the true pathogen positivity rate was 14% (61/444) from bottles with inadequate volume and 11% (36/317) from bottles with adequate volume. Out of 236 positive blood cultures, 139 (59%) were identified as contaminants. A total of 5/761 (0.7%) blood cultures were identified as false positive blood cultures.

Conclusion: Based on the study findings, a step should be taken to discourage single blood culture and to encourage multiple blood cultures for the diagnosis and better patient care. Although, volume of blood is important, inadequate volume did not affect true pathogen positivity rate in present study. Contamination rate of blood cultures is a major concern and regular training of the concerned staff regarding strict asepsis should be implemented.

Keywords: Automated machine, Blood stream infections, Contaminants, True positivity

INTRODUCTION

The BSI are defined as the presence of living microorganisms in the blood. It is a systemic condition that can result in life-threatening sepsis and thus leading to high morbidity and mortality. Blood cultures have become critically important and frequently performed tests to diagnose the aetiology of BSI and sepsis [1]. Positive blood culture results can help clinicians for diagnosis, the targeting therapy against the specific organism (s), and also provide prognostic value [2]. Today many laboratories use modern, automated, continuous-monitoring blood culture systems for the detection of bacterial growth for blood culture [3,4]. Several factors that impact the success of blood cultures by automated system are blood collection time, blood volume, number of blood culture sets and skin disinfection. Several studies mention the amount of blood that is obtained for each blood culture set as the most significant variable [5]. At our hospital, the current method for blood culture is automated detection in BacT/Alert instrument (Biomerieux, France). The BacT/ALERT Microbial Detection System has a colorimetric sensor. The presence of the microorganism in the test sample produces carbon dioxide in the culture medium and reflected light is used to monitor the presence and production of carbon dioxide. It will change the colour at the bottom of the culture bottle from blue-green to yellow. Bottle reflectance is monitored and recorded by the instrument every 10 minutes [5]. Factors affecting the quality of blood culture have not been studied earlier. Through this study, the aim was to assess the effect of the number of blood cultures and volume of blood on positivity rates, contamination rate in blood cultures and rate of false-positive blood cultures by automated systems.

MATERIALS AND METHODS

The present study was a cross-sectional observational study, carried out at Shree Krishna Hospital, Karamsad, Gujarat, India, from May 2019 to July 2019. The study was conducted after the approval of Institutional Ethics Committee (IEC) (IEC/HMPCMCE/105/Faculty/8/29/19). The blood culture bottles (adult and paediatric) received at the Microbiology laboratory of Shree Krishna Hospital, Karamsad were studied for various quality parameters. Minimum one and maximum of three samples were collected from each patient and sent to the Microbiology laboratory within 24 hours' time period.

Inclusion and Exclusion criteria: Blood culture requests for all the indoor and outdoor patients of all age group were included in the study. More than one blood culture sample collected from a single patient having same finding were considered as separate for calculation of numbers of blood cultures, blood volume of culture bottles, contamination rate and false-positive blood culture rate. No specific exclusion criteria was applied for selection of the samples.

Study Procedure

The BacT/ALERT 3D Microbial Detection System (BioMerieux, France) was used for blood culture and BacT/ALERT® FA plus and BacT/ALERT® PF plus culture bottles were employed for adult and paediatric patients to collect the blood for culture under strict aseptic precautions. After collection all bottles was transferred to the pneumatic station of Central Diagnostic Laboratory, a NABL accredited laboratory. After receiving at the pneumatic system, the bottles were then sent to the Microbiology laboratory and loaded into the BacT/ALERT machine as per the standard protocol. Bottles were incubated for five days or until they signalled positive at temperature 37ºC for growth. Signal positive bottles were handled according to standard laboratory protocols for the identification of microorganisms and susceptibility testing using the Vitek 2 Compact system [5].

Following parameters for blood culture bottles were studied:

1. Number of blood cultures: Single, two or three blood cultures collected per patient was calculated and was further analysed in details.
2. Source of blood collection: It was classified as peripheral if the blood was drawn via peripheral venipuncture, central if came from a central venous catheter and arterial if it came from an arterial line.
3. Amount of blood volume in a bottle.

   Adult FA plus: To monitor the blood volume intake into the culture bottle, the target fill-to line on the bottle label was used to assist in estimating a sample volume of approximately 10 mL. If the bottle was filled up to the target fill-to line, then it was considered adequate and if not filled up to the target fill-to line, then it was considered an inadequate volume.

   Paediatric PF plus: Bottle recommended specimen volume was up to 4 mL and the volume collected was monitored by means of 4 mL incremental marking on the bottle label. If the bottle was filled up to 4 mL incremental marking, then it was considered an adequate volume and if not filled up to the 4 mL incremental marking then it was considered an inadequate volume. Adequately and inadequately filled bottles were further analysed in details.
4. Contamination rate: The contaminant was defined as the growth of skin contaminants e.g. *Bacillus* spp., *Corynebacterium* spp., *Propionibacterium* spp., and *Micrococcus* spp. regardless of a positive result. Coagulase-negative staphylococci (CoNS) grown from single/multiple blood culture was clinically correlated to decide whether it was a true pathogen or a skin contaminant.
5. False-positive blood cultures: As per the protocol, all positive bottles were smeared and subcultured. If the smear was negative and there was no growth in subculture after 48 hours, it indicated a possible false positive. The bottle was reloaded into the instrument based on smear findings until the growth of subculture or redesignation as positive. If the same bottle was negative at the end of five days, it was considered as false positive as per the kit insert (BacT/ALERT 3D User Manual Version B.25-page no.4-21).

STATISTICAL ANALYSIS

Demographic characteristics of the patient, admission ward, the source of blood culture, number of culture bottles for each patient, and microbiological result parameters were extracted from the laboratory information system. All the data was entered and analysed in Microsoft Excel 2010.

RESULTS

A total of 761 blood culture bottles from 604 patients were received at the Microbiology laboratory. Amongst them, 354 (58.6%) blood culture bottles were received from males and 250 (41.4%) were from females. Out of 604 patients, maximum blood cultures were received from 0-10 years of the age group 182 (30%) followed by 78 (13%) from 21-30 years and 75 (12%) from 51-60 years.

Out of 761 blood cultures, 236 (31%) were positive blood cultures. True pathogen positivity rate was 41.1% (97/236) and the contamination (CoNS and *Bacillus* spp.) rate was 58.9% (139/236). However, 69% (525/761) blood cultures were negative. Maximum blood cultures, 691/761 (91%) were drawn from peripheral line. Comparison of isolation rate in peripheral, central and arterial lines is shown in [Table/Fig-1].

Location	Total n	No organism n (%)	True pathogen n (%)	Contaminant n (%)
Peripheral line	691	479 (69)	81 (12)	131 (19)
Central line	47	33 (70)	7 (15)	7 (15)
Arterial line	23	13 (57)	9 (39)	1 (4)
Total	761	525 (69)	97 (13)	139 (18)

[Table/Fig-1]: Comparison of isolation rate in peripheral, central, and arterial lines (N=761).

Out of 761 blood cultures, true pathogens were isolated more from two blood cultures 56/306 (18%) as compared to single and three blood cultures requests as shown in [Table/Fig-2]. As shown in [Table/Fig-3], a maximum 212/234 (91%) single blood cultures requests were from paediatric patients while two blood cultures were requested from 284/527 (54%) adults. Two blood culture requests were received more from ICUs than wards. Isolation of gram-positive cocci (GPC), gram-negative bacilli (GNB) and yeast were more in two blood culture requests 56/306 (18%) than single blood culture 41/449 (9%). However, contaminants grew more from single blood cultures 83/449 (18%) than from two blood cultures 55/306 (18%). As shown in [Table/Fig-4], isolation of GNB was more from two blood cultures than single blood cultures in ICU patients,

Number of blood culture/s	Number of patients	Total blood culture bottles received n (%)	Positive for true pathogen n (%)
Single	449	449 (59)	41 (9)
Two	153	306 (40.2)	56 (18)
Three	02	06 (0.8)	0
Total	604	761	97

[Table/Fig-2]: True pathogen positivity rate according to number of blood culture bottles received per patient (single/multiple) (N=761).

Variables	Total N=761 (%)	Single blood culture N=449 (%)	Two blood cultures N=306 (%)	Three blood cultures N=6 (%)
Adult	527 (69)	237 (44.9)	284 (53.8)	6 (1.13)
Paediatric	234 (31)	212 (90.5)	22 (9.4)	0
Peripheral line	691 (90.8)	435 (63)	253 (37)	3 (0.4)
Central line	47 (6.17)	7 (14.8)	38 (80.8)	2 (4.2)
Arterial line	23 (3)	7 (30.4)	15 (65.2)	1 (4.3)
ICU*	271 (35.6)	78 (28.7)	187 (69)	6 (2.2)
PICU†	32 (4.2)	30 (94)	2 (6.2)	0
NICU‡	66 (8.7)	66 (100)	0	0
Paediatric ward	98 (12.8)	96 (98)	2 (2)	0
Medical ward	263 (34.6)	162 (61.5)	101 (38.4)	0
Surgical ward	31 (4.1)	17 (55)	14 (45)	0
No organism	525 (68.9)	325 (62)	195 (37)	5 (1)
GPC§	24 (3.1)	9 (37.5)	15 (62.5)	0
GNB‖	69 (9.1)	29 (42)	40 (58)	0
Yeast	4 (0.5)	3 (75)	1 (25)	0
Contaminants	139 (18.3)	83 (60)	55 (39)	1 (1)

[Table/Fig-3]: Distribution of blood cultures according to number of blood culture bottles received (single/multiple) (N=761).
*Intensive care unit, † Paediatric ICU, ‡Neonatal ICU, §Gram-positive cocci, ‖Gram-negative bacilli

whereas, GPC was isolated more from single blood cultures in paediatric patients. The contamination rate was more in single blood cultures of paediatric patients. Details of the true pathogens and contaminants isolated from positive blood cultures is shown in [Table/Fig-5]. The most common organism was *Escherichia coli* 20/97 (21%) followed by *Klebsiella pneumoniae* 18/97 (19%) and *Staphylococcus aureus* 11/97 (11%). Among the 139 contaminants, 97/139 (70%) were CoNS and 42/139 (30%) were *Bacillus* spp. Out of 236, a total of 105 (45%) CoNS were isolated where only 8 (8%) CoNS were true pathogens and 97 (92%) were confirmed as contaminants.

Variables	Numbers n (%)	No organism n (%)	GPC n (%)	GNB n (%)	Yeast n (%)	Contaminants n (%)
Single blood culture (N=449)						
Adult	237 (44.9)	169 (71.3)	2 (0.9)	18 (7.6)	1 (0.4)	47 (19.8)
Paediatric	212 (90.5)	156 (73.6)	7 (3.3)	11 (5.1)	2 (1)	36 (17)
Peripheral line	435 (96.9)	314 (72.1)	8 (1.8)	28 (6.4)	3 (0.6)	82 (18.8)
Central line	7 (1.6)	6 (85.7)	0	0	0	1 (14.2)
Arterial line	7 (1.6)	5 (71.4)	1 (14.2)	1 (14.2)	0	0
ICU	78 (17.4)	54 (69.2)	2 (2.5)	7 (9)	0	15 (19.2)
PIMC/PICU	30 (6.7)	24 (80)	1 (3.3)	2 (6.6)	0	3 (10)
NICU	66 (14.7)	52 (78.7)	2 (3)	6 (100)	2 (3)	4 (6)
Paediatric ward	96 (21.4)	63 (65.6)	3 (3.1)	4 (4.1)	0	26 (27)
Medical ward	162 (36)	121 (75)	0	9 (5.5)	0	32 (20)
Surgical ward	17 (3.8)	11 (65)	1 (5.8)	1 (5.8)	1 (5.8)	3 (17.6)
Two blood cultures (N=306)						
Adult	284 (93)	179 (63)	15(5.2)	36 (13)	1 (0.3)	53 (19)
Paediatric	22 (7)	16 (72.7)	0	4 (18.1)	0	2 (9)
Peripheral line	253 (82.7)	163 (64.4)	12 (4.7)	29 (11.4)	1 (0.3)	48 (19)
Central line	38 (12.4)	25 (65.7)	3 (7.8)	4 (10.5)	0	6 (15.7)
Arterial line	15 (4.9)	7 (46.6)	0	7 (46.6)	0	1 (6.6)
ICU	187 (61)	117 (62.5)	9 (4.8)	33 (17.6)	1 (0.5)	27 (14.4)
PIMC/PICU	2 (0.6)	2 (100)	0	0	0	0
NICU	0	0	0	0	0	0
Paediatric ward	2 (0.6)	2 (100)	0	0	0	0
Medical ward	101 (33)	66 (65.3)	6 (6)	7 (7)	0	22 (21.7)
Surgical ward	14 (5)	8 (57.1)	0	0	0	6 (43)
Three blood cultures (N=06)						
Adult	6 (100)	5 (83.3)	0	0	0	1 (16.6)
Paediatric	0	0	0	0	0	0
Peripheral line	3 (50)	2 (66.6)	0	0	0	1 (33.3)
Central line	2 (33.3)	2 (100)	0	0	0	0
Arterial line	1 (16.6)	1 (100)	0	0	0	0
ICU	6 (100)	5 (83.3)	0	0	0	1 (16.6)

[Table/Fig-4]: Association of number of blood cultures (single/multiple) with the organisms isolated (N=761).

During study period, 317/761 (42%) blood cultures had adequate volume and 444/761 (58%) blood cultures had inadequate volume as shown in [Table/Fig-6]. Overall, true pathogen positivity rate from bottles with inadequate and adequate volume was 61/444 (14%) and 36/317 (11%), respectively. In paediatrics, 2/14 (14%) true pathogens were from blood cultures with inadequate volume.

[Table/Fig-7] shows that inadequate volume was more in ICU 205/271(76%) followed by medical ward while adequate volume was more in paediatric ward 95/98 (97%) followed by NICU. The isolation rate of GPC, GNB, and contaminants was 14/24 (58%), 45/69 (65%), and 92/139 (66%) from inadequate volume cultures, respectively.

As shown in [Table/Fig-8], contaminants grew more in bottles with inadequate volume particularly from ICU, PIMC/PICU, and medical wards. True pathogens were more commonly seen in samples received from ICU 52/271(19%) followed by NICU 10/66 (15%) and surgical ward 3/31 (10%) whereas contaminants were more commonly isolated

Organism	Numbers of isolates	% Out of true pathogens (n=97)
True pathogens		
Gram positive cocci (N=24)		
Staphylococcus aureus	11	11
Staphylococcus epidermidis	4	4
Staphylococcus hominis	3	3
Staphylococcus haemolyticus	1	1
Streptococcus pyogenes	2	2
Streptococcus pneumoniae	1	1
Streptococcus mitis	1	1
Enterococcus faecium	1	1
Yeast (N=4)		
Candida albicans	2	2
Cryptococcus laurentii	2	2
Gram negative bacilli (N=69)		
Escherichia coli	20	21
Klebsiella pneumoniae	18	19
Salmonella typhi	2	2
Salmonella paratyphi A	6	6
Acinetobacter spp.	6	6
Pseudomonas spp.	4	4
Citrobacter sedlakii	1	1
Enterobacter cloacae	1	1
Shigella sonnei	1	1
Aeromonas hydrophilia	3	3
Brevundimonas diminuta	1	1
Brucella melitensis	1	1
Burkholderia cepacia	1	1
Ralstonia	1	1
Stenotrophomonas maltophilia	2	2
Sphingomonas paucimobilis	1	1
Contaminants (N=139)		
Coagulase Negative Staphylococci	97	70
Bacillus spp.	42	30

[Table/Fig-5]: Organisms isolated from positive blood cultures (N=236).

Variables	Volume	Numbers n (%)	Positive for true pathogen n (%)
Adult	Adequate	97 (13)	14 (14)
	Inadequate	430 (56)	59 (13)
Paediatric	Adequate	220 (29)	22 (10)
	Inadequate	14 (2)	02 (14)

[Table/Fig-6]: Association of blood volume with a true pathogen positivity rate (n=761).

Variables	Total N=761 (%)	Adequate volume N=317 (%)	Inadequate volume N=444 (%)
Adult	527 (69.3)	97 (18.4)	430 (81.5)
Pediatric	234 (30.7)	220 (94)	14 (5.9)
Peripheral line	691 (90.8)	288 (41.6)	403 (58.3)
Central line	47 (6.2)	17 (36.1)	30 (63.8)
Arterial line	23 (3)	12 (52.1)	11 (47.8)
ICU	271 (35.6)	66 (24.3)	205 (75.6)
PIMC/PICU	32 (4.2)	26 (81.2)	6 (18.7)
NICU	66 (8.7)	63 (95.4)	3 (4.5)
Paediatric ward	98 (12.9)	95 (97)	3 (3)
Medical ward	263 (34.5)	58 (22)	205 (77.9)
Surgical ward	31 (4)	9 (29)	22 (71)
No organism	525 (68.9)	234 (44.5)	291 (55.4)
GPC	24 (3.2)	10 (41.6)	14 (58.3)
GNB	69 (9)	24 (34.7)	45 (65.2)
Yeast	4 (0.5)	2 (50)	2 (50)
Contaminants	139 (18.3)	47 (33.8)	92 (66.1)

[Table/Fig-7]: Distribution of blood cultures according to volume (adequate/inadequate) (N=761).

Variables	Numbers n (%)	GPC n (%)	GNB n (%)	Yeast n (%)	Contaminants n (%)
Adequate volume (N=317)					
Adult	97 (30.6)	3 (3)	10 (10.3)	1 (1)	14 (14.4)
Paediatric	220 (69.4)	7 (3.1)	14 (6.3)	1 (0.4)	33 (15)
Peripheral line	288 (90.9)	9 (3.1)	17 (5.9)	2 (0.6)	45 (15.6)
Central line	17 (5.4)	0	2 (11.7)	0	2 (11.7)
Arterial line	12 (3.8)	1 (8.3)	5 (41.6)	0	0
ICU	66 (20.8)	2 (3)	9 (13.6)	0	3 (4.5)
PIMC/PICU	26 (8.2)	1 (3.8)	1 (3.8)	0	1 (3.8)
NICU	63 (19.9)	2 (3.1)	5 (8)	1 (1.5)	4 (6.3)
Paediatric ward	95 (29.9)	3 (3.1)	4 (4.2)	0	25 (26.3)
Medical ward	58 (18.3)	1 (1.7)	5 (8.6)	0	12 (20.6)
Surgical ward	9 (2.8)	1 (11.1)	0	1 (11.1)	2 (22.2)
Inadequate volume (N=444)					
Adult	430 (96.8)	14 (3.2)	44 (10.2)	1 (0.2)	87 (20.2)
Paediatric	14 (3.2)	0	1 (7.1)	1 (7.1)	5 (35.7)
Peripheral line	403 (90.8)	11 (2.7)	40 (9.9)	2 (0.4)	86 (21.3)
Central line	30 (6.7)	3 (10)	2 (6.6)	0	5 (16.6)
Arterial line	11 (2.4)	0	3 (27.2)	0	1 (9)
ICU	205 (46.1)	9 (4.3)	31 (15.1)	1 (0.4)	40 (19.5)
PIMC/PICU	6 (1.3)	0	1 (16.6)	0	2 (33.3)
NICU	3 (0.7)	0	1 (33.3)	1 (33.3)	0
Paediatric ward	3 (0.7)	0	0	0	1 (33.3)
Medical ward	205 (46.1)	5 (2.4)	11(5.3)	0	42 (20.4)
Surgical ward	22 (5)	0	1 (4.5)	0	7 (31.8)

[Table/Fig-8]: Association of blood culture volume with the organisms isolated (n=761).

from specimens received from the surgical ward 9/31 (29%), paediatric ward 26/98 (27%) and medical ward 54/263 (21%).

Out of 761 blood cultures, 241 were signal positive. Out of 241, 236 blood cultures were positive for the growth of the organisms and five blood cultures were smear-negative and did not grow any organism after 48 hours of incubation. All five bottles were loaded again in the BacT/ALERT incubation for five days according to standard protocol. Therefore, in present study, 5/761 (0.6%) were false-positive blood cultures.

DISCUSSION

Blood culture represents a critical tool for detecting the presence of living organisms in the blood [2]. Through this study, various parameters affecting the quality of blood culture using the BacT/ALERT 3D system were assessed.

Peripheral venipuncture is the preferred site to obtain blood. Where venous access is a problem in critical patients, sampling from central venous catheter or an arterial line can be performed [6]. Present study analysed that more samples were received from peripheral lines than other lines as compared to other studies. The study by Gonsalves WI et al., found blood cultures were drawn from peripheral venipuncture, central venous catheter, and an arterial line 51%, 44%, 5%, respectively. They did not find any significant statistical difference between the rates of contamination among the various sites of blood draw [7]. A study conducted by Venturelli C et al., found that 76% blood cultures were collected from peripheral venipuncture and 24% from central catheter venepuncture [8]. Stohl S et al., demonstrated a higher yield of true pathogens in cultures drawn at the time of central line insertion with another study [9]. A recent meta-analysis suggests that for better sensitivity and negative predictive value atleast one culture should be taken from the central venous catheter [6]. Though cultures taken from indwelling lines are often unable to differentiate between colonisation or true pathogen causing infection. Furthermore, the disinfection of these devices may be more difficult than the disinfection of the skin [9]. Present study have not analysed whether the central and arterial cultures in this study were drawn from indwelling lines or at the time of central or arterial line insertion. Overall positivity rate (including true pathogens and contaminants) was more from arterial lines in present study study which was similar to the findings of Gonsalves WI et al., [7].

Present study assessed the role of the number of blood cultures in the outcome of blood cultures. At our hospital, a minimum of one and a maximum of three blood cultures can be sent within 24 hours period. Present study received 74.4%, 25.3%, 0.3% requests for single blood cultures, two blood cultures, and three blood culture bottles, respectively. Although multiple blood cultures were encouraged, the practice of sending only one blood culture still exists. In present study, the true pathogen positivity rate was 9% from single blood cultures and 18% was from two blood cultures which are significantly higher than single cultures. Three blood culture bottles were sent in only two requests but none of them was positive for the true pathogen. In present study, isolation of GPC and GNB was common in two blood cultures. Lee A et al., reported 93% and 87% isolation rates of *S. aureus* only with the first sample [3]. As compared to single blood cultures, the isolation rate of true pathogens increases with two blood culture requests from ICU [10]. Improvement in organism isolation rate with an increase in the number of samples is extremely important for early and accurate clinical diagnosis and management of patients. There were no studies to compare present study findings.

The volume of blood that was obtained for each culture set is the single most important variable in recovering microorganisms from patients with BSI [11]. An optimal recovery of bacteria and fungi from blood depends on culturing an adequate volume of blood. For an adult, the 20-30 mL volume of blood is the recommendation for culture [12]. However, obtaining the optimum volume of blood from infants and children is not well prescribed in literatures. In present study, blood cultures with inadequate volume were more than the adequate volume. In contrast to this, Gonsalves WI et al., reported that they received adequate volume (60%) of blood culture bottles for culture [7]. As described in the literature, there are practical constraints when collecting blood from paediatric patients and paediatricians are unable to collect large volumes of blood [13,14]. However, inadequate volume was more in adults as compared to paediatrics in present study. Adequacy of volume was studied as per the manufacturer's instructions by visually comparing the target fill in line in adults and by 4 mL incremental markings in paediatrics in the present study. In the present study, true pathogen positivity rate was more from bottles with inadequate volume than the adequate volume (14% versus 11%). The higher yield true pathogen positivity with lower volumes of blood can also depend on the condition like higher age, higher severity of the patient's condition, diabetes mellitus, and the absence of antimicrobials at the moment the blood cultures [12]. In present study, volume did not affect the true pathogen positivity rate in adult patients. Present study observed that more true pathogens were isolated from paediatric blood cultures with inadequate volume than adequate volume (14% versus 10%) which was in contrast to the findings of Gonsalves WI et al., [7].

The spectrum of organisms isolated in present study was similar to other studies.71% were gram-negative bacilli, 25% were gram-positive cocci, and 4% were yeast isolates. As compared to previous studies, gram-negative isolates were more and gram-positive cocci were less in present study [6,15-19] The number of fungal isolates was also less as compared to other studies [15,19]. The most common organism was *Escherichia coli* (21%) followed by *Klebsiella pneumoniae* (19%) and *Staphylococcus aureus* (11%) which differ in various studies [8,17-19]. Study conducted by Shrinivasan M et al., conclude that significant factors associated with blood culture positivity were increasing age of the child and a higher blood volume inoculated for a culture with fever episodes in children [20]. Current study findings suggest that apart from volume, other variables might be affecting the true pathogens positivity rate.

Published literature has mentioned that blood culture contamination rates should not exceed 3% [6]. The cost of blood culture contamination often exceeds the cost of performing the test. The increased use of intravascular devices and the practice of taking cultures through them are also important when considering contamination rates. Rapid staff turnover, lack of on-going training, and workload may contribute to a higher contamination rate [2,6]. Similar to the published study, [21] present study found that 18% of contaminants grew as CONS and *Bacillus* spp. more from peripheral lines (19%) which was very high than the internationally accepted range. This finding was discordant with Stohl S et al., [9]. who found central line cultures remained consistently higher than that of venipuncture cultures. Various strategies have been implemented to decrease blood culture contamination rates e.g. training of staff about aseptic collection technique, feedback concerning contamination rates, implementation of blood culture collection kits, and dedicated phlebotomy team [2,6]. The best possible solution can be dedicated phlebotomy to address preanalytical issues of blood culture including number of cultures, skin antisepsis, blood volume etc.

False-positive instrument signal, defined as a bottle flagged positively by the system, though not containing any micro-organism. These false-positive bottles require quick handling and re-incubation into the blood culture system [8]. In present study study, a total of 5 bottles were false positive. A study conducted by Kim SC et al., found skin contaminations were 4.9% and instrument false positives were 1.3% [22]. There are many causes of false-positive results by instrument include bottles contain a high level of leukocyte counts, over-filled bottles, and/or errors in incubation or bottles are monitored with an inappropriate algorithm [21].

Limitation(s)

The study was conducted for three months only and various parameters like the timing of venipuncture, skin antisepsis and antibiotic treatment before sampling might have affected the results. The blood volume in the culture bottles was compared visually as per the manufacturer's instructions. The weight of blood culture bottles before and after the collection was not measured which might have given more accurate results and might have affected present study findings.

CONCLUSION(S)

Based on the study findings a step should be taken to decrease the single blood cultures and encourage multiple blood cultures for diagnosis and better patient care. Inadequate volume did not affect the true pathogen positivity rate. Contamination of blood cultures is a major concern and regular training of the concerned staff should be taken to address the problem.

REFERENCES

[1] Lin HH, Liu YF, Tien N, Ho CM, Hsu LN, Lu JJ. Evaluation of the blood volume effect on the diagnosis of bacteremia in automated blood culture systems. J Microbiol Immunol Infect. 2013;46(1):48-52.
[2] Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006;19(4):788-02.
[3] Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? J Clin Microbiol. 2007;45(11):3546-48.
[4] Bengtsson J, Wahl M, Larsson P. Assessment of the BacT/Alert blood culture system: Rapid bacteremia diagnosis with loading throughout the 24 h. Clinical Microbiol Infect. 1998;4(1):33-37.
[5] Dunne WM Jr, Nolte FS, Wilson ML, Cumitech 1B. Blood Cultures III Coordinating, Hindler JA. ASM Press. Washington, DC. 1997.
[6] Dreyer AW. Blood culture systems: From patient to Result. In: Azevedo L, editor. Sepsis. An ongoing and significant challenge. Rijeka: InTech; 2012. Ch-15.
[7] Gonsalves WI, Cornish N, Moore M, Chen A, Varman M. Effects of volume and site of blood draw on blood culture results. J Clin Microbiol. 2009;47(11):3482-85.
[8] Venturelli C, Righi E, Borsari L, Aggazzotti G, Busani S, Mussini C, et al. Impact of pre-analytical time on the recovery of pathogens from blood cultures: Results from a large retrospective survey. PLoS One. 2017;12(1):e0169466.
[9] Stohl S, Benenson S, Sviri S, Avidan A, Block C, Sprung CL, et al. Blood cultures at central line Insertion in the Intensive care unit: Comparison with peripheral venepuncture. J Clin Microbiol. 2011;49:2398-03.
[10] Elantamilan D, Lyngdoh VW, Khyriem AB, Rajbongshi J, Bora I, Devi ST, et al. Comparative evaluation of the role of single and multiple blood specimen in the outcome of blood cultures using BacT/ALERT 3D (automated) blood culture system in tertiary care hospital. Indian J Crit Care Med. 2016;9:01-06.
[11] Baron EJ, MP Weinstein, WM Dunne, JRP Yagupsky, DF Welch, DM Wilson, et al. Blood cultures IV. Coordinating ed., EJ Baron. ASM Press, Washington. DC. 2005.
[12] Bouza E, Sousa D, Rodríguez-Créixems M, Lechuz JG, Muñoz P. Is the volume of blood cultured still a significant factor in the diagnosis of bloodstream infections? J Clin Microbiol. 2007;45:2765-69.
[13] Tenney JH, Reller LB, Mirrett S, Wang WL, Weinstein MP. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. J Clin Microbiol. 1982;15;558-61.
[14] Neves L, Marra AR, Camargo TZS, Santos MC, Zulin F, Silva PC, et al. Correlation between mass and volume of collection blood with positivity of blood cultures. Bio Med Central. 2015(8):01-07.
[15] Kennedy GT, Barr JG, Goldsmith C. Detection of bacteremia by the continuously monitoring BacT/Alert system. J Clin Pathol. 1995;48(10):912-14.
[16] Lambregts MMC, Bernards AT, van der Beek MT, Visser LG, de Boer MG. Time to positivity of blood cultures supports early re-evaluation of empiric broad-spectrum antimicrobial therapy. PLoS ONE. 2019;14:01-12.
[17] Pan F, Zhao W, Zhang H. Value of time to positivity of blood culture in children with bloodstream infections. Canadian J Infect Dis Med Microbiol. 2019;01-07.
[18] Janapatla RP, Yan JJ, Chien ML, Chen HM, Wu HM, Wu JJ. Effect of overnight storage of blood on bacterial detection time in the BACTEC 9240 blood culture system. J Microbiol Immunol Infect. 2010;43(2):126-32.
[19] Dargere S, Cormier H, Verdon R. Contaminants in blood cultures: Importance, implication, interpretation and prevention. Clin Microbiol Inf. 2018;24:964-69.
[20] Srinivasan M, Sindhu KN, Ramanujam K, Ramasamy RK, Subramaniam S, Ganesan SK, et al. Factors predicting blood culture positivity in children with enteric fever. The J Infect Dis. 2021;224(Supplement_5):S484-93.
[21] Lamy B, Dargere S, Arendrup MC, Parienti JJ, Tattevin P. How to optimize the use of blood cultures for the diagnosis of bloodstream infection? A stat-of-the art. Front Microbiol. 2016;7:01-13.
[22] Kim SC, Kim S, Lee DH, Choi SR, Kim JS. Effect of blood volume in standard Anaerobic blood culture bottles of the BacT/ALERT 3D system used for the detection of pathogens and time to detection. PLoS One. 2015;10:01-08.

PARTICULARS OF CONTRIBUTORS:

1. Technician, Department of Microbiology, Bhaikaka University, Anand, Gujarat, India.
2. Assistant Professor, Department of Microbiology, Pramukhswami Medical College, Shree Krishna Hospital, Bhaikaka University, Karamsad, Gujarat, India.
3. Professor, Department of Microbiology, Pramukhswami Medical College, Shree Krishna Hospital, Bhaikaka University, Karamsad, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Naimikaben Patel,
C-60, Sona Township (Old Mill Compound), Near Kansa Cross Road Opposite to Doctor House, Visnagar-384315, Gujarat, India.
E-mail: drnaimikapatel@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Apr 08, 2022
- Manual Googling: May 14, 2022
- iThenticate Software: May 25, 2022 (16%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 28, 2022**
Date of Peer Review: **Apr 20, 2022**
Date of Acceptance: **May 28, 2022**
Date of Publishing: **Aug 01, 2022**