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Original Article

Assessment of Bacterial Profile and Antimicrobial Susceptibility Pattern of Bacterial Isolates from Blood Culture in Tertiary Level Paediatric Hospital of Nepal

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Abstract

Introduction: Bloodstream infection is a major cause of morbidity and mortality which requires antibiotic treatment. Antimicrobial resistance is an emerging serious public health threat in both developed and developing countries. Children are more susceptible to infections requiring an appropriate choice of antibiotic based on blood culture. This study aims to investigate the bacteriological profile and antibiotic sensitivity pattern of blood culture isolates and compare the yield of bacterial growth between Brain Heart Infusion Broth (BHIB) or BD BACTEC culture media.

Methods: A total of 12,795 blood samples were sent for bacteriological culture either for BHIB or BACTEC techniques, 10994 and 1801 samples respectively. Chi-square test was used for showing association between BACTEC and BHIB among isolates.

Results: The findings showed that the BACTEC method detected more positive isolates than the BHIB method. The rate of isolation was found highest among children under five years. The most common pathogens isolated were Staphylococcus species (28.1%), Staphylococcus aureus (25.6%), Acinetobacter species (12%), Pseudomonas species (8.2%), Klebsiella species (6.6%), CONS (4.4%), Escherichia coli (4.4%), Salmonella Typhi (3.5%), Enterobacter species (3.2%) and Streptococcus species (0.3%).

Conclusions: Staphylococcus aureus was the commonest isolate identified in the current study. BACTEC culture method detected the higher percentage of isolates than BHIB method.

Introduction

Sepsis is a serious infection caused due to toxins made by the bacteria that cause the immune system to attack the body's organs and tissues.[1] Neonates and young children are at high risk of infections due to their vulnerable characteristics.[2] Sepsis is the important cause of child mortality in low and middle-income countries in the range of 100 to 250 deaths per 1000 which is ten times higher than high-income countries.[3] The delayed treatment of infections can even lead to life-threatening conditions which depict the important role of antimicrobials in the timely treatment of infections.[4] Appropriate choice of antibiotics based on blood culture is necessary to manage the infections in newborns and children.[5] Other factors such as probable cause of infection, age, gender, risk factors, and antimicrobial susceptibility pattern are also responsible for empirical treatment.[6] Detection of blood infection causing agents is done through

inoculation of blood in different culture media. Brain Heart Infusion Broth (BHIB) or BD BACTEC are culture media that detect bacterial growth through conventional and automated systems respectively.[7] However, antimicrobial resistance has been a challenging issue in the sector of medicine in the present context.[8] This highlights the need to study blood pathogens and antibiotic sensitivity patterns. This study aims to investigate the bacteriological profile, antibiotic susceptibility pattern and compare the yield of bacterial growth between BHIB or BD BACTEC culture media.

Methods

A retrospective cross-sectional study was conducted in Kanti Children's Hospital, Maharajgunj, Kathmandu, Nepal after taking ethical clearance from the Institutional Review Committee. Documented laboratory records of all the blood culture samples received in the laboratory (which included neonates and children upto14 years) from July 2019 to July 2020 were the samples included in this study. Blood was collected using aseptic technique. Blood samples were drawn at the time of admission before the start of antibiotics whenever possible. Blood was inoculated either in BHIB or in the BD BACTEC culture media. The use of conventional or automated technique depended on the clinician's decision. Blood samples obtained in BHIB were incubated aerobically for 48 hours at 35 ± 2°C after which subcultures were done onto Blood agar and MacConkey agar. Blood agar was incubated at 35 ± 2°C in a candle jar whereas MacConkey plates were incubated aerobically at 35 ± 2°C, for the next 24 hours. Blood samples obtained in BD BACTEC TM Peds Plus / FAerobic vials were placed into BD-BACTEC FX40 blood culture system. It was removed from the system for subculture when the indicator signaled for positive growth and subcultures were done as described above. Vials those were sterile in the BD BACTEC system usually gave a negative signal after 72 hours. Identification and characterization of isolates was done by standard microbiological techniques using the conventional method of characterization.[9] Antimicrobial susceptibility test was performed by commercially available discs on Mueller Hinton Agar by Kirby-Bauer disk diffusion method by taking 0.5 MacFarland turbidity standard and interpretation of discs as Sensitive (S), Intermediate (I) and Resistant (R) were followed as per CLSI guidelines 2019.[10]

Gram positive organisms were tested against; Penicillin-G (P) (10 units), Ampicillin (AMP) (10 μg), Ampicillin/Sulbactam (A / S) (10 / 10μg), Ampicillin/ Cloxacillin (AX) (10 μg), Cefalexin (CN) (30 μg), Cefixime (CFM) (5 μg), Cefazolin (CZ) (30 μg), Ceftriaxone (CTR) (30 μg), Ceftazidime (CAZ) (30μg), Cefotaxime (CTX) (30 μg), Cefepime (CPM) (30μg), Amikacin (AK) (30 μg), Gentamicin (GEN) (10 μg), Ofloxacin (OF) (5 μg), Ciprofloxacin (CIP) (5 μg), Levofloxacin (LE) (5 μg), Bacitracin (B) (10 units), Cotrimoxazole (COT) (25 μg), Linezolid (LZ) (30 μg), Clindamycin (CD) (2μg). Furthermore, Staphylococcus colonies were screened for the susceptibility towards methicillin using Cefoxitin (CX) (30 μg) disc.

Gram negative organisms were tested against; Ampicillin (AMP) (10 μg), Cefixime (CFM) (5 μg), Ceftriaxone (CTR) (30 μg), Ceftazidime (CAZ) (30 μg), Cefepime (CPM) (30 μg), Imipenem (IPM)(10μg), Meropenem (MRP) (10 μg), Piperacillin (PI) (100 μg), Piperacillin and Tazobactam combination (PIT) (100 / 10 μg), Ceftazidime and Clavulanic acid combination (CAZ) (30 / 10 μg), Aztreonam (AT) (30 μg), Amikacin (AK) (30 μg), Gentamicin (GEN) (10 μg), Tobramycin (TOB) (10 μg), Ofloxacin (OF) (5 μg), Ciprofloxacin (CIP) (5 μg), Levofloxacin (LE) (5 μg), Nalidixic acid (NA) (30 μg), Cotrimoxazole (COT) (25 μg), Chloramphenicol (C) (25 μg), Azithromycin (AZM) (15 μg), Linezolid (LZ) (30 mcg] as per the need of the identified organisms in both negative and positive category after gram staining were placed onto the MHA agar plates. The data was analyzed using SPSS version 20.0. Univariate analysis such as frequency, percentage, was used for bacterial species. Chi-square test was used for showing association between BACTEC and BHIB among isolates. P value less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

There was a total of 12,795 blood samples sent for bacteriological culture which included both BHIB and BACTEC techniques representing 10994 and 1801 samples respectively.

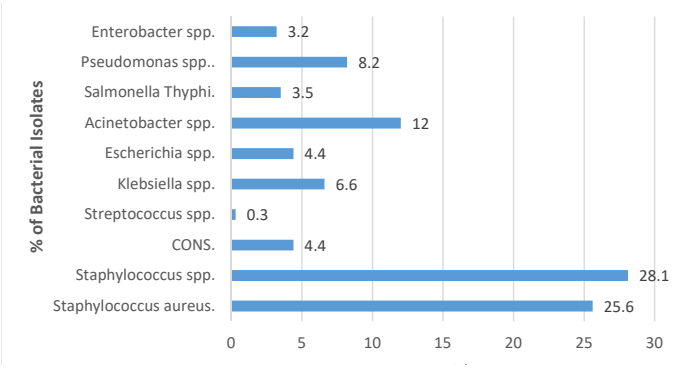

Figure 1: Bacterial profile of isolates from blood culture

Figure 1 shows the bacterial profile of isolates from blood culture. Table 1 demonstrates the age groups, sex and collection place of the study population. Table 2 shows the age distribution frequency of bacterial species isolated from patients with positive blood cultures. Table 3 shows the overall frequency and rate of BACTEC and BHIB positivity detected from blood culture samples. Table 4 demonstrates the association of BACTEC and BHIB blood culture detection among the bacterial isolates.

Table 5 depicts the demographic characteristics of the patients by gram positive and negative organisms. Table 6 shows multidrug resistance profile of bacterial isolates from blood culture. Table 7 explores the resistance pattern of bacterial isolates against different antibiotics

Table 1: Background Characteristics of the patients having blood culture positive (N = 317)

Indicators	Frequencyb (N = 317)	%
Age		
1 - 28 days	136	42.9
1 - 12 months	100	31.5
4 years	51	16.1
5 - 9 years	20	6.3
10 - 14 years	10	3.2
Sex		
Male	203	64.0
Female	114	36.0
Unit		
In patient	88	27.8
ER / Out patient	229	72.2

Table 2: Showing sex and age distribution frequency of bacterial species isolated from patients having blood culture positive (N = 317)

Microorganism	1 - 28 day	1 - 12 months	1 - 4 years	5 - 9 years	10 - 14 years	Total
Staphylococcus aureus	21 (27.6%)	32 (42.1%)	12 (15.8%)	7 (9.2%)	4 (5.3%)	76
Staphylococcus spp.	42 (51.2%)	18 (22.0%)	16 (19.5%)	4 (4.9%)	2 (2.4%)	82
CONS	5 (35.7%)	7 (50.0%)	1 (7.1%)	1 (7.1%)	0 (0.0%)	14
Streptococcus spp.	0 (0.0%)	0 (0.0%)	1 (100%)	0 (0.0%)	0 (0.0%)	1
Klebsiella spp.	15 (78.9%)	3 (15.8%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	19
Escherichia coli	7 (53.8%)	5 (38.5%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	13
Acinetobacter spp.	12 (37.5%)	14 (43.8%)	4 (12.5%)	2 (6.3%)	0 (0.0%)	32
Salmonella Typhii	0 (0.0%)	1 (10.0%)	4 (40.0%)	2 (20.0%)	3 (30.0%)	10
Pseudomonas spp.	13 (54.2%)	8 (33.3%)	3 (12.5%)	0 (0.0%)	0 (0.0%)	24
Enterobacter spp.	5 (62.5%)	2 (25.0%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	8
Enterococcus spp.	6 (75.0%)	1 (12.5%)	1 (12.5%)	0 (0.0%)	0 (0.0%)	8
Citrobacter spp.	0 (0.0%)	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	2

Table 3: BACTEC and Conventional detection frequency and rate among total culture samples

Culture Method	Total	%
Automated (BACTEC) (N = 1801)	112	6.21
Conventional (BHIB) (N = 10994)	205	1.86
Total (N = 12795)	317	2.47

Table 4. Comparison of BACTEC and BHIB among isolates (N = 317)

Bacterial isolates	BACTEC (N = 112) (%)	BHIB (N = 205) (%)	p-value
Staphylococcus aureus	33 (29.5)	48 (23.4)	0.027
Staphylococcus spp.	36 (32.1)	53 (25.9)	
CONS	8 (7.1)	6 (2.9)	
Streptococcus spp.	0 (0.0)	1 (0.5)	
Klebsiella spp.	3 (2.7)	18 (8.8)	
Escherichia coli	4 (3.6)	10 (4.9)	
Acinetobacter spp.	6 (5.4)	32 (15.6)	
Salmonella Typhii	7 (6.3)	4 (2.0)	
Pseudomonas spp.	8 (7.1)	18 (8.8)	
Enterobacter spp.	4 (3.6)	6 (2.9)	
Enterococcus spp.	3 (2.7)	7 (3.4)	
Citrobacter spp.	0 (0.0)	2 (1.0)	

Table 5. Demographic characteristics by gram positive and negative organisms

Variables (N = 317)	Gram Positive N = 195 (%)	Gram Negative N = 122 (%)	p-value
Age			0.250
1 - 28 days	76 (39.0)	60 (49.2)	
1 - 12 months	63 (32.3)	37 (30.3)	
1 - 4 years	34 (17.4)	17 (13.9)	
5 - 9 years	16 (8.2)	4 (3.3)	
10 - 14 years	6 (3.1)	4 (3.3)	
Sex			0.609
Male	127 (65.1)	76 (62.3)	
Female	68 (34.9)	46 (37.7)	

Table 6. Multidrug resistance profile of bacterial isolates from blood culture (N = 317)

Bacterial isolate	Number of MDR organism
Staphylococcus aureus	42 / 81 (52%)
Staphylococcus spp.	31 / 89 (35%)
CONS	8 / 14 (57%)
Klebsiella spp.	19 / 21 (95%)
Escherichia coli	10 /14 (71%)
Acinetobacter spp.	21 / 38 (55%)
Pseudomonas spp.	20 / 26 (77%)
Enterobacter spp.	3 /10 (30%)
Citrobacter spp.	2 / 2 (100%)

MDR= Multidrug resistant

Table 7. Resistance pattern of bacterial isolates against different antibiotics

Bacterial isolates	AMP N = 284 (%)	AK N = 308 (%)	A/S N = 108 (%)	AZM N = 11 (%)	AT N = 34 (%)	CN N = 172 (%)	CPM N = 296 (%)
Staphylococcus aureus	77 (27.1)	80 (26.0)	16 (15.4)	-	-	75 (43.6)	75 (25.3)
Staphylococcus spp.	78 (27.5)	86 (27.9)	6 (5.8)	-	1(2.9)	82 (47.7)	85 (28.7)
CONS	14 (4.9)	13 (4.2)	-	-	-	14 (8.1)	14 (4.7)
Streptococcus spp.	1 (0.4)	1 (0.3)	-	-	-	-	1 (0.3)
Klebsiella spp.	20 (7.0)	20 (6.5)	18 (17.3)	-	13 (38.2)	-	21 (7.1)
Escherichia coli	13 (4.6)	13 (4.2)	9 (8.7)	-	7 (20.6)	-	14 (4.17)
Acinetobacter spp.	35 (12.3)	37 (12.0)	34 (32.7)	-	8 (23.5)	-	35 (11.8)
Salmonella Typhii	11 (3.9)	11 (3.6)	3 (2.9)	11 (100)	-	-	7 (2.4)
Pseudomonas spp.	18 (6.3)	26 (8.4)	9 (8.7)	-	4 (11.8)	-	24 (8.1)
Enterobacter spp.	8 (2.8)	10 (3.2)	8 (7.7)	-	-	-	9(3.0)
Enterococcus spp.	7 (2.5)	9 (2.9)	1 (1.0)	-	-	1(0.6%)	9(3.0)
Citrobacter spp.	2 (0.7)	2 (0.6)	-	-	1 (2.9)	-	2 (0.7)

Table 7 Contd

Bacterial isolates	CTX N = 193 (%)	CTR N = 137 (%)	CFM N = 26 (%)	C N = 6 (%)	CIP N = 302 (%)	COX N = 160 (%)	COT N = 288 (%)	CAZ N = 73 (%)
Staphylococcus aureus	58 (30.1)	32 (23.4)	6 (23.1)	-	74 (24.5)	67 (41.9)	73 (25.3)	1 (1.4)
Staphylococcus spp.	55 (28.5)	33 (24.1)	1 (3.8)	-	86 (28.5)	69 (43.1)	84 (29.2)	1 (1.4)
CONS	10 (5.2)	4 (2.9)	-	-	14 (4.6)	11 (6.9)	13 (4.5)	-
Streptococcus spp.	-	1 (0.7)	-	-	1 (0.3)	1 (0.6)	1 (0.3)	1 (1.4)
Klebsiella spp.	20 (10.4)	15 (10.9)	4 (15.4)	-	20 (6.6)	-	19 (6.6)	4 (5.5)
Escherichia coli	10 (5.2)	10 (7.3)	4 (15.4)	-	14 (4.6)	1 (0.6)	13 (4.5)	6 (8.2)
Acinetobacter spp.	14 (7.3)	18 (13.1)	1 (3.8)	2 (33.3)	38 (12.6)	1 (0.6)	36 (12.5)	30 (41.1)
Salmonella Typhii	2 (1.0)	10 (7.3)	9 (34.6)	4 (66.7)	10 (3.3)	-	10 (3.5)	3 (4.1)
Pseudomonas spp.	11 (5.7)	6 (4.4)	-	-	23 (7.6)	7 (4.4)	20 (6.9)	23 (31.5)
Enterobacter spp.	6 (3.1)	4 (2.9)	-	-	10 (3.3)	1 (0.6)	9 (3.1)	3 (4.1)
Enterococcus spp.	5 (2.6)	3 (2.2)	1 (3.8)	-	10 (3.3)	2 (1.3)	8 (2.8)	-
Citrobacter spp.	2 (1.0)	1 (0.7)	-	-	2 (0.7)	-	2 (0.7)	1 (1.4)

Table 7 Contd

Bacterial isolates	GEN N = 100 (%)	IPM N = 88 (%)	LE N = 35 (%)	LZ N = 12 (%)	MRP N = 238 (%)	NA N = 2 (%)	OF N = 229 (%)	PI N = 25 (%)
Staphylococcus aureus	23 (23.0)	28 (31.8)	6 (17.1)	2 (15.4)	58 (24.4)	-	66 (28.8)	-
Staphylococcus spp.	10 (10.0)	16 (18.2)	1 (2.9)	2 (15.4)	71 (29.8)	-	59 (25.8)	-
CONS	-	2 (2.3)	-	-	12 (5.0)	-	7 (3.1)	-
Streptococcus spp.	-	-	-	-	1 (0.4)	-	1 (0.4)	-
Klebsiella spp.	13 (13.0)	10 (11.4)	14 (40)	-	13 (5.5)	-	18 (7.9)	-
Eschericiacoli	9 (9.0)	7 (8.0)	6 (17.1)	-	7 (2.9)	-	12 (5.2)	-
Acinetobacter spp.	5 (5.0)	10 (11.4)	3 (8.6)	-	33 (13.9)	-	26 (11.4)	2 (8.0)
Salmonella Typhii	3 (3.0)	-	-	-	6 (2.5)	2 (100)	10 (4.4)	-
Pseudomonas spp.	24 (24.0)	13 (14.8)	2 (5.7)	-	23 (9.7)	-	15 (6.6)	23 (92.0)
Enterobacter spp.	2 (2.0)	2 (2.3)	2 (5.7)	-	7 (2.9)	-	7 (3.1)	-
Enterococcus spp.	10 (10.0)	-	-	8 (61.5)	5 (2.1)	-	7 (3.1)	-
Citrobacter spp.	1 (1.0)	-	1 (2.9)	-	2 (0.8)	-	1 (0.4)	-

Table 7 Contd

Bacterial isolates	PIT N = 283 (%)	TOB N = 28 (%)
Staphylococcus aureus	71 (25.1)	1 (3.6)
Staphylococcus spp.	83 (29.3)	-
CONS	13 (4.6)	-
Streptococcus spp.	1 (0.4)	-
Klebsiellaspp	20 (7.1)	-
Escherichia coli	14 (4.9)	1 (3.6)
Acinetobacter spp.	36 (12.7)	1 (3.6)
Salmonella Typhii	5 (1.8)	-
Pseudomonas spp.	23 (8.1)	25 (89.3)
Enterobacter spp.	10 (3.5)	-
Enterococcus spp.	5 (1.8)	-
Citrobacter spp.	2 (0.7)	-

Discussion

The present study provided information on bacteriological analysis and antibiotic sensitivity pattern of blood culture isolates among children of under 15 years. In this study, the rate of isolation was found highest among the children of under five years of age. Similar results were depicted in study conducted in Nigeria.[11]

In present study, BACTEC method detected more positive isolates (6.21%) than conventional method. Similar findings was detected in study where 14.9% growth in bacterial isolates was detected through Bactec method.[12] Similarly, another study also showed that Bactec method detected 24.1% of positive isolates than the conventional method.[13] Similar finding was observed in another study as well.[14]

Among the various pathogens, the most common pathogen isolated in blood culture was Staphylococcus spp (28.1%) followed by Staphylococcus aureus (25.6%), Acinetobacter spp (12%), Pseudomonas spp (8.2%), Klebsiella spp (6.6%), CONS (4.4%), Escherichia coli (4.4%), Salmonella Typhi (3.5%), Enterobacter spp (3.2%) and Streptococcus spp (0.3%). Similar prevalence of pathogens were identified in another study from Nepal where majority were positive for Staphylococcus aureus (65%), followed by Escherichia coli, Klebsiella pneumoniae, Streptococcus pneumoniae and Streptococcus viridence.[15] Similar findings was observed in a study from Ethiopia where Staphylococcus aureus 13 (23.2%) was the most frequent pathogen followed by Serratia marcescens, CONS, Klebsiella spp., and Salmonella spp.[16] Salmonella Paratyphi A and Salmonella Typhi were the major Gram negative organisms causing bloodstream infections in another study from Kathmandu, Nepal.[17]

The high prevalence of Staphylococcus aureus in this study and other related studies might be due to hospital settings. This might be due to the colonization of this organism from the patient's flora, transmission through staff hands, air, surgical procedure, inanimate objects, the longer period of hospital stays, etc. In addition to this, the variations in the prevalence of organisms in different studies may be due to epidemiological differences of etiological agents, prior use of antibiotics, as well as presence or absence of risk factors.

Staphylococcus aureus (41.9%) and Staphylococcus spp. (43.1%) had high prevalence of resistance in Cloxacillin. But resistance in Staphylococcus aureus was seen against Penicillin (92.3%) followed by Ampicillin (84.6%), Co-trimoxazole (61.5%), and Tetracycline (53.8%) in another study from the present institute.[15] Similarly, Staphylococcus aureus was found to be most sensitive to chloramphenicol (88.8%) followed by Amikacin (87.5%), Ofloxacin (76.5%), Ciprofloxacin (72%), and least sensitive to Cloxacillin, Ampicillin and Penicillin in another study.[15]

Klebsiella spp. and Escherichia coli were least found isolates in our study. However, Klebsiella pneumoniae and Escherechia coli were the most common enterobacterial clinical isolates in study conducted in Mexico.[17] This difference might be related to geographical variation and / or seasonal variation. Klebsiella had high prevalence of resistance to Colistin (39.1%) in another study from Ethiopia which was different to our study where Klebsiella spp was resistant against Gentamicin and Ceftriaxone.[16]

Klebsiella spp, Escherichia coli, Acinetobacter spp, Pseudomonas spp, Enterococcus spp, were isolated more in conventional culture compared to BACTEC (P = 0.027) in the present study. However, in another study from our neighbor India, Acinetobacter spp, Pseudomonas spp, Escherichia coli, Staphylococcus aureus, Streptococcus spp, Enterococcus spp, were more isolated in BACTEC was as compared to conventional techniques (P = 0.024).[18] This difference may be explained by different set ups in different geographic regions.

This study has the major limitation being a retrospective and single centric study. Hence, these findings need to be substantiated with more larger, prospective and multi centric studies.

Conclusions

The present study showed 2.47% positive culture for the blood culture samples. Staphylococcus aureus was the commonest isolate. BACTEC culture method yielded more isolates than BHIB method. Most of the isolates were multi-drug resistant depicting antimicrobial resistance as challenge to modern medicine.

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