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Multi-Drug Resistant, Extended Spectrum β-Lactamase and Carbapenemase Producing Bacterial Isolates from Blood Culture and Associated Factors among Children Suspected of Bloodstream Infection at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia

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Research Article

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ABSTRACT

Background: Bloodstream Infections (BSI) due to bacterial pathogens are a major cause of morbidity and mortality among pediatric patients. Emergence of drug resistance from first line to last line antibiotics among bacterial pathogens is another issue of public health concern. In Ethiopia, particularly in Tikur Anbessa specialized hospital, antimicrobial resistance reports are progressively increasing in children with sepsis. Therefore, the study aimed at determines the magnitude of multidrug resistance bacterial isolates from children.

Methods: A cross-sectional study was conducted from September 2017 to June 2018 among pediatric patients with febrile illness aged less than five years at Tikur Anbessa Specialized Hospital (TASH). Three-hundred forty blood samples were collected and processed following standard microbiological techniques. Each sample was incubated with automated BacT/ALERT system for initial growth indicator and followed by identification of bacteria with conventional methods. Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion method and the E-Test to obtain the Minimum Inhibitory Concentration (MIC) for vancomycin.

Results: A total of 137 (40.2%) bacterial pathogens were isolated from pediatric patients. Of these isolates, 46% were gram positive and 54% were gram negative bacteria. The predominant microorganisms isolated from blood samples were *Klebsiella pneumoniae* (31.4%), *Staphylococcus aureus* (21.2%), coagulase negative *Staphylococcus* species (10.9%) and *Acinetobacter* species (8.0%). Among *K. pneumoniae* isolates, 90.7%, 30.2%, 7.0%, 32.5% and 34.8% were Multi-Drug Resistane (MDR), Extended Drug Resistance (XDR), Pan-Drug Resistance (PDR), Extended Spectrum Beta Lactamase (ESBL) and Carbapenem Resistant Entobacteralces (CRE) respectively. The frequency of Methicillin Resistant *Staphylococcus aureus* (MRSA) was 55.2%. Among clinical conditions, high grade fever (AOR=3.2; (1.4, 7.3)), previous hospitalization (AOR=25.0, 95% CI (5.0, 111.1)) and complication of bacteremia for development of sepsis/septic shock/ septicemia (AOR=20.0; 95% CI (6.25, 50.0)) were independent risk factors for positive blood culture (P<0.05).

Conclusion: In our study, *K. pneumoniae* and *S. aureus* are common pathogens associated with bloodstream infections. The existence of MDR, CRE and ESBL

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producing isolates calls for intervention measures for infection prevention and antibiotic stewardship in TASH and beyond.

Keywords: Blood culture; Bloodstream infection; Children; Multidrug resistance; Antibiotic stewardship

INTRODUCTION

Bloodstream Infections (BSI) are one of the major causes of morbidity and mortality globally; roughly 200,000 cases of bacteremia occur every year with mortality rates ranging from 20 to 50% [1]. BSI comprises 10-20% of nosocomial disease and is the eighth leading cause of mortality in the United States with some 17% causing death. In sub-Saharan nations including Ethiopia, septicemia is a significant cause of illness and death in young people with a mortality rate approaching 53% making it a noteworthy medical issue in low and middle-income nations.

In many publications so far documented a wide range of bacteria have been described in febrile patients counting gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Neisseria meningitidis*, *Haemophilus influenzae*, and gram positive bacteria such as Coagulase Negative *Staphylococci* (CoNS), *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Enterococcus faecium*. The diagnosis of these infections can be confirmed by blood culture, which is routinely available in selected hospitals in low-income countries [2,3].

BSI increases the death rate, causes outpatients to remain in hospital emergency, and increases the cost of providing human services [4].

Numerous studies have found that inadequate empirical therapy of bacteremia infections are associated with adverse outcomes including increased mortality and increased emergence of drug resistance [5,6].

During the previous several decades, opposition to the use of antimicrobials has expanded, and the points of view are concerning [7]. The proper use of antibiotics is well understood in the western world but this learning is deficient in low income African countries [8].

Recent studies on the outcome of sepsis in Africa are almost non-existent although there are a few reports on antimicrobial resistance concerning patients admitted to hospitals [9]. While community-acquired infections may have lower levels of resistance [10].

In Ethiopia, the resource situation has not allowed antimicrobial resistance to be prioritized as a major public health concern despite the obvious needs [11]. The aim of this study was to determine the magnitude of multidrug-resistance bacterial pathogens among blood culture specimens from under five children in a tertiary care hospital setting using an automated BacT/ALERT instrument.

MATERIALS AND METHODS

Study setting

The study was conducted at the Tikur Anbessa Specialized Hospital (TASH) which is the teaching hospital for the College of Health Sciences at Addis Ababa University. TASH is the largest specialized hospital in Ethiopia with over 700 beds, and serves as a training center for undergraduate and postgraduate medical students, pharmacists, medical laboratory personnel, and other health related professions who determine and promote solutions for health problems in the community and the nation (http://www.aau.edu.et/chs/tikur-anbessa-specialized-hospital/background-of-tikur-anbessa-hospital/).

Study design and period

A cross-sectional study was conducted from September 2017 to June 2018 to determine the burden of bacterial profiles and antimicrobial susceptibility patterns among under five children suspected of acute febrile illness in Tikur Anbessa Specialized Hospital in Addis Ababa. A convenient sampling technique was employed to recruit study participants.

Inclusion and exclusion criterias

Children aged under five years including neonates with fever, those diagnosed with sepsis, severe sepsis and septic shock were included in the study. All children who gave blood samples were volunteers with parental permission to participate in the study. Those patients who were afebrile and took antibiotics within the last 7 days during data collection time were excluded.

Sample size calculation

The sample size for the study was determined using a single population proportion determination formula. The study considered prior prevalence and antibiotic resistance data in septicemia patients demonstrated 27.9% bacterial isolation and 5% margin of error [12]. Accordingly,

$n= (Z_{\alpha/2})^2 (pq)/d^2$

Where,
n=sample size,
$Z\alpha_{/2}$=level of confidence,
P=diarrhea prevalence
$q=1-p/d^2$=margin of error (0.05):
$n=z^2\times p\times q/d^2$, p=0.279, q=0.721, d=0.05, $Z_{\alpha/2}$=1.96; $1.962\times0.279\times0.721/0.052=309$.

Considering a 10% non- response rate, a total of 340 children patients were enrolled in the study.

Data collection procedure

A standardized questionnaire was used to collect socio-demographic characteristics such as, gender, age, and clinical presentation (fever, vomiting), and household income. Patients visiting outpatient departments (pediatric and general medicine) and those admitted through inpatient units were investigated for bloodstream infections by their unit physicians. Selection criteria included having fever (≥ 38˚C) or the presence of any clinical symptoms compatible with infection.

Specimen collection

A venous blood culture specimen was taken using aseptic technique by cleansing the collection site with 70% alcohol followed by 10% povidone-iodine solution and were collected by trained laboratory personnel. Between 2.5-5 ml of blood was collected and inoculated in aerobic 30 ml BacT/ALERT PF Plus pediatric bottles with a blood to broth ratio of 1:10. At least 2 sets of blood cultures were collected within 30 minutes interval from a patient with suspected bacteremia prior to the initiation of antimicrobial therapy.

Culture isolation and identification

The initial BacT/ALERT culture bottles were incubated in an automated BacT/ALERT® 3D instrument (bioMérieux, Inc., France) at 37°C with 5% CO_2 for 5 days for primary isolation. Two aerobic blood culture bottles were used for each patient and growth in both bottles were considered a positive culture. Microbial growth was detected by the instrument and subsequently subcultured on Sheep Blood Agar (BAP), Chocolate Agar (CAP), and MacConkey Agar (MAC) plates (Oxoid Ltd, UK) and incubated for bacterial isolation at 37°C for 18-24 hours. The MAC plates were incubated aerobically while CAP and BAP plates were incubated in a microaerophilic atmosphere (5-10% CO_2) using a candle jar. A preliminary gram stain result was reported to clinician after 24 hours and a final negative result was reported at the end of 7th day of incubation. Growth was examined for colonial morphology including size, consistency, shape, hemolysis and ability to ferment lactose. For gram negative isolates, conventional biochemical tests were performed for identification [13].

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Antibiotic susceptibility testing

The methods were carried out in accordance with CLSI guidelines. Bacterial suspensions were prepared with 0.85% normal saline to obtain 0.5 McFarland standard density required for antibiotic susceptibility testing. The isolates were tested against the following antibiotic panel (oxoid) commonly used for gram positive bacteria including clindamycin (2 µg), cefoxitin (30 µg), penicillin (10 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg), erythromycin (15 µg), oxacillin (1 µg), ampicillin (10 µg), and vancomycin (30 µg). For gram negative bacteria, tobramycin (10 µg), amoxicilin-clavulanate (20/10 µg), amikacin (30 µg), gentamycin (10 µg), ampicillin (10 µg), piperacillin-tazobactam (100/10 µg), cefepime (30 µg), ceftriaxone (30 µg), ceftazidime, ciprofloxacin (5 µg), meropenem (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), were tested. Kirby-Bauer's disc diffusion method was used for susceptibility of the isolates on Muller Hinton agar and 5% sheep blood with Muller Hinton agar.

Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant Staphylococcus aureus isolates were detected phenotypically by the cefoxitin disk (30 µg) method. *S. aureus* isolates are considered methicillin resistant when the zone of inhibition for cefoxitin was ≤ 21 mm in diameter. Similarly, inducible clindamycin resistance was detected in *S. aureus* by disk approximation using clindamycin (2 µg) and erythromycin (15 µg) on Mueller–Hinton agar plates. After overnight incubation, isolates with flattened zone of inhibition adjacent to the erythromycin disk (referred to as a "D" zone) were considered to exhibit inducible clindamycin resistance.

Vancomycin Resistant *Staphylococcus aureus* (VRSA)

The MIC of vancomycin was determined by E-test a broth microdilution method using Mueller–Hinton broth as recommended by the VRSA was considered to be resistant at ≥ 16 µg/mL and VISA are resistant to vancomycin at concentrations from 4-8 µg/ml and vancomycin susceptible Staphylococcus aureus VSSA with MIC ≤ 2 µg/mL.

Detection of Carbapenem Resistance Enterobacteriacae (CRE)

All carbapenem resistance or intermediate isolates were phenotypically confirmed for the presence of carbapenemase using the modified Carbapenem Inactivation test (mCIM) to identify CRE.

Detection of extended Spectrum Beta-Lactamase (ESBL)

Initial screening for ESBL used the diameters of zones of inhibition produced by ceftazidime (30 µg), ceftriaxone (30 µg) and cefotaxime (30 µg) measured within the CLSI screening criteria. The breakpoints that indicate ESBL production are ceftazidime ≤ 22 mm, ceftriaxone ≤ 25 mm and cefotaxime ≤ 27 mm. Phenotypic detection of ESBL production was confirmed by conducting a double disk synergy test and combined disk test.

Combined Disk (double disk potentiate) Test (CDT): Ceftazidime (30 µg) disk and cefotaxime (30 µg) disks were used alone and in combination with clavulanic acid (30 µg/10 µg) for phenotypic confirmation of the presence of ESBLs. A ≥ 5 mm increase in zone diameter for either of the cephalosporin disks and their respective cephalosporin/clavulanate disks were interpreted as an ESBL producer.

Double Disk Synergy Test (DDST): The test isolate was spread onto a Mueller–Hinton agar plate. ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg) and amoxicillin/clavulanic acid (20/10 µg disks were placed at distances of 20 mm (edge to edge) from the amoxicillin-clavulanate disk placed in the middle of the plate. After 24 h incubation, an enhanced zone of inhibition between either of the cephalosporin antibiotics and the amoxicillin/clavulanic acid disc is interpreted as a positive test.

Operational definitions

- **Sepsis**: It is a serious condition in which the body responds improperly to an infection. The infection-fighting processes turn on the body, causing the organs to work poorly. It's sometimes called septicemia.
- **Early Onset Neonatal Sepsis (EONS):** The clinical feature of sepsis occurs within the first week of life.

- **Late Onset Neonatal Sepsis (LONS):** The clinical feature of sepsis occurs between 7–28 days of life.

Statistical analysis

Statistical Package for the Social Sciences (SPSS versions 20.0) was employed to analyze the data descriptive statistics including cross tab, frequency and proportion were used.

Data quality control

Sterility of culture media was checked by incubating overnight at 35°C–37°C without specimen inoculation. Performance of media used was checked by supporting growth of standard strains. Standard reference strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were used for quality control throughout the study for culture and antimicrobial susceptibility test.

RESULTS

Among a total of 340 participants, 122 (35.9%) were males and 218 (64.1%) were females. The mean age of pediatric patients who participated in this study was 1.04 ± 1.0 (SD) years. Of the study patients, 76 (22.4%) were from pediatric OPD and 181 (53.2%) inpatient ward and 83 (24.4%) ICU ward. The proportion of culture positive patients in the ICU 59/83 (71.1%), inpatient 66/181 (36.5%) and pediatric OPD 10/76 (13.2%) were identified (Table 1).

Table 1. Distribution of socio-demographic and clinical condition of pediatric patients suspected of blood stream infection in TASH, 2018.

Variables	Frequency of study participants n (%)
Sex	
Male	122 (35.9)
Female	218 (64.1)
Age group	
Neonates (<28 days)	111 (32.6)
Infants (<1 years)	115 (33.8)
Children(<5 years)	114 (33.5)
Clinical condition	
CHF	29 (8.5)
Hospital acquired infection	43 (12.6)
Early onset of neonatal sepsis	71 (20.8)
Late onset of neonatal sepsis	48 (14.1)
Sepsis	102 (30.0)
Meningitis	3 (0.8)
Neuroblastoma	5 (1.5)
Community acquired pneumonia	7 (2.0)
Endocarditis	11 (3.2)
Neutropenic fever	9 (2.6)
Others	12 (3.5)
Unit of diagnosis	
ICU	83 (24.4)
Impatient	181 (53.2)
Pediatric OPD	76 (22.4)

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Duration of admission	
1-2 days	61 (17.9)
3-4 days	76 (22.4)
5-6 days	41 (12.0)
=>7 days	84 (24.7
Cause of high fever	
Suspected bacteremia	340 (100)
Malaria	0 (0)
Others (viral, fungal…)	0 (0)
Symptom of BSI	
High grade fever only	267 (78.5)
Mild fever and others	73 (21.5)
Antibiotics taken before 07 days	
Yes	148 (43.5)
No	192 (56.5)
History hospital acquired infection	
Yes	52 (15.3)
No	288 (84.7)
Complication of BSI	
Yes	92 (27.0)
No	248 (73.0)

Positive blood culture

The distribution of positive blood culture among patients with different clinical diagnoses suspected of having BSI were; endocarditis 7/11 (63.6%), hospital acquired infection 26/43 (60.5%), sepsis 49/102 (48%) and late neonatal sepsis 21/48 (44%).

Blood stream infection and associated factors for positive blood culture

Analysis of data using logistic regression model showed that among the clinical condition, high proportion of positive blood culture was isolated in hospital acquired infection and endocarditis however no significant association between clinical condition and blood culture ($p>0.05$). The duration of admission in the Hospital for those admitted longer duration (≥ 7 days) showed high proportion of positive blood culture compared to short duration (1-2 days) but no significant association for positive BSI with p value>0.05 and AOR=0.28(0.06, 1.21)). Among clinical conditions, high grade fever only (AOR=3.2; (1.4, 7.3)), previous HAI (AOR=25.0, 95% CI (5.0, 111.1)) and complication of bacteremia leading to sepsis/septic shock (AOR=20.0; 95% CI (6.25, 50.0)) were independent risk factors for positive blood culture ($P<0.05$). Children with high grade fever were about three times more likely infected with bacteria compared to patients having mild fever and other clinical symptoms (chills, fast heartbeat, shivering and vomiting) (Table 2).

Table 2. Factors affecting positive blood culture.

Variables	Blood culture result		Bivariate	Multivariate
	Positive n (%)	Negative n (%)	COR (95% CI)	AOR (95% CI)
Sex				
Male	55 (45.1)	67 (54.9)	1	
Female	80 (36.7)	138 (63.3)	1.4 (0.9,2.2)	

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Age group				
Neonates (<28 days)	39 (35.1)	72 (64.9)	1.5 (0.9,2.6)	
Infants (<1 years)	44 (38.3)	71 (61.7)	1.3 (0.7,2.2)	
Children(<5 years)	52 (45.6)	62 (54.4)	1	
Clinical condition				
CHF	8 (27.6)	21 (72.4)	2.1 (0.4,9.8)	
Hospital acquired infection	26 (60.5)	17 (39.5)	0.5 (0.1,2.2)	
Early onset of neonatal sepsis	16 (22.5)	55 (77.5)	2.7 (0.6,11.4)	
Late onset of neonatal sepsis	21 (43.8)	27 (56.2)	1.0 (0.2,4.3)	
Sepsis	49(48.0)	53 (52.0)	0.8 (0.2,3.4)	
Mengitidis	0 (0)	3 (100)	-	
Neuroblastoma	1 (20.0)	4 (80.0)	3.2 (0.2,41.2)	
Community acquired pneumonia	2 (28.6)	5 (71.4)	2.0 (0.2,16.3)	
Endocarditis	7 (63.6)	4 (36.4)	0.4 (0.0,2.7)	
Neutrophenic fever	4 (44.4)	5 (55.6)	8.8 (0.7,100.2)	
Othrs	1 (8.3)	11 (91.7)	1	
Unit of diagnosis				
ICU	59 (71.1)	24 (28.9)	0.06 (0.02,0.14)	
Impatient	66 (36.5)	115 (63.5)	0.25 (0.12,0.53)	
Pediatric OPD	10 (13.2)	66 (86.8)	1	
Duration of admission				
1-2 days	10 (16.4)	51 (83.6)	1	
3-4 days	30 (39.5)	46 (60.5)	0.30 (0.13,0.68)*	0.76 (0.25,2.29)
5-6 days	21 (51.2)	20 (48.8)	0.18 (0.07,0.46)*	0.45 (0.13,1.58)
=>7 days	63 (75.0)	21 (25.0)	0.06 (0.02,0.15)*	0.28 (0.06,1.21)
Cause of high fever				
Suspected bacteremia	135 (39.7)	205 (60.3)	-	
Malaria	0 (0)	0 (0)		
Others (viral, fungal…)	0 (0)	0 (0)		
Symptom of BSI				
High grade fever only	82 (30.7)	185 (69.3)	5.9 (3.3,10.6)*	3.2 (1.4,7.3)**
Mild fever and others	53 (72.6)	20 (27.4)	1	1
Antibiotics taken before 07 days				
Yes	49 (33.1)	99 (66.9)	1	
No	86 (44.8)	106 (55.2)	0.6 (0.3,0.9)	
History of hospital acquired infection				
yes	49 (94.2)	3 (5.8)	50.0 (12.5,125.0)*	25.0 (5.0,111.1)**
No	86 (29.9)	212 (70.1)	1	1
Complication of BSI				
Sepsis/septic shock	87 (94.6)	5 (5.4)	100.0 (33.3,200.0)*	20.0 (6.25,50.0)**
No sepsis/septic shock	48 (19.4)	200 (80.6)	1	1

Bacterial pathogens

A total of 137 (40.2%) bacterial pathogens were isolated from 340 paired blood sample bottles. Among positive blood culture results, 63 (46%) isolates were gram positive bacteria while 74 (54%) were gram negative bacteria. *K. pneumoniae* had the highest isolation incidence of 43 (31.4%), *S. aureus* accounted for 29 (21.2%) CoNS 15 (10.9%) and *Acinetobacter* species 11(8.0%). Co-infection from *P. aeruginosa* species and *K. oxytoca* were identified in one patient (Table 3).

Table 3. Distribution of bacteria pathogens isolated from blood stream infection among suspected septicemia patients in TASH.

Bacterial isolates	Frequency (%)
Gram positive bacteria	63 (46)
Staphylococcus aureus	29 (21.2)
Coagulase negative staphylococcus	15 (10.9)
Enterococcus spp.	11 (8.0)
Viridian group streptococcus	5 (3.6)
Streptococcus pneumoniae	3 (2.2)
Gram negative bacteria	74 (54)
Klebsiella pneumoniae	43 (31.4)
Acinitiobactor species	11 (8.0)
Klebsiella oxytoca	6 (4.4)
Escherichia coli	5 (3.6)
P. aeruginosa	5 (3.6)
Entrobacter clocae	1 (0.7)
Citrobacter spp.	1 (0.7)
Salmonella spp.	2 (1.4)
Total	137 (100)

Antimicrobial susceptibility testing

Trends of antibiotics prescribed were assessed prior to blood sample collection before 7 days and 148 (43.5%) participants took antibiotics empirically and of these 49 (33.1%) were culture positive during the study. Ampicillin and gentamicin were the most common empirically prescribed antibiotics.

The predominant gram positive bacteria *S. aureus* isolates were resistant to oxacillin 16 (55.2%), penicillin 16 (55.2%), trimethoprim-sulphamethoxazole 11 (37.9%) and were sensitive to erythromycin 21 (72.4%), clindamycin 19 (65.5%). Coagulase negative *Staphylococcus* isolates were resistant to trimethoprim- sulphamethoxazole 2 (13.3%), oxacillin 1 (6.7%), and penicillin 1 (6.7%) however they were both sensitive to oxacillin and penicillin 14 (93.3%). *Enterococcus* spp. were resistance to ampicillin 2 (18.2%) but sensitive to vancomycin 11 (100%). Viridans Group Streptococci (VGS) were completely sensitive to all tested antimicrobials *i.e.,* clindamycin, ampicillin, penicillin and erythromycin. Furthermore, isolates of *Streptococcus pneumoniae* were resistance to trimethoprim-sulphamethoxazole 1 (33.3%) and completely sensitive to penicillin, clindamycin, ampicillin, erythromycin and augmentin (Table 4).

Table 4. Antimicrobial susceptibility of gram positive bacterial isolates associated with bloodstream infections among pediatric patients in TASH.

Gram positive bacterial isolates		Antimicrobial susceptibility pattern							
		SXT	CN	AMP	OXA	P	E	VAN	AGU
S. aureus (n=29)	R%	11 (37.9)	10 (34.5)	NA	16 (55.2)	16 (55.2)	8 (27.6)	NA	NA
	S%	18 (62.1)	19 (65.5)	NA	13 (44.8)	13 (44.8)	21 (72.4)	NA	NA

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Coagulase negative staph. (n=15)	R%	2 (13.3)	0 (0)	NA	1 (6.7)	1 (6.7)	0 (0)	NA	NA
	S%	13 (86.7)	15 (100)	NA	14 (93.3)	14 (93.3)	15 (100)	NA	NA
Enterococcus spp. (n=11)	R%	NA	NA	2 (18.2)	NA	NA	NA	0(0)	NA
	S%	NA	NA	9 (81.8)	NA	NA	NA	11 (100)	NA
Viridans streptococci (n=5)	R%	NA	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA	NA
	S%	NA	5 (100)	5 (100)	NA	5 (100)	5 (100)	NA	NA
S. pneumoniae (n=3)	R%	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	0 (0)
	S%	2 (66.7)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	NA	3 (100)
Note: P: Penicillin; AMP: Ampicillin; SXT: Cotrimoxazole (trimethoprim+sulphamethoxazole); CN: Clindamycin; ox: Oxacillin; VAN: Vancomycin; E: Erythromycin; AGU: Augmentin									

Klebsiella pneumoniae showed resistance to trimethoprim-sulphamethoxazole 39/43 (90.7%). These isolates were susceptible to meropenem 27/43 (62.8%) and piperacillin-tazobactam 25/43 (58.1%). All *Acinetobacter* species were highly resistant to ceftazidime 11/11 (100%), cefepime 10/11 (90.9%), 72.7% for both meropenem and ciprofloxacin. *P. aeruginosa* demonstrated 2/4 (50%) resistance to anti-pseudomonal antibiotics *i.e.* gentamycin, ciprofloxacin, cefepime, amikacin and ceftazidime but were (75%) susceptible to meropenem and piperacillin-tazobactam. *Salmonella* species were 100% susceptible to ciprofloxacin, ceftriaxone, and ampicillin and less susceptible to cotrimoxazole 1/2 (50%) (Table 5).

Table 5: Antimicrobial susceptibility of gram negative bacterial isolates associated with blood stream infection among pediatric patients in TASH.

Gram negative bacteria isolates		Antibiotic susceptibility pattern											
		SXT	GEN	CIP	CRO	AMP	MEM	FEP	AMK	TORB	PZT	AGU	CAZ
E. coli (n=5)	R%	5 (100)	3 (60.0)	5 (100)	4 (80.0)	4 (80.0)	0 (0)	4 (80.0)	0 (0)	4 (80.0)	3 (60.0)	3 (60.0)	5 (100)
	S%	0 (0)	2 (40.0)	0 (0)	1 (20.0)	1 (20.0)	5 (100)	1 (20.0)	5 (100)	1 (20.0)	2 (40.0)	2 (40.0)	0 (0)
Pseudomonas aeruginosa (n=4)	R%	NA	2 (50.0)	2 (50.0)	NA	NA	1 (25.0)	2 (50.0)	2 (50.0)	1 (25.0)	1 (25.0)	NA	2 (50.0)
	S%		2 (50.0)	2 (50.0)			3 (75.0)	2 (50.0)	2 (50.0)	3 (75.0)	3 (75.0)		2 (50.0)
K. pneumoniae (n=43)	R%	39 (90.7)	38 (88.4)	38 (88.4)	37(86.0)	NA	16 (37.2)	38 (88.4)	22 (51.2)	38 (88.4)	18 (41.9)	37(86.0)	37(86.0)
	S%	4 (9.3)	5 (11.6)	5 (11.6)	6 (14.0)		27 (62.8)	5 (11.6)	20 (46.5)	5 (11.6)	25 (58.1)	6 (14.0)	6 (14.0)
Klebsiella oxytoca (n=6)	R%	5 (83.3)	3 (50.0)	4 (66.7)	6 (100)	NA	2 (33.3)	6 (100)	3 (50.0)	4 (66.7)	3 (50.0)	6 (100)	6 (100)
	S%	1 (16.7)	3 (50.0)	2 (33.3)	0 (0)		4 (66.7)	0 (0)	3 (50.0)	2 (33.3)	3 (50.0)	0 (0)	0 (0)
Acinetobacter spp. (n=11)	R%	NA	9 (81.8)	11 (72.7)	NA	NA	8 (72.7)	10 (90.9)	6 (54.5)	9 (81.8)	6 (54.5)	NA	11 (100)
	S%		2 (18.2)	3 (27.3)			3 (27.3)	1 (9.1)	5 (45.5)	2 (18.2)	5 (45.5)		0 (0)
Enterobacter cloacae (n=1)	R%	1 (100)	1 (100)	1 (100)	1 (100)	NA	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	NA	1 (100)
	S%	0 (0)	0 (0)	0 (0)	0 (0)		1 (100)	0 (0)	1 (100)	0 (0)	0 (0)		0 (0)
Citrobacter (n=1)	R%	1 (100)	0 (0)	0 (0)	0 (0)	NA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	0 (0)

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	S%	0 (0)	1 (100)	1 (100)	1 (100)		1 (100)	1 (100)	1 (100)	1 (100)	1 (100)		1 (100)
Salmonella spp. (n=2)	R%	1 (50.0)	NA	0 (0)	0 (0)	0 (0)	NA	NA	NA	NA	NA	NA	NA
	S%	1 (50.0)		2 (100)	2 (100)	2 (100)							
Note: SXT: Sulphamethoxazol-trimethoperem/cotrimosazole; GN: Gentamycin; CIP: Ciprofloxacillin; CRO: Ceftriaxone; AMP-Ampicillin; MEM: Meropenem; FEP: Cefepime; AMK: Amikacin; CAZ: Ceftazidime, Torbomycin, Piperacillin-Tazobactam, AGU: Augmentin/Amoxycillin-Clavulanic acid, NA: Not applicable													

Methicillin resistant *Staphylococcus aureus*: In the susceptibility testing of *S. aureus* isolates using cefoxitin disc, 16 (55.2%) were resistant to oxacillin. Therefore the prevalence of MRSA was 55.2% of the isolates. Minimum Inhibitory Concentration (MIC)/E tests performed among *S. aureus* isolates for vancomycin susceptibility showed 1 (3.4%) isolate recovered from the blood of a patient had a MIC of 4 µg/ml and was confirmed as a VISA isolate. For the remaining 28 isolates, vancomycin MICs ranged from 0.5 to 1.0 µg/ml. However, no VRSA isolates were detected.

Carbapenem resistant *Enterobacterales*: Out of 59 Enterobacterales isolates, 18 (30.5%) were resistant to carbapenem antibiotics by producing carbapenemase, phenotypically confirmed by mCIM of which 41 (69.5%) were sensitive. The predominant carbapenem resistance isolate for *Enterobacterales* species in our study was *Klebsiella pneumoniae* 27.1% (n=16/59) and *Klebsiella oxytoca* 3.4% (n=2/59). Other gram negative non-*Enterobacterales* isolates capable of developing carbapenem resistance were identified in *Acinetobacter* species 12.2% (n=9/74) and *Pseudomonas aeruginosa* species 1.3% (n=1/74) of all gram negative isolates.

ESBL producing *Enterobacterales*: 59/74 (79.7%) of *Enterobacterales* isolates were suspected ESBL producing organisms of which *K. pneumoniae* 16/59 (27.1%) and *E. coli* 1/59 (1.7%) were ESBL producers.

Combined Disk (double disk potentiate) Test (CDT): The overall prevalence of ESBL producing *Enterobacterales* was 28.8% (n=17/59). Among the suspected 17 isolates, 100% (n=17/17) were phenotypically confirmed for ESBL using the combination disk method; *K. pneumoniae* 100% (n=16/16) and *E. coli* 100% (n=1/1) were confirmed positive for ESBL.

Double Disk Synergy Test (DDST): All isolates (n=17) were further tested for ESBL production by the double disk synergy procedure, another phenotypic confirmatory method. The double disk synergy method indicated 82.3% (n=14/17) were confirmed for ESBL producing *Enterobacterales*. Thus, 100% (n=17/17) of *K. pneumoniae* isolates were positive by the reference (CDT) method, 82.3% (n=14/17) were positive by this method while 17.6% (n=3/17) were negative. The *E. coli* 100% (n=1/1) was ESBL positive with concordance conducted by two methods.

Multi-drug resistance isolates

Out of 29 *S. aureus* isolates 16 (55.2%) of them were MDR by virtue of being MRSA which was equivalent to resistance in ≥ 3 classes of antimicrobial categories and 8 (27.6%) were XDR (non-susceptible to ≥ 1 agent in all but ≤ 2 categories). Half of the isolates in *P. aeruginosa* were MDR and XDR accounted 50%. Among *K. pneumoniae* isolates 39 (90.7%), 13 (30.2) and 3 (7.0) were MDR, XDR and PDR respectively (Table 6).

Table 6. Rate of multidrug resistance among isolates from BSI in TASH.

Bacterial isolates	Multidrug resistance pattern (%)				
	MDR	XDR	PDR	ESBL	CRE
S. aureus (n=29)	16 (55.2)	8 (27.6)	0	-	-
E. coli (n=5)	5 (100)	0	0	1 (20)	0 (0)
Pseudomonas aeruginosa (n=4)	2 (50)	2 (50)	0	-	1 (25)
Klebsiella pneumoniae (n=43)	39 (90.7)	13 (30.2)	3 (7.0)	14 (32.5)	15 (34.8)
Klebsiella oxytoca (n=6)	6 (100)	2 (33.3)	0	0	2 (33.3)
Acinetobacter spp. (n=11)	10 (90.9)	5 (45.5)	0	0	8 (72.7)
Enterobacter cloacae (n=1)	1 (100)	0	0	0	0
TOTAL	79/137 (57.6)	30/137 (21.9)	3/137 (2.2)	15/59 (25.4)	26/74 (35.1)
Note: MDR: non susceptible to ≥ 1 in ≥ 3 antimicrobial categories, XDR: non-susceptible to ≥ 1 agent in all but ≤ 2					

categories. PDR: non-susceptible to all antimicrobial agents listed [14].

DISCUSSION

Bloodstream infection in pediatric patients associated with febrile illness is a major public health problem particularly in low-middle income countries where high child morbidity and mortality rate exist. Timely detection of bacteremia in blood culture is a promising diagnostic tool established to rule out bacteremia and determine its antimicrobial susceptibility profile which is necessary for clinicians to decide appropriate antibiotic therapy, which ultimately decreases the emergence of drug resistance [15].

The overall prevalence of BSI based on significant bacterial growth in blood cultures was 40.2% of isolates which was in agreement with the study in Gondar, Ethiopia 39.5% and other similar studies conducted in African countries such as in Egypt 40.7% and Tanzania 38.9% and in India by Zakariya et al., 41.6% and Khanal et al., (27) has reported 44% of positive blood cultures [16-20].

The results in the present study were higher than previous studies conducted in Addis Ababa, Ethiopia 13.0% and 27.9%, and other African counties such as Tanzania 7.7% and Ghana 19.9%. The difference was due to the high number of patients in ICU and inpatients rather than outpatient departments where high nosocomial infection contributed to greater exposure. Additionally, we used the more sensitive automated system for recovery of bacteria while other studies used conventional methods. However our isolation rate was lower than the study in Nigeria which was 47.6%.

Gram negative bacteria were predominantly isolated from BSI compared to gram positive bacteriai (54% versus 46%) which were comparable with previous study in Addis Ababa, Ethiopia (51.8% *vs.* 46.4%) and elsewhere in India 51.82% *vs.* 46.56%, 51.7% *vs.* 44.8%, Nepal 55.2% *vs.* 44.8%, 56% *vs.* 44% 29 but it was inconsistent with a USA study by Larru et al., 22% *vs.* 72% 30 and in South Africa by Crichton et al., 40.7% *vs.* 59.3%.

In our study, *S. aureus* and CoNS were among the major gram-positive bacteria and accounted for 21.2% and 10.9% which were inconsistent with a previous study in Gondar, Ethiopia which isolated *S. aureus* 42%, CoNS 26%. However, it was nearly similar with the study published by Anjum et al., isolated *S. aureus* 19.7% and CoNS 7.23% and in India 27.37% *S. aureus* and 20.1% CoNS was reported. Our result was lower compared to the study conducted in Ghana which showed *S. aureus* 66.7%, CoNS 17.6%.

Among gram-negative bacteria, *K. pneumoniae* 31.4% isolates were predominant followed by *Acinetobacter* species 8.7%. This was supported by a studies in Jimma, Ethiopia 31.4%, in African countries in Kenya 13%, Ghana 26% , Bouaké, Central Côte d'Ivoire 22.5% and in Asia such as in India by 25.8%, 30.5% in Brazil, by Berezina et al., Vietnam 20%. However, it was different compared to other countries such as in India by Kante et al., Indonesia by Murni et al., where the most frequently isolated pathogen in BSI was *P. aeruginosa* species other than *K. pneumoniae*. This might be due to difference prescription practices for empirical treatment of patients before blood culture and different clinical patient management in pathogens causing nosocomial infection across the counties. In our tertiary care hospital setting, the response to nosocomial infections did not include patient isolation in the ward which further increased the survival of high drug resistant bacteria including *K. pneumoniae*.

A polymicrobial infection in our study was isolated in a single patient and etiologies both were from gram negative bacteria that tends to increase the severity of the diseases which is in agreement with previous study even though some microbiologists consider polymicrobial growth as contamination, sepsis should be clinically correlated.

The trend of empirical treatment in our study 43.5% and the most prescribed antibiotics were ampicillin, gentamicin, and ciprofloxacin and third generation cephalosporins (most commonly ceftriaxone); ampicillin and gentamicin were the most common combination antibiotics used. This was supported by a previous study in Tamale, Ghana.

The rate of antimicrobial resistance in gram positive and gram negative isolates ranged from 0%-55.2% and 0%-100% respectively. In our gram positive isolates, ampicillin was 100% effective for *S. pneumoniae*, *Streptococcus viridans* and 81.8% for *Enterococcus* spp. In addition cotrimoxazole, which demonstrated a high sensitive against *S. aureus* 62.1%, CoNS

86.7% and *S. pneumoniae* 66.7%. However, *S. aureus* showed 55.2% resistance to oxacillin and penicillin. Accordingly, the prevalence of MRSA 55.2% was comparable with previous study in Jimma, by Balta and Fetene 51.8%.

In our study, among MRSA isolates 1 (3.4%) was vancomycin intermediate *S. aureus*. However, no VRSA isolates were detected. The result was in agreement with the study by Wang et al., in Los Angeles but inconsistent to the report from CDC reported by Miller et al., in which two isolates were VRSA. This was in fact due to variation in sample size.

The antimicrobial susceptibility of *K. pneumoniae* isolates demonstrated high level of resistance to cotrimoxazole (90.7%) and gentamycin (88.4%), and were sensitive to meropenem (62.8%), piperacillin-tazobactam (58.1%) which was consistent with the studies by Zenebe et al., who reported 100% resistance to cotrimoxazole, in Bahir dar, Ethiopia by Hailu et al., cotrimosazole 77.1% and gentamicin 71% while in India the resistance of cotrimoxazole and gentamycin done by Kumar et al., were 88%, 67% respectively. It was also comparable in Kaneti Childrens Hospital, Nepal by Kari et al., reported least sensitive to cotrimoxazole and gentamycin. The most effective antibiotics were 3rd and 4th generation cephalosporins, quinolones and carbapenem antibiotics which also showed resistance and is a concern for the treatment of BSI in septic pediatric patients.

The second most predominant GNB isolates in our study were *Acinetobacter* species that were resistant to most tested antimicrobials including ceftazidime 100%, cefepime 90.9% gentamycin 81.8%, tobramycin 81.8% ciprofloxacin 72.7%, meropenem 72.7% and was comparable with other previous studies where high resistance in *Acinetobacter* species. However, our result was high rate of resistance compared to the study conducted in South India by Zakariya et al., meropenem 100% sensitive, while 67% were sensitive to gentamicin, ceftriaxone, ciprofloxacin, ceftazidime and amikacin reported. This may be explained as our study had many isolates and might be due to inappropriate use of meropenem as the first line treatment since most of isolates are from ICU patients.

The overall prevalence of MDR in our study was 57.6% of which most of them were gram-negative bacteria with a very high resistance to beta-lactam antibiotics. This result is supported by the previous study in Ethiopia. *Klebsiella* isolates 91.8% were MDR, and 90.9% of *Acinetobacter* isolates were MDR. This was consistent with the study in north India.

The degree of carbapenem resistance *Enterobacterales* was 30.5% comparable with study conducted in Tanzania 35%. The most carbapenem resistance was detected in 72.2% isolates of *Acinetobacter* spp. and in 62.8% of *K. pneumoniae*. This was inconsistent with a study in north India 64%, 92% respectively.

The prevalence of ESBL-producing *Enterobacterales* in our study is 25.4%. Among *K. pneumoniae* isolates 14 (32.5%) and *E. coli* isolates 1 (20.0%) were ESBL-producers which is in line with the study conducted in south India by Zakariya et al., 32.0% and in Mali by Sangare et al., 29.4%.

LIMITATION OF THE STUDY

Even though our study identifies numerous bacteria pathogens causing BSI in pediatrics under five years, we were unable to isolate other possible pathogens including anaerobic bacteria due to lack of anaerobic culture set up and infrastructure

CONCLUSION

We found that gram negative isolates, *K. pneumoniae*, *Acinetobacter* species and among gram positive isolates *S. aureus* were the most frequent multidrug resistant bacteria isolated from blood stream infection of children under five years at TASH. The majority of antibiotic results including last line demonstrate elevated resistance. The prevalence of MDR, XDR, PDR, ESBL and CRE among *K. pneumoniae* isolates were 90.7%, 30.2%, 7.0% 32.5% and 34.8% respectively. Moreover, the frequency of MRSA among *S. aureus* was 55.2%.

DECLARATION

The author's declare that the study is their original work

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted after it was approved by the department of Medical Laboratory Sciences research and ethics review committee (DRERC), school of Allied health sciences, College of Health Sciences, Addis Ababa University (Ref. no: 132645/18) . An informed consent was obtained from mother /guardian before collection of blood specimens and results were used in the management of patients. Written consent was sought for the study and any information related with the patient result and clinical history was kept confidential.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

The data is available in first author and can provide when necessary.

COMPETING INTERESTS

We declare the is no competing interest.

FUNDING

Not available.

AUTHORS' CONTRIBUTIONS

MM, topic selection, designed the study protocol, participated in data collection, performed analysis, interpretation and wrote the research thesis, ZA, and ME wrote the first and final draft of the manuscript for publication, KD, advised and approved the research topic selection, provide the inputs during analysis and interpretation of the whole research paper. All authors read and approved the final manuscript.

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