Multi-drug resistant, extended spectrum beta-lactamase and carbapenemase producing bacterial isolates among children under five years old with suspected bloodstream infection in a specialized hospital in Ethiopia: Cross-sectional study

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Research article

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Abstract

Background

Bloodstream infections due to bacterial pathogens are a major cause of morbidity and mortality among pediatric patients. Emergence of drug resistance in high classes to antibiotics among the bacterial pathogens is another issue of public health concern. Therefore, this study was conducted to determine multi-drug resistant, extended spectrum β-lactamase and carbapenemase producing bacterial isolates among suspected bloodsteam infection patients in children under five years of age at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Methods

A cross-sectional study was conducted from September 2017 to June 2018 among pediatric patients with febrile illness under five years of age at Tikur Anbessa Specialized Hospital. Three hundred and forty blood samples were collected and processed following standard microbiological techniques and blood culture was performed using a BacT/Alert instrument in combination with conventional methods for identification. Antimicrobial susceptibility testing of the isolates was performed using the Kirby-Bauer disc diffusion method to determine the minimum inhibitory concentration (MIC).

Result

A total of 137 (40.2%) bacterial pathogens were isolated from 340 pediatric patients suspected of bloodstream infection with febrile illness. Of these isolates, 54% were Gram negative bacteria. Among gram negative isolates 43 (31.4%) were identified as *Klebsiella pneumonia*e and 8.7% *Acinetobacter* species were the most frequently isolated pathogens. *Klebsiella pneumoniae* isolates were 88.4 % (38/43) MDR, 32.5% (14/43) ESBL, and 37.2 %(16/43) CRE .

Conclusion

In this study, highly resistant *Klebsiella pneumoniae* are common pathogen associated with BSI. Extended spectrum beta-lactamase (ESBL) producing strains were common in *Klebsiella* species and *Escherichia coli* isolates. Since most of isolates exhibit multidrug resistance, in vitro antimicrobial susceptibility testing is mandatory. A strengthened antimicrobial surveillance system and antimicrobial stewardship programs are necessary for better selection of antibiotics in addition to improved infection prevention practices in hospital settings.

Background

Bloodstream infections 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]. Bloodstream infection comprises 10-20% of every nosocomial disease and is the eighth leading cause of mortality in the United States with some 17% causing death [2]. In sub Saharan nations including Ethiopia,

septicemia is a significant cause of illness and death in young people with death rate approaching 53% making it a noteworthy medical issue in under-developed countries [3].

In many studies, a wide range of bacteria have been described in febrile patients including 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 few Hospitals in developing countries [4, 5].

Bacterial pathogens isolated from bloodstream infection are a major source of critical patient morbidity and mortality. The effect of explicit etiologic agents on BSI persistent have a major impact; BSI increases the death rate, causes outpatients to remain in hospital emergency, and increases the cost in providing human services [6,7].

The timely and appropriate use of antibiotics is currently the best way to treat bacteremia. However, many bacterial pathogens have become resistant to antibiotic regimens and are a serious public health concern with global economic and social implications. Antibiotic resistance is a growing problem in resource limited countries like Ethiopia. In Ethiopia, the unregulated and over-the-counter sale of antimicrobials without a prescription, mainly for self-treatment of suspected infection in humans, and to a lesser extent for use in animals inevitably leads to the emergence and rapid dissemination of resistance [8]. Many studies have found that inadequate empirical therapy of bacteremia infections are associated with adverse outcomes including increased mortality and increased emergence of drug resistance [9-10].

During the previous several decades, opposition to the use of antimicrobials has expanded, and the points of view are disturbing [11]. The proper use of antibiotics is well understood in the western world but this learning is deficient in developing African countries [12-14].Recent studies on the outcome of sepsis in Africa are almost non-existent although there are a few reports. The most concerning reports on antimicrobial resistance concern patients admitted to hospitals [15], while community- acquired infections may have lower levels of resistance [16].

In Ethiopia, the resource situation has not allowed antimicrobial resistance to be prioritized as a major public health concern despite the obvious needs [17]. The aim of this study was to identify and determine multi-drug resistant, extended spectrum β-lactamase and carbapenemase producing bacterial isolates among blood culture specimens from pediatric patients under five years of age fromTikur Anbessa Specialized Hospital using an automated BacT/Alert instrument.

Methods

Study setting

The study was conducted at the Tikur Anbesa Specialized Hospital (TASH) which is the teaching hospital for the College of Health Science 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, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists, and others who lead the solution for health problems of the community and the nation. With more than 70 percent of childhood deaths attributable to communicable diseases and malnutrition, Ethiopia's healthcare resources have been primarily focused on the treatment and prevention of diseases such as malaria and diarrhea [18].

Study design and period

A cross-sectional study was conducted from September 2017 to June 2018 to identify the bacterial profiles and antimicrobial susceptibility patterns among septicemia in under five patients with acute febrile illness in Tikur Anbessa Specialized Hospital in Addis Ababa.

Inclusion and Exclusion criteria

Children aged under five years including neonates with fever, those diagnosed with sepsis, severe sepsis and septic shock. All children who gave blood samples were volunteers with parental permission to participate in the study. Those patients who were a febrile under five years and patients who had taken antibiotics within the last 7 days were excluded.

Sample size calculation

The sample size for the study was determined using a single population proportion formula. The study considered prior prevalence and antibiotic resistance data in septicemia patients at Tikur Anbesa Specialized Hospital which demonstrated 27.9% bacterial isolation (19) with a 95% level of confidence and 5% margin of error. n = $(Z\alpha)^2 (pq)/ d^2$ where: n = sample size, Zα/2 = level of confidence, P = diarrhea prevalence q = 1-p d2 = margin of error (0.05): $n= z^2 * p * q / d^2$, p=0.279, q=0.721, d=0.05, Zα/2=1.96; $1.96^2*0.279*0.721/0.05^2$=309. Considering a 10% non- response rate, a total of 340 children patients were enrolled in the study.

Sampling technique

The study subjects were selected using convenient sampling technique from all patients attending Tikur Anbessa Specialized Hospital among children under five with febrile illness clinically diagnosed at pediatric OPD, ICU and inpatient pediatric wards admitted during the study period. Venipuncture was employed for those children that fulfilled the inclusion criteria.

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 the onset of fever (>37◦C) or the presence of any clinical symptoms compatible with infection.

Blood sample 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. About 2.5-5ml of blood was collected and inoculated in an aerobic 30ml BacT/ALERT PF Plus pediatric bottles with a blood to broth ratio of 1: 10-1:30. At least 2 sets of blood cultures were collected 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 automated BacT/ALERT® 3D instrument at 37^{o}C with 5% CO_2 for 5 days for the 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 sub cultured on 5% sheep blood agar, chocolate agar, and MacConkey Agar plates (Oxoid Ltd, UK) and incubates for bacterial isolation at 37^{o}C for 18-24 hours. The MacConkey agar plate was incubated aerobically while chocolate and blood agar plates were incubated in a microaerophilic atmosphere (5-10% CO_2) using a candle jar. A negative result was checked by a gram stain and a final subculture at the end of 5th day before reporting as a negative result. Growth was examined for colonial morphology including size, consistency, shape, hemolysis and ability to ferment lactose. For gram negative isolates, convectional biochemical test were performed [20].

Antibiotic Susceptibility Test

A pure colony of the bacterial isolate was mixed with 0.85% normal saline and adjusts to obtain a 0.5 McFarland standard density required for antibiotic susceptibility testing. Bacterial isolates were tested against the following antibiotic panel commonly used - for gram negative bacteria: tobramycin (10µg), amoxicilin-clavulanate (20/10µg), amikacin (30µg), gentamycin (10µg), ampicillin (10µg), piperacillin-tazobactam (100/10µg), cefotaxime (30µg), cefepime (30µg), ceftriaxone (30µg),ciprofloxacin (5µg), impenem/meropinem (10µg),trimethoprim- sulfamethoxazole (1.25/23.75µg) were tested. The Kirby-Bauer disk diffusion method was used for susceptibility testing using Muller Hinton agar and the standard interpretative zone size criteria from the current CLSI standards [21].

Detection of Carbapenem Resistance

All carbapenem resistance or intermediate isolates were phenotypically confirmed for the presence of carbapenemase using the modified carbapenem inactivation test (mCIM) to identify carbapenem resistant *Enterobacteriacae* (CRE) isolates as recommended by CLSI [21].

Detection of extended spectrum beta-lactamase

Initial screening for ESBL used the diameters of zones of inhibition produced by ceftazidime (30µg), ceftriaxone (30µg) and cefotaxime (30µg) measured within the CLSI screening criteria. These breakpoints that indicate ESBL production are ceftazidime ≤22mm, ceftriaxone ≤ 25 mm and cefotaxime ≤ 27mm. Phenotypic detection of ESBL production was confirmed by conducting a double disk synergy test and combined disk test according to CLSI guideline [21].

Combined disk (double disk potentiate) test (CDT)

Ceftazidime (30 µg) disk and cefotaxime (30µg) 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. According to CLSI, this method is used as a reference phenotypic method for comparing double disk synergy method [21].

Double Disk Synergy test (DDST)

The test isolate was spread onto a Mueller–Hinton agar plate. Ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30µg), aztreonam (30µg) and amoxicillin/ clavulanic acid (20/10 µg disks were placed at distances of 20 mm (edge to edge) from the amoxicilin-clavulanate acid 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 disk is interpreted as a positive test [21].

Data quality assurance

Media was prepared as per manufacturer instructions and the laboratory Standard Operating Procedures (SOP) was strictly followed. Media were within expiration date and quality control parameters were used as defined in the CLSI standards.

Use ATCC control strain for each isolated bacterium included *E. coli* 25922, *Pseudomonas aeruginosa* 27853, *and H. influenzae* 10479, *K.pnuemoniae* 700603, *S.typhimurium* 13311. The results were reported on a log sheet and isolates were stored at -80 0c in skim milk.

Data analysis and interpretation

Statistical package for social science (SPSS) versions 20.0 was employed to analyze the work and to make inferences on the frequency of occurrence of the bacterial pathogens associated with febrile illness and to show resistance patterns. Descriptive statistics to analysis used frequency, proportions graphs, and crosstabs and odds ratio. Bivariate analysis was performed for each factor associated with bloodstream infection. Regression analysis was conducted to identify associated factors and how they are associated with dependent variables .The strength of association was presented by odds ratio and 95% confidence interval and p-value of <0.05 was considered as statistical significant association.

Results

Socio-demographic characteristics

Among the study participants 122 (35.9%) were males and 218 (64.1%) were females resulting in an overall female to male ratio of 1.7:1. The mean age of pediatrics participated in this study was 1.04±1.0 (SD) years. In regarding to patients location 76 (22.4%) were from pediatric OPD and 181(53.2%), 83(24.4%) from inpatient ward and ICU ward respectively. 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%) patients were identified (**Table 1)**.

Clinical features

Patients showed different presumptive clinical diagnoses before confirmed their BSI in blood culture, most of were sepsis 102 (30.0%) followed by early onset of neonatal sepsis /EoNS/ 71 (20.9%), late onset neonatal sepsis/LoNS 48(14.1%) and hospital acquired infection 43(12.6%). However, the distribution of positive blood cultures among patients with different clinically diagnosed diseases suspected of having BSI showed that among clinical disease in endocarditis 7/11(63.6%), hospital acquired infection 26/43(60.5%), sepsis 49/102 (48%) and late neonatal sepsis 21/48 (44%) high positive blood culture were identified as shown (**Figure1)**.

Bacteria pathogens among blood stream infection

Of 340 paired blood sample bottles, a total of 137(40.2%) bacterial pathogens were isolated from pediatric patients suspected of BSI with febrile illness. Among positive blood culture results about 54% were Gram negative bacteria with *Klebsiella pneumoniae* having the highest incidence of 31.4% followed by *Acinitiobacter* species (8.7%). Double infection from *Pseudomonas species* and *Klebsiella oxytoca* were identified in one patient as shown (**Figure2)**.

Antimicrobial susceptibility testing

Trends in prescribing antibiotics were assessed prior to blood sample collection before 7 days and 148 (43.5%) participants received antibiotics empirically of which 49(33.1%) were culture positive during the study. Ampicillin and gentamicin were among the most common empirically prescribed antibiotics. Culture isolation of 74 gram negative bacteria were tested with 12 antibiotics and demonstrated that the most prescribed antibiotics cotrimoxazole, gentamicin, and ciprofloxacin showed high resistance.

Antimicrobial susceptibility pattern of Gram negative bacterial isolates

A total of 74 gram negative isolates with exception of *Salmonella* species, were tested with beta-lactam antibiotics, fluoroquinolones, aminoglycosides, and carbapenems. The predominant gram negative isolate from BSI was *Klebsiella pneumoniae* which showed resistance to ampicillin (100%) and cotrimosazole (90.7%) but the isolates were susceptible to meropenem (62.8%) and Piperacillin-Tazobactam (58.1%). All *Acinetobacter* species were highly resistance to cefepime (100%), ceftazidime (90.9%), 72.7% for meropenem and ciprofloxacin. *Pseudomonas* species also showed 50% resistance to

anti-pseudomonal antibiotics gentamycin, ciprofloxacin, cefepime, amikacin and ceftazidime but was 75% susceptible to meropenem and piperacillin-tazobactam. Both *Salmonella* species were completely susceptible to ciprofloxacin, ceftriaxone and ampicillin but 50% of the isolates we resistant to cotrimoxazole [Table 2].

Multi-drug resistant isolates

Sixty two (83.7%) of the isolates were shown to be resistant to three or more classes of the antibioties considered as multidrug resistance organisms. *Pseudomonas aeruginosa* showed that two (50%) of the isolates exhibit resistance to three antibiotic classes. In *Klebsiella pneumoniae*, 38(88.4%) of the isolates were MDR. Among eleven *Acinetobacter species* 8(72.2%) of the isolates were resistance to more than three classes of antimicrobials. The least isolate of gram negative bacteria was *Enterobacter cloacae* 1 (100%) exhibits high MDR which was resistance to eight or more antibiotics. However there was no MDR in *Citrobacter* and *Salmonella species* (Table 3).

Carbapenem resistant *Enterobacteriaceae* (CRE)

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

Extended spectrum beta-lactamase producing *Enterobacteriaceae*

Screening tests showed a total of 74 gram negative bacteria 59(79.7%) *Enterobacteriaceae* isolates were suspected ESBL producing organisms. *Klebsiella pneumoniae* 27.1% (n=16/59) and *Escherichia coli* 1.7% (n=1/59) were among gram negative *Enterobacteriaceae* isolates identified as ESBL producers.

Combined disk (double disk potentiate) test (CDT): The overall prevalence of ESBL producing *Enterobacteriaceae* was 28.8% (n=17/59). Among the suspected 17 isolates, 100% (n=17/17) were phenotypically confirmed as ESBL using combination disk method, with *K. pneumoniae* 100% (n=16/16) and E. coli 100% (n= 1/1) were positive. This result was used since the CLSI standard recommends this technique as a reference for other phenotypic methods. This test result was compared to the findings of the double disk method.

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

Discussion

Blood stream infection (BSI) in pediatric patients associated with febrile illness is a major public health problem especially in developing countries where there are high child morbidity and mortality rates. Accordingly, the timely detection of bacteremia in blood cultures is a promising diagnostic tool established to rule out bacteremia. The determination of the antimicrobial susceptibility profile is necessary for clinicians to determine appropriate empirical therapy, which ultimately decreases the emergence of drug resistance [22]. The present study included 340 pediatric patients under five years of age clinically diagnosed with different diseases suspected of causing bacteremia. Even though there was no statistically significant association for endocarditis (63%), hospital acquired infection (60%) and sepsis (48%) patients accounted for the highest proportion of positive blood cultures [Table1].

In this study, overall prevalence of bloodstream infection based on significant bacterial growth in the blood cultures obtained from suspected patients was 137 (40.2%). This was in agreement for other studies which demeonsrated a prevalence range of 35%-45% in line with the study done in Gondar, Ethiopia was 39.5% [23] and other similar studies conducted in African countries such as in Egypt 40.7% [24] and Tanzania 38.9% [25] as well as in India by Zakariya et al., 41.6% [26] and Khanal et al., [27] who reported 44% positive blood cultures. Meanwhile, the present study was higher than the studies conducted in Addis Ababa, Ethiopia 13.0% [28], 27.9% [23], and other African counties such as Tanzania 7.7% [29] and Ghana 19.9% [30]. The difference between these studies might be due to differences patient status in which our study included more patients from ICU and inpatients than outpatients. Furthermore blood cultures were performed by using the more sensitive automated BacT/ALERT system. However we have isolated bacteria lower than the studies in Nigeria 47.6% [31], this was due to the patient status in which others only include inpatient and also isolated anaerobic bacteria.

Among the total isolates, 54% gram negative bacteria caused blood stream infections in children which is in keeping with the previous study done in Addis Ababa, Ethiopia 51.8% [23], India 51.8% [32], Kabul, Afghanistan 51.7%[33], and Nepal 55.2%, 56% [34] respectively. However it was higher compared to the study done in USA by Larru., et al., 22%[35] and in South Africa by Crichton et al., 40.7% [36], this was due to difference in socioeconomic , geographical and infection control mechanisms.

In this study, the most common causes of bloodstream infections were gram-negative bacteria, in particular *Klebsiella pneumoniae* 31.4 % followed by *Acinitobacter* species 8.7%. This was supported by the study done in Jimma Ethiopia 31.4% [37], Kenya 13%[38], Ghana 26% [39], Bouaké, central Côte d'Ivoire 22.5%[40] in Asian such as in India by 25.8%, 30.5%[41,42] in Brazil, Latin America [43], Vietnam 20% [44] where the most common isolate was *Klebsiella pneumoniae*. However the predominant GNB isolation rate varied from country to country where in India by Kante et al., [45], Indonesia by Murni et al., [46] frequently isolated pathogen in BSI was Pseudomonas other than *Klebsiella pneumoniae* in the same age group. This might be explained by different practices in prescribing antibiotics for empirical treatment prior to blood culture collection and differences in the management of nosocomial infections across counties. Furthermore, in our hospital setting, nosocomial infections did not receive the necessary

infection control and preventative measures. Which further increase the survival of highly drug resistant bacteria including *Klebsiella pneumoniae.*

A polymicrobial infection in our study was found in a single patient and both gram negative isolates tended to increase the severity of the diseases. This is in agreement with a previous study even though some microbiologists consider polymicrobial growth as contamination, but nonetheless sepsis should be clinically correlated [47].

The trend of empirical treatment in our study 43.5% and the most prescribed antibiotics were ampicillin, gentamicin, ciprofloxacin and third –generation cephalosporin (most common ceftriaxone) in which ampicillin and gentamicin were the most common combined drugs used. This was supported by the previous study in, Ghana [48].

The antimicrobial susceptibility of *Klebsiella pneumoniae* isolates gave high levels of resistance to ampicillin(100%), cotrimoxazole (90.7%) and gentamycin (88.4%), despite of sensitive to meropenem (62.8%), Piperacillin-Tazobactam (58.1%) which was consistent with the studies by Zenebe et al [49] 100%resistance to ampicillin and Cotrimosazole, in Bahirdar ,Ethiopia by Hailu et al.,[50] ampicillin 91.4%,cotrimosazole 77.1% and gentamicin 71% while in India the resistance of ampicillin, cotrimosazole and gentamycin done by Kumar et al.,[41] were 97%,88%,67% respectively. it was also comparable in Kaneti children Hospital, Nepal by kari et al [51] reported 100% resistance to ampicillin and least sensitive to Cotrimosazole and Gentamycin. The more potent drugs of the 3rd and 4th generation cephalosporin, quinolones and carbapenem antibiotics also showed resistance which is a concern for treatment of BSI in pediatrics with septicemia.

The second most predominant isolates in our study were Acinetobacter species which demonstrated resistance to most antimicrobials tested: ceftazidime 100%, cefepime 90.9%, gentamycin 81.8%, tobramycin 81.8%, ciprofloxacin 72.7%, meropenem 72.7% which was comparable with other previous studies [52, 53]. However our result gave a higher rate of resistance compared to a study conducted in South India, [54] in which isolates were meropenem 100% sensitive, while 67% were sensitive to gentamicin, ceftriaxone, ciprofloxacin, ceftazidime and Amikacin. This is due to our greater number of isolates and might be due to the inappropriate empirical use of meropenem as the first line treatment since most of isolates are from ICU patients in our hospital.

The overall prevalence of multidrug resistance isolates in our study was 83.7% Gram-negative bacteria with a high resistance to beta-lactam antibiotics. This result is supported by a previous study in Ethiopia [19]. Among *Klebsiella* species 87.7 %(43/49) and *Acinetobacter* 72.2% were the predominant MDR species which was consistent with the study in north India [55].

The present study identified carbapenem resistant Enterobacteriaceae (CRE) with a rate of 30.5% comparable with a study conducted in Tanzania 35% [56]. Most carbapenem resistance was detected in 72.2% of *Acinetobacter* isolates and 62.8% of *Klebsiella pneumoniae*.

The prevalence of ESBL-producing *Enterobacteriaceae* in our study is 25.4%. Among 43 *Klebsiella pneumoniae* isolates 14(32.5%) and 5 *E.coli* isolates 1(20.0%) are ESBL-producers. This is in keeping with the study conducted in south India by Zakariya et al., 32.0% [54] and in Mali by Sangare et al., 29.4% [57] for ESBL producing *Klebsiella pneumoniae*.

Limitation of the study

Even though our study identifies numerous bacteria pathogens causing BSI in pediatrics under five years, we could not able to isolate other possible pathogens including anaerobic bacteria. Due to supply shortage molecular characterization was not performed.

Conclusions

Blood stream infection in pediatric patients caused by Gram negative organisms with high antibiotic resistance were a treat of children. Among the dominate gram negative isolates *Klebsiella pneumoniae* and *Acinitobacter* species were multidrug resistant to 3rd and 4th generation cephalosporin, quinolones, aminoglycosides, and carbapenem. The prevalence rates of MDR 83.7%, CRE 30.5% and ESBL 25.4% are high in gram negative bacterial isolates. in this study and are of major concern: duration of hospitalization, a history of hospital acquired infections and complications of clinically suspected septicemia with high grade fever were statistically significant and associated with positive blood culture in pediatric patients ($p<0.05$).

Based on our findings, we recommend that blood culture should be taken for pediatric patients prior to antimicrobial therapy with most sensitive BacT/Alert machine. Clinicians should avoid prescribing last line antibiotics first for pediatrics in ICU.

Since the majority of antibiotics even last line antibiotics alarmingly resistance, laboratory based testing should be routinely conducted so as to provide the clinician definitive laboratory data and therefore enable improved patient management.Confirmed nosocomial infection requires patient isolation and is mandatory to minimize transmission of resistance genes including CRE, MDR and ESBL producing organisms in hospitalized patients.

Strengthening of antimicrobial surveillance system and antimicrobial stewardship programs are necessary for better management of antibiotics selection in addition to infection prevention practices in hospital settings.

Abbreviations

ATCC- American type of culture collection, **BSI**-blood stream infection, **CLSI**-clinical laboratory standard institute, **CRE**-carbapenem resistance Enterobacteriaceae, **ESBL**-extended spectrum beta lactamase, **GNB** –gram negative bacteria, **ICU**-intensive care unit, **MDR** –Multi drug resistance.

Declarations

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, 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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Tables

Due to technical limitations, tables 1-3 are only available as a download in the supplemental files section.

Figures

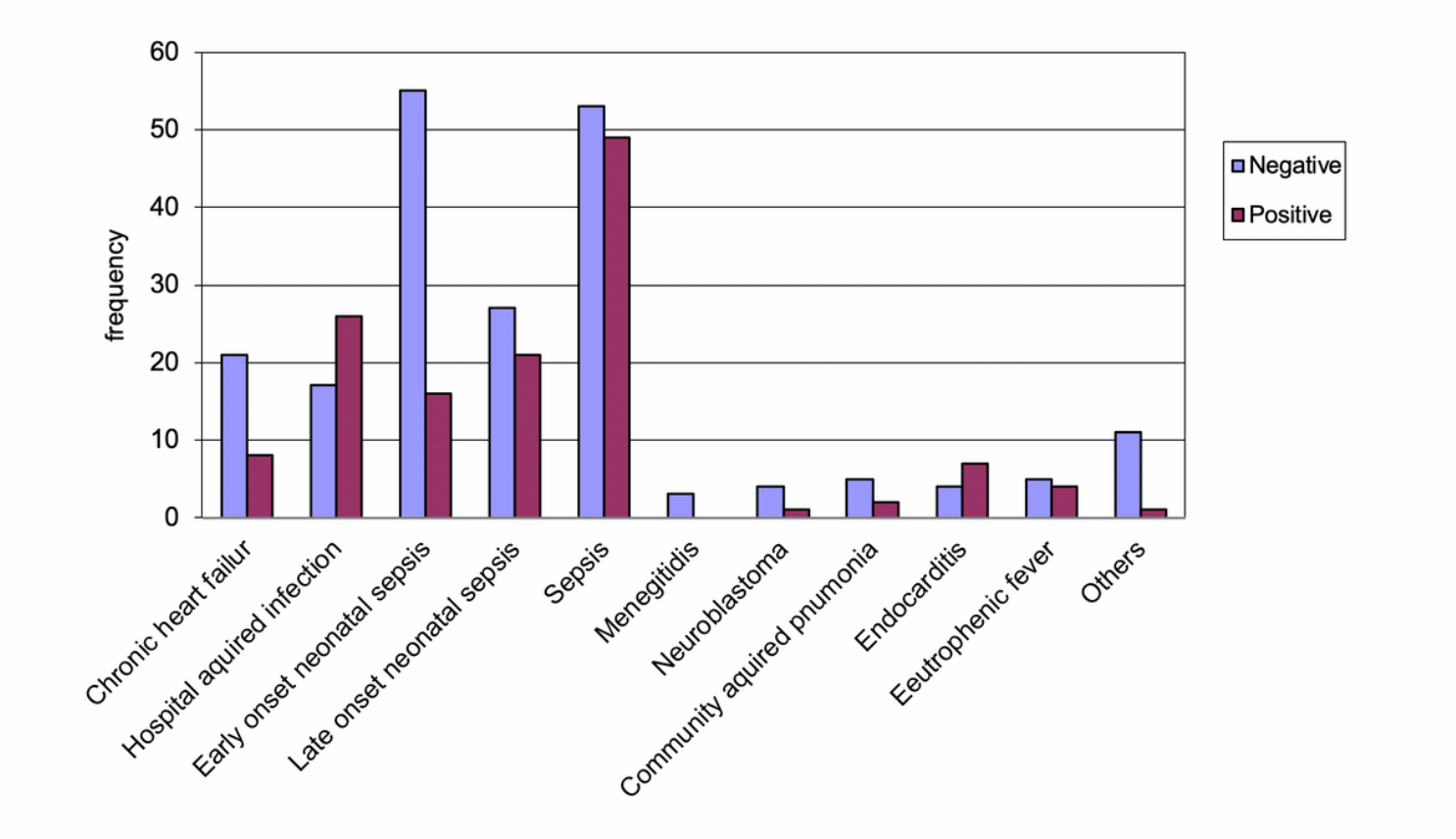

Figure 1

Distribution of clinical condition of patients among positive blood culture from blood stream infection suspected septicemia in Tikur Anbesa Specialized Hospital, 2018

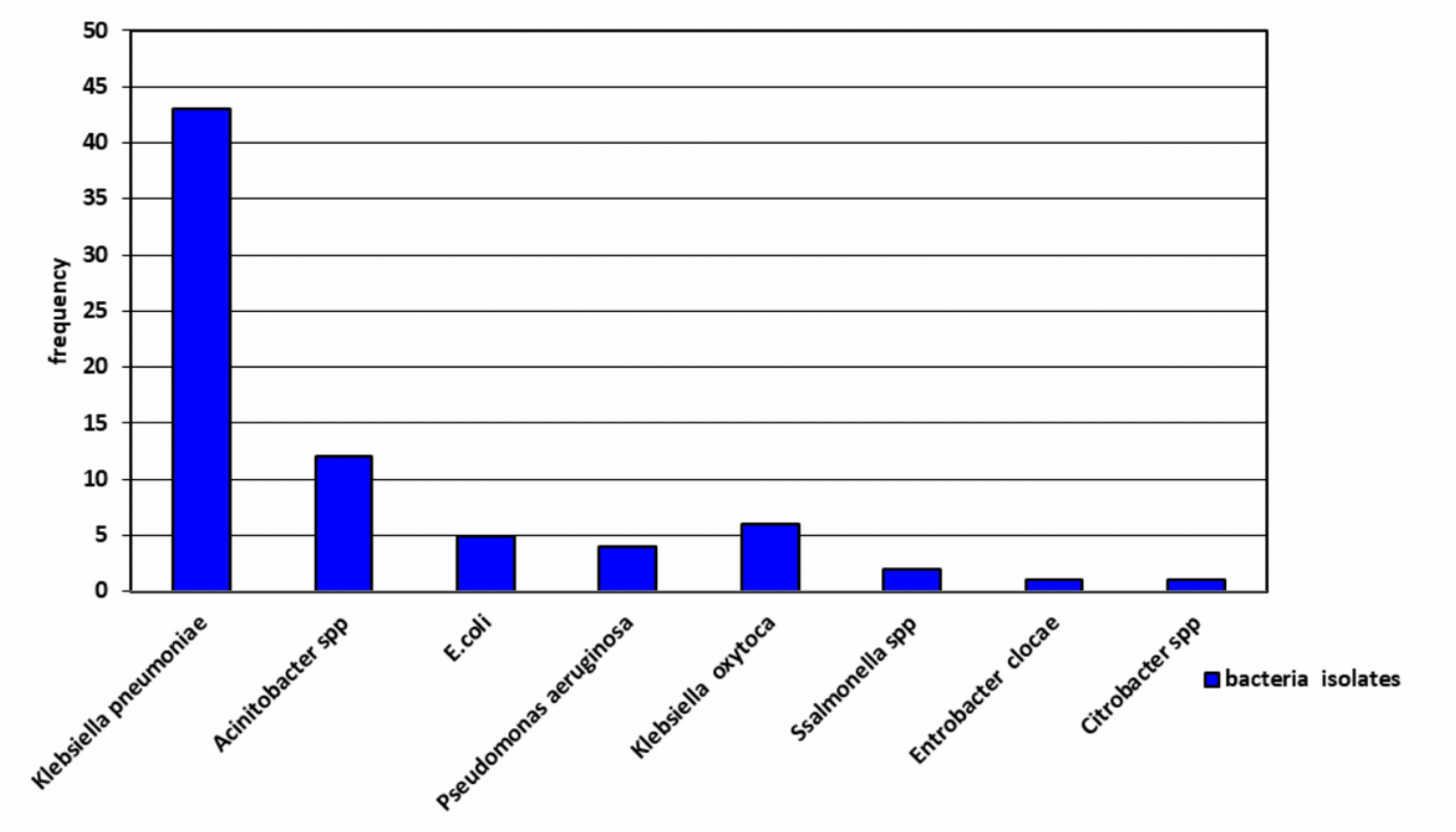

Figure 2

Distribution of bacteria pathogens among positive blood culture isolated from blood stream infection suspected of septicemia patients in Tikur Anbesa Specialized Hospital, 2018