

Prevalence and antimicrobial susceptibility of bacterial isolates from blood samples referred to National Reference Laboratory, Ethiopia

Amete Mihret Teshale*, Negga Asamene Abera, Rajiha Abubeker Ibrahim, Surafel Fentaw Dinku, Abebe Aseffa Negeri, Meseret Asefa Oda, Degefu Beyene Gobeze, Elias Seyoum Derbe, Tesfa Addis Kefyalew, Yonas Mekonnen Gebeyehu, Eyasu Tigabu Seyoum, Sintayehu Abate Temesgen

¹Ethiopian Public Health Institute, Clinical Bacteriology and Mycology National Reference Laboratory

² Ethiopian Public Health Institute, Ethiopian Journal of Public Health and Nutrition

*Corresponding author: amete2004@gmail.com, P.O. Box:-1242, Addis Ababa, Ethiopia, Tel: +251-913773888

Abstract

Background: Blood stream infections are very common public health problem that are known to cause high case-fatality rates. Globally, approximately 200,000 cases of bacteraemia occur annually with mortality rates ranging from 20-50%. Early diagnosis and treatment of these infections can make the difference between life and death.

Objective: The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of bacterial isolates recovered from blood samples.

Methods: This is a retrospective study that used data on blood culture test performed from 2010 to 2015 at Clinical Bacteriology and Mycology Laboratory of Ethiopian Public Health Institute. Data was entered and analyzed using SPSS version 21. Frequency and percentage of variables was calculated. Chi-square test was used to show association between variables.

Results: Of the 1327 blood samples processed, 156 (11.8%) of them were cultures positive for bacterial growth. Compared to other age groups, patients with age ranging from one month to seven years were highly affected. The most frequently isolated bacteria were Coagulase negative *Staphylococcus* 48(30.8%), *S.aureus* 31(19.9%), and *Klebsiella pneumoniae* 20(12.8%). Coagulase negative *Staphylococcus* isolates were less susceptible to penicillin and cotri-moxazole and alarmingly susceptible to clindamycin. Enterobacteriaceae have shown good susceptibility to chloramphenicol. Very close to 50% of the tested isolates were found to be Multi-drug resistant.

Conclusion: The findings from this study demonstrate that the prevalence of bacteremia is considerably high and the antimicrobial resistance pattern of bacterial isolates is very alarming. Continuous monitoring of bacterial pathogens and their antimicrobial resistance pattern is necessary for proper treatment of patients and also for better prevention and containment of antimicrobial resistance.

Key words: Blood stream infections, antimicrobial susceptibility, bacterial isolates

Introduction

Bacteremia is considered as a leading cause of severe and challenging bacterial infection which could progress rapidly to death if prompt and effective antibiotic therapy is not undertaken (Kasper et al. 2008; Ndir et al. 2016). Microbial invasion of the bloodstream have serious immediate consequences including shock, multiple organ failure, disseminated intravascular coagulation (DIC) and death (Manjunth et al. 2015). In United States alone, blood stream infections (BSIs) are reported to occur more than 200,000 times annually, with associated mortality rates of 35 to 60% (Weinstein et al. 1997; Martin et al. 2003; Wisplinghoff et al. 2004). The epidemiology of BSIs in Africa is poorly documented (Dramowski et al. 2015). The available studies reported high case-fatality rate associated with BSIs, mainly caused by Gram-negative bacteria, and this is 2-fold higher than that of malaria (43.5% versus 20.2%) (Ndir et al. 2016). Moreover, 18.1% mean mortality rate was reported in a meta-analysis on community acquired BSIs in Africa (Onken et al. 2015). Despite lack of good estimate of the net global health burden of antimicrobial resistant bacteria, this problem is widely regarded as one of the major public health concern of the 21st century

(Woolhouse et al. 2016). Antibiotics resistance is a growing problem in developing countries like Ethiopia (Dagnew et al. 2013). Numerous BSI bacterial pathogens are becoming less susceptible to wide ranges of antimicrobial classes (Patel et al. 2011; Barati et al. 2009). Clinical significance and changing spectrum of microbial pathogens necessitate for the strengthening of antimicrobial resistance surveillance systems. Such a program will play a critical role in guiding physicians toward appropriate agents for use in the treatment of both community and hospital acquired BSIs, as well as identifying changing patterns of etiologic agents and drug susceptibility (Patel et al. 2011). Therefore, the purpose of this study is to identify bacterial etiologies of BSIs and determine their susceptibility pattern.

Materials and Methods

Study Design and data source: A retrospective study on blood culture samples referred to National Clinical Bacteriology and Mycology Reference Laboratory of Ethiopian Public Health Institute (EPHI) was done from 2010-2015. Patient information such as age, sex, patient identification number, pathogen isolated, antibiotic resistant pattern of the isolates, referring health facilities were included in the study.

Laboratory procedures: A total of 1327 blood samples, in Tryptic Soy Broth, were received at EPHI for routine culture test during the study period. After overnight incubation (35±2°C), blood culture bottles were sub-cultured blindly to Chocolate and if there was growth indication, Sheep Blood (Oxoid, England) and MacConkey (Oxoid, England) agar plate were used in addition to Chocolate agar (Oxoid, England). Then, plates were incubated at 35±2°C with 5% CO₂. Colonies on plates with bacterial growth were identified by colony morphology, gram staining and appropriate biochemical tests. Antimicrobial susceptibility test was done manually using disc diffusion technique on Muller Hinton (Oxoid, England) Agar. Interpretation was done according to Clinical and Laboratory Standard Institute guideline.

Data management and analysis: Data was entered and cleaned using Microsoft excel. Analysis was done by SPSS version 21 (Armonk, NY: IBM Corp). Frequency and percentage for each variable were calculated. Chi-square test was used to show presence of association between variables. P-value ≤0.05 was considered statistically significant.

Ethical consideration: This study was ethically cleared and approved by IRB of EPHI.

Results

Socio-demographic characteristics: A total of 1327 patient results were retrieved and analyzed in the study, of which 669 (50.4%) and 658 (49.6%) were

female and male, respectively. Patients were in the age range of one month to 90 years with mean age of 3.8 years (SD±2.264). Predominant number of the patients were within age group of one month to seven years and 24 to 31 years and each accounts for 298 (22.5%). Majority of the study patients were from Addis Ababa area (85.1%).

Distribution of bacterial isolates with sex and age:

Out of the total specimens processed, 156 (11.75%) showed bacterial growth, of which, 79 (50.97%) were females. Gram positive and gram negative bacteria accounted for 93 (59.7%) and 63 (40.3%), respectively. Coagulase negative *staphylococcus* were the predominant bacteria [48 (30.76%)] representing more than 50% of all gram positive bacterial isolates identified. Fifty five (59.1%) of gram positive bacterial were isolated from female patients. The lowest numbers of bacterial isolates detected were *Bacillus* spp, *P.aeruginosa*, *S.sonnii* and *Salmonella* spp (one organism each) and all were isolated from male patients. Among gram negative bacterial isolates, 48 (76.2%) were in the family enterobacteriaceae dominated by *Klebsiella pneumoniae*. The remaining 15 (23.8%) were non-lactose fermenters with *Acinetobacter* spp being dominant. Thirty seven (58.7%) gram negative bacteria were isolated from male patients. High number of gram positive (50%) and negative (67.2%) bacteria were isolated from patients with age ranging from one month to seven years (Table-1).

Table 1: Bacterial isolates distribution from blood culture of patients with age at National Reference Laboratory, Ethiopia, 2010-2015.

Age group	Gram positive bacteria N (%)	Gram negative bacteria N (%)	Total N (%)
1month-7yrs	47(50.5)	43(67.2)	90(57.1)
8-15yrs	4(4.3)	6(9.4)	10(6.4)
16-23yrs	11(12.0)	2(3.1)	13(8.3)
24-31yrs	15(16.3)	5(7.8)	20(12.8)
32-39yrs	3(1.9)	1(1.6)	4(2.6)
40-47yrs	3(1.9)	2(3.17)	5(3.2)
48-55yrs	4(4.3)	3(4.7)	7(4.5)
56-63yrs	3(1.9)	0(0)	3(1.9)
64-71yrs	2(2.2)	0(0)	2(1.3)
>71yrs	1(1.1)	1(1.6)	2(1.3)
Total	93(100)	63(100)	156(100)

Age of patients was significantly associated with prevalence of bacterial isolates (P=0.0059) with pediatrics being more affected than adults (Table-1). Isolation of blood pathogens reached its peak (59.6%) in the year 2014 (Fig-1).

Antimicrobial susceptibility pattern: Coagulase negative *staphylococcus* and *Staphylococcus aureus* were less susceptible to penicillin and cotri-moxazole. On the other hand, these isolates were alarmingly

susceptible to clindamycin. *Klebsiella pneumoniae* was less susceptible to ceftriaxone and gentamicin (Table 2).

Resistances to three or more antibiotics were considered as multi-drug resistance (MDR). Accordingly, 77(49.4%) of the bacterial isolates were MDR. The rate of MDR in Gram positive and negative bacterial isolates was 42(45.2%) and 35(55.6%) respectively (Table 3).

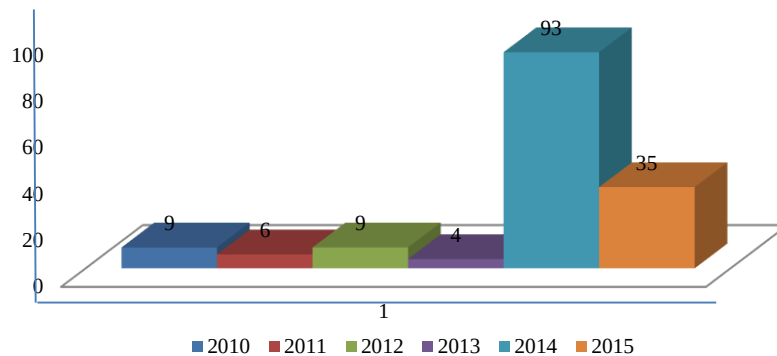


Fig 1: Trend of bacteria isolation from blood culture at National Reference Laboratory, Ethiopia, 2010-2015

Table 2: Antibigram of common bacterial isolates from blood, at National Reference Laboratory, Ethiopia, 2010-2015

Organism	No Strains	%Susceptibility								
		P	O	CN	SXT	CL	E	TE	AMC	C
CONs	48	29.7	31.6	56.3	25	72.9	43.8	45.8	-	-
<i>S.aureus</i>	31	9.7	38.8	83.8	45.2	83.8	70.9	41.9	-	-
<i>K.pneumoniae</i>	20	-	-	20	68	-	-	-	45	90

P: Penicillin, O: Oxacillin, SXT: Sulfamethoxazole-Trimethoprim, CL: Clindamycin, E: Erythromycin, TE: Tetracyclin, CN: Gentamicin, AMC: Augmentin, C: Chloramphenicol, CR: Ceftriaxone, CIP: Ciprofloxacin, (-): Antibiotic not done

Table 3: Multi-Drug Resistance pattern of bacterial isolates, from blood at National Reference Laboratory, Ethiopia, 2010-2015.

Organism	R0 N (%)	R1 N (%)	R2 N (%)	R3 N (%)	R4 N (%)	R5 (%)	TOTAL
CONs	8(16.8)	6(12.5)	6(12.5)	9(18.7)	9(18.7)	10(20.8)	48
<i>S.aureus</i>	7(22.6)	9(29.0)	4(12.9)	5(16.1)	4(12.9)	2(6.5)	31
<i>K.pneumoniae</i>	2(10)	2(10)	1(5)	3(15)	8(40)	4(20)	20

R0: Isolates not resistant for tested antibiotics, R1: Resistance to one of tested antibiotics, R2: Resistance to two of tested antibiotics, R3: Resistance to three of tested antibiotics, R4: Resistance to four of tested antibiotics, R5: Resistance to five of tested antibiotics

Discussion

In this study, the overall prevalence of bacterial isolates from blood culture was 11.8%. This finding was lower than a report from Pakistan, which is 16% (Fayyaz et al. 2015), but it is higher than a report from Jimma (8.8%) (Zenebe et al. 2011). Other studies conducted in Ethiopia have also reported a higher prevalence (28%) rate (Wasihun et al. 2015; Nigussie et al. 2015). This discrepancy may be due to difference in sample size and prior antibiotic treatment. Among all bacterial isolates, gram positives particularly coagulase negative staphylococcus were the predominant similar to a study in Pakistan (Fayyaz et al. 2015) which might be due to central venous catheterization (duration > 10 days), nasal canula, gastrointestinal tract, exposure to antibiotics, and prolonged hospitalization (Emma, 2013). Other studies conducted in Ethiopia have also shown similar results (Nigussie et al. 2015; Kumalo et al. 2016). The finding was also in line with a report from other study done elsewhere that documented gram positive and negative bacterial isolates accounting for 54.1% and 38.2% respectively (Riedel et al. 2008). *Klebsiella*

pneumoniae (12.8%) were the predominant bacterial isolates from gram negatives. Similar finding was reported from a study conducted in Iran (Barati et al. 2009). Among the antibiotics tested for gram positive bacteria, vancomycin was the most effective antibiotics with all the tested isolates being 100% susceptible. *S.aureus* isolates were 90.3% resistant to penicillin which was in agreement with studies conducted elsewhere (Qureshi et al. 2011; Barati et al. 2009). Higher rate of resistance to ampicillin was noted for *E.coli* (93.3%) which was in accordance with the study conducted in Iran (Barati et al. 2009). This drug has been commonly overused in outpatients for many years; hence, high resistance rate is expected. The rate of MDR *Acinetobacter* is increasing in many parts of the world and poses a serious therapeutic dilemma. In this study, the rate of MDR *Acinetobacter* isolates was 100% of which 80% were resistance to five and more of tested antibiotics which is similar with another study conducted in Iran (100%) (Ranjbar et al. 2007) but higher than another report of different season from Iran (20.43%) (Barati et al. 2009).

Conclusions

Blood stream infection was found to be increasing from year to year, mostly affecting pediatrics age (1month-7years) with increased resistance to commonly prescribed antibiotics. Gram positive organisms were the predominant causes in our setting. Penicillin resistance *Staphylococcus* species and ceftriaxone resistance Gram negative organisms are increasing. Screening of *S.aureus* for MRSA and gram negative rods for ESBL is required. Vancomycin and gentamicin yielded excellent in vitro activity against Gram positive organisms. This study demonstrated that the prevalence of bacteremia is considerably high and the antimicrobial resistance pattern of bacterial isolates is very alarming. Continuous monitoring of bacterial pathogens and their antimicrobial resistance pattern is necessary for proper treatment of patients and also for better prevention and containment of antimicrobial resistance. Lastly, we emphasize that empiric therapy should be guided by local susceptibility data when available; however, in the absence of such information, surveillance data can help with therapeutic choices.

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