Bacterial blood stream infections and antibiogram among febrile patients at Bahir Dar Regional Health Research Laboratory Center, Ethiopia

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ABSTRACT

Bacterial blood stream infections (BSIs) are a common cause of morbidity and mortality. Prevailing data on bacterial species causing BSI and their antibiogram are essential for proper management of patients. A retrospective study was conducted on blood culture results that had been processed from March 2013 to January 2015 at Bahir Dar Regional Health Research Laboratory Center. In January 2015, data on age, sex of patients and bacterial isolates with antibiogram were extracted from registration log book. Blood stream causing bacteria were identified according to standard operational procedure for blood culture. Antimicrobial susceptibility tests were performed according to Kirby-Bauer disc diffusion methods. A total of 561 blood specimens were requested for blood culture. Of these, 220 (39.2%, 95% CI: 35.3-43.4%) blood cultures had aerobic bacterial growth. Gram negative bacterial isolates constituted 115 (52.3%) of the isolated bacteria. *Staphylococcus aureus* 50 (22.7%), coagulase negative staphylococci 35(15.9%), *Klebsiella pneumoniae* 35 (15.9%), *Escherichia coli* 19 (8.6%), *Pseudomonas aeruginosa* 15 (6.8%) and *Acinetobacter* species 13(5.9%) were the most dominant isolates. Overall, drug resistance for gram positive bacteria were 7 to 61% and for gram negatives 6.9 to 82.6%. Among the gram positive bacteria, high resistance levels were observed against penicillin (61%) and oxacillin (52.9%). The gram negative bacterial isolates showed 66 to 82.6% resistance to ampicillin, ceftriaxone and trimethoprim-sulfamethoxazole. The present study revealed that bacterial blood stream infections linked with high levels of drug resistance would pose a challenge in treatment of patients with BSIs. Hence, blood culture with antibiotic susceptibility tests could play key role for appropriate treatment of patients with bacterial blood stream infection.

Key words: Blood culture, Bloodstream infection, antibiogram, Ethiopia

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INTRODUCTION

Bloodstream infections (BSIs) cause self-limiting infections to life threatening sepsis and account for significant mortality and morbidity worldwide. Blood stream infection accounts for 10-20% of all nosocomial infections and is the eighth leading cause of mortality (James *et al*., 2002). In sub-Saharan countries including Ethiopia BSI is an important cause of illness and death in children, the mortality rate approaches 53% which makes it a significant health problem in developing countries (Mehdinejad *et al*., 2009).

Bloodstream infections are often complicated with syndromes associated with septic shock (Balk, 2000). Bacteria present in circulating blood whether continuously or intermittently are a threat to every organ in the body usually (Vanitha *et al*., 2012). Individuals with bacteremia may develop septicemia, a life-threatening condition in which multiplying bacteria release toxin in to the blood stream and trigger the release of cytokines, causing fever, chills, malaise and lethargy, with difficulty in breathing especially in children (Shahla *et al*., 2009; Ehwarieme *et al*., 2011).

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Development of resistance to antimicrobial agents further adds complication to its proper treatment outcome. Studies from Ethiopia and worldwide have reported an increased antimicrobial resistance among bacterial isolates causing BSIs (Usha *et al*., 2007; Kaistha *et al*., 2009; Kingsley *et al*., 2013). In different studies a wide range of organisms have been isolated in BSIs such as *Acinetobacter* species, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Neisseria meningitidis*. Furthermore, gram positive bacteria such as coagulase negative staphylococci (CoNS), *Staphylococcus aureus*, enterococci, and alpha-hemolytic streptococci (Atul *et al*., 2007; Anu *et al*., 2010 Kavitha *et al*., 2010).

Blood culture in order to isolate pathogens and determine drug sensitivity of the isolates remains the main stay of definitive diagnosis and management of BSIs (Shahla *et al*., 2009). Early identification of bacteria causing BSI and their antimicrobial susceptibility is essential for rapid administration of antimicrobial therapy to patients with BSIs. This has shown to improve treatment outcomes (Munson *et al*., 2003).

In Ethiopia, a few studies documented data on bacterial blood stream infections and antimicrobial resistance profiles of blood stream causing bacterial species (Tizazu Zenebe *et al*., 2011; Mulat Dagnew *et al*., 2013; Araya Gebreyesus *et al*., 2015). However, data on bacterial blood stream infections are not documented in the study area. Therefore, this study was conducted to determine the bacterial gents and antimicrobial susceptibility profiles of blood stream infections among patients referred to Bahir Dar Regional Health Research Laboratory Center, North West Ethiopia.

METHODS

Study settings

A retrospective study was conducted on blood culture results that had been processed from March 2013 to January 2015 at the Bahir Dar Regional Health Research Laboratory Center. In January 2015, data on age, sex of patients and bacterial isolates with their antibiogram were extracted from laboratory registration records of using a standard data extraction sheet. Bahir Dar Regional Health Research Laboratory Center is the technical arm of Amhara Regional Health Bureau currently providing culture and susceptibility tests, MDR-TB culture and molecular laboratory techniques like real time PCR and quality assurance services to Felege Hiwot referral Hospital, nearby health centers, private hospitals and clinics.

Culture and identification

According to the standard operational procedures for blood culture, 5ml and 2ml blood samples were aseptically collected from adults and children, respectively. The vein puncture sites were cleaned with 70% alcohol and dried before collection of blood sample. Collected blood samples were inoculated onto Tryptic Soy Broth (Oxoid, UK) in 1:5 to 1:10 proportions and incubated at 37ºC. Overnight incubated culture bottles were sub-cultured onto sheep blood agar, chocolate blood agar and MacConkey agar. Negative blood culture results would be followed till fifth days up by examining the daily before issued results.

Preliminary identification of the isolates was made on agar based on macroscopic colony characteristics. Gram-negative rods were identified by series of biochemical tests such as Indole, Simon's citrate,

Urea, Triple sugar iron (TSI), lysine iron, and motility. Gram-positives were identified based on their preference of growth on blood agar plate followed by catalase, coagulase, bacitracin and optochin tests (Wikler *et al*., 2007).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was performed according to Kirby-Bauer disk diffusion method on Mueller Hinton agar plates (Oxoid, UK) (Wikler *et al*., 2007). The antimicrobials tested were obtained from Oxoid Ltd. UK with the following concentrations: ampicillin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), trimethoprim/sulphamethoxazole 1.25/23.75 µg), pepracillin (100 µg), gentamicin (10 µg), tetracycline (30 µg), vancomycin (30 µg), penicillin (10IU), clindamycin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg), and oxacillin (30 µg). Grades of susceptibility were determined after incubation at 35^0C for 24 hours according to Clinical Laboratory Standards Institute (CLSI) (Wikler *et al*., 2007).

Quality control

The standard reference strains *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used to check the potency of antimicrobial discs, to control drug susceptibility testing procedures and for identification of bacterial species. The absence of bacterial growth in un inoculated media was done by randomly taking the prepared culture media and incubating over nigh to see for any growth.

Data analysis

Data were analyzed using Statistical Package for Social Sciences version 20 Software (*IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp*). The chi-square test was employed to compare the proportion of bacterial isolates with patients' age and sex and p-value of less than 0.05 was considered as statistical significance.

Ethical statements

Ethical clearance was obtained from Amhara Regional Health Bureau Institutional Review Board (IRB) at Bahir Dar Regional Health Research Laboratory Center to get permission for scientific publication of the data.

RESULTS

From March 2013 to January 2015, a total of 561 blood specimens were requested for culture and antimicrobial susceptibility tests. Among these, 289 (51.5%) were from females patients. The median age of patients was 15.5 years with a range of 4 days to 75 years. Overall, 220 (39.2%) of blood specimens had aerobic bacterial growth. Among aerobic culture positives, 203 (92.3%) were monomicrobial and 13 (5.9%) were polymicrobial growth.

Table 1 depicts blood culture results with patient characteristics. Regards to sex, 104 (38.2%) male patients had blood culture positive while 116 (40.1%) females had blood culture positive. Statistically significant associations were between age and status of blood culture (P=0.001). Highest percentage 115 (70.1%) of microbial isolates were reported in the age group of patients less than one year.

Table 2 demonstrates bacterial species isolated from blood cultures. *S. aureus* was the predominate isolate with 50 (22.7%) followed by coagulase neg-

Table 1. The distribution of blood culture results by patients' age and sex (Mar 2013-Jan, 2015)

Variables	Blood culture results		P value
	Culture negative N (%)	Culture positive N (%)	
Age (Years)			
<1	49 (14.4)	115 (70.1)	0.001
1-12	96 (66.7)	48 (33.3)	
13-18	47 (73.4)	17 (26.6)	
>18	149 (78.8)	40 (21.2)	
Total	341 (60.8)	220 (39.2)	
Sex			
Male (n=272)	168 (61.8)	104 (38.2)	0.645
Female (n=289)	173 (60)	116 (40)	
Total	341(60.8)	220 (39.2)	

ative staphylococci (CoNS) 35 (15.9%), *K. pneumoniae* 35 (15.9%), *E. coli* 19 (8.6%), *P. aeruginosa* 15 (6.8%) and *A. baumanni* 13 (5.9%). Gram negative bacterial isolates constituted 115 (52.3%) of all isolates.

The antimicrobial susceptibility profiles of gram positive and gram negative isolates are shown in Table 3 and 4. Among the gram positive bacteria, high resistance level was observed against penicillin (61%) and oxacillin (52.9%). The gram

Table 2. Frequency of bacterial species isolates from blood culture (Mar 2013-Jan, 2015)

Bacterial Group	Number (%)
Gram positive bacteria	
Staphylococcus aureus	50 (22.7)
Coagulase negative staphylococci	35 (15.9)
Eenterococcus faecalis	6 (2.7)
Others	10 (4.5)
Gram negative bacteria	
Escherichia coli	19 (8.6)
Acinetobacter baumanni	13 (5.9)
Klebsiella pneumoniae	35 (15.9)
Eenterobacter clonae	8 (3.6)
Pseudomonas aeruginosa	15 (6.8)
Proteus mirabilis	5 (2.3)
Proteus vulgaris	1 (0.5)
Others	19 (8.6)

Table 3. Antimicrobial resistance profiles of gram positive bacteria from blood cultures (Mar 2013-Jan, 2015)

Bacterial species	Antimicrobial resistance N (%)								
	OX	CIP	SXT	VA	TE	P	C	DA	CN
S. aureus	33 (66)	10 (20)	20 (48.7)	NT	48 (96)	39 (78)	25 (50)	7 (14)	NT
CoNS	13 (37.1)	3 (8.5)	13 (37.1)	NT	2 (5.7)	17 (48.5)	9 25.7)	3(8.5)	NT
E. fecalis	NT	NT	NT	1 (16.6)	NT	NT	1 (16.6)	NT	1 (16.6)
S. pneumoniae	NT	NT	NT	0 (0)	NT	0 (0)	NT	NT	NT
S. viridians	NT	NT	NT	0 (0)	NT	0 (0)	NT	1 (25)	NT
S. pyogenes	NT	0 (0)	0 (0)	1 (33.3)	NT	2 (66.6)	1(33.3)	1 (33.3)	NT
Micrococcus spp.	0 (0)	0 (0)	NT	NT	NT	0 (0)	0 (0)	0 (0)	NT
Total (n=101)	46 (52.9)	13 (14.4)	23 (26.1)	2 (14.3)	6 (7)	58 (61)	36 (37.5)	12 (12.8)	1 (16.6)

Key: OX=Oxacillin; CIP=ciprofloxacin; SXT=Trimethoprim+Sulphamethoxazole; VA=Vancomycin; TE=Tetracycline, P=Penicillin; C=Chloramphenicol; DA= Clindamycin; CN= Gentamycin; NT=Not tested

negative organisms showed 66-82.6% resistance to ampicillin, ceftriaxone and trimethoprim-sulfamethoxazole. The overall the range of resistance in gram positive and negative bacteria are shown in table 4.

DISCUSSION

Studies show that BSIs are frequently encountered by clinicians mainly in developing countries. BSI is one of the major infections for frequent antibiotic use (Vanitha *et al*., 2012; Mulat Dagnew *et al*., 2013). In the present study, blood culture was one of the frequently requested specimens for culture and antimicrobial susceptibility tests. This indicates that BSI is a common health problem of the community which could partly explained by 39% prevalence of blood stream infection.

The blood culture positivity rate in this study is higher compared to other studies in Ethiopia which reported 18.2% and 8.8% positivity rate (Tizazu Zenebe *et al*., 2011; Mulat Dagnew *et al*., 2013). The isolation rate of bacteria in the present study is also higher than studies reported in other parts of the world like in South India (8.39%), Iran 5.6%, Nigeria 19.3% and 20.02% and 9.44%

Table 4. Antimicrobial resistance profiles of gram negative isolates from blood cultures (Mar 2013-Jan, 2015)

Bacterial species	Antimicrobial resistance N (%)								
	AMP	CRO	CIP	SXT	AK	CN	C	PRL	CAZ
E. coli	14 (73.7)	12 (63.1)	5 (26.3)	15 (78.9)	0 (0)	10 (52.6)	3 (15.7)	NT	NT
Acinetobacter spp	10 (76.9)	10 (76.9)	2 (15.3)	8 (61.5)	1 (7.6)	7 (53.8)	9 (69.2)	NT	NT
K. pneumoniae	32 (91.4)	26 (74.2)	10 (28.5)	27 (77.1)	2 (5.7)	25 (71)	16 (45.7)	NT	NT
E. clonae	8 (100)	7 (87.5)	1 (12.5)	4(50)	2 (25)	6 (75)	6 (75)	NT	NT
P. aeruginosa	15 (100)	NT	2 (13.3)	NT	1 (6.6)	3 (20)	NT	4 (26.6)	8 (53.3)
P. mirabilis	3 (60)	3 (60)	0 (0)	1 (20)	0 (0)	0 (0)	2 (40)	NT	NT
P. vulgaris	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NT	NT
Salmonella spp	3 (60)	3 (60)	1 (20)	4 (80)	0 (0)	2 (40)	1 (20)	NT	NT
Providencia spp	2 (66.6)	1 (33.3)	0 (0)	1 (33.3)	0 (0)	1 (33.3)	0 (0)	NT	NT
K. ozenae	2 (50)	2 (50)	1 (25)	2 (50)	0 (0)	2 (50)	1 (25)	NT	NT
K. rhinose	2 (100)	2 (100)	1 (50)	1 (50)	0 (0)	1 (50)	1 (50)	NT	NT
Citrobacter spp	4 (100)	2 (50)	1 (25)	3 (75)	2 (50)	3 (75)	3 (75)	NT	NT
Total (n=115)	95 (82.6)	68 (68)	24 (20.8)	66 (66)	8 (6.9)	60 (52.1)	27 (27)	4 (26.6)	8 (53.3)

Key: AMP=Ampecillin; CRO=Ceftriaxone; AK=Amikacin; PRL=Pepracillin; CAZ=Ceftazidime

in India (Usha *et al*., 2007; Kalantar *et al*., 2008; Mehdinejad *et al*., 2009; Ehwarieme *et al*., 2011; Vanitha *et al*., 2012; Sumita *et al*., 2014). The possible explanation for these differences could be differences in age range of patients, administration of antibiotics before blood culture, laboratory procedure, geographical location, seasonal variation and epidemiological difference of the etiological agent (Pitout and Laupland, 2008).

In the present study, gram negative bacteria were more frequently isolated than gram positives. This

conforms to a study in Lahore which reported 60% of gram negative and 40% of gram positives (Majda *et al*., 2011). Likewise, in India 52.82% gram negative and 46.56% gram positive bacteria were isolated in blood stream infections (Amit *et al*., 2014). In contrast, 31% of gram negative and 69% of gram positive bacteria were reported from Gondar (Mulat Dagnew *et al*., 2013) and 39.1% gram negative and 60.9% gram positive from Jimma, Ethiopia (Tizazu Zenebe *et al*., 2011). The predominant isolate of this study was *S. aureus* (22.7%) which is comparable with other studies (Vanitha *et al*., 2012; Kalpesh *et al*., 2014; Sumita *et al*., 2014). Coagulase negative staphylococci (CoNS) have been considered as non pathogens believed that it could be skin flora specially when isolated in a single blood culture. But, their role as pathogens was reported in different studies as an important cause of morbidity and mortality in immune suppressed individuals (Usha *et al*., 2007; Shahla *et al*., 2009; Tizazu Zenebe *et al*., 2011).

The rate of antibiotic resistance in this study is comparatively higher than previous reports in Ethiopia (Tizazu Zenebe *et al*., 2011; Mulat Dagnew *et al*., 2013) and other studies conducted elsewhere in the world (James *et al*., 2002; Amit *et al*., 2014). A high prevalence of antibiotic resistance was noticed in this study especially among gram negative bacteria. This might be due to none judicious use of antibiotic in health facilities and easy availability of drugs in the market (Bayeh Abera *et al*., 2014).

Amongst the gram positive bacteria, high resistance was observed to penicillin (61%) and oxacillin (52.9%) which is almost consistent with a study by Kalantar *et al*. (2008) that recorded about 41% resistance to oxacillin and 60% for penicillin. Furthermore, high resistance among gram negative was seen against ampicillin (82%), ceftriaxone (68%) and thrimetoprim-sulfamethoxazole (66%) which is concordant with the study finding by Mehdinejad *et al*. (2009).

Among gram-positive bacteria, *S. aures* showed high resistance level (48-96%) against trimetoprim-sulphamethoxazole, oxacillin, penicillin and tetracycline which are commonly prescribed antimicrobial agents in the area (Bayeh Abera, 2014). This result was similar with a report in other studies in Ethiopia (Tizazu Zenebe *et al*., 2011; Araya Gebreyesus *et al*., 2015) and in Nigeria (Ehwarieme *et al*., 2011). However, clindamycin and ciprofloxacin were effective against *S. aureus*. In Ethiopia, uncontrolled over the counter sale of antimicrobial agents, mainly for self treatment without health professionals prescription would inevitably lead to emergence and rapid dissemination of antibiotic resistance in the community (Bayeh Abera *et al*., 2014).

CONCLUSION

This study showed that bacterial blood stream infections are common in all age groups of febrile patients. However, bacterial blood culture positivity rate was higher in febrile children below 1 year than other age groups. Overall, bacterial isolates in blood culture showed high levels of antibiotic resistance against commonly prescribed antimicrobials. Hence, blood culture with antibiotic susceptibility tests could play key role for appropriate treatment of patients with blood stream infection.

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