ORIGINAL ARTICLE

BACTERIAL CAUSES OF ACUTE FEBRILE ILLNESSES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERNS IN ADULT PATIENTS ATTENDING FELEGE-HIWOT REFERRAL HOSPITAL, NORTHWEST ETHIOPIA: A CROSS SECTIONAL STUDY

Mequanint Bezie[1] , Moges Tiruneh[2] , Belay Anagaw[2], Mulat Dagnew[2]*

ABSTRACT

Background: *Bacterial etiologies of acute febrile illnesses (AFIs) are common causes of hospital admission and death in Africa, including Ethiopia. The very limited resources and the great diversity of etiologies of AFIs in the tropical regions of Africa are critical challenges to establishing definite diagnosis, treatment, prevention and control of infectious diseases.*
Objective: *The aim of this study was to assess the bacterial causes of AFI and their susceptibility to antimicrobial agents among acute febrile illness patients.*
Methods*: A cross sectional study was conducted on 137 patients suspected acute febrile illness at Felege Hiwot Referral Hospital, from February1 to May 30, 2013. Data on socio-demography were collected using a pre-tested structured questionnaire. Blood samples were collected and inoculated into brain heart infusion. Blood culture and antimicrobial susceptibility tests were done according to the Clinical Laboratory Standard Institute guideline. Data were entered and analyzed using SPSS version 16.*
Results*: Of the total 137 blood cultures, 24 (17.5%) were positive for six different types of bacteria. The isolates were Coagulase negative Staphylococcus 8(5.8%) followed by K. pneumoniae 7 (5.1%) and S. pyogens 5 (3.6%). Enterococcus species and Enterobacter species accounted 1(0.7%) each. The isolates showed high rates of resistance to most antibiotics tested. The range of resistance rates for Gram positive bacteria was from 0% to 64.3% and for Gram negative from 0% to 100%. All Gram negative bacteria were multidrug resistant.*
Conclusions*: This study showed a high prevalence of bacterial pathogens in acute febrile illness suspected patients with high rate of resistance to most commonly used antibiotics. Therefore, early diagnosis and treatment of bacterial infections based on culture and drug susceptibility are crucial to reduce bacterial causes of acute febrile illness. A rational use of antibiotics should be practiced in order to minimize the spread of drug resistant bacteria.*

Key words*: Acute febrile illness, bacteriaial etiologies, Antimicrobial susceptibility.*

INTRODUCTION

Acute febrile illness (AFI) is defined as a disease characterized by a rise in body temperature by more than 38 $^{0}_{C,}$ resulting perhaps from an infectious process (1). Most febrile illnesses may have manifestations, like fever, loss of appetite, general malaise, myalgia and arthralgia, chills, rigors, headache, cough, vomiting, convulsion, hepatomegaly, splenomegaly, rash, and neck stiffness (2).

Fever is a common presenting symptom to the Emergency Department and is attributable to a wide range of clinical diseases or infection. Frequently, most fevers resolve without treatment, but some may also indicate a potentially fatal illness (3).

Acute febrile illnesses are the leading cause for admissions to hospitals and deaths in Ethiopia (4). The agents of human febrile illnesses can vary by region and country (5). In Africa, population-based studies which examine the etiology of ambulatory fevers, using a systematic approach are very limited (6).

[1] Department of Medical Laboratory, Debre Tabor, College of Health Sciences, [2] Department of Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, the University of Gondar
Corresponding author: *Mulat Dagnew, Email: dagnewmulat@gmail.com*

Thus however, the etiologies are not well characterized. A syndromic-based disease surveillance provides a useful methodology to systematically identify and document the causes of acute febrile illnesses (7). The provision of diagnostic facilities and the awareness of the community on the prevalence of non-malaria febrile illnesses are very low in the continent (8).

Therefore, diagnosing AFIs in many African countries remains challenging for a variety of reasons, including delayed recognition and reporting of outbreaks, need to test for multiple potential pathogens, lack of adequate diagnostic facilities and methods in field laboratories, and inability to collect optimal specimen types (e.g. blood cultures) (9).

As AFIs are nonspecific, especially during the early stages of onset, misdiagnosis and mistreatment can occur often. If patients are treated without definitive diagnosis, there will be a misuse of drugs which results in drug resistance (4). Many bacterial pathogens become resistant to antibiotic regimens, leading to serious public health concerns with economic and social implications throughout the world (10). Although, a timely and appropriate use of antibiotics is currently the only way to treat bacterial causes of acute febrile illnesses, the infections caused by multidrug resistant organisms are more likely to prolong hospital stay, increase the risk of death, and require treatment with more expensive antibiotics (10).

Researches in Ethiopia documented that there is a high bacterial drug resistance to commonly used antibiotics, mainly due to lack of a national guideline for antibiotic use and the absence of good laboratory facilities to do antimicrobial drug susceptibility tests. As a result, clinicians use empirical ways to treat their patients; there also is a high self-treatment of humans and animals without the prescriptions of doctors. All these lead to the emergence and rapid dissemination of resistance (11, 12,13, 14). Research findings have reported that inappropriate treatments of BSI aggravate patient mortality and the emergence of drug resistant strains (14,15)

Only a few studies examined the organisms involved in BSI and their susceptibility patterns among patients hospitalized in the Inpatient and Outpatient Department (OPD) (11,12, 13,14,15).

However, no studies have been published in the study area, Bahir Dar,where malaria is a common problem & acute febrile patients attend the health institutions now and then although some of the patients are negative for malaria and sometimes wrongly treated with malaria drugs. Therefore, this study aimed to find out the aetiologic agents that cause febrile illnesses due to bacteria and their drug susceptibility patterns in the OPD.

There is a scarcity of data on bacterial causes of febrile illnesses and their drug susceptibility patterns at hospital OPDs in the study area. Therefore, this study set out to assess the bacterial causes of AFI and their susceptibility to antimicrobial agents among patients with acute febrile illnesses at Felege Hiwot Referral Hospital.

METHOD

Study design, area, period and population: A cross-sectional study was conducted from February 1 to May 30, 2013, at Felege Hiwot Referral Hospital. The hospital is located in Bahir Dar, 578km northwest of Addis Ababa and provides services to over

five million inhabitants. Bahir Dar, the capital of the Amhara National Regional State, is on latitude $11^0$36'N and longitude $37^0$24'E, / 11.833; 39.6 with an elevation of 1840 meters above sea level. All clinically confirmed acute febrile illness patients at the OPD of Felege Hiwot Hospital, Bahir Dar, during the study were the population death with.

Inclusion criteria: Patients ≥18 years old and had fever (axillary temperature of ≥38°C) were included.

Exclusion criteria: Patients who were not febrile but took antibiotic during the last two weeks of their visits to the hospital were excluded.

Sample size and sampling techniques: The sample size was 124, determined based on prevalence rate 8.8% (11) and calculated using 5% marginal error and 95% confidence interval of certainty ($\alpha = 0.05$). The actual sample size for the study was computed using the single population proportion formula. By assuming a 10% non-response rate, the final sample size of 137 was obtained. Patients who were ≥18 years old and had fever (axillary temperature of ≥38° C) at the Outpatient Department (OPD) during the study were selected by using the simple random sampling technique. Patients who fulfilled the selection criteria at the OPD were taken randomly by using the lottery method.

Socio-demographic, clinical data, and sample collection: After getting a written consent from each study participant, a pre-tested structured questionnaire was used to collect data on socio-demographic characteristics. The skin for the veni-puncture was prepared by cleansing vigorously with a 70% alcohol. Then, the site was cleansed using cotton swab soaked in a 2% tincture of iodine. This antiseptic preparation was then allowed to air dry at least for 30 seconds. Using a sterile needle and syringe, vein puncture was performed; then a 5ml blood sample was collected and transferred into a bottle containing a sterile brain heart infusion (BHI) broth (Oxoid, LTD). A minimum blood-to-broth ratio of 1 in 10 was maintained (16, 17).

Bacterial isolation and identification: The blood culture broths were incubated at 37^oC and checked for signs of bacterial growth daily up to 7 days. Bottles were examined for visible evidences of such bacterial growth as turbidity, haemolysis, and gas bubbles. Bottles which showed signs of growth were further processed for Gram stain, and sub-culture was made onto Blood agar (BA) (Oxoid, LTD), MacConkey agar (MAC) (Oxoid, LTD), and Chocolate agar (CA) (Oxoid, LTD), and was incubated at 37^oC for 24 hours. Chocolate agar was incubated at 370c in a candle jar for 24 hours. Blood culture broths with no bacterial growth were sub-cultured on BAP, CAP, and MacConkey agar plates, before reported as negative (17,18).

The identification of organisms was based on Gram reactions, colony morphology, and biochemical characteristics. Biochemical and sensitivity tests, like Catalase, Coagulase, Bacitracin, Novobiocin, and Optochin were done for Gram positive bacteria (Oxoid, LTD). Besides, a series of biochemical tests, like hydrogen sulphide production (H_2S), indole, urease, citrate, LDC (Lysine decarboxylase), gas production and carbohydrate fermentation, using triple sugar iron agar (TSI) were carried out for Gram negative bacterial species identification (Oxoid, LTD). The species of the bacteria were identified and recorded based on their specific biochemical nature observed in the biochemical tests (17,18).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing of the isolates for different antimicrobial agents was carried out on Muller Hilton media (Oxoid, LTD), using the agar disc diffusion technique as per the National Committee for Clinical Laboratory Standards (CLSI) guide line (19). Two or three pure colonies were picked up with a sterile wire loop and inoculated in about 3ml of sterile nutrient broth. This suspension was incubated at 37^{o}_{C} up to 3 hours until growth was ascertained by the turbidity that matched the turbidity of 0.5 McFarland standards.

A sterile cotton swab was dipped into the suspension of the isolate in the broth and, squeezed free from excess fluid against the side of the test tube. The test organisms were uniformly swabbed over the Muller-Hilton surface and exposed to a concentration gradient of antimicrobial agents, diffusing from the antimicrobial agents an impregnated paper disk into the agar medium. The medium was then inverted and incubated at 37^{o}_{C} for 24 hours.

The antibiotic discs (all from Oxoid, LTD) used for the susceptibility testing were penicillin G (10 IU), amoxicillin (20µg), tetracycline (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), trimethoprim+sulphamethazole (25µg), gentamicin(10µg), ceftriaxone (30µg), erythromycin (10µg), methicillin (5µg), clindamycine, piperacillin (100µg) and nalidixic acid (30µg). The criterion used to select the antimicrobial agents tested was based on the availability and frequency of prescriptions for the management of bacterial infections in Ethiopia.

Results were read after 24 hours incubation at 37^{o}_{C}, and the diameters of the growth inhibition around the discs were measured and interpreted as sensitive and resistant. Intermediate results were considered as resistant. In this study, multi drug resistance was defined as simultaneous resistance to two or more antimicrobial agents.

Data quality control and management: The questionnaire was pretested on patients at the Bahir Dar Health Center. All culture media were prepared, following the manufacturers instructions. The performance of each prepared media was checked by reference strains. The prepared media was checked for sterility by incubating 5% of it at 37^{O}_{C} for one day. If there was growth of organisms on this 5% , the batch was discarded. The reference strains used as control were *E. coli* (ATCC 25922), and *S. aureus* (ATCC 25923). All steps of the procedure were done aseptically. To standardize the density of the inoculum of the bacterial suspension for susceptibility, a 0.5 McFarland standard was used (19).

Data processing and analysis: Data were entered and analyzed using SPSS (Statistical Package for Social Sciences) version 16. Pearson's chi-square and Fisher exact test were used to see the existence of associations between dependent and independent variables. Fisher exact was used in a two by two table when one of the expected frequencies was less than five. A p-value of <0.05 was considered to be statistically significant.

Ethical considerations: Ethical approval was obtained from the Ethical Committee of the School of Biomedical & Laboratory Sciences, the University of Gondar. Written consents were taken from each study participant. Participants were given full right to continue or withdraw from the study. Information obtained at each course of the study was kept confidential by keeping data secretly. Participants were treated based on culture results and antimicrobial susceptibility patterns as per the hospital guideline.

RESULT

Socio-demographic characteristics: A total of 137 patients with acute febrile illness were included in the study. Of the study participants, 84 (61.3%) were females. The age of the respondents ranged from 18-68 years. Seventy-three (53.3%) of the participants were illiterate, and 41(30%) had secondary school and above education. Seventy-nine (57.7%) patients were rural dwellers (Table 1).

Table 1: Socio-demographic characteristic of AFI patients attending Felege Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia, February 1 to May 30, 2013

Socio-demographic characteristic	Number (%)
Sex	
Male	53(38.7)
Female	84(61.3)
Age	
18-39	110(80.3)
40-60+	27(19.7)
Educational status	
Illiterate	78(56.9)
Literate	59(43.1)
Marital status	
Unmarried	78(56.9)
Married	59(43.1)
Residence	
Urban	58 (42.3)
Rural	79(57.7)
Total	**137(100)**

Magnitude of Bacterial isolates: Of the 137 blood cultures, a total of 25 bacterial species were isolated from 24 different cases, with a culture positivity rate of 17.5% (Fig. 1). Out of the 25 bacteria, two bacteria (CoNS and *Enterococcus* species) were isolated from one patient. The predominant bacteria isolated from blood culture were Coagulase negative Staphylococcus (CoNS) 8 (5.8%), followed by *K. pneumoniae* 7 (5.1%) and *S. pyogens* 5 (3.6%). The Gram positive and Gram negative bacteria constituted 14 (56%) and 11 (44%) of the culture isolates, respectively.

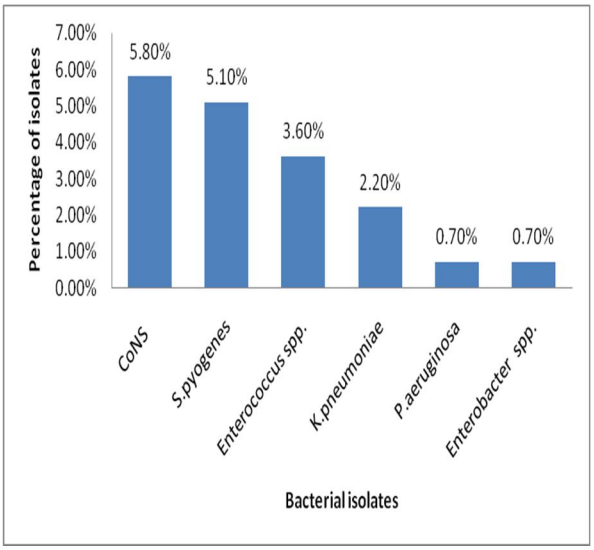

Figure 1: *Bacterial isolates from blood culture of patients with AFI attending Felege Hiwot Referral Hospital, Bahir Dar town, northwest Ethiopia, February 1 to May 30, 2013*

Antimicrobial resistance patterns of bacterial isolates: The range of resistance for Gram positive bacteria was from 0% to 64.3%. A high level of resistance was observed to chloramphenicol and tetracycline, 64.3% each. All Gram positive isolates were 100% sensitive to ceftriaxone, followed by erythromycin and ciprofloxacin, 92.9% each. Coagulase negative *Staphylococci* were (87.5%) resistant to chloramphenicol, (75%) to gentamycine and (62.5%) to tetracycline. *Streptococcus pyogenes* were (60%) resistant to tetracycline. All *Streptococcus pyogenes* were sensitive to penicillin (Table 2).

Table 2: Antibiotic resistance pattern of Gram positive bacterial isolates

Blood bacterial isolate	Resistance antibiogram	Number*
CoNS (8)	C	1
	C, GM, TE	1
	C, GM, TE	1
	C,GM, TE, MET,	1
	C, GM, TE, MET, AMP	1
	C, GM, TE, MET, AMP, P, SXT	1
	C, GM, TE, MET, AMP, P, SXT	1
S. pyogens (5)	TE	1
	TE, SXT,	1
	TE, SXT, C, MET, GM	1
Enterococus spp. (1)	P, AM, SXT, E, DA, C, MET	1

The range of resistance of Gram negative isolates was from 9.1% to 100%. Gram negative bacteria showed 100% resistance to naldixic acid, tetracycline, and chloramphenicol. *Klebsiella pneumoniae* showed 100% resistance to clindamycin, chloramphenicol, tetracycline and naldixic acid. *Pseudomonas aeruginosa* showed 100% resistance to naldixic acid, trimethoprim + sulphamethazole, gentamicin, chloramphenicol, and tetracycline. *Enterococcus* species was 100% resistant to chloramphenicol, tetracycline, and naldixic acid (Table 3).

Multiple drug resistance patterns of the isolates: All Gram negative bacterial isolates were multidrug resistant (resistant to two and more antibiotics). Five distinct antibiogram (resistance patterns) were observed in CoNS. One S. *pyogenes* isolate was resistant to five drugs (Table 2 &3). All the *K.pneumoniae* isolates were multidrug resistant varying from four to ten antibiotics. Likewise, two and one *P.aeruginosa* isolates were resistant to six and nine antibiotics, respectively. Single isolates of *Enterococcus* and *Entrobacter* species were resistant to eight & seven drugs, respectively (Table 3).

Table 3: Antibacterial resistance pattern of Gram negative bacterial isolates

Blood bacterial isolate	Resistance antibiogram	Number*
K. pneumoniae (7)	DA,C,NA, TE	1
	DA, C, NA, TE	1
	DA,C,NA, TE	1
	DA,C,NA, TE, GM,	1
	DA,C,NA, TE, GM, SXT, PRL	1
	DA,C,NA, TE, GM, SXT, CRO	1
	DA,C, NA, TE, GM, SXT, AMX	1
P. aeroginosa (3)	C, GM, TE, NA, SXT, PRL	1
	C, GM, TE, NA, SXT, PRL	1
	C, GM, TE, NA, SXT, PRL, CIP, AMX	1
Enterobacter spp.(1)	DA, SXT, C,TE, CIP, NA, PRL	1

Key: *P=Penicillin G, AMX=Amoxicillin, DA=Clindamycin, E= Erythromycin, TE=Tetracycline, C= C chloramphenicol, CIP=Ciprofloxacin, SXT=Trimethoprim + Sulphamethazole, GM= Gentamicin, CRO= Ceftriaxone, MET= Methicillin, CoNS= Coagulase negative staphylococcus Number*=number of resistance strain*

DISCUSSION

Acute febrile illness is the most common cause of morbidity and mortality in tropical and subtropical countries (1). Infections with AFI agents cause unrecognized deaths in malaria endemic areas and where there is lack of good laboratory facilities (1). AFI is common in the current study area, Bahir Dar, where due to lack of blood culture facility AFI patients are diagnosed only by blood smear and sero-

logical tests. If these tests are negative, patients are treated by antimalarial drugs. So this research is timely and appropriate for describing the bacterial cause of AFI. In the study, blood stream bacterial infection was not found to be a common cause of acute febrile illness in the Outpatient Department of Felege Hiwot Hospital. In the study, 24 (17.5%) of the blood cultures were positive for different bacterial species. This finding is similar to that of a study done in Gondar (14). On the other hand, the finding was relatively lower compared to the findings of some previous studies done at Tikur Anbessa, Mekele, and Gondar hospitals, Ethiopia, which reported 21.4, 28, and 24.2%, respectively (11, 13,15), In addition, Nepal reported (20, 21, 22) and India and Malawi (23, 24).

However, this finding was greater when compared to those of other studies done in Nepal (25) and Jimma (8.8%) (12). The differences in the reported prevalence rates in the various studies may be due to variations in techniques of bacteria isolation in blood, socioeconomic status, seasons, climate conditions, personal and community hygiene, study population, geographical location, infection control polices among nations, and the years in which the surveys were conducted (12, 13,14).

In our study, Gram positive and Gram negative bacteria accounted for 56% and 44% of the isolates, respectively. In similar studies done in Addis Ababa (62.6% & 37.4%) Jimma (60.9% and 39.1%), Mekele (72.2% and 27.8 %), Gondar (69% and 31%), Gondar (70.2%, 29.8) of the isolates were Gram positive and Gram negative , respectively (11, 12,13,14, 15). On the contrary, Gram negative bacteria have been reported as the commonest cause of bacteremia compared with Gram positive bacteria in acute febrile patients in developing countries, according to studies in India (76% and 24%) and Nepal (89.19% and 10.8%), respectively (23, 26). The possible explanation for the difference in the proportion of Gram negative and Gram positive bacteria may be variations in geographical locations and epidemiological patterns of etiological agents (13).

In the present study, CoNS were the most frequently isolated bacteria though lower when compared with the result obtained in Jimma (26.1%) (12). A study in Malaysia also showed that CoNS were the most common organisms isolated; accounting for 33.0% of the total blood culture isolates (27). Although long considered nonpathogenic as members of the normal human skin and mucosa, CoNS have lately turned out to be significant etiologic agents causing nosocomial infections, and their increasing incidence in different infections, like bacteremia related to plastic indwelling devices, the central nervous systems shunt infections, valve endocardiatis, urinary tract infections have been reported, and they have also been an important cause of morbidity and mortality among immunocopromised individuals (28,29). In addition, their increasing resistance to antibacterial drugs evoked a deepening concern about infections involving them (28).

In this study, the second predominant isolates were *K. pneumoniae* (5.1%), lower than the 14.99% detected in India (23). *Streptococcus pyogenes* was the third largest isolated bacteria accounting for 3.6% of the total isolates; it was lower when compared with the finding of a study done in Jimma (13%) (21). However, it was higher than the rate obtained in India (1.24%) (30). In our work, *P. aeruginosa* accounted for 2.2% of the total isolates which is comparable to 10.7%, the result of a study done in La-

hore (31). Next to *P. aeruginosa,* the least isolated were *Enterobacter* and *Enterococcus* species each accounting for 0.7%. The isolation rate for *Enterococcus* species (0.7%) in this study was lower than the rate (2.35%) obtained in India (23).

The in vitro antimicrobial susceptibility profile of the etiological agents of bacteremia has revealed that there was a growing emergence of multi-drug resistant microbes. The overall range of resistance for Gram positive bacteria was from 0% to 64.3% which is comparable to those of studies in Jimma, Mekele, and Gondar which noted0-85.7% and 0-83%, 23.5-58.8%, respectively (22,32,33). Among the antibiotics used for susceptibility testing for Gram-positive isolates, ceftriaxone was very effective (100%), followed by ciprofloxacin and erythromycin (92.9%) each. The sensitivity rate of ciprofloxacin (92.9%) in our study is comparable with the result of the study done in Nigeria (32). However, this finding is higher than the result (76.6%) of a study done in Iran (33). Other studies reported that ciprofloxacin was found to be an effective drug for Gram positive bacteria (22, 32, 33), whereas a high resistance was observed to chloramphenicole and tetracycline, 64.3% each (Table1). A research done in Malaysia showed an increased tetracycline resistance of 81.8% (34).

The range of resistance for Gram negatives was from 0 to 100%. The finding of this study is similar to those of a Jimma, Mekele, & Gondar which was 0-100%, 0-100% & 20-100%, respectively (12, 13, 14). Gram negative bacteria showed a 100% resistance against naldixic acid and chloramphenicol, 81.8% to clindamycine, 63.6% to gentamicin and trimethoprim+ Sulphamethazole. Even though increased resistance of gentamycin (63.6%) was obtained in this study, a study done in Nigeria showed a sensitivity rate of 70.7% (32). Ceftriaxone was the most effective (90.9%) against Gram negative bacteria, followed by amoxicillin and ciprofloxacin each with 81.8% effectiveness (Table 4). So, ceftriaxone is recommended for the management of bacteremia patients. The increased effectiveness of ciprofloxacin (81.8%) in this study was comparable with that of a study (82.9%) in India (30).

Most Gram positive and all Gram negative isolates were resistant to more than two antibiotics in this study. The frequency of multidrug resistance is alarmingly high. The increased resistant blood isolates in this study may be a signal of indiscriminate and continuous use of sub-therapeutic doses of commonly available antimicrobials both in the veterinary and public health sectors (34). None of the antibiotics used singly showed high sensitivity to all Gram-negative bacteria, so a combination of two or more drugs is recommended to cover the broad range of possible pathogens which may be difficult to distinguish clinically. This may prevent the emergence of resistance as they may have additive or synergistic antimicrobial activity (30).

This study has no doubt some potential limitations. First, anaerobic bacteria were not investigated due to limited laboratory facilities. Second, the study period was so short that there were seasonal variations; the number of the participants was too small to examine the bacterial causes of acute febrile illness. Thirdly, the sensitivity of blood culture is less than 100%. Therefore, the use of a single blood culture bottle may have contributed to low detections of bacteria (35). Bacterial etiology, such as *Neisseria meningitides* and *Salmonella typhi* that cause febrile illness were not investigated in this study.

CONCLUSION

Our study showed a high prevalence of bacterial pathogens in acute febrile illness suspected patients with a high rate of resistance to most commonly used antibiotics. Therefore, early diagnosis and treatment of bacterial infections based on culture and drug susceptibility are crucial to reduce bacterial causes of acute febrile illnesses. A rational use of antibiotics should be practiced in order to minimize the spread of drug resistant bacteria. A further longitudinal study is recommended to address the proper etiology of acute febrile illnesses.

Authors' contributions: MB, the primary researcher, conceived the study, designed, participated in data collection, and conducted data analysis. MD assisted in data collection, interpreted results, reviewed initial and final drafts of the manuscript and finalized the manuscript for publication. MT and BA assisted in data collection, interpreted results, and reviewed initial and final drafts of the manuscript. All authors read and approved the final manuscript.

Conflict of interest: The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENT

We acknowledge the University of Gondar for funding the study. We greatly appreciate Felege Hiwot Hospital staff and Bahir Dar Regional Laboratory Microbiology staff for thier cooperation during the study. We are also grateful to the patients who participated in the study.

REFERENCE

1. Tadesse H, Tadesse K. The etiology of febrile illnesses among febrile patients attending Felegeselam Health Center, northwest Ethiopia. *American Journal of Biomedical and Life Science*.2013;1(3):58-63
2. Peters RP, Zijlstra EE, Schijffelen MJ, Walsh AL, Joaki G, Kumwenda JJ *et al.* A prospective study of bloodstream infections as cause of fever in Malawi: clinical predictors and implications for management. *Trop Med Int Health*. 2004; 9 (8):928-34.
3. Pondei K, Kunle-Olowu OE, Peterside O. Patterns of acute febrile illness in children in a tertiary health institution in the Niger Delta Region of Nigeria. *J Med and Medi Scien*.2012; 3(11): 734-740.
4. Abebe A, Yalemtsehay M, Damte S, Eden E. Febrile illnesses of different etiology among outpatients in four Health Centers in Northwestern Ethiopia. *Jpn J Infect Dis*. 2009, 62 (2), 107-110.
5. Afifi S, Azab M, Yossef FG, Saaka H, Wasfy M, Mansour H *et al*. Hospital based surveillance for acute febrile illness in Egypt: A focus on community-acquired blood stream infections. *Am J Trop Med Hyg. 2005;* 73(2): 392–399.
6. Bajpai G, Bichile S, Lata S. Mortality analysis of patients of acute febrile illness during Monsoon in a Tertiary care Hospital of Mumbai. *Infec Dis Clin Pract*. 2008; 16(5): 294-297.
7. Kasper MR, Blair PJ, Touch S , Sokhal B,Yasuda CY, Williams M, *et al* . Infectious Etiologies of Acute Febrile Illness among Patients Seeking Health Care in South-Central Cambodia. *Am J Trop Med Hyg*. 2012; 86(2): 246 –253.

8. Ari MD, Guracha A, Fadeel MA, Njuguna C, Njenga MK , Kalani R, et al. Challenges of establishing the correct diagnosis of outbreaks of acute febrile illnesses in Africa: The case of a likely *Brucella* outbreak among Nomadic Pastoralists, Northeast Kenya. *Am J Trop Med Hyg.*2011; 85(5): 909–912.
9. Foundation for innovative new diagnostics: Acute febrile syndrome strategy, Geneva. 2012. *www.finddiagnostics.org.* Accessed on January 13, 2013.
10. Laxminarayan R, Heymann D. Challenges of drug resistance in the developing world. *BMJ.* 2012, 344:1-4.
11. Asrat D, Amanuel YW. Prevalence and antibiotic susceptibility pattern of bacterial isolates from blood culture in Tikur Anbassa Hospital, Addis Ababa, Ethiopia. *Ethiop Med J.* 2001, 39 (2):97-104.
12. Tizazu Z, Subbaram Kannan, Daniel Yilma, Beyene G. Invasive bacterial pathogens and their antibiotic susceptibility patterns in Jimma University Specialized Hospital, Jimma, and Southwest Ethiopia. *Ethiop J Health Sci.* 2011; 21(1): 1-8.
13. Gebreyesus A, Negash L, Aregawi S, Asmelash T, Luel A, Dejenie T *et al.* Bacteriological profile and drug susceptibility pattern of blood culture isolates among febrile patients in Mekele hospital ,Northern Ethiopia. *Springer plus* 2015;4:314
14. Ali J, Kebede Y: Frequency of isolation and antimicrobial susceptibility pattern of bacterial isolation from blood culture in Gondar UniversityHospital. *Ethio Med J* 2008, 46(2):155–161.
15. Dagnew M, Yismaw G, Gizachew M, Gadisa A, Abebe T, Tadesse T et al. Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia. *BMC Res Notes* 2013; 6:283
16. Blood culture bottle for use in the culture of microorganisms: A qualitative test for the detection of microorganisms in blood. *Guide of Lab Proc.* 1989. 1-12. Available from URL: http://www.bd.com/ds/technicalCenter/clsi/clsi-septicheck.pdf. Accessed on January 11, 2013.
17. CDC. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. Atlanta, Georgia, USA; 2003.
18. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd ed. NY. Cambridge University Press. 2006, 2: 48-70.
19. Clinical Laboratory Standard Institute. Methods for determining bactericidal activity of antimicrobial agents. Tentative Guidelines, M100-TNCLSI, Villanova. 2011.
20. Easow JM, Dhungel BA, Chapagain B, Shivananda PG. Blood stream infections among febrile patients attending a teaching Hospital in Western Region of Nepal. J *Austr Med*. 2010; 39 (10): 633-637.
21. Sharma NP, Peacock SJ, Phumratanaprapin W, Day N, White N, Pukrittayakamee S**.** A Hospital -based study of blood stream infections in febrile patients in Dhulikhel Hospital Kathmandu University teaching Hospital, Nepal**.** *SE Asian J Trop Med Publ Heal.* 2006, 37(2): 351-354.
22. Suttinont C, Losuwanaluk K, Niwatayakul K, Hoontrakul S, Intaranongpai D, Silpasakorn S, Suwancharoen D, Panlar P, Saisongkorh W, Rolain JM, Raoult D, Suputtamongko L. Causes

of acute, undifferentiated, febrile illness in Rular Thailand: Results of a prospective observational study. *Ann Trop Med and Parasito.* 2006; 100 (4): 363-370.

23. Mehta M, Dutta P, Gupta V. Antimicrobial Susceptibility Pattern of Blood Isolates from a Teaching Hospital in North India. *Jpn J Infect Dis.* 2005; 58: 174-176.
24. Chibald LK, Nwanyanwu O, Tokars J, McDonald LC, Kazembe P, Reller LB, et al. A Hospital -Based prevalence survey of bloodstream infections in febrile patients in Malawi: Implications for Diagnosis and Therapy. *JID*. 2000; 181:1414 -1420.
25. Amatya NM, Shrestha B, Lekhak B. Etiological agents of bacteraemia and antibiotic susceptibility pattern in Kathmandu Model Hospital. *JNMA J Nepal Med Assoc*. 2007, 46(167):112-8.
26. David RM, Christopher WW, Mark DZ. The Etiology of Febrile Illness in Adults Presenting to Patan Hospital in Kathmandu, Nepal. *Am J Tro. Med Hyg.* 2004; 70(6): 670–675.
27. Karunakaran R, Raja NS, Peng Ng K, Navaratnam P. Etiology of blood culture isolates among patients in a multidisciplinary teaching hospital in Kuala Lumpur. *J Microbiol Immunol Infect*. 2007; 40:432-437.
28. Longauerova A. Coagulase negative Staphylococci and their participation in pathogenesis of human infections. *Brati LekLis*. 2006; 107(11-12):448-452.
29. Johannes H, Donald AG. Coagulase-negative staphylococcus: Role as pathogens. *Ann Rev Med.*1999; 50:223- 36.
30. Arora U, Devi P. Bacterial profile of blood stream infections and antibiotic resistance pattern of Isolates. *JK Scie.* 2007; 9 (4): 186-190.
31. Qureshi M, Aziz F. Prevalence of microbial isolate in blood cultures and their antimicrobial susceptibility profiles. *Biomedica.* 2011; 27: 136 –139.
32. Nwadioha SI, Nwokedi E, Kashibu E, Odimayo MS, Okwori EE. A review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian tertiary Hospital. *Afr J Microbiol Res.* 2010; 4 (4):222-225.
33. Mehdinejad, M, Khosravi, AD, Morvaridi A. Study of prevalence and antimicrobial susceptibility pattern of bacteria isolated from blood cultures**.** *Bio Sci J*. 2009; 9(3): 249-253.
34. Ayobola ED, Egbule, Sochi O, Omonigho O. Study of prevalence and antimicrobial susceptibility of blood culture bacterial isolates. *Malay J Micro*. 2011; 7(2):78-82.
35. Lee A, Mirrett S, Reller LB, Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? *J Clin Microbiol* 2007; 45:3546–3548.