

ORIGINAL ARTICLE

Bacteria isolate distribution and factors associated with cultures in Hakim Gizaw Hospital, Debre Berhan, EthiopiaTizazu Zenebe^{1*}, Yosef Temesgen², Biniam Mekonnen³, Getachew Bizuneh⁴

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**ABSTRACT**

Background: Bacterial infections are linked to one in eight global deaths. In low-income countries like in Ethiopia with conflict-affected settings, due to the presence of many barriers to infection prevention and control, bacterial infection problem is more severe and there is scarce epidemiological data to inform better preventive and treatment strategies. The present study aimed to determine distribution of bacterial isolates and associated factors among patients presented to Hakim Gizaw Hospital (HGH).

Methods: A cross-sectional study was conducted in HGH, Debre Berhan, Ethiopia, between January and December 2024. All patients presented to HGH with diagnosed bacterial infections were eligible for the study. Socio-demographic data and clinical data were collected using standardized questionnaire. Following standard operating procedure, all collected clinical samples were investigated in microbiology laboratory. All the data generated were analyzed using BM SPSS Statistics version 27.0.

Results: A total of 252 participants, majority living in rural area of Debre Berhan were included in the analysis. The mean age of the study participants was 28.9 (with median age 28) with almost equal distribution for male (50.8%) and female (49.2%). A total of 280 clinical samples (224 single-type and 28 two types of samples (11.1%)) were included in the laboratory analysis. Among 252 participants, only 103 (103/252, 40.9%) with 95% CI =0.348, 0.470 were culture positive, regardless of positive for one or two bacterial isolates. Gram positive bacteria (65/115, 56.5%) were the predominant compared to Gram negative bacteria (50/115, 43.5%). Occupational status [AOR: 6.771 (1.414, 32.427)] and chief complaints [AOR: 0.101 (0.023, 0.432)] showed association with culture positivity.

Conclusion: A high bacterial infection was found in the present study. High concerns and timely tailored public health interventions are urgently required for minimizing the problems.

Keywords: Bacterial infections; Bacterial isolates; Culture positivity; Antimicrobial resistance; Hakim Gizaw Hospital; Ethiopia.

INTRODUCTION

Due to the wide range of resistance against antimicrobial medicines many common human diseases and medical conditions are under threat [1]. Some of the commonest childhood diseases in developing countries such as pneumonia, other respiratory infections, and dysentery can no longer be cured with many older antibiotics or medicines [1]. Currently, bacterial infections linked to one in eight global deaths [2]. Five leading pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were responsible for 54.9% of deaths among the investigated bacteria [3].

Several factors including the infectivity of an organism, the pathogenicity, virulence of the organism (propensity to cause disease), and host factors such as genetic makeup, nutritional status, age, duration of exposure to the organism, and coexisting illnesses will determine development of bacterial infections and disease [4]. In low-income countries, with conflict-affected settings, due to the presence of many barriers to infection prevention and control (IPC) in hospitals [5], bacterial infection problem is more severe. The presence of fragility of IPC programmes in these settings contributes to the spread of bacterial pathogens with unprecedented rates. Hospital environments without adequate and effective interventions can become epicentres of disease transmission [6]. In Africa, infectious diseases are predominantly influenced by vulnerable populations, social inequality, poor health delivery systems, inadequate funding, poor microbiology services, and limited technical experts [7]. All these lead to scarce epidemiological data to inform better preventive and treatment strategies, that contribute to the rise in the burden of bacterial infections.

In Ethiopia, there is limited diagnostic capacity and surveillance program for providing bacterial isolate distribution among different clinical samples. There is 63.8% of an overall prevalence of antimicrobial use, and most treatments are empiric (96.7%) in Ethiopia [8]. Poor diagnosis and treatment practice is a problem in the country [9]. There is no data providing distribution of bacteria isolates in clinical samples, and patient characteristics. This research aimed to determine distribution of bacterial isolates and associated factors.

MATERIALS AND METHODS

Study design and area: A cross-sectional study was conducted in Hakim Gizaw Hospital (HGH), Debre Berhan, Ethiopia. The study was conducted from January 2024 to December 2024. HGH is Debre Berhan University teaching hospital established in 2022 to serve the surrounding community and provide practical training. The hospital has 128 beds across nine wards.

Study population: All patients who arrived at HGH with clinical characteristics suggesting bacterial infection were the study population. Socio-demographic data and clinical information, depending on the patient's illness and clinical presentation, were recorded using a standard structured questionnaire (Supplement file 1). Those patients who took antimicrobial treatment before culture and who were not recommended for culture tests were excluded.

Sample size determination: All the patients who visited HGH and who were diagnosed for potential infectious diseases were eligible to the present study. The sample size was determined based on a previous study [10], using $d=0.04$. Accordingly, with 5% response rate, a total of 252 eligible patients were included in this study.

Data collection tool and techniques: Socio-demographic data, clinical features, laboratory results and patient management for each clinical case were collected with structured questionnaire (Supplement file 1). Socio-demographic detailed clinical and microbiological findings were documented. The clinical examination was conducted among study participants by clinicians and/or residents during the specimen collection. All clinical procedures were conducted per the hospital protocol.

Data collection: All eligible patients presenting to HGH during the study period were consecutively enrolled until the required sample size was achieved. Clinicians were oriented on the data collection procedures, and sociodemographic and clinical information was collected using a structured questionnaire.

Specimen type, Collection, Preparation, Transport and Storage: We collected the potential samples based on the site of infection (blood, urine, stool, urethral discharge, swab from ulcer, venous blood and other body fluids) following standard operating procedures (SOPs). Specimens were labelled with all the necessary information and transported using appropriate transport medium to microbiology laboratory.

Bacteria isolation and identification: Isolation and identification of bacteria were done based on standard microbiological procedure [11, 12]. The collected samples were first inoculated onto primary culture media based on the nutritional requirement of a particular pathogen according to SOPs. Briefly, urine specimens were inoculated onto CLED agar (HiMedia, India) and incubated aerobically at 37°C for 18-24 hours. Blood specimens were inoculated into Tryptic Soy Broth (TSB) (Oxoid, UK) and incubated aerobically at 37°C for up to seven days. CSF

samples were inoculated on BAP, MAC, and CAP with aerobic incubation at 35-37°C. CAP was supplemented with 5 % carbon dioxide to facilitate the growth of fastidious organisms. Similarly, other samples were also inoculated following standard microbiological procedures. Subculture was done on nutrient agar, and subsequent analysis was done after identifying the species of the bacteria by using standard biochemical tests. And isolates were kept at -800C.

Data analysis: Data were analyzed using SPSS Statistics version 25. Descriptive statistics, including frequencies and proportions, were used to summarize the data. Binary logistic regression was performed for bivariate analysis, and variables with $p < 0.25$ were considered for inclusion in the multivariable binary logistic regression model. The results were presented using crude odds ratios (CORs) and adjusted odds ratios (AORs) with corresponding 95% confidence intervals.

Ethical clearance: The project proposal was reviewed and approved by Institutional Review Board of Astrat Woldeyes Health Science Campus, Debre Berhan University (Ref.No: IRB 01/120/2016; Protocol number: IRB-283).

RESULTS

Socio-demographic data:

A total of 252 participants, 55 from out-patient department, 169 from in-patient department, and 28 from emergency department were enrolled in the present study. Majority of the participants came from Debre Berhan town (59.9%, 151/252), with 61.5% (155/252) of them living in rural areas of Debre Berhan (outside the town). The predominant participants in this study were from the inpatient department (169/252, 67.1%)

and were aged 18-64 years (57.1%, 144/252). The mean age of the participants was 28.9 (with a median age of 28). The distribution of the participants in terms of sex was almost equal for males (50.8%, 128/252) and females (49.2%, 124/252).

Clinical characteristics of the study participants:

The leading main/chief complaints of the participants in this study were pain during urination (18%, 45/252) followed by abdominal pain (13%, 33/252), shortness of breath (11%, 28/252), fever (11%, 28/252), swelling (localized or generalized) (9%, 22/252), abdominal distension (4%, 10/252), diarrhea or/and vomiting (2%, 6/252), and fever and cough (2%, 6/252). Others (23%, 58/252), including failure to gain weight, failure to pass flatus, failure to suck, flank pain, gunshot injury, left-sided body weakness, preterm evaluation, right-sided body weakness, skin rash, loss of consciousness, headache and fever, burn injury, and right toe black discoloration.

The majority of participants had 1-7 days of acute illness and a low hospitalization history (24.2%, 61/252) in the last 90 days. In the present study, the primary admission diagnosis was infectious diseases (46.4%, 117/252) followed by gastrointestinal disorder (12.7%, 32/252), cardiovascular conditions (7.5%, 19/252), neurological conditions (4.8%, 12/252), Orthopaedic conditions (4.4%, 11/252), and others. Others (7.1%, 18/252) include Endocrine/metabolic disorder, genitourinary disorder, respiratory disorder, prematurity, and malnutrition. A total of 48 participants (19.0%, 48/252) were under-gone surgery. The predominant types of surgery done was debridement, incision and drainage (20.8%, 10/48), and followed by Incision and drainage (10.4%, 5/48), appendectomy (8.3%, 4/48), E-

LUST C/S (8.3%, 4/48), retention suture (6.3%, 3/48), choapart Amputation (2.1%, 1/48), cholecystectomy (2.1%, 1/48), emergency laparotomy and appendectomy (2.1%, 1/48), and others. Others (39.6%, 19/48) included Laparotomy+Bilateral ovarian cystectomy, relaparotomy, chest tube insertion, and laparotomy abscess drainage. About 30.6% (77/252) of the participants were with comorbidity (Table 2). The leading comorbidity in this study was congestive heart failure (7.1%, 18/252), followed by chronic pulmonary disease (4.8%, 12/252), Diabetes (3.6%, 9/252), AIDS (2.8%, 7/252), Hemi- or paraplegia (2.8%, 7/252), cancer/leukaemia (2.8%, 7/252), peptic ulcer (2.4%, 6/252), HIV on ART (0.8%, 2/252), malnutrition (1.2%, 3/252), moderate/severe liver disease (0.8%, 2/252), mild liver disease (0.4%, 1/252), and Tuberculosis (0.4%, 1/252).

Clinical samples and culture positivity

A total of 280 clinical samples (224 single- one type, and 28 two-type samples) were included in the laboratory analysis. The main clinical sample in the study was blood (102/280, 39.2%), followed by urine (38/280, 13.6%), blood and other (two sample type; 36/280, 12.9%), abscess and wounds (/280, 7.5%), Urine and other (two sample type; 16/280, 5.7%), Sputum (15/280, 5.4%), Peritoneal fluid (10/280, 3.8%), CSF (7/280, 2.5%), Pleural fluid (7/280, 2.5%), Urethral discharge (7/280, 2.5%), Stool (7/280, 2.5%), and others (Table 1). Others (4.3%) included synovial fluid, tracheal aspiration, vaginal swabs, peritoneal fluid and Stool. Among 252 participants, only 40.9% (95% CI: 34.8%-47.0%) were culture-positive, with 91 clinical samples (91/252, 36.1%) positive for a single organism and 12 clinical samples (4.8%, 12/252) positive for two organisms.

Table 1. Number of bacterial strains isolated by types of clinical samples of the present study done in Debre Berhan Hakim Gizaw Hospital, 2024/2025.

Clinical samples	One strains	Two strains	No organisms
Blood (n=102)	36 (35.3%)	2 (2.0%)	64 (62.7%)
Sputum (n=15)	6(40.0%)	3 (20.0%)	6(40.0%)
Blood and other (n=18)	4 (22.2%)	3(16.7%)	11(61.1%)
Urine and other (n=8)	2(25.0%)	2(25.0%)	4(50.0%)
Others (n= 12)	4 (33.3%)	1(8.3%)	7(58.3%)
Urine (n=38)	14(36.8%)	0(0.0%)	24(63.2%)
CSF (n=7)	1(14.3%)	0(0.0%)	6(85.7%)
Pleural fluid (n=7)	1(14.3%)	0(0.0%)	6(85.7%)
Peritoneal fluid (n=10)	2(20.0%)	0(0.0%)	8(80.0%)
Abscess and wounds (n=21)	12(57.1%)	1(4.8%)	8(38.1%)
Urethral discharge (n= 7)	7(100.0%)	0(0.0%)	0(0.0%)
Stool (n=7)	2(28.6%)	0(0.0%)	5(71.4%)

Note: Others include synovial fluid, tracheal aspiration, vaginal swabs, and peritoneal fluid and Stool.

Bacterial isolation distribution:

The distribution of bacterial isolates by sample types was characterized (Figure 2). *E. coli* was mainly isolated from urine (27.3%), *K.*

pneumoniae from blood (55.6%), and *E. aerogens* from blood (50.0%). From Gram-positive bacteria, *S. aureus* was predominantly isolated from blood (37.8%) and CNS from blood (36.8%).

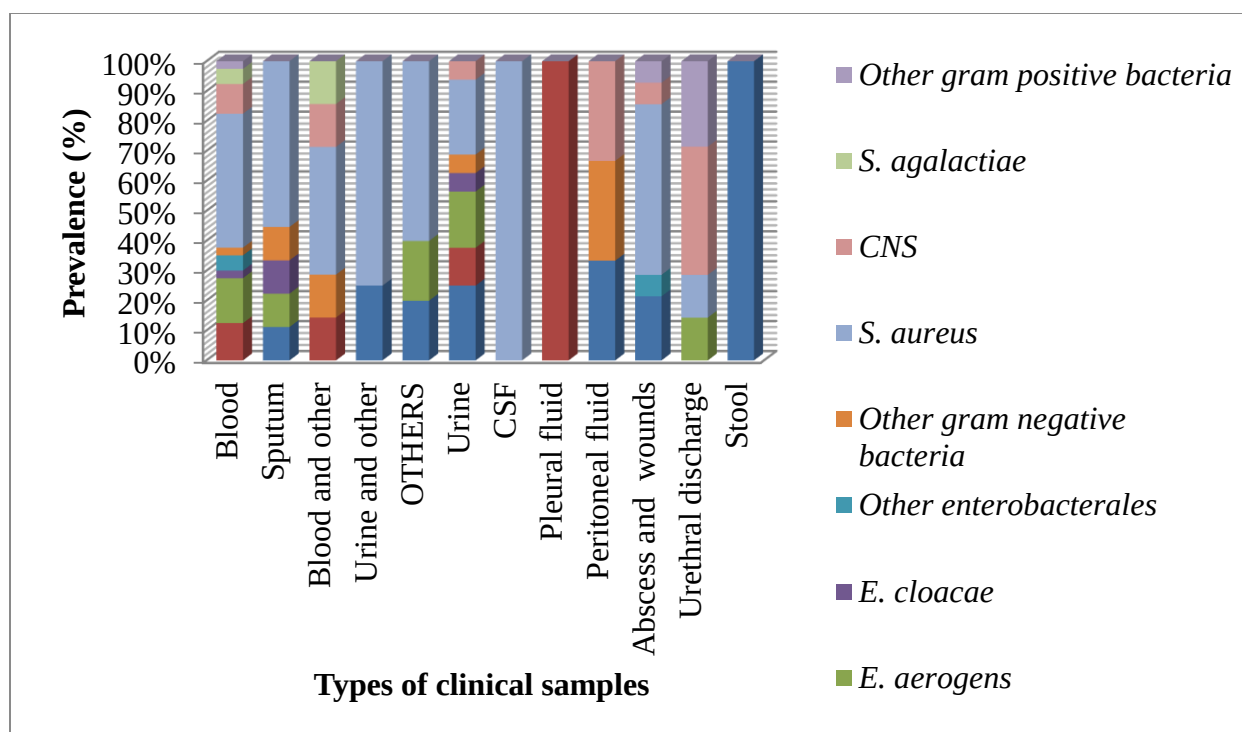


Figure 1. Distribution of bacterial isolates by types of clinical samples in the present study done in Debre Berhan Hakim Gizaw Hospital, 2024/2025. **Note:** Others include synovial fluid, tracheal aspiration, vaginal swabs, and peritoneal fluid and Stool.

Factors associated with cultures:

Culture was predominantly positive among participants with age of 18-64 years (51/103, 49.5%) with p-value=0.028 (Supplement Table S1). Culture positivity was also high in study participants come from in-patient department (70/103, 68.0%) with p-value =0.324. Culture was more positive among others including children not eligible for education (44/103, 42.7%), and illiterate (19/103, 18.4%) based on educational level, with p-value=0.007.

Based on chi-square analysis, age (p-value=0.008), educational level (p-value = 0.007), occupational status (p-value =0.003), hospitalization history (p-value= 0.034), and antibiotic exposure (p-value = 0.008) showed significant statistical association with culture positive. The other variables had p-values >0.05

in the chi-square analysis. Then univariant and multivariate analyses were done for culture positivity. In the final multivariate analysis, variables were selected based on their p-value (≤ 0.25) and previous literature evidence. Occupational status, private employed and others (farming, doing paid work, and non-regular businesses), chief complaints, cough and fever were identified as predictor variables (Supplementary Table S1). Participants who were privately employed and others were more than 6 times [AOR: 6.771 (1.414, 32.427)] and 4 times [AOR: 4.362 (1.169, 16.283)] more likely to be culture-positive compared to unemployed, respectively. Cough [AOR: 0.101 (0.023, 0.432)] and fever [AOR: 0.146 (0.034, 0.633)] showed protective for culture positive.

DISCUSSION

Majority of the study participants (59.9%), came from Debre Berhan mainly (61.5%). Rural populations have closer contact with animals which could increase the risk of exposure to diseases transmitted from animals to humans [13]. They also have exposure to AMR due to many factors including livestock related practice, inadequate diagnostic capabilities, limited healthcare access, and inadequate surveillance systems [14]. On the other hand, the higher population densities and more access healthcare facilities in urban can lead to increased transmission of bacteria and greater antibiotic consumption [14]. Below the bacterial distribution and associated factors have been discussed.

In the present study, the culture positivity was 40.9%, positive to one or two bacterial isolates. Culture positivity rate (or prevalence) was high among participants with age 28 days-1 year (59.4%) and 1-12 years (66.7%) with p -value=0.028. Culture positivity rate was also higher among participants with primary and educational level (41.0%), unemployed (53.8%), and hospitalization (52.5%) and antibiotic exposure (52.3%) history in the last 90 days (p -value <0.05). Infectious disease transmission can be prevented with good infection prevention and control (IPC) practice such as personal hygiene practices [15]. Personal hygiene and other IPC practice could be affected by the participants' characteristics such as educational level [16]. For example, educated people have better knowledge, skill and attitude for prevention of infectious diseases [17]. However, multivariate analysis in the present study found lower odds of culture positivity among participants presenting with cough and fever and private employed and

others (retired, farming, doing paid work, and non-regular businesses) occupational status as high odds of culture positivity. Participants with cough and fever chief complaints were also less likely for culture positive compared to abdominal distension. Both cough and fever can be symptoms of both bacterial and viral infections, and common viral infections like the common cold, influenza (flu), and other respiratory illnesses can cause cough, and fever [18]. All bacterial infections in patients with cough and fever are not routinely cultured and low culture positive rate could be resulted. The abdomen is a significant source of sepsis, often arising from infections within the gastrointestinal tract or abdominal cavity, and can lead to conditions like peritonitis and septic shock which could contribute to culture positive rates [19]. In this study, private employed and others (retired, farming, doing paid work, and non-regular businesses) occupational status (more than four times) were more likely leads to have culture positive compared to unemployed. This could be due to that people in daily business or job activities will have more exposure disease conditions than people who have no daily activities.

Gram-positive bacteria (56.5%) were predominant compared to Gram negative bacteria (43.5%) in the present study. Among 12 classified types of clinical samples in this study, in seven of them, Gram positive bacteria isolation rate was greater than Gram negative (p -value=0.007); many of them were more relevant to the isolation of Gram-positive pathogens, and this may contribute for isolation of more Gram-positive bacteria than Gram negative bacteria. Detection of bacterial isolates from the participants may varied depending on the different factors like sample type, antibiotic

therapy, pathogen type, sample collection and handling, and time to positivity [20]. In line with the findings of the present study, Alemnew et al [21] reported 15.5% Gram-positive and 10.5 % Gram-negative in Ethiopia. Similar reports are also available outside Ethiopia, such as 54.2% Gram-positive and 24.9% Gram-negative in India [22] and 70.7% Gram-positive bacteria and 29.3% Gram-negative bacteria in China [23]. However, many other studies reported inconsistent findings with the present study. Among these studies, 32.2% Gram positive and 67.8% Gram-negative [10], and 16.4% Gram positive and 53.6% Gram-negative [24] bacteria were reported in Ethiopia. In addition, 16% Gram positive and 84% Gram-negative bacteria in Tanzania [25], and 31.1 % Gram-positive and 68.9% Gram-negative in Bangladesh [26] were reported. The discrepancy may be due to difference in types of the sample proportion and study design. In the present study, blood samples were the predominant clinical sample, and a blood culture analysis found 77.4% gram-positive bacteria and 22.6% Gram-negative bacteria in Ethiopia [27] and 70.7% Gram positive bacteria and 29.3% Gram-negative bacteria in China [28]. The high proportion blood sample in this study may contribute to the high prevalence of Gram-positive bacteria.

The present research played a vital role in strengthening HGH microbiology laboratory. Early diagnosis and treatment reduces the risk of long-term complications in patients and prevent further transmission [29]. In resource-limited settings, several challenges include a lack of leadership and advocacy, human resources, career path and retention of staff, national laboratory policy, strategic planning (budgetary concerns), sufficient physical infrastructure, supply chain management, and quality

management systems (quality assurance), which have resulted in inadequate laboratory services [30]. However, the findings of the present study could enable public health players to overcome the abovementioned challenges to have advanced and fruitful microbiology in the hospital setting.

CONCLUSIONS

The participants in this study were mainly residents living in rural areas outside Debre Berhan town, where limited health awareness and health service access are expected. Different bacteria, Gram positive and negative, were isolated from different clinical samples. The predominant bacterial isolates distributed among different clinical samples in this study were *S. aureus*, *E. coli*, *E. aerogens*, and *K. pneumoniae*.

The culture positivity rate was found to be high (40.9%), and this could reflect either the presence of poor infection prevention and control practices or the need for strong interventions in the study area. It was more associated with some predictor variables and may need interventions to address these contributing factors. We recommend a strong infection prevention and control program to prevent infection transmission within the hospitals and conducting advanced and large scale with a reasonable budget, focusing on rapid diagnostic methods and epidemiology of bacterial diseases/infections with their pathogen and AMR profile.

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