ORIGINAL RESEARCH

Aerobic Bacterial Profile and Their Antibiotic Susceptibility Patterns of Sterile Body Fluids Among Patients at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia

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Background: Infections of sterile body fluids are susceptible to serious invasive bacterial infections and critical, with high morbidity and sequelae risk. This study has not been conducted previously in eastern Ethiopia.

Objective: The present study was designed to determine the bacterial profile, associated factors, and their susceptibility to antimicrobial agents of isolates among patients with sterile body fluids at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia.

Methods: Hospital-based cross-sectional study was conducted from April to June 2021 among 204 patients selected using a convenient sampling technique. Data were collected using a pretested structured questionnaire. All consenting patients submitting body fluid specimens for testing at the clinical laboratory were included and analyzed using standard microbiology methods. Antimicrobial susceptibility testing was performed using the disk diffusion method and interpreted as per Clinical and Laboratory Standards Institute guidelines. Data were double entered into Epi data version 4.6, exported, and analyzed using Statistical Package for Social Science version 25. Bivariate and multivariable logistic regressions were used to assess the association between outcome and predictor variables. P-value <0.05 was considered to be statistically significant.

Results: The overall prevalence of bacteria among different sterile body fluid samples was 16.7% (95% CI: 12–22%). Most of the bacterial isolates (70.6%) were Gram-negative bacteria, mainly *K. pneumoniae (26.5%)* and *E. coli (20.6%)*. Multidrug resistance was identified in 76.5% of the isolates. Being inpatient (AOR = 3.59; 95% CI: 1.52, 8.51) and turbid appearance (AOR = 4.35; 95% CI: 1.67, 11.29) were significantly associated with culture growth rate.

Conclusion: The prevalence of bacterial isolates in this study comprises about 17%. Gram-negative bacteria, particularly *K. pneumoniae* and *E. coli,* were the major etiologic agents. Being inpatient and the turbid appearance of the specimen were significantly associated with the culture-positive result. Significant numbers of multidrug-resistant bacteria were isolated, mainly against beta-lactams. Therefore, culture and susceptibility testing should be an integral part of the laboratory investigation.

Keywords: sterile body fluids, bacteria, antimicrobial resistance, Eastern Ethiopia

Introduction

Sterile body fluids (SBF) are fluids in which no microbes exist as normal flora under normal conditions. However, microorganisms can invade sterile body sites[1,2] causing serious invasive diseases, resulting in critical phenomenon.[3,4] In response to infection, fluids may accumulate in any body cavity associated with invasive diseases, such as bacteremia, sepsis, bacterial meningitis, bacterial peritonitis, and other complications.[5] These infections are serious and urgent condition that requires immediate treatment because untreated infections at sterile body sites can lead to severe potentially life-threatening infection throughout the body.[6]

Especially, infections caused by resistant pathogens are persisting to be a significant cause of severe infections across the world, with increasing morbidity and mortality rates.[7] In addition, these infections caused by antimicrobial-resistant bacteria can lead to longer hospital stays, higher medical costs, and increased mortality.[8,9] To a great extent, it is more prevalent in developing countries where health care services are limited, scarce resources, poor hygiene and sanitation, irrational use of antibiotics, and poverty are some of the leading causes of the development of antibiotic-resistant microbes.[10] Similar to other developing countries, Ethiopia also faces similar challenges.[11] Therefore, early diagnosis and appropriate antibiotic treatment are necessary to avoid and reduce complications.[12]

A wide range of bacterial agents have been described as sterile body fluid infections including gram-negatives, such as *E. coli, Klebsiella* spp.,*Acinetobacter* spp.,*Pseudomonas* spp., as well as gram-positive bacteria like *Streptococci* spp., *Neisseria meningitidis, Enterobacter* spp., *Staphylococci* spp., etc.[13,14]

In Ethiopia, there is a scarcity of data on the prevalence, associated factors, and antimicrobial susceptibility patterns of sterile body fluid infections. In particular, this topic has never been addressed in the study area. Therefore, the current study was conducted to determine the bacterial profile and antimicrobial susceptibility pattern of sterile body fluids among patients at HFSUH, Eastern Ethiopia.

Materials and Methods
Study Area and Period
This study was conducted in Harar town at HFSUH from April to June 2021. This hospital was selected because of the large and diverse population they serve. The hospital is now also a teaching facility for Health and Medical Sciences students at Haramaya University.

Study Design and Population
A hospital-based cross-sectional study was conducted among all patients who visited and admitted at HFSUH. Two-hundred and four patients who submitted sterile body fluid specimens for testing to the clinical laboratories at HFSUH from the different specialty wards of the hospital and the outpatient department were included and participated. Blood samples, patients with a history of antibiotic therapy in the preceding two weeks, contaminated samples, and delayed samples for more than 2 hours were excluded.

Sample Size, Sampling Technique and Procedure
The sample size of the study was determined using a single population proportion formula by considering the magnitude of the bacterial from sterile body fluids at Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia, with a prevalence of 14.1%,[13] a 95% confidence level, and a margin of allowable error of 5%. To minimize the errors arising from the likelihood of non-compliance, 10% of the sample size was added. The final sample size was 204. A convenient sampling technique was used until the required sample was reached.

Data and Specimen Collection Methods
Data were collected using pretested structured questionnaires developed after reviewing different literature.[13,15,16] Following informed consent, the individual providing the sample was asked and recorded about demographic information (age, gender, marital status, residence, occupation, income, and educational status) and clinical/sample-related data (hospitalization, specimen type, area of specimen collected, and gross appearances of the specimen), which were collected through a questionnaire-guided face-to-face interview. All specimens were transported to the microbiology and processed within 2 hours of collection.[2]

Microbiological Analysis
Microbiological analysis was performed using a technique described by Cheesbrough tests[17] to isolate and identify bacterial infection from sterile body fluids. The specimen greater than 1 ml was centrifuged at a rate of 2500 revolutions

per minute (rpm) for 10 minutes to concentrate on any organisms, and the sediment was then seeded for culture. However, the specimen less than 1 ml and purulent one was inoculated directly into the culture media.

Collected specimens were cultured onto Blood agar, Mac-Conkey agar for Gram-negative bacteria and chocolate agar for fastidious organisms (HiMedia, India). The inoculated Blood agar and MacConkey agar were incubated at 35–37°C aerobically for 18–24 hours, and inoculated chocolate agar was incubated in a 5% to 10% carbon dioxide-supplemented atmosphere (in a candle). Identification of grown isolates was done based on standard bacteriological techniques, such as colony morphology, Gram staining, and biochemical testing as described by Cheesbrough.[17]

Morphological features of the colonies such as size, shape, pigmentation, and opacity were noted and recorded. Gram staining was performed to distinguish gram-positive from gram-negative bacteria through their shape and arrangement. Biochemical characteristics of gram-positive bacteria were determined by carrying out catalase test, coagulase test, Bile Esculin test, optochin sensitivity test, Novobiocin sensitivity test, bacitracin sensitivity test, Pyrrolidonyl arylamidase (PYR) tests, mannitol test, DNase test, and hemolytic activity on blood agar. Besides, Lancefield grouping system was used for the identification of the Genus *Streptococci* isolates. However, indole production test, citrate utilization test, lactose and glucose fermentation test, urease test, oxidase test, lysine decarboxylase (LDC) test, methyl red/voges proskauer (MR/VP) test, motility test, and hydrogen sulfide gas production test were carried out for the identification of gram-negative bacteria. Identification of *Enterobacterales* and *Pseudomonas* bacterial isolates was confirmed using API 20E/20NE identification kit (BioMerieux, France).[18]

Determination of the Antibiotic Sensitivity Pattern of Bacterial Isolates

Susceptibility of isolates to antimicrobial agents of different classes was assessed by the Kirby-Bauer disk diffusion method in compliance with a commercially prepared antibiotic disc of known concentration with Clinical and Laboratory Standards Institute (CLSI) guidelines on Mueller–Hinton agar standard media[17] and CLSI.[19] Using a sterile wire loop, up to 5 pure colonies were picked and emulsified in 5 ml normal saline (0.85% NaCl) that matched the turbidity of the 0.5 McFarland standard, equivalent to 1.5×10^8 CFU/ml. A drop of the suspension was taken, and a sterile swab was inserted/dipped into the inoculum tube and rotated the swab against the side of the tube using firm pressure, to remove excess fluid. The suspension was swabbed and uniformly distributed over the entire surface of the Mueller Hinton agar plates using a sterile cotton-tipped applicator. Within 15 minutes of inoculation, the appropriate disks impregnated with antimicrobial agents were placed on the surface of agar plates, which were pre-inoculated with the suspension of the microorganisms being tested, and the plates were inverted for incubation to prevent the accumulation of moisture on the agar surface, which would interfere with the interpretation of test results. Finally, the plates were incubated at 37°C for 18 to 24 hours. Similarly, the Mueller-Hinton Agar supplemented with 5% defibrinated sheep blood at 37°C with; 5% carbon dioxide (CO_2) incubation period was used for testing fastidious organisms like streptococci and enterococci.[20] After the incubation, the diameter of the inhibition zone was measured using a scaled ruler (Caliper) to the nearest millimeters and compared against the predefined values provided by CLSI guidelines.[19] Using the Interpretative chart based on CLSI 2020 guidelines, the zone sizes of each antibiotic were interpreted, and the isolate was reported as sensitive (S), intermediate (I), or resistant (R).[19] In this study, multi-drug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.[21] Oxacillin (methicillin) susceptibility to *Staphylococcus aureus* and Coagulase-negative Staphylococci was interpreted using 30 μg cefoxitin as a surrogate test for Methicillin-resistant *Staphylococcus aureus* (MRSA).[19]

Drugs for Gram-Positive Cocci: Ciprofloxacin (CIP, 5μg), gentamicin (GM, 10μg), ceftriaxone (CRO,5μg), erythromycin (ERY, 15μg) clindamycin (CLD, 2μg) trimethoprim/sulfamethoxazole (SXT,1.25/23.75 μg), penicillin (P, 10units), amoxicillin clavulanic acid (AXC, 30μg), vancomycin (VA, 30μg), cefoxitin (FOX, 30μg) (Oxoid, LTD, UK).

Drugs for Gram-Negative Bacilli: Ampicillin (AMP, 10 μg), amoxicillin-clavulanic acid (AMC, 30 μg), ceftriaxone (CRO, 30 μg), ceftazidime (CAZ, 30 μg), amikacin (AMK, 30 μg), gentamicin (GM, 10 μg), meropenem (MEM, 10μg) ciprofloxacin (CIP, 5μg), trimethoprim/sulfamethoxazole (SXT,1.25/23.75 μg) (Oxoid, LTD, UK). These antibacterial discs were selected based on pathogen, local availability, and CLSI recommendations.

Quality Assurance

The questionnaire was initially prepared in the English language and was translated into local languages (Afan Oromo and Amharic) and translated back to English by language experts to assure its consistency. Five percent of the questionnaire (5%) was pretested at Jugel General Hospital to check its consistency and understandability before the actual data collection. The data was double entered by two data clerks into Epi-data. The consistency of the entered data was cross-checked by comparing the two separately entered data.

To maintain the quality of the data, standard operating procedures were followed in the preparation of media and drug susceptibility testing. As an internal quality control, uninoculated media (negative control) were incubated and must remain clear to check for sterility. American Type Culture Collection (ATCC) reference strains, such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and/or *Staphylococcus aureus* (ATCC 25923), were used as positive controls (for culture and monitoring the quality of the reagents). Known strains from United Kingdom External Quality Assessment Scheme (UKNEQA): *E. coli* (UKNEQA 6453) and *S. aureus (*UKNEQA 5958) were used for drug susceptibility testing as well. These standard control strains were obtained from the Child Health and Mortality Prevention Surveillance (CHAMPS) project. The inoculum density of the bacterial suspension was standardized to the 0.5 McFarland standard to perform antibiotic susceptibility testing.

Data Processing and Analysis

All questionnaires were checked for completeness. The collected data were double entered, cleaned using Epi-data (version 4.6) and exported to the Statistical Package for Social Sciences software (SPSS) version 25 for further cleaning and analysis. Descriptive statistical tools were used to summarize the findings. Logistic regression models through bivariate and multivariate logistic regression were used to predict the relationship between dependent and independent variables; to identify factors associated with culture-positive rates. Variables with a p-value ≤0.25 in bivariate logistic regression analysis were moved to a multivariable logistic regression analysis model, which accounts for potential confounding and identification of independent predictors. Crude odds ratio (COR) and adjusted odds ratio (AOR) with their 95% confidence interval (CI) were used to determine the significance of the predictors. Variables with a p-value less than 0.05 in multivariable analysis were taken as significant predictors. In addition, p-values were calculated using fisher's exact test for some variables. The assumption of multivariate logistic regression was checked using Hosmer and Lemeshow goodness-of-fit-test, and a p-value >0.05 was considered a good fit.

Ethical Consideration

Ethical clearance was obtained from Haramaya University Institutional Health Research Ethics Review Committee of the College of Health and Medical Sciences (IHRERC) before the commencement of the study. This study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from each participant before the commencement of data collection. To maintain confidentiality, independent code numbers were given to each study participant. Besides, both study participants and data collectors used sanitizer and worn face masks to protect them from COVID-19.

Result

Socio-Demographic Characteristics

In this study, a total of 204 study patients were recruited and participated. Among enrolled patients, a majority (63.7%) of study participants were males, 32.4% were unable to read and write, 66.2% were rural residents, and 63.8% were married. The median and interquartile range of the age of the study participants was 33 and 56, respectively. Nearly half (47%) of the study participants were in the age group of less than 20 years (Table 1).

Clinical and Sample-Related Characteristics

A total of 204 body fluid samples were collected and investigated. These data showed that more than half of the processed samples were CSF (52.0%) followed by pleural fluid (29.9%). Out of a total of 34 (16.7%) positive cases, half

Table 1 Socio-Demographic Character of the Study Participants of Sterile Body Fluid Infections at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021

Variables	Category	Frequency	%
Age	≤20	96	47.1
	21–40	16	7.8
	41–60	35	17.2
	≥61	57	27.9
Sex	Male	130	63.7
	Female	74	36.3
Residence	Urban	69	33.8
	Rural	135	66.2
Marital Status	Under age*	77	37.7
	Single	29	22.8
	Married	81	63.8
	Widowed	5	4.0
	Divorced	12	9.4
Educational status	Pre-school	62	30.4
	Unable to read and write	66	32.4
	Read and write	19	9.3
	Primary	23	11.3
	Secondary/High school	16	7.8
	College and above	18	8.8
Occupation	Government employee	43	21.1
	Labor worker	2	1.0
	Student	37	18.1
	House wife	8	3.9
	Farmer	80	39.2
	Merchant	26	12.7
	Other	8	3.9
Monthly income	≤1500	110	53.9
	1600–3000	83	40.7
	≥3100	11	5.4

Note: *Non-candidates for marital status.

(50%) of the isolates were identified from CSF sample. The higher culture-positive cases were found in the inpatient sample (61.8%) and a larger proportion (56%) of the isolates were from turbid appearance (Table 2).

Prevalence of Bacterial Isolates

From a total of 204 specimens, 34 were culture-positive, which yielded an overall prevalence of 16.7% (95% CI: 12% to 22%). As shown in Figure 1, the highest frequency and distribution were shown by gram-negative bacteria (24/34, 70.6%). Among these, *K. pneumoniae* (9/24, 37.5%) and *E. coli* (724, 29.2%) were the most predominantly isolated gram-negative bacteria, while Gram-positive cocci constituted the remaining 10 cases (10/34, 29.4%). Of these, *S. aureus* (4/10, 40%) and *Enterococcus* spp. (4/10, 40%) were the main isolates.

Associated Factors

In bivariate regression analysis, age, patient setting, and appearance of the specimen showed a significant association at a p-value of ≤0.25 and were considered as candidates for multivariate logistic regression. Through a multivariable analysis, being inpatient and turbid appearance were significantly associated with culture-positivity rates at p-value <0.05 after adjusting for confounding factors.

Table 2 Total and Positive Cases of Clinical and Sample-Related Characteristics of Sterile Body Fluid Infection Among Patients at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021

Variables	Category	Growth N (%)	No Growth N (%)	Total N (%)
Specimen type	CSF	17 (16)	89 (84)	106 (52.0)
	Synovial	–	3 (100)	3 (1.5)
	Pleural	12 (19.7)	49 (80.3)	61 (29.8)
	Peritoneal	5 (14.7)	29 (85.3)	34 (16.7)
	Total	34 (16.7)	170 (83.3)	204 (100)
Patient setting	Inpatient	21 (22.8)	71 (77.2)	92(45.1)
	Outpatient	13 (11.6)	99 (88.4)	112 (54.9)
	Total	34 (16.7)	170 (83.3)	204 (100)
Ward/outpatient unit	Gynecology-obs	1 (25)	3 (75)	4 (2.0)
	Pediatrics	11 (17.2)	53 (82.8)	64 (31.3)
	Medical	21 (17.4)	100 (82.6)	121 (59.3)
	Surgical	1 (14.3)	6 (85.7)	7 (3.4)
	NICU	–	3 (100)	3 (1.5)
	Isolation center	–	5 (100)	5 (2.5)
	Total	34 (16.7)	170 (83.3)	204 (100)
Specimen appearance	Clear	8 (8)	92 (92)	100 (49.0)
	Turbid	19 (26.8)	52 (73.2)	71 (34.8)
	Bloody	2 (13.3)	13 (86.7)	15 (7.4)
	Other*	5 (27.8)	13 (72.2)	18 (8.8)
Overall total		**34 (16.7)**	**130 (83.3)**	**204 (100)**

Note: *Purulent, yellowish.

In the case of the patient setting, inpatients were 3.59 times (AOR = 3.59; 95% CI: 1.52, 8.51; P = 0.004) more likely to be infected compared to those of outpatients. The odds of being culture-positive rate increased by 4.35 times (AOR = 4.35; 95% CI: 1.67, 11.29; P = 0.003) for those patients who had the turbid appearance of the specimen rather than the counter parts. Even if a high positivity rate was observed among participants with an age group above 60 years, there was no significant difference between the age group and culture-positivity rate (p = 0.14) (Table 3).
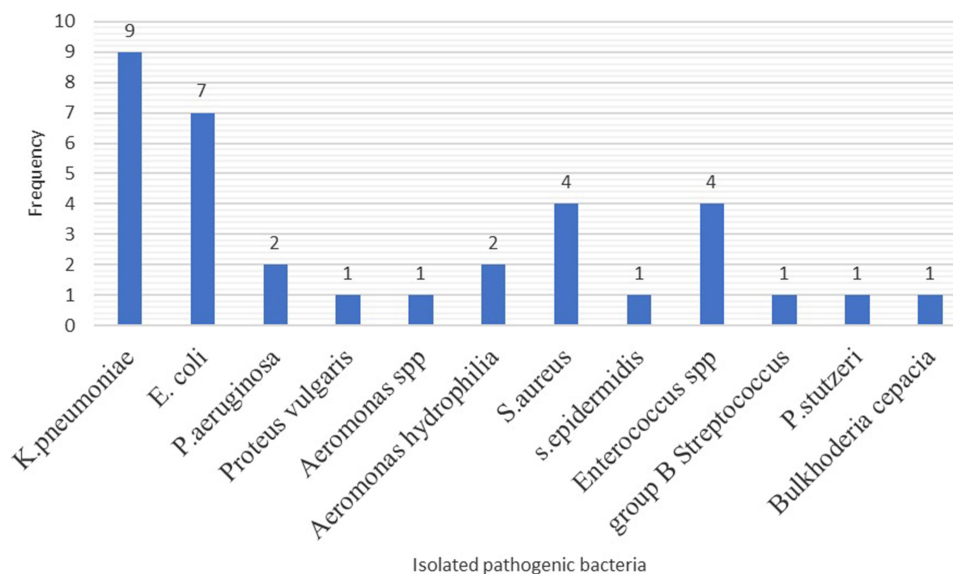
Figure 1 Bacterial isolates from patients diagnosed with sterile body fluid infections at HFSUH, Harar, Eastern Ethiopia at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021.

Table 3 Bivariate and Multivariate Analysis of Factors Associated with Bacterial Infections from Sterile Body Fluids at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021

Variables	Category	Culture Growth N (%)		Bivariant Analysis		Multivariable Analysis	
		Yes	No	COR (95% CI)	P-value	AOR (CI 95%)	P-value
Gender	Male	23(17.7)	107(82.3)	1.23(0.56, 2.69)	0.60		
	Female®	11(15)	63(85)				
Residence	Rural	24(17.8)	111(82.2)	1.27(0.57, 2.85)	0.55		
	Urban®	10(14.5)	59(85.5)				
Age group	≤20	11(11.5%)	85(88.5%0	1.94(0.23, 16.16)	0.54	2.28(0.25, 20.91)	0.46
	41–60	9(25.7%)	26(74.3%)	5.19(0.59, 45.09)	0.135	5.67(0.59, 55.31)	0.13
	≥61	13(22.8%)	44(77.2%)	4.43(0.53, 36.80)	0.168	5.62(0.61, 51.97)	0.13
	21–40®	1(6.3%)	15(93.7%)				
Patient type	Inpatient	21(22.8%)	71(77.2%)	2.25(1.058, 4.796)	0.035	3.59(1.52, 8.51)	**0.004***
	Outpatient®	13(11.6%)	99(88.4%)				
Specimen type	Synovial	–	3 (100)	Fisher's exact test	P=0.89		
	Plueral	12(19.7)	49 (80.3)	F-test=0.68			
	Ascites	5 (14.7)	29 (85.3)				
	CSF	17(16)	89(84)				
Appearance of specimen	Turbid	19(26.8%)	52(73.2%)	4.20(1.72, 10.27)	0.002	4.35(1.67, 11.29)	**0.003***
	Bloody	2(13.3%)	13(86.7%)	1.77(0.34, 9.26)	0.499	1.37(0.25, 7.5)	0.714
	Other	5(27.8%)	13(72.2%)	4.42(1.27, 15.59)	0.021	3.66(0.96, 13.9)	0.058
	Clear ®	8(8%)	92(92%)				

Note: *Statistically significant.
Abbreviations: COR, crude odds ratio; AOR, adjusted odds ratio; CI, confidence interval; ®, reference category.

Antimicrobial Susceptibility Testing

A drug sensitivity test was performed for all[34] bacterial isolates. Isolates have shown various resistance and susceptibility patterns against different antibiotics that were tested. The antimicrobial profile of gram-negative bacteria, ciprofloxacin (87.5%) and meropenem (83.3%) were found to be the most effective drugs against gram-negative isolates. Also, they showed higher sensitivity to amikacin (75%). However, resistance to ampicillin (87.5%), and third-generation cephalosporins such as ceftriaxone (75%) and ceftazidime (75%) were shown among gram-negative isolates.

Among antibiograms of gram-positive bacteria, they (n=10/34) were resistant to penicillin (8/10, 80%), ceftriaxone (8/10, 80%), and erythromycin (6/10, 60%). Meanwhile, amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole exhibited moderate activity (50%) against gram-positive isolates. In the susceptibility pattern of *S. aureus*, trimethoprim-sulfamethoxazole was most effective against all *S. aureus* isolates. By contrast, all *S. aureus* isolates were resistant to cefoxitin, which indicates methicillin resistant *S. aureus* (MRSA). And ampicillin, ceftriaxone, and penicillin were also ineffective against *S. aureus*. In addition, all isolates of *Enterococcus* species were resistant to vancomycin, penicillin, ceftriaxone, and trimethoprim sulfamethoxazole. Moreover, the antibiotic susceptibility patterns of the recovered Gram-positive and Gram-negative isolates are shown in Tables 4 and 5, respectively.

In general, multi-drug resistance (MDR) (resistance to at least one agent in ≥3 classes of antimicrobials) was identified in 76.4% (95% CI: 60% to 92%) of the isolates. Out of the 34 isolates, 26 isolates (76.4%) were MDR. Among these MDR isolates, 19 isolates (73%) were Gram-negative and 7 isolates (27%) were Gram-positive. Based on this finding, penicillin, ampicillin, ceftriaxone, ceftazidime, gentamicin, and trimethoprim-sulfamethoxazole showed a high rate of resistance.

Discussion

Microbial invasion of normally sterile parts of the body can cause systemic illness.[22] It is a life-threatening emergency and any delay in treatment may result in death. In addition, any microorganisms found where no resident microbiota is

Table 4 Antimicrobial Susceptibility Pattern of a Gram-Negative Bacterial Isolate from Sterile Body Fluid Samples at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021

Antimicrobial Classes										
N = 24		Penicillins		Cephalosporin		Fluoroquinolones	Sulfonamides	Carbapenem	Aminoglycosides	
Isolated Organism	Pattern	AMP	AXC	CRO	CTZ	CPR	SXT	MEM	AMK	GN
K. pneumoniae (n = 9)	S	1	4	2	1	8	1	8	4	4
	I	–	4	1	–	–	5	1	3	1
	R	8	1	6	8	1	3	–	2	4
E. coli (n = 7)	S	–	2	–	–	7	1	6	7	1
	I	–	4	–	–	–	2	–	–	–
	R	7	1	7	7	–	4	1	–	6
P. aeruginosa (n = 2)	S	–	–	–	–	1	–	–	2	–
	I	–	–	–	–	–	–	–	–	–
	R	2	2	2	2	1	2	2	–	2
P. stutzeri (n = 1)	S	–	–	–	–	1	–	1	1	1
	I	1	–	–	–	–	–	–	–	–
	R	–	1	1	1	–	1	–	–	–
Aeromonas sp. (n = 1)	S	–	–	–	–	1	1	1	–	–
	I	1	1	1	1	–	–	–	1	–
	R	–	–	–	–	–	–	–	–	1
P. vulgaris (n = 1)	S	–	1	1	1	1	1	1	1	1
	I	–	–	–	–	–	–	–	–	–
	R	1	–	–	–	–	–	–	–	–
A. hydrophilia (n = 2)	S	–	2	–	–	1	–	2	–	1
	I	–	–	1	2	–	1	–	–	–
	R	2	–	1	–	1	1	–	–	1
B. cepacia (n = 1)	S	–	1	–	1	1	–	1	1	–
	I	–	–	–	–	–	–	1	–	–
	R	1	–	1	–	–	–	–	–	1
Total (24, %)	S	1(4.2)	10(41.7)	3(12.5)	3(12.5)	21(87.5	4(16.7)	20(83.3)	18(75)	8(33.3)
	I	2(8.3)	9(37.5)	3(12.5)	3(12.5)	–	9(37.5)	1(4.2)	4(16.7)	1(4.2)
	R	21(87.5)	5(20.8)	18(75)	18(75)	3(12.5)	11(45.8)	3(12.5)	2(8.3)	15(62.5)

Abbreviations: AMP, ampicillin; CRO, ceftriaxone; AMC, amoxicillin-clavulanic acid; GN, gentamicin; SXT, trimethoprim-sulfamethoxazole; CTZ, ceftazidime: MEM, meropenem; AMK, amikacin; CPR, ciprofloxacin; S, sensitive; I, intermediate; R, resistant.

Table 5 Antimicrobial Susceptibility Patterns of a Gram-Positive Bacterial Isolate from Sterile Body Fluid Samples at Hiwot Fana Specialized University Hospital, Harar, Eastern Ethiopia, 2021

		Antimicrobial Classes										
N = 10		**Penicillins**				*Cephalosporin*	*Macrolides*	*Fluoroquinolones*	*Sulfonamides*	*Glycopeptides*	*Aminoglycosides*	*Lincosamides*
Isolates (N)	**Pattern**	**P**	**AMP**	**AXC**	**FOX**	**CRO**	**ERY**	**CPR**	**SXT**	**VA**	**GN**	**CLN**
S. aureus (n = 4)	S	–	–	3	–	–	–	2	4	NA	–	3
	I	–	1	1	–	–	1	–	–	–	2	1
	R	4	3	–	4	4	3	2	–		2	–
Enterococcus spp. (n = 4)	S	–	–	–	NA	–	–	–	–	–	NA	–
	I	–	1	2	NA	–	1	1	–	–	NA	1
	R	4	3	2	NA	4	3	3	4	4	NA	3
S. epidermidis (n = 1)	S	–	1	1	1	1	1	1	–	NA	–	1
	I	1	–	–	–	–	–	–	1	NA	–	–
	R	–	–	–	–	–	–	–	–	NA	1	–
S. agalactiae (n = 1)	S	1	1	1	NA	1	1	1	1	–	–	–
	I	–	–	–	NA	–	–	–	–	1	–	1
	R	–	–	–	NA	–	–	–	–	–	1	–
Total (n = 10, %)	S	1(10	2(20)	5(50)	1 (10)	2(20	2(20	4(40)	5(50)	–	–	4(40)
	I	1(10	2(20)	2(20)	–	–	2(20	1(10)	1(10)	1(20	2(33)	3(30)
	R	8(80	6(60)	2(20)	4(90)	8(80	6(60	5(50)	4(40)	4(80	4(67)	3(30)

Abbreviations: NA, not applicable; AMP, ampicillin; SXT, trimethoprim-sulfamethoxazole; CRO, ceftriaxone; AMC, amoxicillin-clavulanic acid GN, gentamicin; VA, vancomycin; P, penicillin; CPR, ciprofloxacin; FOX, cefoxitin; ERY, erythromycin; CLN, clindamycin; S, sensitive; I, intermediate; R, resistant.

present must be considered significant.[23] More recently, the effectiveness of currently available antibiotics is decreasing globally as a result of increasing resistant strains. As a result, infection caused by resistant agents is difficult; it becomes impossible to treat.[24,25]

In the present study, the overall prevalence of bacterial pathogens was 17% (95% CI: 12–22%). This finding is consistent with previous studies from Mekelle, Ethiopia (20.2%),[16] India (22%),[26] and Brazil (21.3%).[27] In contrast, it is higher as compared to reports from Addis Ababa, Ethiopia (11.5%)[28] and Nepal (10.7%),[29] but lower than other studies in India (36%)[30] and Turkey (25%).[31] These variations can be attributed to differences, in sample processing/laboratory technique (molecular technique versus conventional methods), and the practice of infection control.[12] Other explanations could be variation in sample size and duration of the study.

In this study, the most frequently isolated microorganisms were gram-negative bacteria (70.6%). A similar finding is reported from previous studies in India (71%)[32] and Addis Ababa, Ethiopia (74.6%).[28] In contrast, studies from Mekelle, Ethiopia,[16] Turkey,[31] and India[33,34] reported that gram-positive bacteria were found to be the most common isolates. This difference could be due to different hospital-acquired infections, and different standard infection control precautions.[35,36] Besides, different sample sizes and study areas could be attributed to the difference.

In general, *Klebsiella pneumoniae* and *Escherichia coli* were the bacterial species most frequently isolated, causing sterile bodies infection in this study (47%), which is similar to the findings that have been reported in previous studies done in India,[32] Addis Ababa, Ethiopia,[28] and Nepal.[29] This finding can be explained by their distinctive structure of gram-negative bacteria leading to increasingly resistant to most available antibiotics, or the opportunistic nature of organisms.[37,38]

In the analysis, being inpatient and the turbid appearance of the sample had shown a significant effect (p < 0.05) on the culture-growth rate. Significant association between patient type (hospitalization) and culture-growth (p < 0.05) was observed. The odds of culture-growth were 3.59 times higher in inpatient cases compared to those of outpatients (p = 0.004). The higher rate of culture-positive result among inpatients could be due to secondary sources of infection (cirrhosis, surgical procedures, trauma) and low immune status due to any underlying medical conditions (burn, transplant surgery, and intensive care support.[39] Besides, probably due to nosocomial acquired infections at the time of admission.[36,40]

In relation to the appearance of the specimens, the results indicated that the appearance of the specimens was associated with a culture-positivity rate (P = 0.003). The turbid appearance of the specimen was 4.35 times more likely to be culture-positive than counterpart. This finding is in agreement with the previous studies in Ethiopia.[13] The high detection of bacteria in the turbid appearance can partially be attributed to the rapid changes in the physiology and immunology of the patient as a result of bacteria. Thus, turbidity may indicate the presence of microbes, or a buildup of white blood cells or proteins.[2]

In this study, ciprofloxacin (87.5%), meropenem (83.3%), and amikacin (75%) in the fluoroquinolone, carbapenem, and aminoglycoside class of antibiotics, respectively, were found to be most effective against gram-negative isolates. However, β-lactam antibiotics such as ampicillin (12.5%) and third-generation cephalosporins (25%) were the least effective antibiotics against those of gram-negative agents. This finding is comparable to previous studies in Nepal,[29] India,[32] and Ethiopia.[13,16] The increased rate of beta-lactam drug resistance can be possibly correlated with the frequent use of these antibiotics, easy availability, the practice of self-medication, limited diagnostic facilities, inappropriate antibiotic use, and the issuance of prescriptions without susceptibility data have been considered as the factors associated with this phenomenon.[24,41] In addition, antibiotic resistance can occur naturally such as efflux pumps, alteration of the drug-binding site, membrane permeability, and due to degradation enzymes.[24]

However, a higher prevalence of resistance to the commonly prescribed beta-lactam drugs was observed in the present study among gram-positive isolates, where (80%) of the isolates were resistant to penicillin and ceftriaxone, but half (50%) of the isolates were sensitive to amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole. Similarly, high levels of resistance to beta-lactam agents were reported from previous study in Ethiopia[13,16] and India.[32] This finding could be explained by the production of beta-lactamase and/or the presence of mobile-resistant factors.[24] In addition, prescribing frequency as a result of wide availability of beta-lactams, type antibiotic use, and irrational use of antibiotics could also be the factors for increasing antibiotic resistance.[10,42]

Furthermore, all *S. aureus* isolates (n = 4, 100%) in this study were methicillin resistant (MRSA), which is higher compared to the report from other countries like India, 36.6%,[33] Nepal (30%),[29] and Ethiopia (36.4%).[28] This could be due to geographic variation, differences in infection control practice, and difference in treatments used.[43]

In the present study, high rates of multi-drug resistance (MDR) (76.4%) (95% CI: 60% to 92%) were reported in the current study, particularly against penicillins and third-generation cephalosporin class of antibiotics, and aminoglycosides specifically gentamicin. A similar prevalence rate of MDR was reported from studies in Addis Ababa, Ethiopia (75%)[44] and Mekelle, Ethiopia (90%).[16] But higher than Nepal (30%).[29] There could be many explanations for this difference and increased prevalence including: different bacterial strains, geographic variation, patients' awareness towards the use of the antimicrobials, the difference in infection control practice, the difference in antibiotic prescribing policies, easy availability of drugs without a prescription, and indiscriminate/prolonged use of common antibiotics lead to rapid and extensive spread of antimicrobial resistance.[7,45]

Limitations of the Study

The culture positivity of sterile body fluid was relatively low as the etiology of sterile fluid infections also included anaerobic bacteria, which were not identified in this study. Also, the risk of false-negative results in agar-based culture media is high because only a small number of microorganisms may be present in the specimens.[31,46] To overcome this drawback, innovative techniques using enrichment media and polymerase chain reaction (PCR) are very helpful and recommended.[27]

Conclusion and Recommendations

A variety of gram-positive, as well as gram-negative organisms, were documented, which yielded 17%. Most notably, *Klebsiella pneumoniae* and *Escherichia coli* were the leading causative agents for sterile body area infection. Being inpatient and turbid appearance of the specimen was significantly associated with the culture-growth rate of the infection. The majority (76.5%) of the isolates showed multidrug resistance, especially against beta-lactams and gentamicin, which suggests one of the most important public health problems currently.

Culture and susceptibility testing should be an integral part of routine laboratory tests for the management of infections in patients, and the choice of drugs should be based on results of sensitivity testing. The antibiotic sensitivity profile of this study suggests that ciprofloxacin and amikacin can be the choice of drugs for the infections. However, Beta-lactam antibiotics such as third-generation cephalosporins, ampicillin, and penicillin should be used based on microbiological evidence. The careful prescribing practice or rational use of antibiotics based on sensitivity testing result is needed to stop the tendency to increase antibiotic resistance. Besides, great care should be given to inpatients, as they are more vulnerable to infections at sterile sites and tissues.

The present study was conducted on a limited number of samples; this may not reflect the entire scenarios of the study. This highlights the need for further studies to be conducted on larger sample sizes.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

Authors have no conflicts of interest.

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