

Original article

Bacterial profile and antimicrobial susceptibility pattern of isolates recovered from sterile body fluids referred to the National Reference Laboratory at the Ethiopian Public Health Institute

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Abstract

Introduction: Assessing the presence of bacterial etiologies and their antimicrobial susceptibility patterns is very crucial for proper diagnosis of different infections and controlling antimicrobial resistance in the clinical setting with the ultimate goal of better patient care and outcome.

Objective: To assess bacterial etiologies and the antimicrobial susceptibility pattern of isolates recovered from sterile body fluids from patient specimens referred to National Bacteriology and Mycology Reference Laboratory in Ethiopia.

Method: This is a retrospective study that used routine data from laboratory records from April 1, 2014, to August 30, 2018 from patients in which culture was performed from sterile body fluids in the National Bacteriology and Mycology Laboratory, Ethiopian Public Health Institute. Data were extracted from the laboratory registration book. The data collection sheet was prepared to collect the data and was entered and analyzed using SPSS version 23. Groups were compared using Chi2 and descriptive analysis was performed.

Results: Out of the total 654 body fluid specimens processed in the laboratory, the culture-positive result was documented on 75 (11.5%) of which 19 (25.4%) of them were Gram-positive bacteria and 56 (74.6%) were Gram-negative. The predominant bacteria species isolated were *Escherichia coli* (n=13; 17.3 %), followed by *Acinetobacter* spp. (n=12; 16%), *Klebsiella pneumoniae* (n=10; 13.3 %), *Staphylococcus aureus* (n=10; 13.3%) *Neisseria meningitidis* (n=9; 9.3%). *Pseudomonas* spp. (n=5; 6.3%). The highest bacterial isolation rate was obtained in the age group between 1-10 years old. From the Gram-negative, *Escherichia coli* were the dominant isolates and of which 6 (46.2%) isolates were multidrug-resistant. From the Gram-positives bacteria, *Staphylococcus aureus* was the dominant isolate with one isolate being multidrug-resistant.

Conclusion: Emerging ESKAPE (*Enterobacter species*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter species*, *Pseudomonas species*, and *Enterococcus faecium*) organisms have been isolated in this study and this remains crucial and occurrence of multidrug resistance patterns of bacterial isolates of great public health importance indicating that further researches also must be conducted in different parts of the country.

Keywords: Body fluid, bacterial etiologies, antimicrobial susceptibility testing

Introduction

Body fluids are important in transporting nutrients, regulating body temperature, and assist in the respiration process (Abdinia et al. 2014). Generally, body fluids like cerebrospinal fluid (CSF), pleural, peritoneal, synovial and pericardial fluids are naturally free of microorganisms under normal circumstances. (Deb et al. 2014). Microorganisms like bacteria, fungi, viruses, and parasites may invade and infect the body fluids and results in severe morbidity and mortality (Hasbun et al. 2013). Therefore, early detection and rapid identification of microorganisms infecting body fluids is crucial for the appropriate patient management and helps the clinician to initiate early and more specific treatment and reduced lengths of stay of the patients in the hospital (Sujatha et al. 2015).

In developing nations, bacterial infection and antimicrobial resistance are of great public health concern. For empirical treatment, awareness of local antimicrobial susceptibility pattern and causative bacteria is essential. Moreover, for better management of patients and framing the antibiotic policy, the knowledge of likely prevalent strains along with their antimicrobial resistance pattern is essential (Vishalakshi et al. 2016). CSF is produced by ultra-filtration or secretion and circulates through the ventricles and spinal cord, when compared to with plasma has less protein concentration. Bacterial, fungal and viral infections could result in meningitis. Among these microorganisms', bacterial meningitis is the leading cause of meningitis which often presents acutely in a medical emergency (Greenhow et al. 2014).

Peritoneal effusions are the term of ‘ascites’ refers to the detection and pathologic collection of fluid in the peritoneal cavity (Reginato et al. 2011). Peritonitis is a frequent, and severe complication in cirrhotic patients with ascites, the study described that the risk of Severe Bacterial Peritonitis increased in hospitalized patients with a prevalence of 10% to 30%, although the mortality rate reported up to 90%. (De et al. 2014). Pleural effusions a result of excessive fluid accumulation in the pleural space the appearance is clear, straw-colored, odorless and non-viscous fluids. (Milevoj and Culej 2014) Accumulation of pus in the pleural space remains a very significant cause of childhood mortality and morbidity in children caused by *S.aureus*, *S. pneumoniae*; group *A streptococci* and *H.Influenzae* (Lisboa et al. 2011).

Various microorganisms may be involved in arthritis like viruses, fungi, bacteria such as; *Mycoplasma* species, *S.aureus*, *S.epidermidis*, *S.pyogenes*, *N. gonorrhoeae*, Gram-negative bacilli, and *M. tuberculosis* (Lafi et al. 2010). Assessing the bacterial etiologies and their antimicrobial susceptibility patterns from body fluids is very crucial for clinicians, microbiologists, and pharmacist and policymakers for proper diagnosis of different infections and for prudent antibiotic use. Therefore, this study aimed to assess the bacterial etiologies and their antimicrobial susceptibility pattern from various sterile body fluids from patient specimens referred to National bacteriology and mycology laboratory from April 1, 2014-August 30, 2018. Ethiopian public health institute (EPHI), Addis Ababa, Ethiopia.

Materials and Methods

This was a retrospective study using routine data from laboratory records from April 1, 2014 –August 30, 2018, at the National bacteriology and mycology laboratory of EPHI. In this laboratory, patient samples are analyzed using standard operating procedures starting from sample reception to release according to international and national guidelines. All patients prescribed for sterile body fluids that were diagnosed in the Ethiopian public health Institute, National Bacteriology, and Mycology laboratory for sterile body fluid culture from April 1, 2014 –August 30, 2018, were the study population. Laboratory records of all patient body fluids cultured from sterile body sites in the specified period were included and patients with incomplete laboratory information were excluded. The data collection sheet was prepared to collect the data and was entered and analyzed using SPSS version 23. Groups were compared using Chi2 and descriptive analysis was performed.

Ethics approval was obtained from the EPHI scientific research ethical review Committee. No patient name and Identification number were used. Data was entered in the data extraction sheet from patients’ laboratory record. No patient names were collected. No detailed address was collected. Confidentiality was maintained by keeping data collection forms locked in a secured cabinet, while the electronic data file was kept in a password-protected computer.

Result

Socio-demographic characteristics and culture positivity:

A total of 654 patient’s body fluids were analyzed for isolation, identification and antimicrobial susceptibility testing among which 75 (11.6%) of them were culture positive. Forty-nine (7.5%) culture positives specimens were obtained from female patients while 26 (4%) were from males. The age range of study participants was between 2 days-74

Age in years	Males with positive culture N (%)	Females with positive culture N (%)	Total N (%)
<1 (n=58)	4(6.9)	6(10.3)	10(17.2)
1-10 (n=29)	13(44.8)	4(13.8)	17(55.2)
11-20 (n=13)	8(61.5)	2(15.4)	10(76.9)
21-30 (n=103)	0(0)	1(0.9)	1(0.9)
31-40 (n=64)	10(15.6)	6(9.4)	16(26.5)
41-50 (n=53)	8(15.1)	7(13.2)	15(28.3)
50+ (n=334)	6(1.8)	0(0)	6(1.8)
Total (n=654)	49(7.5)	26(3.9)	75(11.4)

years. The highest bacterial isolation was obtained in the age group between 1-10 years old 17(22.6%) (Table 1).

Table 1: Distribution of bacterial isolates from sterile body fluids

From the total body fluid specimen submitted for laboratory analysis, cerebrospinal fluid (CSF) was the most frequently encountered body fluid accounting for 423(90.3%) followed by pleural fluid 166 (86.1%). (Table-2).

Table 2: Sterile body fluid specimens submitted to Bacteriology and Mycology Reference Laboratory, EPHI from April 1, 2014-

Samples	Total no. of samples	Growth n (%)	No growth n (%)
CSF	423	41(9.7)	382(90.3)
Pleural fluid	166	23(13.9)	143(86.1)
Peritoneal fluid	29	8(27.6)	21(72.4)
Synovial fluid	13	1(7.8)	12(92.2)
Pericardial fluid	1	0(0)	1(100)
Joint Fluid	1	0(0)	1(100)
Ascetic fluid	21	2(9.5)	19(90.5)
Total	654	75(11.5)	579(89.5)

August 30, 2018. Addis Ababa, Ethiopia

Proportion of bacterial pathogens from body fluids:

From the total culture-positive specimens, Gram-negative and Gram-positive isolates constituted 56 (74.6%) and 19 (25.4%) respectively. The

predominant bacteria species isolated comprise *Escherichia coli* 13 (17.3 %), *Acinetobacter* species. 12 (16%), *Klebsiella pneumoniae* 10 (13.3 %), *S*

aureus 10 (10.3%) *Neisseria meningitidis*, 9 (9.3%). *Pseudomonas* species 5 (6.3%) (Table 3).

Table 3: Different organisms isolated from different samples from sterile body fluids referred to Ethiopian Public Health Institute Bacteriology and Mycology Reference Laboratory, from April 1, 2014- August 30, 2018. Addis Ababa, Ethiopia

Organism	CSF N (%)	Pleural fluid N (%)	Peritonea l fluid N (%)	Ascitic fluid N (%)	Synovial fluid N (%)	Total body fluids N (%)	Average from the total positive N (%)
<i>Escherichia coli</i>	4 (0.94)	8(4.8)	1(3.4)	-	-	13(1.99)	13(17.3)
<i>Klebsiella ozenea</i>	-	1(0.6)	-	-	-	1(0.15)	1(1.3)
<i>Klebsiella pneumonie</i>	4(0.95)	5(3)	1(3.4)	-	-	10(1.53)	10(13.3)
<i>Pseudomonas spp.</i>	2(0.5)	2(1.2)	-	-	1(7.7)	5(0.76)	5(6.6)
<i>Acinetobacter spp.</i>	7(1.65)	4(2.4)	1(3.4)	-	-	12(1.83)	12(16)
<i>Entrobacter spp.</i>	1(0.23)	3(1.8)	1(3.4)	-	-	5((0.76)	5(6.6)
<i>Staphylococcus aureus</i>	4(0.94)	-	4(13.8)	2(9.5)	-	10(1.53)	10(13.3)
<i>Haemophilus influenza</i>	2(0.5)	-	-	-	-	2(0.3)	2(2.6)
<i>Enterococcus spp.</i>	3(0.7)	-	-	-	-	3(0.45)	3(4)
<i>Neisseria meningitides</i>	7(1.65)	-	-	-	-	7(1.07)	7(9.3)
<i>Coagulase Negative Staphylococcus</i>	1(0.23)	-	-	-	-	1(0.15)	1(1.3)
<i>β -Hemolytic Strep.(S.pyogen)</i>	2(0.5)	-	-	-	-	2(0.3)	2(2.6)
<i>Proteus spp.</i>	1(0.23)	-	-	-	-	1(0.15)	1(1.3)
<i>S.milleri</i>	1(0.23)	-	-	-	-	1(0.15)	1(1.3)
<i>Streptococcus spp.Viridans Group</i>	2(0.5)	-	-	-	-	2(0.3)	2(2.6)
Total	41(9.7)	23(13.8)	8(27.5)	2(9.5)	1(7.7)	75(11.5)	-

Antimicrobial drug resistance pattern of bacteria isolated from body Fluids: The antimicrobial drug resistance profiles of bacteria ranged from 0 to 100%. The highest resistance rate for Gram-Negative isolates were recorded for Ampicillin 56(100%), Amikacin 38 (68.2%) and Gentamycin 21 (38.5%). *Proteus spp.* hasn't shown any resistance to Chloramphenicol, Ceftriaxone, Gentamycin, and Meropenem. Among the Gram-Positives, bacteria those showed a relatively higher resistance rate were *Enterococcus spp.* to penicillin 3(100%), and *Staphylococcus aureus* to vancomycin 4(36.7%) and Oxacillin 4 (36.4). The non-*Enterobacteriaceae* *Pseudomonas* species and *Acinetobacter* species were resistant to ceftriaxone, Gentamycin, Amikacin and Ceftazidime. Of the bacterial isolated *E.coli* 3(18%)

of *S.aureus* 5 (46.2%) are resistant to three groups of antibiotic while of *E.coli* 7 (54.7%) *Pseudomonas* species 2 (45.5%) of *Acinetobacter* species 5 (40%) of the bacteria are resistant to four or more group of antibiotics, or more drugs belonging to different classes of antibiotics. The level of MDR for Gram-Negative *Enterobacteriaceae*, Gram-Negative non-*Enterobacteriaceae* and Gram-Positive isolates was found to be 20(36.6%) 3 (16.6%) and 6(28.9%) (n=29.8 respectively. *E.coli* was the dominant isolate and of these isolates from Gram-Negative 6 (46.2%) were MDR. From non-*Enterobacteriaceae* *Pseudomonas* species, 3(54.7%) were Multi-drug resistance (MDR). and among the Gram Positives isolates *S.aureus*, were dominant and 2(18 %) were MDR (Table 4).

Antibacterial agent	Gram Negative N (%)				Gram Positive N (%)	Gram Negative non-Enterobacteriaceae			Average resistance
	<i>E.coli</i>	<i>K. pneumoniae</i>	<i>Protus spp</i>	<i>Entrobacter spp</i>		<i>S.aureus</i>	<i>Entero cocci</i>	<i>Pseudomonas spp</i>	
Ampicillin	100	100	100	100	-	-	-	-	100
Amikacin	53.8	36.4	100	40	-	-	-	-	68.2
Amikacin	-	-	-	-	-	-	40	75	57.5
Cefoxitin(Oxacillin)	-	-	-	-	-	-	-	-	-
Ceftriaxone	53.8	72.7	0	40	-	-	-	-	36.4
Ceftriaxone	-	-	-	-	-	-	NA	83.3	83.3
Gentamycin	76.9	72.7	0	60	-	-	-	-	38.5
Gentamycin	-	-	-	-	-	-	60	83.3	71.7
Chloramphenicol	46.1	36.4	0	20	-	-	-	-	23.05
Chloramphenicol	-	-	-	-	33.3	-	-	-	33.3
Ceftazidime	-	-	-	-	36.4	NA	-	-	36.4
Ceftazidime	-	-	-	-	-	-	40	75	57.5
Cefepime	-	-	-	-	40	58.3	-	-	49.2
Penicillin	-	-	-	-	NA	100	-	-	100
Meropenem	46.2	36.4	0	40	-	-	-	-	23.1

Meropenem	-	-	-	-	-	-	40	33.3	36.7
Tobramycin	-	-	-	-	-	-	40	NA	40
Vancomycin (MIC)	-	-	-	-	36.7	36.7	-	-	36.7

Table 4: Resistance patterns of Gram Negative Enterobacteriaceae isolates from different sterile body fluids samples referred to Bacteriology and Mycology Reference Laboratory, Ethiopian Public Health Institute from April 1, 2014- August 30, 2018. Addis Ababa, Ethiopia

Discussion

The overall 11.6% (n=75/654) prevalence of bacteria from fluid was 11.6% relatively lower than the finding from a study conducted in Tikur Anbesa Hospital in Addis Abeba, Ethiopia. (Teklehymanot, et al. 2017) This might be due to that most of the patients have exposure to antibiotics. The predominant organisms were *Escherichia coli* 13(17.3 %), *Acinetobacter* spp. 12(16%), *Klebsiella pneumoniae* 10 (13.3 %), *S aureus* 10 (10.3%) *Neisseria meningitidis* 9(9.3%). *Pseudomonas* species 5 (6.3%) in another study conducted in North India, *Escherichia coli* and *Acinetobacter* spp., each with 19 isolates (5.9%), were predominant (Rouf and Nazir 2019).

Staphylococci and *Escherichia coli* were the most frequent pathogens a study conducted in Ethiopia (Ten Hove, et al, 2017) in a study also conducted in Nepal (Shrestha et al. 2015). The most prevalent pathogens were *E. coli* (28.5%). This can be due to the ability of these pathogens to cause nosocomial infections, poor infection control practice in hospitals, lack of standard facilities, poor sterilization of all gowns and equipment. The detection of *Enterococci*, *Acinetobacter* species, and *Klebsiella pneumoniae* may be associated with this reason. meningitides were the most common pathogens causing meningitis where our report *Neisseria meningitidis* 9 (9.3%) which is in agreement with previous studies in Ethiopia (Mengistu et al. 2013).

Hundred percent resistance level to penicillin was reported in a study in Iran, Namibia and Ethiopia (Abdinia et al. 2014; Teklehymanot et al. 2017; Mengistu et al. 2013). This is similar to our study. This can be due to the emerging resistance characteristics of the gram-positive bacteria. Similarly, *K. pneumoniae*, *E.coli* showed relatively higher resistance 100% to Ampicillin compared to a study done in Iran that reported 87.5% resistance to ampicillin (Reginato et al. 2011). This could be the number of bacterial isolates and differences in the use of antibiotics in each country. From Gram Negatives, 6 (46.2%) were MDR. From non-Enterobacteriaceae *Pseudomonas* species 3(54.7%) were MDR .among the Gram Positives isolates, *S.aureus* 2(18 %) were MDR. The high level of MDR resistance (75%) in

this study is in agreement with a previous study conducted in Gondar, Ethiopia (Mulu et al. 2005). This high MDR level may be due to the inappropriate use of commonly prescribed antibiotics. A previous study in Ethiopia and Iran reported high-level MDR resistance among gram-negative bacteria that is similar to the current study in Iran and Ethiopia (Abdinia et al. 2014; Mulu et al. 2005). Lack to identify the sources of infection and the ward/OPD were taken as limitations or this study.

Conclusion

Emerging ESKAPE (*Enterobacter* species, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas* species, *Enterococcus faecium*) organisms have been isolated in this study and this remains crucial and the occurrence of multidrug resistance patterns of bacteria isolates of great public health importance indicating that farther researches also must be conducted in different parts of the country.

Declaration: The authors declare that this is their original work.

Specific patient benefit: As this is a retrospective study, there is no direct benefit for the study participants. However, this study will provide additional information on bacterial isolates and their antimicrobial susceptibility patterns from sterile body fluids for control program and strategy development in clinical settings in the country.

Competing interests: The authors declare that, they do not have competing interests.

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