Original article

Bacterial isolates and their antimicrobial resistance profile among patients presumptive for meningitis at a referral hospital, northwest Ethiopia

Hiwot Tesera[1], Awoke Derbie[2, 3*], Daniel Mekonnen[2]

Abstract

Background: Bacterial meningitis remains as a major cause of mortality and morbidity in many developing countries, including Ethiopia. Data on the type and antimicrobial resistance profile of the isolates from cerebrospinal fluid (CSF) is limited in Ethiopia and particularly in the study area. Therefore, the aim of this study was to identify bacterial pathogens from CSF and to determine their antimicrobial resistance profile at Felege Hiwot Referral Hospital (FHRH).

Methods and Materials: A hospital-based cross-sectional study was conducted on 176 CSF samples collected from patients presumptive for meningitis at FHRH. Cerebrospinal fluid was collected by an experienced clinician aseptically and inoculated on blood, chocolate and MacConkey agars. Bacteriological culture and identification of the isolates was done following the conventional bacteriological procedure. Antimicrobial susceptibility testing (AST) was performed using the disk diffusion method. Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 23 for Windows. Descriptive statistics were used to present and summarize the findings.

Results: Of the 176 study participants, 112 (63.6%) were males and 70 (39.8%) were infants. The mean age of the study participants was at 14.3 years. Eight (4.5%) CSF samples were found bacteriological culture positive. Of these, gram-negative isolates accounted for five cases (62.5%), including three *E. coli*, and one case each of *K. pneumoniae* and *P. aeruginosa*. The remaining three isolates were *S. aureus.* In this study, the overall multi-drug resistance (MDR) rate was at 75%. Gentamicin and ciprofloxacin were found effective against *S. aureus*. Similarly, gram-negative isolates were found sensitive to ceftazidime and ceftriaxone.

Conclusions: In this study, the bacterial isolation rate from CSF was relatively low (4.5%). *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* were identified and nearly one third of them were found to be multi-drug resistant which should be of concern to relevant stakeholders. A large-scale study is warranted. [*Ethiop. J. Health Dev.* 2020; 34(1):14-21]

Key words: Bacterial meningitis, Cerebrospinal fluid, types of bacterial isolates, antimicrobial resistance

Introduction

Bacterial meningitis (BM) is one of the most severe forms of infectious disease and a common cause of death and disability worldwide (1). BM affects approximately 1.2 million people each year and causes almost 170,000 deaths globally (2). The fatality rate is high, with a survival rate of between 2% and 30% (3). The highest burden of BM occurs in sub-Saharan Africa, known as the meningitis belt, which stretches from Senegal in the west to Ethiopia in the east (1). This area is characterized both by meningococcal hyperendemicity as well as by the intensity of its recurring epidemics, which affect close to 400 million people in 21 countries (4).

The three main organisms that account for over 80% of the world's cases of meningitis are *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib) (5). Other pathogens that are associated with BM include *L. monocytogenes, E. coli, K. pneumonia, Staphylococcus aureus*, *Streptococcus agalactiae, Streptococcus pyogenes, Salmonella* spp., *Pseudomonas aeruginosa, Entrobacter* spp. and *Acintobacter* spp. (6,7).

BM has for a long time been treated with a combination of penicillin/ampicillin and chloramphenicol, and this combination is still the widely recommended first choice in most of African countries. However, the increasing frequency of reports of bacterial resistance to these drugs has raised concerns that this choice may no longer be appropriate (8). Bacterial drug resistance is currently an emerging worldwide problem and a threat to public health, especially in developing countries including Ethiopia, because of the absence of well-equipped bacteriological laboratories and antimicrobial resistance surveillance activities (7). Antimicrobial resistance is changing over time and from place to place for different reasons. Because of the ever-increasing number of drug-resistant strains over time, current information on local pathogens and their drug susceptibility profile is crucial for better management of patients and to monitor the onset of antimicrobial resistance (8-11).

However, despite the challenges associated with BM in Ethiopia, particularly in the present study area, there is quite limited bacteriological data on the field. Therefore, this study was conducted to provide data on the bacteriology of CSF from patients presumptively diagnosed for meningitis.

[1]Student clinic service, Bahir Dar University, Ethiopia Email: hiwottess@gmail.com
[2] Department of medical Microbiology, College of Medicine and Health Sciences, Bahir Dar University, Ethiopia. Email: nigusdaniel@gmail.com
[3*]Centre for Innovative Drug Development and Therapeutic Trials for *Africa* (CDT-Africa), Addis Ababa University, Ethiopia. Email: awe.love2000@gmail.com

Methods and Materials

Study design, setting and period: A hospital-based cross-sectional study was conducted from 01 April to 30 July 2018 at Felege Hiwot Referral Hospital (FHRH) located in Bahir Dar, which is situated about 565km from Addis Ababa, the capital city. The hospital was established in 1952 and provides services in outpatient, inpatient and operation theatres for more than 10 million people in Bahir Dar city and the surrounding zones and regions.

Population and sampling: Using a single population proportion formula at the prevalence of (4.3%), which was reported in Gondar (12), and a 95% confidence level (Z value at alpha 5%), with a margin of allowable error at 3%, our calculated sample size was at 176. We used a quota sampling technique in which the study populations were all patients presumptively diagnosed for meningitis that were visiting the hospital during the data collection period and willing to give CSF for bacteriological analysis. BM is hard to diagnose, as the signs and symptoms are frequently non-specific, particularly in young children. In our study, patients were presumptively diagnosed for meningitis when they were presented with a combination of the following symptoms: fever, poor feeding, vomiting, lethargy, and irritability. In older children, the extra typical signs of neck stiffness, headache and photophobia were also considered. Individuals who had taken antibiotics two weeks before the data collection period were excluded.

Data collection

Socio-demographic and clinical data collection: Data on socio-demographic characteristics of the participants – such as age, sex, residence, type of ward (outpatient, inpatient) – were collected using a structured questionnaire-guided interview. Moreover, laboratory-related data were collected on CSF culture and AST profile.

CSF sample collection: The CSF samples were collected aseptically by an experienced clinician. After collection, samples were delivered to the bacteriology laboratory section of FHRH for analysis within 15 minutes. A sterile wide-bore needle was inserted between the fourth and fifth lumbar vertebrae and the CSF (1ml-4ml) was allowed to drip into a dry sterile container. The volume and gross appearance of CSF (i.e. clearness, bloody or traumatic, cloudy and xanthochromic results) were recorded. For cloudy specimens, gram stain was done before centrifugation; if not, the CSF was centrifuged for gram stain. The CSF was centrifuged for 15 minutes at 2,500 to 3,000 revolutions per minute when the volume was greater than 1ml (9, 5).

CSF culture and isolate identification: All the CSF specimens were inoculated onto blood agar, chocolate agar and MacConkey agar. The blood and chocolate agar plates were incubated at 35-37°C in a candle jar with a capnophilic (5-10% CO_2) environment. MacConkey agar was incubated aerobically and used for the isolation of gram-negative aerobic bacteria. Plates were examined for the presence of any bacterial growth after 18 to 24 hours incubation. Those plates showing no growth were incubated for another 24 hours (9). Identification of the isolates was done based on the standard bacteriological techniques. Colony characteristics, gram reaction, and conventional different panel of biochemical tests were used to identify the isolates. These tests include catalase, coagulase and hemolytic activity on blood agar, for identification of gram-positive bacteria, while indole, citrate, triple sugar iron, urea, oxidase, and motility hydrogen sulphide gas production test panels were used to identify gram-negative bacteria (10,9).

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was carried out using Kirby-Bauer's disk diffusion method on Mueller-Hinton agar, according to Clinical and Laboratory Standards Institute guidelines (11). About three to five pure colonies of each bacterium were picked using a sterile wire loop and emulsified into a tube containing 5ml of nutrient broth (Oxoid). The preparation was mixed thoroughly to make the suspension homogenous.

The suspension of the identified test organism was prepared from similar colonies and the density of suspension was determined by comparing with McFarland 0.5 solutions in order to standardize the size of the inoculums. A sterile swab was dipped into the suspension and squeezed free from excess fluid against the side of test tube. Then, the surface of the Mueller-Hinton agar plate was uniformly flooded with the suspensions and allowed to dry for about 15 minutes and the test organisms were uniformly seeded on the surface of the Mueller-Hinton agar and incubated at 37°C for 18 to 24 hours (9). The diameters of the growth of inhibition were measured and interpreted according to the CLSI guidelines (11).

The following antimicrobial discs were used based on the types of the bacterial isolates – for gram-negatives: ampicillin (AMP: 10µg), ceftazidime (CAZ: 30µg), ceftriaxone (CRO: 30µg), cefotaxime (CTX: 30µg), amoxicillin-clavulanate (AMC: 20/10µg) and piperacillin (PIP: 100µg) (specifically for *P. aeruginosa*); and for gram-positives: penicillin (P:10 unit), clindamycin (DA: 2µg), cefoxitin (CXT: 30µg) and erythromycin (E: 15µg). For both groups of isolates, ciprofloxacin (CIP: 5µg), gentamicin (GEN: 10µg), tetracycline (TE: 30µg), trimethoprim-sulfamethoxazole (SXT: 1.25/23.75µg) and chloramphenicol (CAF: 30µg) were tested (11).

Quality control: The reliability of the study findings was guaranteed by implementing quality control (QC) measures throughout the whole process of the laboratory work. The sterility of culture media was checked by incubating the batch at 35-37°C overnight and was evaluated for possible contamination. The standard reference bacteria strains, such as *S. aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922), were tested weekly as controls on the biochemical tests and agar plates, including Mueller-Hinton agar with antimicrobial discs, to assure testing performance of the potency of

antimicrobial discs. The data were double-checked for completeness and representativeness prior to entry.

Data analysis: Data were entered and analyzed using SPSS version 23 for Windows. Descriptive statistics and data summary measures were used to present the data. The results were presented using tables and graphs. The distribution of the CSF culture result across the included variables was checked using Chi-square test for statistical association. Significance was set at *p*-value <0.05.

Ethical considerations

Ethical clearance was approved by Bahir Dar University, College of Medicine and Health Sciences Institutional Review Board (IRB). Official letters of co-operation were provided to FHRH prior to data collection. Written informed consent (or assent) was obtained from study participants (or their parents/guardians) after explaining the purpose and objective of the study. Confidentiality was maintained and the laboratory results from the study participants were communicated to their physicians for appropriate treatment.

Results

Socio-demographic characteristics of the study participants: A total of 176 study participants were included in this study, of whom 112 (63.6%) were male, the mean age was at 14.3 years (range from 1 day to 68 years), 115 (65.3%) were from urban settings, and 76 (43.2%) were inpatients (Table 1).

Table 1: **Demographic characteristics of the study participants (n=176) attending FHRH from April to July 2018**

Variables	Category	N (%)
Age	<28 days	14 (8)
	28 days to 1 year	56 (31.8)
	1-15 years	43 (24.4)
	≥15 years	63 (35.8)
Gender	Male	112 (63.6)
	Female	64 (36.4)
Residence	Rural	61 (34.7)
	Urban	115 (65.3)
Patient setting	Inpatient	76 (43.2)
	Outpatient	45 (25.6)
	NICU*	55 (31.2)

*NICU: neonatal intensive care unit

Bacterial isolates among culture-positive CSF

Of the 176 study participants, eight (4.5%) CSF samples had bacterial growth, of which five (62.5%) were gram-negative and three (37.5%) were gram-positive (i.e. *S. aureus*). The gram-negative isolates were *E. coli* (n=3), *K. pneumoniae* (n=1) and *P. aeruginosa* (n=1).

The distribution of the CSF culture result across the included variables does not show statistical association (*p*-value > 0.05). Details are presented in Table 2.

Table 2: **Cross-tabulation of CSF culture results with variables at FHRH from April to July 2018**

		CSF culture result			
Variables		**Total n (%)**	**Positive n (%)**	**Negative n (%)**	***X^2, p-value***
Age	<28 days	14 (8)	1 (7.1)	13 (92.9)	2.36, 0.501
	28 days to 1 year	56 (31.8)	4 (7.1)	52 (92.9)	
	1-15 years	43 (24.4)	2 (4.7)	41 (95.3)	
	≥15 years	63 (35.8)	1 (1.6)	62 (98.4)	
Gender	Male	112 (63.6)	6 (5.4)	106 (94.6)	0.5, 0.494
	Female	64 (36.4)	2 (3.1)	62 (96.9)	
Residence	Rural	61 (34.7)	3 (4.9)	58 (95.1)	0.03, 0.863
	Urban	115 (65.3)	5 (4.3)	110 (95.7)	
Patient setting	Inpatient	76 (43.2)	2 (2.6)	74 (97.4)	1.59, 0.453
	Outpatient	45 (25.6)	2 (4.4)	43 (95.6)	
	NICU*	55 (31.2)	4 (7.3)	51 (92.7)	
Total		176 (100)	8 (4.5)	168 (95.5)	

* NICU: neonatal intensive care unit

Antimicrobial resistance profile of the isolates: Two of the three *S. aureus* isolates were found to be resistant to penicillin, cefoxitin, chloramphenicol and tetracycline. Similarly, almost all gram-negative bacteria isolated in this study were resistant to ampicillin. However, they were found to be sensitive to third-generation cephalosporins (ceftriaxone, cefotaxime and ceftazidime) and ciprofloxacin (Table 3).

In this study, six (75%) of the eight isolates were found to be multi-drug resistant (bacterial isolates that were found to be resistant to three or more classes of antibiotics). Two of the three *S. aureus* and four of the five gram-negative isolates were found to be multi-drug resistant (Table 4).

Table 3: **Antimicrobial resistance profile of the bacterial isolates from CSF at FHRH from April to July 2018**

Bacterial isolates	**Number of resistance to antimicrobial agent, n**														
	PEN	CXT	CAF	GEN	ER	SXT	CIP	TTC	CL	AMP	CAZ	PIP	CRO	AMC	CTX
S. aureus (n=3)	2	2	2	0	1	1	0	2	1	-	-	-	-	-	-
E. coli (n=3)	-	-	2	2	-	1	0	2	-	3	0	-	0	2	0
K. pneumoniae (n=1)	-	-	1	1	-	1	1	1	-	1	0	-	0	1	0
P. aeruginosa (n=1)	-	-	-	1	-	-	1	-	-	-	0	1	-	-	-

Key: PEN: Penicillin; CXT: Cefoxitin; CAF: Chloramphenicol; GEN: Gentamicin; ER: Erythromycin; SXT: Trimethoprim-sulfamethoxazole; CIP: Ciprofloxacin; TTC: Tetracycline; CL: Clindamycin; AMP: Ampicillin; CAZ: Ceftazidime; PIP: Piperacillin; CRO: Ceftriaxone; AMC: Amoxicillin-clavulanate; CTX: Cefotaxime.

Table 4: **Multi-drug resistance (MDR) profile of bacterial isolates (n=8) from CSF at FHRH from April to July 2018**

Organism isolated	Degree of resistance							Total multi-drug resistant isolates≥R3
	R0	R1	R2	R3	R4	R5	R6	
E. coli (n=3)	0	1	0	0	1	1	0	2
S. aureus (n=3)	1	0	0	1	0	0	1	2
K. pneumoniae (n=1)	0	0	0	0	0	1	0	1
P. aeruginosa (n=1)	0	0	0	1	0	0	0	1
Total (n=8)	1	1	0	2	1	2	1	6

Key: R0: Susceptible to all antibiotics; R1: Resistant to one antibiotic; R2: Resistant to two antibiotics; R3: Resistant to three antibiotics; R4: Resistant to four antibiotics; R5: Resistant to five antibiotics; R6: Resistant to six antibiotics

Discussion

For most Ethiopians, it is hard to get routine CSF culture and AST as part of routine patient management. Hence, in most cases, patients with a presumptive diagnosis for meningitis would normally be subjected to empirical therapies. Better practices, such as regular pathogen surveillance and AST profiling, are worth knowing about. In the present study, the types of bacterial isolates with their current AST profiles were described for patients presumptive for bacterial meningitis.

A total of 176 study participants were included, of whom 112 (63.6%) were males. The mean age of the study participants was 14.3 years. In addition, about 70 (39.8%) of the study participants were infants, 115 (65.3%) lived in urban settings and 76 (43.2) were inpatients. The proportion of culture-positive CSF was 4.5%. Similar findings were reported in Gondar, Ethiopia, at 4.3-5.6%, and in Nepal, at 4.5% (12,7,13,14). However, our finding was lower than studies done in Namibia, Iran and India, which had CSF culture-positive proportions of 9.6%, 11.4% and 19.1%, respectively (15–17). The reason for the low proportion in our study might be due to our limited sample size, and there might be clinical over-diagnosis of febrile patients for presumptive meningitis. On the other hand, the rate of bacterial isolation in our study is higher than other studies conducted in Nigeria, Iran and Gondar, Ethiopia, with isolation rates of 1.5%, 2.9% and 3.8%, respectively (18–20). This variation might be related to our sample size, or because of geographical or other socio-demographic variations.

The predominant organisms isolated in this study were gram-negative, at five (62.5%). Different studies conducted in the field reported slightly different findings in this regard. For example, Ogeneh and colleagues in Nigeria had a very similar finding to our result (18). In contrast, in studies done in Nepal, Iran, Namibia and Gondar, Ethiopia (15,17,12,7,14), the predominant isolated bacteria were gram-positive. Differences in sample size, study population, study design and geographical variation might explain the disparity.

In this study, the type and frequency of identified isolates were: *S. aureus* (n=3), *E. coli* (n=3), *K. pneumoniae* (n=1) and *P. aeruginosa* (n=1). *S. aureus* and *E. coli* accounted for 75% of the isolates. Closely related findings were reported in Nigeria (18) as well as in Iran, where *S. aureus/*Coagulase-negative staphylococci (CONS) and *E. coli* were the common isolates from CSF (15). These observed differences could be attributed to several factors, such as underlying clinical conditions, virulence factors of the bacterial pathogens and the immune status of the patients.

With regard to the AST profile, in our study we reported different resistance profiles of the isolates for different drugs they were tested for . Currently, pathogens with MDR are becoming a big challenge worldwide, especially in developing countries. The continued drug resistance arises for a number of reasons, such as a lack of well-organized bacteriology laboratories, poor health service set-up and irrational use of antimicrobials (7).

In our study, the antimicrobial resistance level for *S. aureus* and gram-negative isolates ranged from between 0% to 100%. Six of the eight (75%) isolates in this study were multi-drug resistant. Comparable findings at 50% to 62% were reported in Gondar, Ethiopia as well (13, 7). Multi-drug resistant, especially gram-negative, isolates were also reported in Iran identified from CSF sample (21).

In our study, the most effective antibiotics against *S. aureus* were gentamicin and ciprofloxacin (100% sensitivity). This finding is comparable to the study conducted in Gondar, Ethiopia (7). However, two of the three *S. aureus* isolates showed the highest level of drug resistance against chloramphenicol, penicillin, and

tetracycline. Dagnew *et al.*'s study in Gondar reported almost the same figure in this regard (7).

Similarly, gram-negative isolates showed 100% sensitivity for cephalosporins, such as ceftazidime, cefotaxime and ceftriaxone. Similar findings were reported in Gondar, Ethiopia, and in Pakistan (12, 22). However, the study conducted in Iran showed that gram-negative bacteria were found to be resistant to cephalosporins (19,16). The variation could be due to geographical and antimicrobial usage policy differences.

Similarly, in the present study, more than 80% of gram-negatives were resistant to ampicillin. Other studies conducted in Ethiopia and in Iran and Pakistan reported comparable findings (12, 23, 19, 22).

Of the gram-negative isolates, *E. coli* showed 100% sensitivity for ceftazidime, ceftriaxone, and ciprofloxacin. Further, two of the three *E. coli* isolates showed relatively lower sensitivity for trimethoprim-sulfamethoxazole. Other studies in Ethiopia and elsewhere in the world have also reported comparable findings (18, 12, 23). In contrast, all of the *E. coli* isolates showed resistance against ceftazidime, ceftriaxone and ciprofloxacin, according to a study conducted in Iran (24).

Other gram-negative bacteria isolated in our study were *K. pneumoniae*, which was resistant to all antibiotics except cephalosporin. This finding is similar to the results of a study conducted in Addis Ababa, Ethiopia (23). Similarly, *P. aeruginosa* was found to be sensitive for ceftazidime, but resistant to piperacillin, gentamicin and ciprofloxacin, which is similar to the findings of studies conducted in Iran and Addis Ababa, Ethiopia (24, 23). In contrast to our study, a study conducted in India showed that *P. aeruginosa* was resistant to ceftazidime (16).

In general, some differences reported from other studies in terms of the AST profile of the isolates might be attributed to the small sample size we employed and because of geographical and temporal differences. On top of this, differences in antibiotic prescription policies, the absence of guidelines regarding the selection of drugs, the social trend of inappropriate use of commonly prescribed drugs, and differences in infection prevention policy, resource and AST profiling capacity might contribute to different antimicrobial resistance reports around the globe.

Using antibiotics without definitive diagnosis of specific pathogens leads to antimicrobial resistance of bacteria, which is currently an emerging worldwide problem that could be a great public health threat in developing countries such as Ethiopia. Because of the absence of well-equipped bacteriological laboratories in Ethiopia, there is no organized antimicrobial resistance surveillance system.

Conclusions

S. aureus, E. coli, K. pneumoniae and *P. aeruginosa* were the major pathogens isolated from culture-positive CSF in this study. The frequency of MDR was 75%. There is a need for periodic surveillance of the type of bacterial pathogens and their antimicrobial resistance profiles from CSF in the study setting. Further studies with a better design and a larger sample size should be conducted.

Conflict of interest

The authors declare that they have no any competing interests.

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References

1. Gudina EK, Tesfaye M, Adane A, Lemma K, Shibiru T, Pfister HW, *et al.* Challenges of bacterial meningitis case management in low income settings: An experience from Ethiopia. Tropical Medicine & International Health. 2016;21(7):870-8.
2. Owusu M, Nguah SB, Boaitey YA, Badu-Boateng E, Abubakr A-R, Lartey RA, *et al.* Aetiological agents of cerebrospinal meningitis: A retrospective study from a teaching hospital in Ghana. Annals of Clinical Microbiology and Antimicrobials. 2012;11:28.
3. Ceyhan M, Yildirim I, Balmer P, Borrow R, Dikici B, Turgut M, *et al.* A prospective study of eiology of childhood acute bacterial meningitis, Turkey. Emerging Infectious Diseases. 2008:14(7):1089-96.
4. World Health Organization. Enhanced surveillance of epidemic meningococcal meningitis in Africa: A three-year experience. Weekly Epidemiological Record. 2005;80(37):313-20.
5. World Health Organization. Laboratory methods for the diagnosis of meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*. WHO Manual, 2nd ed.; 2011.
6. Choi C. Bacterial meningitis in aging adults. Clinical Infectious Diseases. 2001;33(8):1380-5.
7. Dagnew M, Anagaw B, Motbaynor E, Getie M, Million Y, Mathewos B. Bacterial profile and antimicrobial susceptibility pattern of bacterial meningitis among neonates and children at Gondar University Hospital, northwest Ethiopia. International Journal of Pharmaceutical and Health Care Research. 2013;01(02):46-52.
8. Kiwanuka JP, Mwanga J. Childhood bacterial meningitis in Mbarara Hospital, Uganda: antimicrobial susceptibility and outcome of treatment. African Health Sciences. 2001;1(1):9-11.

9. Cheesbrough M. District laboratory practice in tropical countries. Cambridge: Cambridge University Press; 2006.
10. World Health Organization. Basic laboratory procedures in clinical bacteriology. 2nd ed. WHO: Geneva; 2003.
11. Clinical Laboratory Standard Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests approved standard. 27th edition Available at <https://clsi.org/media/1469/m100s27_sample.pdf >
12. Addis Z, Birhane M, Mathewos B, Tilahun A, Tessema B, Getahun K. Frequency and antimicrobial susceptibility pattern of bacteria isolated from CSF in university of Gondar hospital, north west Ethiopia. International Journal of Pharmaceutical and Health Care Research. 2013;03(01):41-6.
13. Mulu A, Kassu A, Tassema B. Bacterial isolates from cerebrospinal fluids and their antibiotic susceptibility patterns in Gondar University Teaching Hospital, northwest Ethiopia. Ethiopian Journal of Health Development. 2005:19(2):160-4.
14. Pandey P, Jha B, Shrestha A. Etiology of meningitis from patients suspected of meningitis attending Tribhuvan University Teaching Hospital, Kathmandu, Nepal. American Journal of Microbiology. 2015;6(2):21-30.
15. Motamedifar M, Ebrahim-Saraie HS, Mansury D, Nikokar I, Hashemizadeh Z. Prevalence of etiological agents and antimicrobial resistance patterns of bacterial meningitis in Nemazee Hospital, Shiraz, Iran. Archives of Clinical Infectious Diseases. 2015;10(2):e22703.
16. Sudharshan R, Reddy M, Neelima A. Pattern and antibiogram of bacterial meningitis in children at a tertiary care hospital. J Scientific and Innovative Res. 2013;2(6):1012-16.
17. Mengistu A, Gaeseb J, Uaaka G, Ndjavera C, Kambyambya K, Indongo L, *et al.* Antimicrobial sensitivity patterns of cerebrospinal fluid (CSF) isolates in Namibia: Implications for empirical antibiotic treatment of meningitis. Journal of Pharmaceutical Policy and Practice. 2013;6:4.
18. Ogeneh B, Nwokeoma C. Bacteria isolated from the cerebrio-spinal fluid (CSF) of suspected cases of meningitis in Enugu State, Nigeria. Journal of Nursing and Health Science. 2015;4(6):34-8.
19. Rezaeizadeh G, Pourakbari B, Ashtiani MH, Asgari F, Mahmoudi S, Mamishi S. Antimicrobial susceptibility of bacteria isolated from cerebrospinal fluids in an Iranian referral pediatric center, 1998-2008. Maedica (Buchar.). 2012;7(2):131-7.
20. Tegene B, Gebreselassie S, Fikrie N. Bacterial meningitis: A five-year retrospective study among patients who had attended at University of Gondar Teaching Hospital, northwest Ethiopia. Biomedical Research and Therapy. 2015;2(5):270-8.
21. Modi GB, Patel KD, Soni ST, Patel KJ, Mangukiya JD, Jain PS. Bacteriological profile of pyogenic meningitis in tertiary care hospital, Ahmedabad. National Journal of Medical Research. 2012;2(3):313-7.
22. Tajdin F, Rasheed MA, Ashraf M, Rasheed H, Ejaz H, Khan GJ. Antibiotic therapy in pyogenic meningitis in paediatric patients. Journal of the College of Physicians and Surgeons Pakistan. 2013;23(10):703-7.
23. Zewide DAT. Prevalence, aetiology and antimicrobial susceptibility of bacterial neonatal meningitis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Masters of Medicine Degree Dissertation. University of Nairobi; 2011.
24. Abdinia B, Rezaee MA, Oskouie SA. Etiology and antimicrobial resistance patterns of acute bacterial meningitis in children: A 10-year referral hospital-based study in northwest Iran. Iranian Red Crescent Medical Journal. 2014;16(7):e17616.