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FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIA ISOLATED FROM CSF IN UNIVERSITY OF GONDAR HOSPITAL, NORTH WEST ETHIOPIA

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Abstract

Bacterial meningitis remains a common disease worldwide. Information on the relative frequency of the isolation and antibiotic susceptibility patterns of these pathogens is of paramount importance in combating antimicrobial resistance and for successful management of meningitis patients. The aim of this study was to assess the common bacterial isolates and their antimicrobial resistance pattern. Data about 1754 CSF samples delivered for culture from September 2009 to March 2011 was retrospectively collected from microbiology registration book of university of Gondar Hospital laboratory. Bacteria were isolated from 75 patients which makes the isolation rate 4.3%; 3.7% among males and 5.1 % among females. The common bacterial isolates were *S.pneumoniae* (32.8%) followed by *N.meningitdis* (30.2%) and *E.coli* (10.5%). *S. pneumoniae* showed a high level of drug resistance against cotrimoxazole (81.8%), gentamycin (69.6%) and tetracycline (60%). The single isolate of Veridian streptococci was 100% resistant to ciprofloxacin, erythromycin, gentamycin and co-trimoxazol. *N. meningitides* was 95.3% and 57% resistant to cotrimoxazole, and gentamycin respectively while they were sensitive to penicillin (100%) and ceftriaxone (90.9%). *E. coli* showed 100% resistance to cotrimoxzole, tetracycline, ampicllin and naldixic acid but the isolates were 100% sensitive to ceftriaxone. This study concluded that the common bacterial pathogens responsible for meningitis in the study area are still similar to previous reports though there are differences in rate of isolation. These Several bacteria have developed antimicrobial resistance to conventional antibiotic regimes. Large scale studies and continuous assessment of antimicrobial resistance common bacterial isolates should be done so to limit the increasing rate of antimicrobial resistance.

Keywords: Meningitis, CSF, Antimicrobial Susceptibility Pattern.

Introduction

Meningitis is a very serious infection of the meninges that surround the brain and the spinal cord. It is usually caused by viral, bacterial or fungal pathogens. Despite advances in vaccine development and chemoprophylaxis, bacterial meningitis remains a common disease worldwide.

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The disease is more common in developing countries[1]. Bacterial pathogens isolated from the central nervous system (CNS) in patients with symptoms and diagnostic signs of bacterial meningitis are *Streptococcus pneumoniae*, *Streptococcus agalactiae* and other gram-positive cocci, *Haemophilus influenzae*, *Neisseria meningitidis*, and *Escherichia coli*[2].

Meningitis receives a high level of medical, public health and media attention because of its rapid onset and high level of morbidity and mortality. Apart from epidemic, at least 1.2 million cases of meningitis are estimated to occur every year with 135,000 deaths[3].

Despite improvements in antimicrobial therapy and intensive care support, overall mortality rates related to bacterial meningitis of around 20% to 25% have been reported by major centers[4]. The estimated incidence of bacterial meningitis is 0.6 to 4 per 100 000 per year in developed countries, and may be up to ten times higher in other parts of the world[5]. It accounts for an estimated 171 000 deaths worldwide per year[6, 7].

The need to treat infections of the CNS immediately requires empiric choice of an antibacterial agent. For many years β-lactams have comprised the cornerstone of therapy and parenteral third-generation cephalosporins such as ceftriaxone or cefotaxime are most commonly used[8]. Where infection is likely to have occurred from another body site, available laboratory information on identification and antibiogram of the organism can help target therapy appropriately[2].

Determining the etiology and antimicrobial susceptibility profile of invasive pathogens is an important part of clinical practice that can generate data to improve therapeutic decisions and guide prevention strategies. So the aim of this study was to retrospectively assess the bacterial etiologies of meningitis and their antimicrobial susceptibility pattern in Gondar University Teaching Hospital, North West Ethiopia.

Materials and Methods

Study design

This study is a retrospective analysis of 1754 CSF samples send to the microbiology department of Gondar University Teaching Hospital Laboratory from September, 2009 up to March, 2011. Culture results and antimicrobial susceptibility pattern of isolates was collected from the microbiology registration book.

Isolation and Identification of Bacteria

CSF samples were collected as part of the routine clinical management of patients admitted in different wards of the hospital. The samples were collected in sterile containers by attending physicians and delivered to the Bacteriology laboratory within half an hour after collection. The samples were processed following the standard microbiological procedures by inoculating on blood agar, chocolate agar, and MacConkey agar plates (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubating at 35- 37^{o}C for 24-48hrs. Organisms were identified by standard microbiological methods, which included colony morphology, staining and biochemical tests[9].

Antimicrobial susceptibility testing

The antimicrobial susceptibility tests of the isolates was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method using Kibry-Bauer disk diffusion test on Mulle-Hinton agar (Oxoid CM0337 Basingstoke, England). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as quality control organisms[10, 11].

Data analysis

The collected data was cleaned and checked for completeness, entered, complied and analyze by using SPSS version 13. Frequencies and percentages were used to describe findings and results were presented in tables and graphs.

Ethical consideration

The study was ethically cleared by the ethical review committee of department of medical laboratory technology and permission was obtained from the responsible authorities to use the data in the laboratory.

Result

Patient characteristics and rate of bacterial isolation

Cerebrospinal fluid samples from a total of 1754 suspected meningitis cases, 1030 (58.7%) males and 724 (41.3%) females, were submitted for bacteriological investigation from September 2009 to March 2011. The age range of meningitis suspected individuals was from 1 day to 85 years with a median age of 4 years. When these patients are classified at different age groups 39.4%, 33.2% and 27.4% were adults, infants and children respectively. Bacterial pathogens were isolated from 75 patients showing an isolation rate of 4.3%. The isolation rate among males was 3.7% and 5.1 % among females. The prevalence within an age group was higher for children (6.2%), followed by infant (4.3%).

About 60% of the isolates were Gram negative organisms among which *N. meningitis* was the most frequent isolate (30.7%) followed by *E. coli* (10.7%), *H. influenza* (5.3%) and *Salmonella* species (4%). The remaining, 40% of the isolates were Gram positive bacteria which include *S. pneumonia* (33.3%), and *S. aureus* (5.3%). *Acinetobactor*, *Citrobactor*, *Morganella Morgany,* viridian streptococci, *Proteus species*, *Providencia spicies* and *Pseudomonas species* together comprise 10.6% of the isolates.

Table No. 01: Sex and age distribution of common bacterial isolates from CSF, Gondar University Hospital September 2009 – March 2011

Organisms	Number (%)	Patient category and isolates frequency				
		Sex		Age		
		Male N (%)	Female N (%)	Infants N (%)	Children N (%)	Adults N (%)
S .pneumonia	25(32.8%)	13(34.2)	12(31.6)	6(24%)	10(33.3)	9(42.8)
N. meningitides	23(30.2%)	13(34.2)	10(26.3)	4(16)	17(56.7)	2(9.5)
E.Coli	8(10.5%)	2(5.3)	6(15.8)	2(8)	-	6(28.6)
Salmonella Specious	3(4.0%)	2(5.3)	1(2.6)	2(8)	1(3.3)	-
S. aurous	4(5.3%)	3(7.9)	1(2.6)	1(4)	1(3.3)	2(9.5)
H. influenza	4(5.3%)	1(2.6)	3(7.9)	3(12)	1(3.3)	-
Acinetobacter	2(2.6%)	1(2.6)	1(2.6)	2(8)	-	-
Citrobactor	1(1.3%)	-	1(2.6)	-	-	1(4.8)
Morganella Morgany	1(1.3%)	1(2.6)	-	1(4)	-	-
S. Viridian	1(1.3%)	1(2.6)	-	1(4)	-	-
Proteus species	1(1.3%)	-	1(2.6)	1(4)	-	-
Pseudomonas Specious	1(1.3%)	1(2.6)	-	1(4)	-	-
Providencia Specious	1(1.3%)	-	1(2.6)	1(4)	-	-
Total	76(100%)	38(50%)	38(50%)	25(32.9)	30(39.5)	21(27.6)

Antimicrobial susceptibility pattern of isolated bacteria

Among Gram positive organisms *S. pneumoniae* showed a high level of drug resistance against cotrimoxazole (81.8%), gentamycin (69.6%) and tetracycline (60%) but they were 100% sensitive to ampicillin. *S.aureus* also showed 100% resistance to cotrimoxazole and tetracycline but were sensitive to chloramphenicol and gentamycin. The single isolate of Veridian streptococci was 100% resistant to ciprofloxacin, erythromycin, gentamycin and co-trimoxazol.

From Gram negative bacteria isolated *N. meningitides* was 95.3% and 57% resistant to cotrimoxazole, and gentamycin respectively while they were sensitive to penicillin (100%) and ceftriaxone (90.9%). All isolates of *H. influenzae* were 100% sensitive to ampicillin, ceftriaxon, chloramphinicol, ciprofloxacilin, erythromycin, norfloxacilin, and kanamycin while they were resistant to gentamycin (66.7%). *E. coli* showed 100% resistance to cotrimoxzole, tetracycline, ampicllin and naldixic acid but the isolates were 100% sensitive to ceftriaxone. *Salmonella* species were 100% resistant to ceftriaxon, chloramphinicol, gentamycin, ampicillin and tetracycline while they were 100% sensitive to ciprofloxacilin and norfloxacilin.

Acinetobacter isolates were 100% resistant to amoxicillin, gentamycin, norfloxacillin, penicillin, cotrimoxazol, and kanamycin and 100% sensitive to naldixic acid and ceftriaxon. Citrobactor isolates were 100% resistant to chloramphinicol and tetracycline and it was 100% sensitive to amoxicillin, ampicillin, ceftriaxon, gentamycin and cotrimoxazol. Isolates of *Proteus* species and *M. morgani* were 100% resistant to amoxicillin, ampicillin and cotrimoxazole. *Pseudomonas* species was resistant only for tetracycline but sensitive to the rest of the drugs tested.

Table No. 02: Antimicrobial resistance of commonly isolated bacteria from CSF in university of Gondar Hospital (September 2009- March 2011)

Organism	AMX (%)	AMPI (%)	CRO (%)	CAF (%)	CIP (%)	E (%)	CN (%)	NOR (%)	PG (%)	SXT (%)	TE (%)
N.meningitidis **(n=23)**	25	ND	0	48	55	14.3	575	12.5	0	95.5	20
S.pneumoniae **(n=25)**	9.1	0	4.8	4.8	23.3	27.8	69.6	21.4	18.2	81.8	60
E.coli **(n=8)**	80	100	ND	25	14.3		33.3	20		100	100
Salmonella Spp. **(n=3)**	ND	100	100	100	0	ND	100	0	ND	66.7	100
S.aureus **(n=4)**	50	50	25	0	50	33.3	0	ND	33.3	100	100
H.influenzae **(n=4)**	50	0	0	0	0	ND	66.7	0	33.3	50	ND
Acinobacter Spp **(n=2)**	100	ND	0	50	50	ND	100	100	100	100	50
Citrobacter Spp **(n=1)**	0	0	0	100	0	0	0	0	ND	0	100
M.morgani **(n=1)**	100	100	0	0	0	0	0	0	0	100	100
Varidian Streptococci (n=1)	0	100	0	0	100	100	100	0	0	100	ND
Proteus **Spp. (n=1)**	100	100	100	100	100		100	100	ND	ND	ND
Providentia Spp. **(n=1)**	100	100	100	100	ND	ND	100	ND	100	100	ND
Pseudomonas Spp **(n=1)**	0	0	0	0	0	0	0	0	0	0	0

Key: AMX= Amoxcillin; AMPI= Ampicillin; CRO=Ceftriaxone; CAF= Chloramphenicol; CIP= Ciprofloxacin; E= Erythromycin; CN= Gentamycin; NOR= Norfloxacin; PG= Penicillin; SXT= Cotrimoxazole; TE= Tetracycline; ND = Not done.

Discussion

The finding of this retrospective study showed that *N.meningitides*, *S.pneumonia* and *H.influenzae* were the most common isolated pathogens from meningitis patients. Despite the differences in the isolation rate the common isolates in this study were similar with the previous reports from the study area and elsewhere in the world Gondar, Egypt, and Niger[1, 12, 13]. On the other hand *S.aureus* and *K.pneumoniae* were reported as predominant isolates in other studies[14, 15]. The overall isolation rate of bacteria in the present study, 4.3%, is lower than the previous studies conducted in Gondar[1] and in Egypt[12] which reported an isolation rate of 5.6 % and 7.1% respectively. *S.pneumoniae, N. meningitides* and *H. influenza* were the causes of 68.4% cases of bacterial meningitis and this was lower than the previous study conducted in Gondar which was 81% [1]. It was also lower than the report from Egypt and Togo which reported an isolation rate of 85% and 72.2% respectively[12, 16, 17]. The differences were also observed in the isolation rate across different age groups when compared to studies from other areas[7, 18, 19]. These differences in the isolation rate may be associated with prior exposure to antibiotics, over clinical diagnosis of meningitis, the time of the studies conducted during the epidemic and near to the epidemic season or laboratory procedures followed.

Antimicrobial resistance of *S. pneumoniae, N. Menigitidis* and *H. influenza* to commonly prescribed antibiotics has become common now a day[2, 20]. Several of other bacteria also developed antimicrobial resistance to conventional antibiotic regimens and emerging multidrug resistance strains[21]. A study conducted in the same area of the country reported that *S. pnenumoniae* isolated from pediatric patient was resistant to amoxicillin (43%), gentamycin (14%), cotrimoxazol (43%), tetracycline (43%) and chloramphinicol (57%)[22]. The results of the current study has also indicated higher rates of resistance to cotrimoxazol (81.8%), gentamycin (69.6%) and tetracycline (60%) which contributes to the difficulties in treating bacterial meningitis. *S.pneumonia* showed a lower resistance to chloramphinicol, cefriaxon and erythromycin and higher resistance rate for cotrimoxazole as compared to a report from Egypt[12]. A report from Nepal and elsewhere in the world indicated 52% and 60% resistance to cotrimoxazole, respectively, which were lower than the current report[3, 6]. On the other hand a relatively low level of resistance to ciptofloxacin (23%) was observed when compared to a study from Mumbi which reported 33.3% resistance [19]. Even though there was a difference in resistance to ampicillin H.influenzae isolates were 100% sensitive to ceftriaxone both in our study and the Egyptian report among children isolates[24]. Not only *S.pneumoniae* showed increased resistance to commonly prescribed drugs, *N.meningitidis* is also among the isolates with high level of resistance though there is a difference when compared to a resistance reported elsewhere [18]. Unlike to a report from USA no methicillin resistance *S.aureus* was found in our study [14]. Even though there are geographical differences in the drug resistance pattern of bacteria, the problem is increasing at an alarming rate. The reason why the drug resistant increased is not well known but it might be due to the absence of guide lines regarding the selection of drugs, information about drug resistance are not well communicated and the social trend of inappropriate use of a commonly prescribed drug.

Since clinicians will likely initiate antimicrobial therapy prior to the microbiological characterization of the infecting agent and resistance surveillance plays an important role in helping to understand trends in predominant pathogens and the impact of resistance on empiric choice. From this point of view, this study concluded that the common bacterial pathogens responsible for meningitis in the study area are still similar to previous reports though there are differences in rate of isolation. These Several bacteria have developed antimicrobial resistance to conventional antibiotic regimes and emerging multidrug resistance strains. This urges the need of wider area studies and the development of updated information about pathogens so as to combat drug resistance strains.

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