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Full Length Research Paper

Etiology and antimicrobial susceptibility profile of bacteria isolated from patients with meningitis

Ghulam Jilany khan[1]*, Fauzia Tajdin[1,2], Nadeem Reyaz[3]

[1]Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore.
[2]Department of Microbiology, The Children's Hospital and Institute of Child Health, Lahore.
[3]Department of Pathology, University of Health Sciences, Lahore.

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The aim of this prospective study was the determination of etiology and antimicrobial susceptibility profile of bacteria. This study includes 276 culture positive cases of patients aging from newborn to 15 years age group. The method involves the pathogen identification and antibiotic sensitivity testing. The pathogens were isolated from CSF samples of the admitted patients, identified and purified by selective culturing methods, which were subjected to active growth, during which sensitivity to different antibiotics was checked and measured with area marked by the zone of inhibition as well as by Clinical Laboratory Standards interpretations (CLSI). Gram negative bacteria were found in 145 patients and most occurring bacteria were *Pseudomonas spp* (23.7%), *E.coli* (6.5%), 10.5% of *Klebsiella pneumoniae*, *Enterobacter spp*, *Proteus spp*, *Acinetobacter baumanii* and *Pseudomonas aeruginosa*. In this study, gram negative bacteria were found to be the major cause of bacterial meningitis, majority of which is resistant to antibiotics used in common practice that needs careful selection of drugs for the treatment.

Keywords: Bacterial meningitis, Cerebrospinal fluid, Sensitivity pattern.

INTRODUCTION

Meningitis is an inflammation of brain membranes and spinal cord. These membranes are collectively known as meninges and these membranes provide protection against external hazards and various microorganisms (Saez-Llorens and McCraken, 2003). This bacterial infection has high mortality rate if untreated (Tunkel *et al.,* 2004) because in this infection host defense mechanism of meninges to fight against bacteria becomes week or no more. In 1805, meningococcal disease for first time got world attention in Geneva (Greenwood, 2006) but the active agent of meningococcal meningitis was noticed by Anton Weicheslbaum in 1887.

H influenzae is the main important causative organism, especially in those who are not vaccinated and ranging in age of two months to two years. (Martin *et al*., 2004) while among the age group 2 years to 21 years *Neisseria meningitidis* and *Streptococcus pneumoniae* are most common pathogens (Saez-Llorens and GH, 2008) and less frequent organisms are *Listeria monocytogenes*, *Staphylococcus aureus* (in infections of skull fractures), *Cryptococcus neoformans* (in immunosuppressed) and *Mycobacterial Tuberculosis*. In adults over 50 years *Listeria monocytogenes* is an important cause (Beek *et al*., 2004). With the introduction of pneumococcal vaccine, pneumococcal meningitis has been decreased in adults and children (Hsu *et al*., 2009, Brouwer *et al.,* 2010). The highest risk with *S.*

*Corresponding Author E-mails: u4574904@hotmail.com; Contact: +92-333-4574904.

pneumoniae infection is below to the age group of two years. In developing countries, *E.coli* and other gram negative enteric *bacilli* such as *Klebsiella*, *Enterobacter* and *Salmonella* are also important causes of meningitis (Osrin *et al*., 2004).

For the determination, isolation and identification of causative agent, lumbar puncture should be performed (Beek *et al*., 2004). All microorganisms that cause bacterial meningitis are treated with Third generation Cephalosporins in adults except tuberculosis and fungal meningitis, however in older age Ampicillin is usually added with third Generation Cephalosporins as they are mostly affected with *Listeria monocytogenes* and infection due to *Listeria monocytogenes* are usually covered with Ampicillin (Chaudhuri *et al.,* 2008). Age of patient is an important factor for the selection of empiric antibiotic therapy as in neonates (of age less than 28 days) third generation Cephalosporins are not recommended because they cause hyperbillirubinemia. In children of 2-3 weeks Ampicillin is given with cefotaxime or amino glycosides as initial antibiotic therapy (Saez-Llorens and McCraken, 2003). Once the organism is identified, empiric therapy is changed with specific antibiotic therapy. IV infusions of Dopamine or Dobutamine are administered in case of hypotension or shock and sometimes Dexamethasone is as an adjuvant therapy to reduce inflammation of meninges (Gans and beek, 2002).

The drug resistant organisms have resulted in increased cases of meningitis which emerged as a threat for the quality of treatment. Ceftriaxone resistant *Streptococcus pneumoniae* is now a major global concern. Reports of Ceftriaxone resistant *Haemophilus influenzae* and *Neisseria meningitidis* are still rare although WHO emphasizes upon need of improvements in the availability of adequate facilities in regional and national laboratories to document and maintain a record regarding drug resistance and the implementation of longitudinal surveillance programmes to detect changes in resistant pattern (Scarborough and Thwaits, 2008). Proper diagnosis and accurate treatment of bacterial meningitis is a major problem in children especially in developing countries (Brouwer*et al.,* 2010). Therefore, determination of etiology and antibiotic resistant pattern of pathogens is important to improve the therapeutic success of antibiotics (Saha *et al.,* 2005).

MATERIAL AND METHOD

A prospective study was designed and executed to determine the etiology and the bacteria sensitivity to antibiotics in meningitis against antibacterial agents which are usually recommended. The procedure adopted was identification of pathogenic organism and the susceptibility evaluation of antibiotic by using Kirby Bauer Disk Diffusion method. Antibiogram of commonly used antibiotics in disease was carried with Pathogens obtained from cerebrospinal fluid of the patients.

Patient Selection

376 patients were carefully chosen and processed for their microbiology study. Out of these patients, 276 presented positive culture as confirmed by cytology, biochemistry and microbiology with no previously received treatment for meningitis as shown by following in the medication history.

CSF Sample collection/ Lumber puncture

Out of many patients reported with different diseases in the hospital during the period of study 376 patients showed clinical signs (such as vomiting, neck rigidity, bulging fontanelle, reduced consciousness, sudden onset of fever, headache, convulsions and irritability) of meningitis, the CSF samples of these 376 patients were collected by the trained practitioners and phlebotomists and were referred to laboratory of the microbiology department.

Bacteria Isolation and identification

Gross appearance of CSF was noted and then the each sample was centrifuged for 20 minutes at a speed of 2000 rpm. The obtained sediment was further processed following the standard microbiological procedures by inoculating on different agar plates made by Oxide USA while the supernatant after the centrifugation was discarded. These agar plates were blood agar using mammalian blood like sheep, chocolate agar and MacConkey agar and were prepared as instructions given by the manufacturer. The blood and MacConkey agar plates were incubated at 35-37^{o}C aerobically. The chocolate agar plate was incubated by putting in a CO_2 incubator, which provided 5-10% CO_2 concentration to create a carboxyphilic condition for fastidious bacteria. After 18-20 hours of incubation, the plates were examined for the presence of bacterial colonies. Identification of organisms was done with the standard microbiological techniques including staining, biochemical tests, assessment of morphology of the colonies and serological tests (WHO, 2007) and growth characteristics such as Catalase test, oxidase test, Gram's staining, coagulase test, diagnostic discs, and growth enhancing factors, API 20NE, API NH and API 20E (Murrey *et al.,* 1999). Binax NOW kit was used for the identification of *Streptooccus pneumoniae* (Moisi *et al.,* 2009).

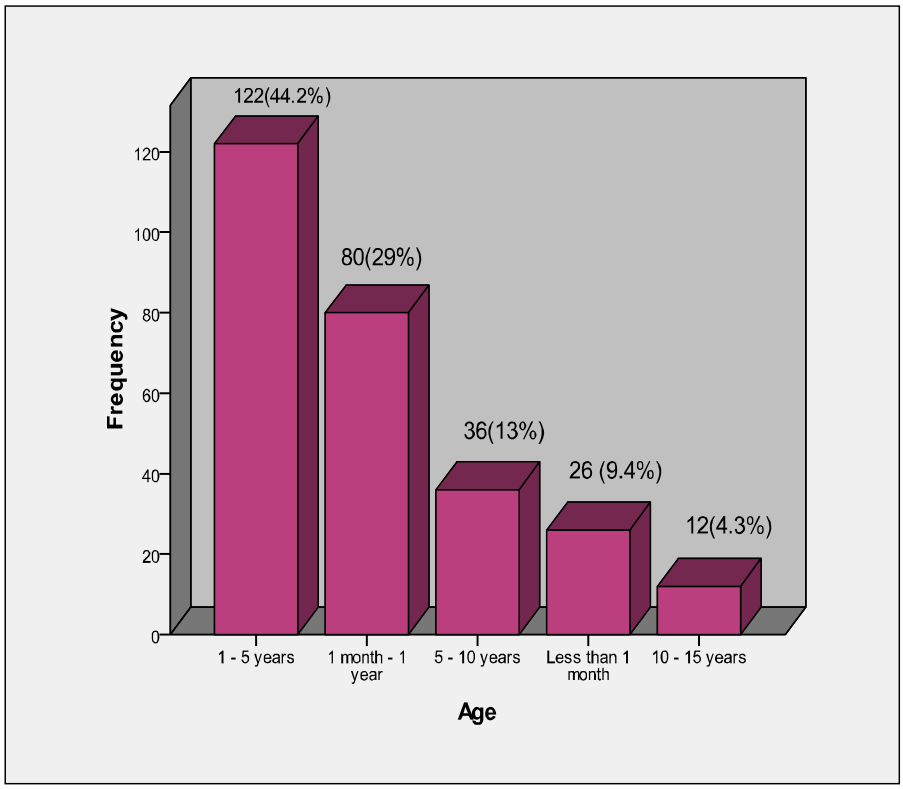

Figure 1. Frequency of occuring of disease in patients of different age groups

Antibiotic Susceptibility Testing

An approved method (by the Clinical Laboratory Standards (CLSI, 2010) and World Health Organization (WHO, 1977)) of Disc diffusion technique was used for antibiotic sensitivity testing (Bauer and Kirby, 1966). Appropriate sensitivity discs (already taken out from the refrigerator and kept at room temperature for 1-2 hours prior to use so that the possibility of water condensation can be vanished) of Vancomycin (VAN), Penicillin (PEN), Oxacillin (OXA), Meropenem (MEM), Ciprofloxacin (CIP), Chloremphenicol (CHM), Cefuroxime (CXM), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Cefepime (FEP) Cefoparazone and Sulbactam (SCF), Ampicillin (AMP), Amoxicillin-Clavulanic acid (AMC) and Amikacin (AKC), manufactured by Difco Laboratories, USA were used during the studies to determine the *in-vitro* susceptibility of microorganism to different antimicrobials.

Statistical analysis

The quantitative data was checked for completeness, coded and fed into SPSS version17.
This study was approved by Ethical Review Board of University of Veterinary and Animal Sciences and The Children Hospital Lahore.

RESULTS

The results of this study were analyzed by descriptive statistics. All scrutinized patients (276) were analyzed on the basis of their ages. Five different age groups of children were made. Frequency of occurrence of disease in the age group of less than one month (neonates) was 26 (9.4%) while the highest frequency was observed in 3rd group (one year to five) which was 44.2% (Figure 1). There were 191 (69.2%) male and 85 (30.8%) female patients which showed that bacterial meningitis was twice common in males as compared to females. In different age groups, fever was highly prevalent in all age groups followed by seizures. A bulging fontanelle and headache was less common in all age groups except in patients of age group 1 month to 1 year. Altered consciousness level was proved to be less prevalent in children of ages 5 to 15 year (Table 1).

Out of 276 culture positive patients, gram negative bacteria were identified in 145 (52.5%) patients and gram positive bacteria were identified in 131 (47.5%). The gram negative bacteria isolated were lactose fermenting which were *Haemophilus influenzae, Nesseria meningitiditis, E.coli, Klebsiella pneumoniae, Enterobacter spp, Enterobacter cloaceae* and non lactose fermenting bacteria which were *Proteus spp, Pantoea species, Pseudomonas species, Serratia marcescens,*

Table 1. Clinical manifestations in patients of pyogenic meningitis in different Age Groups

Symptoms	Less than 1 month %age	1 month to 1 year %age	1-5 year %age	5-10 year %age	10-15 year %age	Total
Fever	13.77	44.4	27.8	12.5	1.38	276
Seizure	13.6	46.9	27.27	10.6	1.5	253
Altered Conscious level	18.2	40.9	27.3	13.6	0	169
Bulging Fontanelle	0	70	10	20	0	38
Neck Rigidity	12.5	37.5	34.4	12.5	3.12	123
Vomiting	17.5	15	27.5	15	0	153
Irritability	17.4	50	26.1	6.5	0	176
Headache	0	29.4	47.1	23.5	0	65

Table 2. Bacteria isolated from patients of bacterial meningitis

Gram positive Bacteria	Frequency	Percent	Gram negative Bacteria	Frequency	Percent
Enterococcus spp	2	0.7	*Haemophilus influenzae*	2	0.7
non hemolytic Streptococci	1	0.4	*Klebsiella pneumoniae*	29	10.5
Group D Streptococcus	2	0.7	*Pseudomonas aeruginosa*	9	3.3
Streptococcus pyogenes	2	0.7	*Enterobacter spp*	9	3.3
Staphylococcus aureus	25	9.1	*Citrobacter fraundii*	4	1.4
Coagulase negative Staphylococcus	79	28.6	*E. coli*	18	6.5
Alpha hemolytic Streptococci	1	0.4	*Proteus spp*	2	0.7
Streptococcus species	2	0.7	*Pseudomonas stutzeri*	2	0.7
			Neisseria meningitidis	1	0.4
			Serratia marcescens	3	1.1
			Stentrophomonas maltophilia	9	3.3
			Pseudomonas spp	35	12.7
			Enterobacter cloaceae	1	0.4
			Pantoea spp	1	0.4
			Burkholderia cepacia	1	0.4
			Serratia Texa	1	0.4
			Enterobacter sakazakii	1	0.4
			Moraxella catarrhalis	1	0.4
			Pseudomonas flourescenes	1	0.4
Total			276 100%		

Citrobacter freuaundii, Pseudomonas stutzeri, Acinetobacter baumanii, Burkholderia cepacia, Serratia texa, Enterobacter sakazakii (Table 2). The isolated gram positive bacteria were *Staphylococcus aureus, Coagulase negative Staphylococcus, Streptococcus pneumoniae, Streptococcus pyogenes, Alpha hemolytic Streptococci, Group D Streptococcus* (Table 2). Of these isolates, coagulase negative staphylococci were most frequently occurred bacteria (28.6%) followed by *Pseudomonas species, Staphylococcus aureus, Klebsiella pneumoniae* and *E.coli*.

Susceptibility profile

Susceptibility pattern of different antibiotics against these isolated bacteria were studied. The antimicrobial drug resistance profile of bacterial isolates showed that *S.*

Table 3. Drug resistance pattern of Gram positive bacteria isolated from bacterial meningitis patients admitted in the Children Hospital Lahore

Sr No.	Organism No. (n)	Frequency (%) of resistant Drugs to be Tested						
		AMP	OXA	PEN	CIP	VAN	AKC	AMC
1	*Staphylococcus aureus*	21	16	21	9	0	2	---
	(n=25)	(84)	(64)	(84)	(36)	(0)	(8)	
2	*CoNS*	70	65	69	38	3	6	---
	(n= 79)	(88.6)	(82.3)	(87.3)	(48.1)	(3.8)	(7.6)	
3	*Streptococcus Pneumoniae*	5	---	3	3	1	8	1
	(n= 18)	(27.8)		(16.7)	(16.7)	(5.6)	(44.4)	(5.6)
4	*Streptococcus Pyogenes*	1	--	1	1	0	1	1
	(n=2)	(50)		(50)	(50)	(0)	(50)	(50)
5	*Streptococcus spp*	0	---	0	0	0	1	0
	(n=2)	(0)		(0)	(0)	(0)	(50)	(0)
6	*Enterococcus spp*	1	0	1	1	0	1	---
	(n=2)	(50)	(0)	(50)	(50)	(0)	(50)	
7	*α Hemolytic Streptococcus pneumoniae*	0	---	0	0	0	1	0
	(n=1)	(0)		(0)	(0)	(0)	(100)	(0)
8	*Gp D Streptococcus*	0	---	0	0	0	1	1
	(n=1)	(0)		(0)	(0)	(0)	(100)	(100)
9	*Non Hemolytic Streptococcus*	1	---	1	0	1	0	0
	(n=1)	(100)		(100)	(0)	(100)	(0)	(0)
Total n=131		99 (75.6)	81 (61.8)	96 (73.3)	52 (39.7)	5 (3.8)	21 (16)	3 (2.3)

CoNS: Coagulase negative Staphylococcus; AMP: Ampicillin; OXA: Oxacillin; PEN: Penicillin; CIP: Ciprofloxacin; VAN: Vancomycin; AKC: Amikacin; AMC: Co Amoxiclav.
Note: empty box showed no reaction of antibiotic

aureus showed high resistance against strains of Ampicillin(84%) and penicillin (84%) followed by oxacillin (64%) while least resistance against ciprofloxacin, amikacin and no resistance against vancomycin was observed. *Coagulase negative staphylococcus* showed highest resistance against Ampicillin (88.6%), penicillin (87.3%) and oxacillin (82.3%) while showed least resistance against Ciprofloxacin, Vancomycin and Amikacin. Similarly strains of S*treptococcus pneumoniae* showed highest resistance against Amikacin and Ampicillin and least against other antibiotics co-Amoxiclav, Penicillin, Ciprofloxacin and Vancomycin as given in Table 3. The other isolated gram positive strains were found in very less numbers so their susceptibility pattern cannot be explained properly (Table 3).

The gram negative bacteria in this study showed varied resistance against most commonly used antibiotics. As described in Table 4, the strains of *E.coli* showed highest resistance against commonly used antibiotics Ceftriaxone, Cefotaxime, Cefuroxime, Ceftazidime and Co-Amoxiclav while *Pseudomonas aeruginosa* showed highest resistance against these commonly used antibiotics. The strains of *Klebsiella pneumoniae, Enterobacter spp, Acinetobacter baumanii, H. Influenzae, and Nesseria meningitiditis* and other isolated strains of this study showed multiple drug resistance against commonly used antibiotics like Cephalosporins and Co-Amoxiclav while least resistance against Ciprofloxacin, Meropenem, Amikacin and the combination of Sulbactam and Cefoparazone and Chloremphenicol is observed. The *Acinetobacter baumanii* also showed highest resistance 78.6% against Chloremphenicol (Table 4).

Out of all Gram positive bacterial isolates 74% showed multi drug resistance (MDR) against at least to two to five more drugs. Antibiogram (Table 5) of Gram positive bacterial isolates showed that 24.4% and 29.8% of them were resistant to three and four tested drugs respectively. About 28% of *Staphylococcus aureus* and approximately 39.2% of *Coagulase negative Staphylococcus* were also resistant to four out of six drugs tested. Similarly, about 91.7% of Gram negative bacterial isolates showed MDR against to ten drugs tested. On the other hand, antibiogram (Table 6) of Gram negative isolates revealed that 23.4 % of them were resistant to five drugs tested. Out of all tested drugs 15.8%, 13.1%.and 12.4% were found resistant against each six, seven and eight drugs respectively. Similarly 55.6% of *Pseudomonas aeruginosa* were resistant to five, 37.1% of *Pseudomonas species* to five, 22.2% of *E.coli* to five and 26.7% *Klebsiella pneumoniae* to seven drugs tested (Table 6).

Table 4. Drug resistance patterns of Gram negative bacteria isolated from bacterial meningitis patients admitted in The Children Hospital Lahore

Sr No.	Organism No.	Frequency (%) of resistant Drugs to be Tested									
		CIP	CHM	AMC	SCF	CRO	CTX	CAZ	CXM	MEM	AKC
1	*E.coli*	8	8	13	5	16	16	13	16	7	9
	(n=18)	(44.4)	(44.4)	(72.2)	(27.8)	(88.2)	(88.2)	(72.2)	(88.2)	(38.8)	(50)
2	*Pseudomonas Spp*	2	20	30	4	25	26	6	23	2	8
	(n=35)	(5.7)	(57.1)	(85.7)	(11.4)	(71.4)	(74.3)	(17.1)	(65.7)	(5.7)	(22.9)
3	*Pseudomonas aeruginosa*	0	4	8	0	9	7	0	9	0	1
	(n=9)	(0)	(44.4)	(88.9)	(0)	(100)	(77.8)	(0)	(100)	(0)	(11.1)
4	*Pseudomonas stutzeri*	0	0	0	2	0	0	0	2	0	0
	(n=2)	(0)	(0)	(0)	(100)	(0)	(0)	(0)	(100)	(0)	(0)
5	*Pseudomonas*	0	0	1	0	0	1	0	1	0	0
	flourescenes (n=1)	(0)	(0)	(100)	(0)	(0)	(100)	(0)	(100)	(0)	(0)
6	*Klebsiella pneumoniae*	13	9	25	9	28	27	27	27	5	16
	(n=30)	(43.3)	(30)	(83.3)	(30)	(93.3)	(90)	(90)	(90)	(16.7)	(53.3)
7	*Acinetobacter baumanii*	6	11	10	2	12	11	8	11	4	5
	(n=14)	(42.8)	(78.6)	(71.4)	(14.3)	(85.7)	(78.6)	(57.1)	(78.6)	(28.6)	(35.7)
8	*H. Influenzae*	1	1	1	1	2	2	1	1	0	2
	(n=2)	(50)	(50)	(50)	(50)	(100)	(100)	(50)	(50)	(0)	(100)
9	*Neisseria Meningitiditis*	0	0	0	0	1	1	1	1	0	1
	(n=1)	(0)	(0)	(0)	(0)	(100)	(100)	(100)	(100)	(0)	(100)
10	*Enterobacter spp*	2	5	6	2	6	6	5	6	2	1
	(n=9)	(22.2)	(55.6)	(66.7)	(22.2)	(66.7)	(66.7)	(55.6)	(66.7)	(22.2)	(11.1)
11	*Enterobacter Cloaceae*	0	0	1	0	1	1	1	1	0	0
	(n=1)	(0)	(0)	(100)	(0)	(100)	(100)	(100)	(100)	(0)	(0)
12	*Enterobacter sakazakii*	0	1	1	1	1	1	0	1	0	0
	(n=1)	(0)	(100)	(100)	(100)	(100)	(100)	(0)	(100)	(0)	(0)
13	*Stentrophomonas*	2	4	9	2	8	6	3	8	7	3
	Maltophilia (n=9)	(22.2)	(44.4)	(100)	(22.2)	(88.9)	(66.7)	(33.3)	(88.9)	(77.8)	(33.3)
14	*Citrobacter freundii (n=4)*	0	2	2	0	2	2	2	2	0	2
		(0)	(50)	(50)	(0)	(50)	(50)	(50)	(50)	(0)	(50)
15	*Proteus spp*	0	2	2	0	2	2	2	2	0	2
	(n=2)	(0)	(100)	(100)	(0)	(100)	(100)	(100)	(100)	(0)	(100)
16	*Pantoea spp*	0	0	1	0	1	1	1	1	0	1
	(n=1)	(0)	(0)	(100)	(0)	(100)	(100)	(100)	(100)	(0)	(100)
17	*Serratia marcescens*	2	1	2	0	3	3	2	3	0	2
	(n=3)	(66.7)	(33.3)	(66.7)	(0)	(100)	(100)	(66.7)	(100)	(0)	(66.7)
18	*Serratia Texa*	0	0	1	0	0	0	0	1	0	1
	(n=1)	(0)	(0)	(100)	(0)	(0)	(0)	(0)	(100)	(0)	(100)
19	*Moraxella catarrhalis*	1	0	1	1	1	1	0	1	0	0
	(n=1)	(100)	(0)	(100)	(100)	(100)	(100)	(0)	(100)	(0)	(0)
20	*Burkholderia cepacia*	1	1	0	1	1	1	1	0	0	0
	(n=1)	(100)	(100)	(0)	(100)	(100)	(100)	(100)	(0)	(0)	(0)
Total		37	69	115	28	119	115	73	118	27	54
(n=145)		(25.5)	(47.6)	(79.3)	(19.3)	(82.1)	(79.3)	(50.3)	(81.4)	(18.6)	(37.2)

CIP: Ciprofloxacin; CHM: Chloremphenicol; AMC: Co Amoxiclav; SCF: Sulbactam+ Cefoparazone; CRO: Ceftriaxone; CTX: Cefotaxime; CAZ: Ceftazidime; CXM: Cefuroxime; MEM: Meropenem; AKC: Amikacin

Table 5. Antibiogram of gram positive bacteria isolated from CSF of bacterial meningitis patients

Organism No.	Antibiogram No (%) of resistance					Total
Gram +ve	**R2**	**R3**	**R4**	**R5**	**R6**	
Staphylococcus aureus	7	6	7	1	Nt	21
(n=25)	(28)	(24)	(28)	(4)		(84)
CoNS	9	24	31	6	Nt	70
(n= 79)	(11.4)	(30.4)	(39.2)	(7.6)		(88.6)
Streptococcus pneumoniae	2	1	Nt	Nt	Nt	3
(n= 18)	(11.1)	(5.6)				(16.7)
Streptococcus pyogenes	Nt	Nt	Nt	1	Nt	1
(n=2)				(50)		(50)
Streptococcus spp	Nt	Nt	Nt	Nt	Nt	0
(n=2)						(0)
Enterococcus Spp	Nt	Nt	1	Nt	Nt	1
(n=2)			(50)			(50)
α Hemolytic Streptococcus pneumoniae	Nt	Nt	Nt	Nt	Nt	0
(n=1)						(0)
Gp D Streptococcus	Nt	Nt	Nt	Nt	Nt	Nt
(n=1)						
Non Hemolytic Streptococcus	Nt	1	Nt	Nt	Nt	1
(n=1)		(100)				(100)
Total	18	32	39	8	Nt	97
(n=131)	(13.7)	(24.4)	(29.8)	(6.1)		(74)

R2: resistant to 2 drugs; R3: resistant to 3 drugs; R4: resistant to 4 drugs; R5: resistant to 5 drugs; R6: resistant to 6 drugs;

Table 6. Antibiogram of gram negative bacteria isolated from CSF of bacterial meningitis patients

	Antibiogram No (%) of resistance									Total
Gram -ve	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**R9**	**R10**	
E.coli	Nt	Nt	3	4	2	3	3	Nt	2	15
(n=18)			(16.7)	(22.2)	(11.1)	(16.7)	(16.7)		(11.1)	
Pseudomonas species	3	6	3	13	5	2	Nt	Nt	Nt	32
(n=35)	(8.6)	(17.1)	(8.6)	(37.1)	(14.3)	(5.7)				(91.4)
Pseudomonas aeruginosa	1	1	2	5	Nt	Nt	Nt	Nt	Nt	9
(n=9)	(11.1)	(11.1)	(22.2)	(55.6)						(100)
Pseudomonas stutzeri (n=2)	1	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	1
	(50)									(50)
pseudomonas flourescenes	Nt	1	Nt	Nt	Nt	Nt	Nt	Nt	Nt	1
(n=1)		(100)								(100)
Klebsiella pneumoniae	1	Nt	1	5	7	8	4	3	Nt	29
(n=30)	(3.3)		(3.3)	(16.7)	(23.3)	(26.7)	(13.3)	(10)		(96.7)
Acinetobacter baumanii	1	2	1	Nt	2	1	5	1	Nt	14
(n=14)	(7.1)	(14.3)	(7.1)		(14.3)	(7.1)	(35.7)	(7.1)		(100)
H. Influenzae	Nt	Nt	Nt	1	1	Nt	Nt	Nt	Nt	2
(n=2)				(50)	(50)					(100)
Neisseria Meningitiditis	Nt	Nt	Nt	1	Nt	Nt	Nt	Nt	Nt	1
(n=1)				(100)						(100)
Enterobacter spp	Nt	Nt	1	1	Nt	1	2	1	Nt	6
(n=9)			(11.1)	(11.1)		(11.1)	(22.2)	(11.1)		(66.7)
Enterobacter Cloacea (n=1)	Nt	Nt	Nt	Nt	1	Nt	Nt	Nt	Nt	1
					(100)					(100)
Enterobacter sakazakii (n=1)	Nt	Nt	Nt	1	Nt	Nt	Nt	Nt	Nt	1
				(100)						(100)
Stentrophomonas	Nt	Nt	3	3	Nt	1	2	Nt	Nt	9
Maltophilia (n=9)			(33.3)	(33.3)		(11.1)	(22.2)			(100)
Citrobacter freundii (n=4)	Nt	Nt	Nt	Nt	1	1	Nt	Nt	Nt	2
					(25)	(25)				(100)

Table 6. continue

Proteus spp (n=2)	Nt	Nt	Nt	Nt	Nt	1 (50)	1 (50)	Nt	Nt	2 (100)
Pantoea spp (n=1)	Nt	Nt	Nt	Nt	1 (100)	Nt	Nt	Nt	Nt	1 (100)
Serratia marcescens (n=3)	Nt	Nt	1 (33.3)	Nt	Nt	1 (33.3)	1 (33.3)	Nt	Nt	3 (100)
Serratia Texa (n=1)	Nt	1 (100)	Nt	Nt	Nt	Nt	Nt	Nt	Nt	1 (100)
Moraxella catarrhalis (n=1)	Nt	Nt	Nt	Nt	1 (100)	Nt	Nt	Nt	Nt	1 (100)
Burkholderia cepacia (n=1)	Nt	Nt	Nt	Nt	1 (100)	Nt	Nt	Nt	Nt	1 (100)
Total (n=145)	7 (4.8)	11 (7.6)	15 (10.3)	34 (23.4)	23 (15.8)	19 (13.1)	18 (12.4)	5 (3.4)	2 (1.4)	133 (91.7)

R2: resistant to 2 drugs; R3: resistant to 3 drugs; R4: resistant to 4 drugs; R5: resistant to 5 drugs; R6: resistant to 6 drugs; R7: resistant to 7 drugs; R8: resistant to 8 drugs; R9: resistant to 9 drugs; R10: resistant to 10 drugs;

DISCUSSIONS

During this study, out of 376 suspected patients of meningitis 276 were culture positive. Mostly gram negative bacteria were isolated. The etiological agents of bacterial meningitis are difficult to isolate due to improper handling, limited laboratory facilities and prior use of antibiotics before lumber puncture (Mengistu *et al.,* 2013). The male to female ratio of bacterial meningitis patients was 191 (69.2%) and 85 (30.8%). This showed that males suffered from bacterial meningitis were twice as compared to females, similar findings were also reported by Farag and his colleagues in 2005 (Farag *et al.,* 2005) and Dash and his colleagues (Dash *et al.,* 2008). The maximum number of cases (44.2%) were seen in the age group of 1 month to 1 year followed by 29% in the age group of 1 month to 1 year with no significant difference in other age groups was found. 2nd age group is more susceptible to disease due to lack of maternal antibodies as reported by molyneux and his colleagues (Molyneux *et al.,* 2011). This age group is more susceptible to disease due to improper hygienic conditions, congested environments.

The predominance of gram negative bacteria was also reported as major etiological agents of bacterial meningitis (Saez-Llorens and McCraken, 2003). The relationship between patient's age and culture report was evaluated by Chi Square test. It was suggested that in the ages of less than 1 month *Staphylococcus aureus, Coagulase negative Staphylococci*, *E.coli, Klebsiella pneumoniae, Enterobacter species* and *Serratia marcescens* were found to be more prevalent organisms according to this study as also observed in our study (Tunkel *et al.,* 2004). *Klebsiella pneumoniae* and *Serratia marcescens* were found to be most important pathogens for neonatal meningitis in other countries (Alharthi *et al.,* 2000). While in patients of ages 1 month to 1 year, *E.coli, Pseudomonas species, Pseudomonas aeruginosa, Streptococcus pneumoniae, Coagulase negative Staphylococci,* and *Staphylococcus aureus* were more prevalent. *Coagulase negative staphylococci, Stentrophomonas maltophilia*, and *Pantoea spp* were the causative organisms for the disease. Among the age group of 2 years to 21 years *Neisseria meningitides* and *Streptococcus pneumonia* were most common pathogens and less frequent organisms are *Listeria monocytogenes*, *Staphylococcus aureus* (in infections of skull fractures), *Cryptococcus neoformans* (in immunosuppressed) and M*ycobacterial Tuberculosis*. 80% cases of bacterial meningitis in adults are due to *Nesseria meningitides* and *Streptococcus pneumoniae* (Beek *et al.,* 2006) but these worldwide isolated strains *Nesseria meningitidis* and *Haemophilus influenzae* type B of bacterial meningitis were not isolated in a very high number in our country.

There is an increased resistance development trend in pathogens against traditional empirical therapy regime and this antimicrobial resistance varies from organism to organism. The strains of *Haemophilus influenza* were proved to be susceptible to all antibiotics. The most gram negative bacteria *E.coli, Klebsiella pneumoniae* and others were found more resistant except *Pseudomonas aeruginosa*, *Pseudomonas spp* (Saez-Llorens and McCraken, 2003). Cephalosporins were proved to be less effective in this study and it was also shown in Jones study (Jones *et al.,* 2004).

CONCLUSION

The present study documents the major etiological agents of pyogenic meningitis are gram negative bacteria. These bacteria developed increased resistance against Cefotaxime, Penicillin, Ceftriaxone, Cefuroxime and Ampicillin. The isolated organism's rate in this study was comparable to other similar studies carried out in developing countries. However, the bacterial isolates

identified from studied patients were terribly resistant for commonly available and prescribed antimicrobial drugs. Therefore, antibiotics such as Ampicillin, Penicillin, Chloremphenicol, Ceftriaxone, Cefuroxime, and Ceftazidime are not the drug of choice for treating patients with bacterial meningitis. From the antibiotic profile Amikacin and Vancomycin can be recommended in gram positive bacteria. Meropenem and combination of Cefoparazone and Sulbactam proved to be more susceptible antibiotics from antimicrobial profile but due to less penetration of Meropenem to blood brain barrier it should not be recommended.

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REFERENCES

Alharthi AA, Khalid AD, Asindi AA, Bello CS (2000). Neonatal meningitis. Saudi Med. J. 21(6):550-553.

Bauer AW, Kirby WMM (1966). Antibiotic susceptibility testing by a single disc method. Am. J. Clin. Pathol. 45: 493.

Beek VD, Gans DJ, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M (2004). "Clinical features and prognostic factors in adults with bacterial meningitis". N. Engl. J. Med. 351 (18): 1849–1859.

Beek VDD, Gans DJ, Tunkel AR, Wijdicks EF (2006). Community-acquired bacterial meningitis in adults". N. Engl. J. Med. 354 (1): 44–53.

Brouwer MC, Tunkel AR, Beek DVD (2010). Epidemiology, Diagnosis, and Antimicrobial Treatment of Acute Bacterial Meningitis. Clin. Microbiol. Rev. 23(3): 467-492.

Chaudhuri A, Martinez MP, Martin PM (2008). EFNS guideline on the management of community-acquired bacterial meningitis: report of an EFNS Task Force on acute bacterial meningitis in older children and adults. Eur. J. Neurol. 15 (7): 649–59.

Clinical and Laboratory Standards Institute (CLSI) (2010). Performance standards for antimicrobial susceptibility tests 20th ed. approved standard, CLSI document M100-S20, vol. 30. Wayne, PA: CLSI.

Dash N, Panigrahi D, Khusaibi SA, Awaidy SA, Bawikar S (2008). Acute Bacterial meningitis among children under 5 years of age in Oman: a retrospective study during 2000-2005. J. Infect. Devel. Count. 2(2): 112-115.

Farag HF, Abdul Fatteh MM, Youssri AM (2005). Epidemiological, clinical and prognostic profile of acute bacterial meningitis among children in Alexandria, Egypt. Ind. J. Med. Microbiol. 23(2): 95-101.

Forbes BA, Sahm DF, Bailey AS, Scott S (2002). Diagnostic Microbiology. 11th ed. St. Louis: Mosby 229-250.

Gans JD, Beek VD (2002). Dexamethasone in adults with bacterial meningitis. N. Engl. J. Med. 347:1549-1556.

Greenwood B (2006). "100 years of epidemic meningitis in West Africa – has anything changed?" Tropical Medicine and International health: TM and IH 11, (6): 773–80.

Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, Farley MM, Jorgensen JH, Lexau CA, Petit S, Reingold A, Schaffner W, Thomas A, Whitney CG, Harrison LH (2009). "Effect of pneumococcal conjugate vaccine on pneumococcal meningitis". N. Engl. J. Med. 360 (3): 244–256.

Jones ME, Draghi DC, Karlowsky JA, Sahm DF, Bradley JS (2004). Prevalence of antimicrobial resistance in bacteria isolated from central nervous system specimens. Ann. Clin. Microbiol. 3:3.

Martin M, Casellas JM, Madhi SA, Urquhart TJ, Delport SD, Ferrero F, Chamany S, Dayan GH, Rose CE, Levine OS, Klugman KP, Feikin DR (2004). Impact of Haemophilus influenzae type b conjugate vaccine in South Africa and Argentina. Pediatr. Infect. Dis. J. 23: 842– 784.

Mengistu A, Gaeseb J, Uaaka G, Ndjavera C, Kambyambya K, Indongo L, Kalemeera F, Ntege C, Mabirizi D, Joshi MP, Sagwa E (2013). Antimicrobial sensitivity patterns of cerebrospinal

fluid (CSF) isolates in Namibia: implications for empirical antibiotic treatment of meningitis. Journal of Pharmaceutical Policy and Practice, 6:4.

Molyneux E, Nizami SQ, Saha S, Huu KT, Azam M, Bhutta ZA, Zaki R, Weber MW, Qazi SA (2011). 5 versus 10 days of treatment with ceftriaxone for bacterial meningitis in children: a double-blind randomised equivalence study. Lancet. 377: 1837-1845.

Moïsi JC, Saha SK, Falade AG, Njanpop-Lafourcade BM, Oundo J, Zaidi AK, Afroj S, Bakare RA, Buss JK, Lasi R, Mueller J, Odekanmi AA, Sangaré L, Scott JA, Knoll MD, Levine OS, Gessner BD (2009). Enhanced diagnosis of pneumococcal meningitis with use of the Binax NOW immunochromatographic test of Streptococcus pneumoniae antigen: a multisite study. Clin. Infect. Dis. 1;48(2): 49-56.

Murrey PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (1999). Manual of Clinical Microbiology. 7th ed. ASM Press.

Osrin D, Vergnano S, Costello A (2004). Serious bacterial infections in newborn infants in developing countries. Curr. Opin. Infect. Dis. 17: 217– 224.

Saez-Llorens X, McCracken GH (2003). Bacterial meningitis in children. Lancet. 361: 2139 – 8.

Saez-Llorens XM, GH XM (2008). “Acute bacterial meningitis beyond the neonatal period,” in *Long: Principles and Practice of Pediatric Infectious Diseases Revised Reprint*, S. Long, Ed., pp. 284–291, Churchill Livingstone, Philadelphia, Pa, USA, 3rd edition.

Saha SK, Baqui AH, Darmstadt GL, Ruhulamin H, Hanif M, Arifeen ES, Oishi K, Santosham M, Nagatake T, Black RE (2005). Invasive Haemophilus Influenzae type b diseases in Bangladesh with increased resistance to antibiotics. Pediatr. J. 146(2): 227-233.

Scarborough MG, Thwaits E (2008). the diagnosis and management of acute bacterial meningitis in resource poor settings. Lancet Neurol. 7(7): 637-48.

Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, Whitley RJ (2004). Practice guidelines for the management of bacterial meningitis. Clin. Infect. Dis. 39, 1267-84.

World Health Organization (2007). Standardized treatment of Bacterial meningitis in Africa on epidemic and non epidemic situations. Available at: www. who.int/csr/resources/publications/.