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BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ACUTE MENINGITIS IN A TERTIARY CARE HOSPITAL, EAST DELHI, INDIA.

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Abstract: **Introduction: Bacterial meningitis is considered a medical emergency with high morbidity and mortality. The classical triad of meningitis is fever, headache, and stiffness of the neck and when accompanied by lethargy, stupor, or seizure activity, the presentation is highly suggestive of bacterial meningitis. The present study aims to investigate the causative agents of bacterial agents of acute meningitis in a tertiary care hospital in East Delhi, India. Material and Methods: This retrospective observational study was conducted in Department of Microbiology, University college of Medical Sciences, New Delhi. Data from January 2018 to December 2018 was retrieved for CSF samples sent for microbiological investigation were included. Cerebrospinal fluid (CSF) samples were sent to Microbiology department during this period from**

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various wards and ICU (both paediatric and adult patients) diagnosis suggestive of with suspected acute bacterial meningitis (ABM) in the hospital. The study was approved by institutional ethical committee. Result: Total of 1100 CSF samples were submitted for microbiological examination. Culture came out positive in 183 samples. Of this 108 (59%) were males and 75(41%) were females. Sample submitted ranged from age new-born to 75-year-old. Majority of samples were from children less than 1 month of age. Similarly, samples submitted from Neonatal Intensive care unit (NICU) were highest. Conclusion: Meropenem is the drug of choice for Gram-negative pathogens and vancomycin is the drug of choice for Gram positive pathogens. The emergence of multidrug resistance is posing the major challenge in the treatment of patients with limited options of effective antibiotics.

Keywords: **Acute Meningitis, Bacterial Meningitis, Community Acquired Infection, Hospital Acquired Infection, Multidrug Resistance.**

I. INTRODUCTION

Bacterial meningitis is considered a medical emergency with high morbidity and mortality [1,2,3]. It can result in death or permanent debilitation such as brain damage, hearing loss, and learning disabilities [4]. The classical triad of meningitis is fever, headache, and stiffness of the neck and when accompanied by lethargy, stupor, or seizure activity, the presentation is highly suggestive of bacterial meningitis [5]. The illness may present as either that of an acute fulminant illness that progresses rapidly in a few hours or that of a subacute infection that gets progressively worse over several days [6]. The mortality rate reported from many Asian countries including India range from 16–32 % [7,8,9,10].

The causes of bacterial meningitis vary with age group. The causative agents also differ depending on the source of infection whether community or hospital acquired. The classically known causes of acute meningitis which are acquired from community are Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis, Group B Streptococci, Listeria monocytogenes and Escherichia coli. The profile of organism differs in hospital acquired meningitis with predominance of Gram-negative bacilli (Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and E. coli) followed by Staphylococcus aureus, coagulase-negative Staphylococcus and Enterococcus faecium [11,12]. Under the current vaccination program in India, Pneumococcal and H. influenza vaccine have been included. This has shifted the epidemiology of causative agents of meningitis. Hence a constant review of organisms causing bacterial meningitis is needed. Identification of isolates via automated methods have shown the diversity of organism found to be associated with CNS infections. There is a need to understand the role of such organisms as true pathogen or contaminants. The present study aims to investigate the causative agents of bacterial agents of acute meningitis in a tertiary care hospital in East Delhi, India

II. MATERIAL AND METHODS

This retrospective observational study was conducted in Department of Microbiology, University college of Medical Sciences, New Delhi. Data from January 2018 to December 2018 was retrieved for CSF samples sent for microbiological investigation were included. Patient demographic data including along with direct microscopy and organism isolated was retrieved and analysed. Cerebrospinal fluid (CSF) samples were sent to Microbiology department during this period from various wards and ICU (both paediatric and adult patients) diagnosis suggestive of with suspected acute bacterial meningitis (ABM) in the hospital. The study was approved by institutional ethical committee.

Sample Processing

Cerebrospinal fluid (CSF) samples submitted were processed using standard laboratory protocol. Heaped up smear were made and Gram staining done for the presence of pus cell, microorganism or any fungal element. Direct cultures were performed on 5 percent sheep blood agar, Chocolate agar and MacConkey agar (Himedia). Blood and Chocolate agar plates were incubated in candle jar and MacConkey agar in aerobic environment. Plates read after 24 hours and any growth obtained was processed accordingly. Bacterial identification and Antibiotic sensitivity testing were done by MicroScan Walk Away system (Siemens Healthcare Diagnostics, West Sacramento, CA). Enrichment was done on day 1 in Brain heart infusion broth for 24 hours and subculture the next day on all three-culture media mentioned above. Any new growth obtained was processed as per standard microbiology protocols. The quality control strains used while staining for gram negative and positive organisms were E. coli ATCC 25922 and S. aureus ATCC 29213. Antimicrobial sensitivity test was performed on Mueller Hinton agar by the Kirby Bauer disk diffusion method [13].

III. STATISTICAL ANALYSIS

The data was analysed using descriptive statistics, Microsoft Excel was used to import the data for analysis, and SPSS version 11 was used.

IV. RESULTS

Total of 1100 CSF samples were submitted for microbiological examination. Culture came out positive in 183 samples. Of this 108 (59%) were males and 75(41%)

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were females. Sample submitted ranged from age new-born to 75-year-old. Figure 1 showed that majority of isolates were from children less than 1 month of age. Samples from paediatric age group were predominant in the study set. Similarly, samples submitted from Neonatal Intensive care unit (NICU) were highest (Table 1.). Microscopic examination showed organism in only 59 samples (shown in figure 2). The reports of direct microscopy were urgently communicated to respective units for case management. The results of microscopy and culture were found to be concordant when single type gram finding seen. Single isolates were recovered in case where multiple organisms were seen in microscopy.

Gram negative isolates were more as compared to Gram Positive in culture finding in comparison to microscopy where almost equal type of result observed.

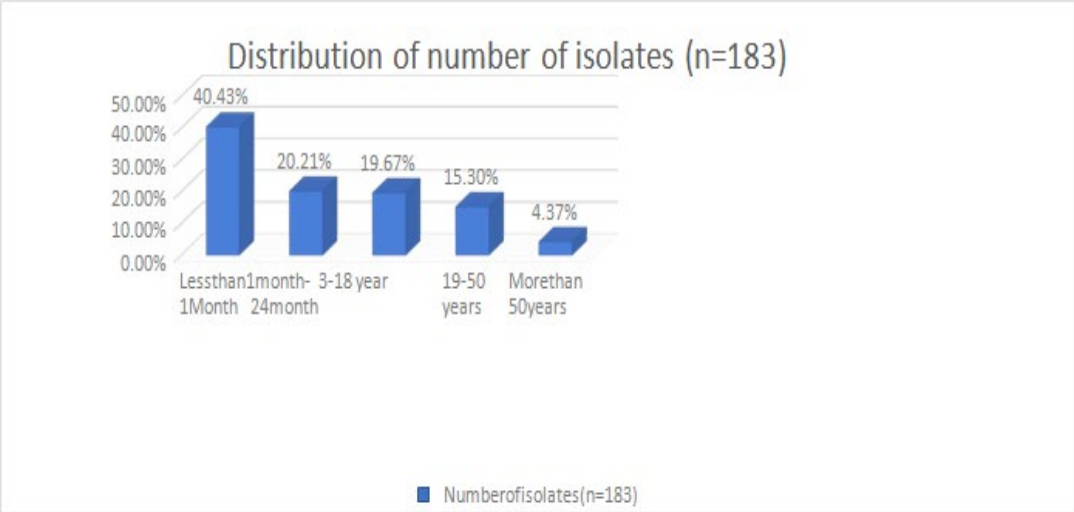

Figure 1. Distribution of Culture positives isolates according to age criteria

Table 1. Distribution of Culture positives isolates according to the Department Ward/ICU

Department Ward/ICU	**Number of isolates (n= 183)**	**Percentage**
Adult (n=48)		
ICU	5	2.73
Medicine ward	25	13.66
Neurosurgery ward	18	9.84
Children (n=135)		
Paediatric Ward	53	28.96
Paediatric ICU	19	10.38
Neonatal ICU	63	34.43

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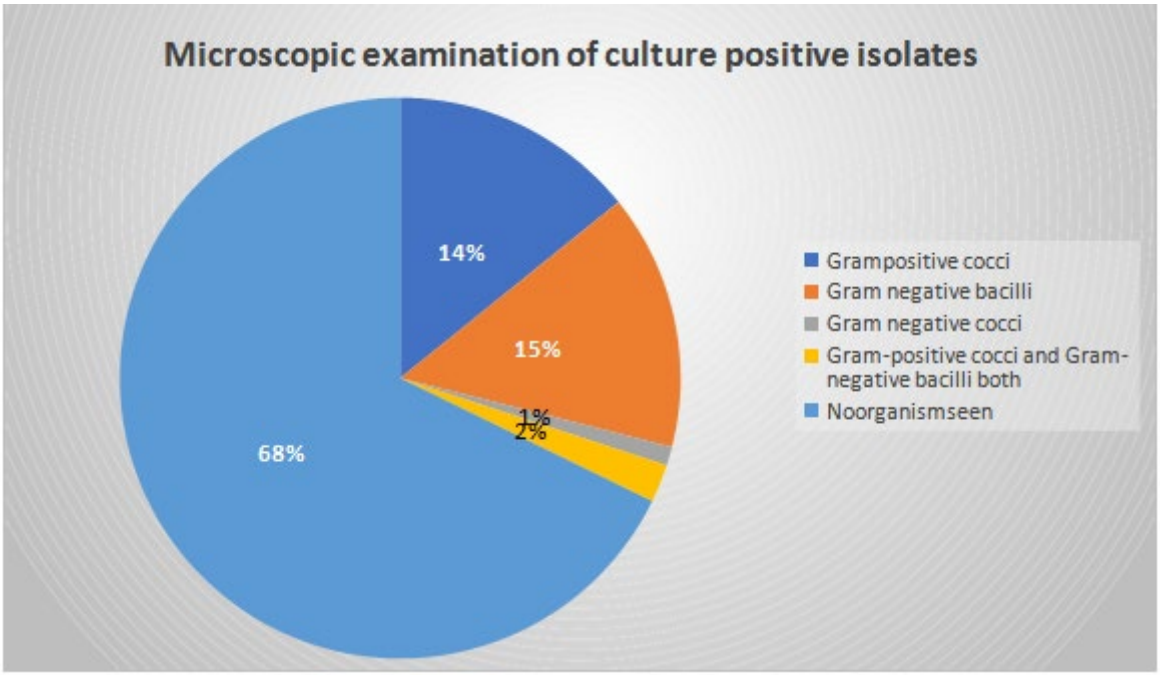

Figure 2. Gram Staining microscopic examination of culture positive isolates

Table 4 (a). Distribution of Gram-positive isolates

Gram positive organism	No of isolates (n=80)	Percentage
Enterococcus spp.	33	41.25
Staphylococcus aureus	20	25
Staphylococcus haemolyticus	7	8.75
Staphylococcus epidermidis	7	8.75
Staphylococcus capitis	3	3.75
Staphylococcus xylosus	2	2.5
Streptococcus pneumoniae	2	2.5
Staphylococcus hyicus	1	1.25
Staphylococcus simulans	1	1.25

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Table 4 (b). Distribution of Gram-negative isolates

Gram Negative organism	**No of isolates (n=103)**	**Percentage**
Acinetobacter baumannii	59	57.28
Escherichia coli	14	13.59
Klebsiella pneumoniae	11	10.68
Pseudomonas aeruginosa	7	6.80
Citrobacter spp.	4	3.88
Burkholderia spp.	1	0.97
Proteus vulgaris	2	1.94
Achromobacter xylosoxidans	1	0.97
Enterobacter cloacae	1	0.97
Proteus mirabilis	1	0.97
Sphingobacterium spiritivorum	1	0.97
Stenotrophomonas maltophilia	1	0.97

Table 5: Distribution of predominant organisms in various age groups.

Age	**Organisms**	**Total number of isolates (n=183)**
<1 month	Acinetobacter spp.	26
	Enterococcus spp.	18
	Staphylococcus spp.	18
	E coli	6
	Proteus	3
	Streptococcus pneumoniae	1
1-24 month	Acinetobacter spp.	14
	Enterococcus spp.	7
	Staphylococcus spp.	8
	E coli	2
	Pseudomonas spp.	3
	Klebsiella spp.	2
	Citrobacter spp.	1
3-18 years	Acinetobacter spp.	11
	Enterococcus spp.	8
	Staphylococcus spp.	5
	E coli	3
	Pseudomonas spp.	2
	Klebsiella spp.	3
	Achromobacter spp.	1
	Burkholderia spp.	1
	Stenotrophomonas spp.	1
	Sphingomonas spp	1
19-50 years	Acinetobacter spp.	7
	Enterococcus spp.	2
	Staphylococcus spp.	7
	E coli	3

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	Pseudomonas spp.	2
	Klebsiella spp.	6
	Citrobacter spp.	1
>50 years	Acinetobacter spp.	1
	Enterococcus spp.	1
	Staphylococcus spp.	1
	E coli	1
	Streptococcus pneumoniae	3

Table 6 (a): Antibiotic Susceptibility Pattern of Gram-Positive Bacilli (GPB)

ics	**E. coli (n=14)**				**A. baumannii (n=59)**				**K. pneumoniae(n= 11)**				**P. aeruginosa(n=7)**			
	Sensitive		**Resistant**		**Sensitive**		**Resistant**		**Sensitive**		**Resistant**		**Sensitive**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Gentamicin	9	64.29	5	35.71	29	49.15	30	50.85	8	72.73	3	27.27	4	57.14	3	42.86
Amikacin	10	71.43	4	28.57	21	35.59	38	64.41	9	81.82	2	18.18	5	71.43	2	28.57
Ceftazidime	-		-		-		-		-		-		5	71.43	2	28.57
Ciprofloxacin	9	64.29	5	35.71	30	50.85	29	49.15	8	72.73	3	27.27	4	57.14	3	42.86
Meropenem	14	100.00	0	0.00	34	57.63	25	42.37	7	63.64	4	36.36	5	71.43	2	28.57
Imipenem	10	71.43	4	28.57	39	66.10	20	33.90	8	72.73	3	27.27	5	71.43	2	28.57
Piperacillin tazobactam	12	85.71	2	14.29	38	64.41	21	35.59	7	63.64	4	36.36	6	85.71	1	14.29
Cefotaxime	9	64.29	5	35.71	50	84.75	9	15.25	7	63.64	4	36.36	-		-	
Cotrimoxazole	11	78.57	3	21.43	45	76.27	14	23.73	4	36.36	7	63.64	7	100.00	0	0.00

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Table 6 (b): Antibiotic Susceptibility Pattern of Gram-negative Cocci (GNC)

Antibiotics	Gram-Negative Cocci Organisms															
	Enterococcus spp. (n=33)				S. aureus (n=20)				CONS (n=23)				S. pneumoniae (n=2)			
	Sensitive		Resistant		Sensitive		Resistant		Sensitive		Resistant		Sensitive		Resistant	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ciprofloxacin	16	48.48	17	51.52	17	85	3	15	14	60.87	9	39.13	2	100	0	0
Erythromycin	15	45.45	18	54.55	8	40	12	60	9	39.13	14	60.87	2	100	0	0
Clindamycin	-		-		13	65	7	35	16	69.57	7	30.43	1	50	1	50
Linezolid	33	100.00	0	0.00	19	95	1	5	23	100.00	0	0.00	-	-	-	-
Tetracycline	19	57.58	14	42.42	19	95	1	5	19	82.61	4	17.39	0	0	2	100
Vancomycin	31	93.94	2	6.06	20	100	0	0	23	100.00	0	0.00	2	100	0	0
Teicoplanin	26	78.79	7	21.21	20	100	0	0	23	100.00	0	0.00	-	-	-	-
Cotrimoxazole	-	-	-	-	19	95	1	5	16	69.57	7	30.43	1	50	1	50
High level gentamicin	30	90.91	3	9.09	-	-	-	-	-	-	-	-	-	-	-	-
Cefoxitin	-	-	-	-	13	65	7	35	9	39.13	14	60.87	-	-	-	-

V. DISCUSSION

Bacterial meningitis remains the major problem of concern as it needs immediate attention. Its management requires prompt clinical suspicion with rapid microbiological confirmation. Both are essential to decrease the morbidity and mortality associated with central nervous system infections. It is essential to collect correct samples before starting antibiotic regimen. The final laboratory diagnosis depends on culture and sensitivity testing of the CSF sample. Hence it directs the management of meningitis depending upon the identification of the types of organisms that causes the disease and the selection of an effective antibiotic against the organism in question [14]. Etiological agents of bacterial meningitis are also changing its order and antibiotic susceptibility pattern throughout the world due to effective implementation of vaccines and rational use of antibiotics. In our study, a total of 183 CSF isolates from all age groups were analysed with the age of the patients range from min 1day−62 years. Among these, 108 (59%) were males and 75(41%) were females respectively. Infection with bacterial meningitis was significantly associated with males as reported by studies conducted in India [15]. Patients with acute bacterial meningitis classically present with fever, headache, meningism's and signs of cerebral dysfunction. These symptoms are found in more than 85% of patients [16,17]. In our study, where most of the cases (60%) were aged less than 2 years. These findings were in accordance with a study of Saudi Arabia [18]. Microscopic examination of gram-stained, concentrated CSF was reported to be highly sensitive and specific in early diagnosis of bacterial meningitis [19,20]. In this study the gram-stained CSF smears correctly detected 32% of the cases, which correlates with the findings of other authors [19,21,22]. Of the 183 organisms isolated from microscan automated system, Acinetobacter spp. (32.24%) were the predominant organism in all age groups except in age >50 years where Staphylococcus spp. was the dominant organism [Table 5]. In neonates, Group B Streptococcus (GBS) and E. coli were the main pathogen [23]. However, in our study GBS was not found. In our study, Acinetobacter baumannii was the most common Gram-negative organism followed by E. coli, K. pneumoniae and P. aeruginosa, finding is in contrast with various studies [24,25,26] where E. coli was the common pathogen. Similarly, Enterococcus spp. was the predominant Gram-positive organism followed by Staphylococcus aureus. This finding coincides the finding of the study by Khan et al. [27]. We found that all Gram-positive isolates were 100% susceptible to most of the antibiotics tested. However, S. aureus was only susceptible to vancomycin teicoplanin (100

%) and was resistant to other antibiotics tested. this finding agrees with the finding of Mani et al. [26]. In the case of the Gram-negative organism, the most susceptible antibiotic was meropenem for E. coli (100 %), P. aeruginosa (71%), K. pneumoniae (63 %) and A. baumannii (57 %) followed by piperacillin and tazobactam for E. coli (85%), P. aeruginosa (85 %), A. baumannii (64%) and K. pneumoniae (63%) and; these findings are in accordance with previous Indian studies [28, 29]. Similarly, amikacin was the most susceptible to K. pneumoniae (81%), E. coli (71 %), P. aeruginosa (71 %) and A. baumannii (35 %). Our finding was concurrent with the finding of Mengistu et al. [29].

VI. CONCLUSION:

In bacterial meningitis, Gram-negative organisms are the predominant isolates and are multidrug resistant. Meropenem is the drug of choice for Gram-negative pathogens and vancomycin is the drug of choice for Gram positive pathogens. The emergence of multidrug resistance is posing the major challenge in the treatment of patients with limited options of effective antibiotics. Hence, antibiotic stewardship should be strictly implemented to prevent developing multidrug resistance and to update the existing knowledge of the antibiotic susceptibility pattern.

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