Pak. J. Biotechnol. Vol. 21(2), 387–391, ***2024.*** http://doi.org/10.34016/pjbt.2024.21.02.922

Research Article

OPEN ACCESS

Available on http://www.pjbt.org
Pakistan Journal of Biotechnology
(PJBT)
(P-ISSN: 1812-1837 and E-ISSN: 2312-7791)

BACTERIOLOGICAL ANALYSIS AND ANTIBIOTIC RESISTANCE TRENDS IN BACTERIA ISOLATED FROM CEREBROSPINAL FLUID OF SUSPECTED BACTERIAL MENINGITIS PATIENTS AT HYDERABAD, SINDH.

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Article Received 13-02-2024, Article Revised 15-04-2024, Article Accepted 20-05-2024.

ABSTRACT

Meningitis is the inflammation of the meninges, the membranes that surround the brain and spinal cord. It is a devastating infectious disease with a high morbidity and mortality rate worldwide. The present study aimed to study the bacteriological profiling of CSF samples and the evaluation of antibiotic susceptibility patterns of common bacterial isolates of CSF. A total of 681 CSF samples were collected from patients suspected of bacterial meningitis through Lumbar puncture and processed for bacteriological analysis. Bacterial isolation and identification were carried out by using conventional microbiological methods including cultural, microscopic, biochemical, and immunological tests. Gender-wise distribution revealed that 59.32% (n=404) of samples were from male and 40.68% (n=277) belonged to female patients. The culture positivity of CSF samples showed that out of 681 samples 5.73% (n=39) yielded bacterial growth, while 94.27% (n=642) were sterile. It was observed that microbial infection of CSF was more common in patients aged below one year accounting for 44.93%. Gram-negative bacteria were more common with 87.18% while the Gram-positive bacteria were less prevalent in CSF samples with 12.82% prevalence. *Klebsiella pneumoniae* was the dominant with 30.77% of total isolated bacteria, followed by Pseudomonas spp. and *E. coli*. Antimicrobial sensitivity testing data revealed that Colistin, Piperacillin/Tazobactam and Meropenem were highly effective against *K. pneumoniae*, *E. coli* and Pseudomonas spp., respectively. In summary, the proper diagnosis and empirical treatment of bacterial meningitis can be achieved by AST and culturing CSF specimens.

Keywords: CSF, Bacterial meningitis, Antibiotic susceptibility, *E. coli, Klebsiella pneumoniae*

INTRODUCTION

Bacterial meningitis (BM) is a devastating infectious disease that significantly increases neurological morbidity and mortality rates worldwide (Liu *et al.*, 2016). Each year 7.0 and 22.3 cases of BM per 100,000 children less than five years of age have been reported to occur in China (Y. Li *et al.*, 2014). An estimated 3.0–10.6% of cases resulted in death (Svendsen, Ring Kofoed, Nielsen, Schønheyder, & Bodilsen, 2020), while a comparatively high percentage of survivors experience neurological problems (Hsu *et al.*, 2018). Bacterial meningitis has been associated with a high prevalence of morbidity and mortality in developing countries (Oordt-Speets, Bolijn, van Hoorn, Bhavsar, & Kyaw, 2018). Over 236000 meningitis deaths and roughly 2.5 million new cases were reported globally in 2019 (Schiess, Groce, & Dua, 2021). In the US, Europe, and many other developed countries, the BM in children under five years age is mainly reported to cause by *N. meningitidis*, *H. influenzae* type b, *S. pneumoniae*, *S. agalactiae*, and *L. monocytogenes*. To minimize the disease and its consequences, early diagnosis and appropriate antibiotic treatments are crucial, for reducing neurological sequelae, including hearing disability, mental disorders, seizures, and changes in behaviour (Svendsen *et al.*, 2020). The delays in diagnosis and treatment may contribute to high morbidity and mortality rates of bacterial meningitis (Richardson, Louie, Louie, & Simor, 2003).

The causative organisms and their antimicrobial resistance patterns mostly determine the choices of empirical antimicrobial drugs in clinical practice. For the individual affected with BM, prompt and efficient antibiotic use is of paramount importance in achieving an effective outcome (Khan *et al.*, 2017). Different types of microorganisms have been reported to cause BM, however, the etiology differs depending on geographical location, time, and the cases (Okike *et al.*, 2014; Tsolenyanu *et al.*, 2019). According to data collected from England and Wales between 2004 and 2011, *N. meningitidis*, *S. pneumoniae*, and *S. aureus* were the main pathogens causing BM (Okike *et al.*, 2014).

Although CSF culture is the gold standard for diagnosing acute bacterial meningitis, it only yields positive results in 70–85% of patients who did not receive antibiotic therapy before lumbar puncture. The culture's results often aren't accessible for 48 hours. Additionally, poor storage, transportation, and cultural practices contribute to a comparatively low positive rate of CSF culture. However, since cultured bacteria provide information on antibiotic susceptibility, subtyping, the production of antigens to be included in future vaccinations, and the pathophysiology of isolates, culture should continue to be the gold standard. The sensitivity of Gram stain is low (10–93%, depending on the organism and whether or not antibiotics were administered before CSF collection), but it is quicker and has suitable specificity (Poplin, Boulware, & Bahr, 2020). The current study aimed to evaluate the bacteriological profiling and antibiotic resistance patterns of bacteria from CSF samples at Hyderabad, Sindh, Pakistan.

MATERIALS AND METHODS

A cross-sectional, prospective, study was carried out for one year at the Institute of Microbiology, University of Sindh, Jamshoro. CSF specimens from patients with suspected bacterial meningitis from Sindh province were collected from January 2020 to December 2020. All the CSF specimens were collected by a trained officer at the health facility. Clinical specimens of CSF of suspected bacterial meningitis patients were collected through the lumber puncture method after obtaining verbal informed consent from patients or the parents of minor (under 17 years old) patients. After the collection of samples, their physical appearance was recorded and then the samples were initially identified based on Gram-staining analysis. The specimens were then cultured on Blood Agar, Chocolate Agar and MacConkey's Agar for isolation and identification of bacterial pathogens of CSF. Pure cultures of bacteria were further identified based on morphological, cultural, and biochemical characteristics according to standard methods (Cheesbrough, 2006). Antibiotic susceptibility testing (AST) was carried out using the Kirby-Bauer disk diffusion method as per Clinical and Laboratory Standards Institute-2020 guidelines using commercially available antibiotic discs (Oxoid, UK) (Table 4a, b, c).

RESULTS

A total of 681 CSF samples were collected and processed for bacteriological analysis. Gender-wise distribution revealed that 59.32% (n=404) of samples were from male and 40.68% (n=277) belonged to female patients. The physical appearance of CSF was recorded, and the samples were processed for the detection of bacterial growth. The male samples were comparatively higher than the females. All samples were inoculated on culture media for isolation and identification of bacterial isolates of CSF. Data showed that 5.73% (n=39) of the samples yielded bacterial growth of both groups of bacteria, while 94.27% (n=642) were sterile as no growth was observed on the culture plates.

Age and Gender wise distribution of all samples: All samples were distributed in 10 years age wise groups. The data revealed that the majority of samples belonged to the age group under one year age (Table 1) followed by 2 to 10 years and gradually decreased in succeeding higher age groups (Table 2).

Table 1. Month-wise frequency of CSF positive samples of less than one year age

Sample	Total Male		Male (-ve)		Male (+ve)		Total Female		Female (-ve)		Female (+ve)	
Age Months	(n=)	(%)	(n=)	(%)	(n=)	(%)	(n=)	(%)	(n=)	(%)	(n=)	(%)
1M	56	8.22	52	7.64	4	0.59	38	5.58	38	5.58	0	0
2M	23	3.38	21	3.08	2	0.29	17	2.50	15	2.20	2	0.29
3M	31	4.55	29	4.26	2	0.29	10	1.47	9	1.32	1	0.15
4M	11	1.62	10	1.47	1	0.15	3	0.44	2	0.29	1	0.15
5M	10	1.47	10	1.47	0	0	6	0.88	6	0.88	0	0
6M	13	1.91	13	1.91	0	0	6	0.88	5	0.73	1	0.15
7M	10	1.47	8	1.17	2	0.29	5	0.73	5	0.73	0	0
8M	8	1.17	8	1.17	0	0	5	0.73	5	0.73	0	0
9M	10	1.47	10	1.47	0	0	6	0.88	6	0.88	0	0
10M	5	0.73	5	0.73	0	0	1	0.15	1	0.15	0	0
11M	3	0.44	3	0.44	0	0	2	0.29	2	0.29	0	0
12M	15	2.20	15	2.20	0	0	12	1.76	11	1.62	1	0.15

Data analysis revealed that the majority of positive culture were obtained from the age group of 1 to 12 months age, with highest cases in the age of less than one month (Table 1), followed by 2 to 10 years (Table 2). The infection rate was higher in males as compared to females.

Table 2. Overall frequency of age and gender-wise CSF positive samples

Samples	Total		Male culture (-ve)		Male Culture (+ve)		Female culture (-ve)		Female Culture (+ve)	
Age (years)	**(n=)**	**(%)**	**(n=)**	**(%)**	**(n=)**	**(%)**	**(n=)**	**(%)**	**(n=)**	**(%)**
1-12 months	306	44.93	184	27.02	11	1.62	105	15.42	6	0.88
02-10	233	34.21	111	16.30	7	1.03	107	15.71	8	1.17
11-20	56	8.22	36	5.29	1	0.15	17	2.50	2	0.29
21-30	44	6.46	19	2.79	2	0.29	22	3.23	1	0.15
31-40	15	2.20	13	1.91	0	0	2	0.29	0	0
41-50	11	1.62	8	1.17	0	0	3	0.44	0	0
51-60	9	1.32	7	1.03	0	0	2	0.29	0	0
61-Above.	7	1.03	5	0.73	0	0	1	0.15	1	0.15
Total	**681**	**100**	**383**	**56.24**	**21**	**3.08**	**259**	**38.03**	**18**	**2.64**

Gender-wise analysis of CSF samples of less than one-year-old patients: The data showed that 44.93% of total samples belonged to less than one year old age group, with male BM patients accounting higher ratio of 28.63% and females 16.30%. The remaining all cases of 2 years old and above were 55.07% (Figure. 1).

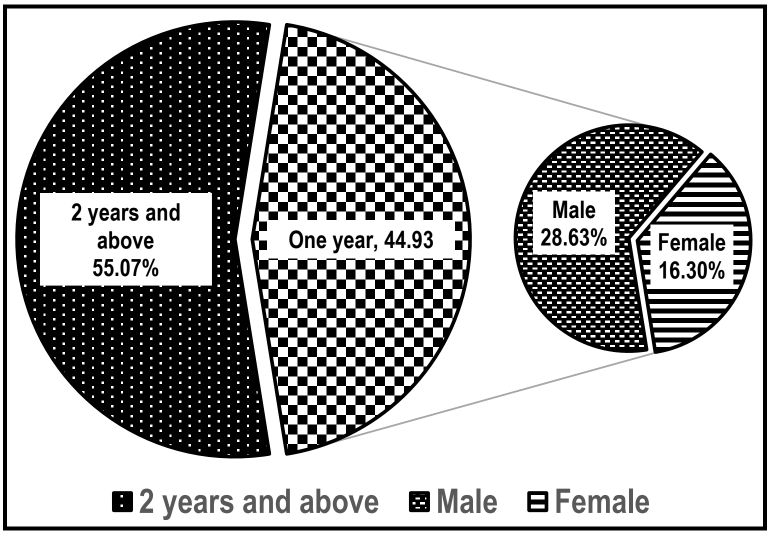

Figure 1: Pie chart demonstrating the overall distribution of positive CSF samples of suspected bacterial meningitis.

Percentage distribution of all isolated bacteria: The clinical isolates of CSF were initially subjected to Gram staining, the data demonstrated that 12.82% of CSF samples were infected with Gram-positive bacteria, while 87.18% yielded the growth of various Gram-negative bacteria (Table 3). The Gram-positive bacteria included Coagulase-negative Staphylococci (CoNS), *S. aureus* and Enterococcus spp.

Table 03. Distribution of clinical isolates of CSF of this study

Gram-positive bacteria				
Name	**Male**	**Female**	**Total (=n)**	**%**
Enterococcus spp.	1	0	1	2.56
S. aureus	1	1	2	5.13
CoNS	1	1	2	5.13
Total	**3**	**2**	**5**	**12.82**
Gram-negative bacteria				
Acinetobacter spp.	1	0	1	2.56
E. coli	3	5	8	20.51
Klebsiella pneumoniae	7	5	12	30.77
Pseudomonas spp.	6	3	9	23.08
Proteus mirabilis	0	2	2	5.13
Neisseria meningitidis	1	1	2	5.13
Total	**18**	**15**	**33**	**87.18**

Among the Gram-negative bacteria, *K. pneumoniae* were highly prevalent with 30.77% followed by Pseudomonas spp. (23.08%), *E. coli* (20.51%), *P. mirabilis* (5.13%) and *N. meningitidis* (5.13%), with the least prevalence of Acinetobacter spp. 2.56%, while the Gram-positive bacteria *S. aureus* and CoNS accounted for 5.13% each and Enterococcus spp. were least prevalent with 2.56% of all bacteria isolated from CSF samples.

Antibiotic resistance patterns of common bacterial isolates of CSF: Antibiotic susceptibility profiling of the most common bacterial isolates of CSF demonstrated that *K. pneumoniae* were highly sensitive to Colistin (100%), followed by Meropenem (66.67%). Cephalosporins Ceftriaxone, Cefixime and Cefuroxime were the least effective antibiotics against *K. pneumoniae* with 25% efficacy (Table 4 A). *E. coli* strains were highly resistant to Cefixime and Ampicillin while 87.5% were sensitive to Piperacillin/Tazobactam. Pseudomonas spp. were comparatively sensitive to all tested antibiotics with varying resistance patterns (Table 4 B). All of Pseudomonas spp. exhibited 77.78% sensitivity to Meropenem, Gentamicin, Piperacillin/Tazobactam, and Colistin (Table. 4C).

Table 4. (A): AST Patterns of Klebsiella pneumoniae (n=12)

Antibiotics disc/Potency	Sensitive (%)	Resistant (%)
Ceftriaxone (30µg)	25	75
Meropenem (10µg)	66.67	33.33
Cefixime (5µg)	25	75
Cefuroxime (30µg)	25	75
Piperacillin / Tazobactam (36µg)	58.33	41.67
Colistin (10µg)	100	0
Ampicillin (10µg)	16.67	83.33

Table 4. (B): AST patterns of E. coli (n=8)

Antibiotics disc/Potency	Sensitive (%)	Resistant (%)
Amikacin (30µg)	37.50	62.50
Aztreonem (30µg)	25	75
Ciprofloxacin (10µg)	37.50	62.50
Meropenem (10µg)	75	25
Ampicillin (10µg)	0	100
Cefixime (5µg)	0	100
Amoxycillin/Clavulanic Acid (30µg)	12.50	87.50
Piperacillin / Tazobactam (36µg)	87.50	12.50

Table 4. (C): AST patterns of Pseudomonas spp. (n=9)

Antibiotics disc/Potency	Sensitive (%)	Resistant (%)
Aztreonem (30µg)	66.67	33.33
Ciprofloxacin (10µg)	66.67	33.33
Meropenem (10µg)	77.78	22.22
Ceftazidime (30µg)	55.56	44.44
Gentamicin (10µg)	77.78	22.22
Piperacillin / Tazobactam (36µg)	77.78	22.22
Colistin (10µg)	77.78	22.22

DISCUSSIONS

The present study reported the bacteriological analysis of CSF samples collected from patients suspected of bacterial meningitis at Hyderabad, and the antibiotic resistance patterns of bacterial isolates. A previous study regarding meningitis surveillance in African regions revealed that *S. pneumoniae* and *H. influenzae* were the two main causes of meningitis from 2011 to 2016 (Tsolenyanu *et al.*, 2019). On the contrary, in a study conducted in a province in southwest China, *Escherichia coli* and *S. pneumoniae* were reported to cause 46.0% of the BM cases from 2012 to 2015 (Jiang *et al.*, 2017) The present study demonstrated *K. pneumoniae*, Pseudomonas spp. and *E. coli* were the main bacterial agents responsible for the majority of BM cases in Sindh. *N. meningitidis* was the least prevalent bacteria while no cases of BM caused by *S. pneumoniae* and *H. influenzae* were recorded. A previous study conducted at Hyderabad reported that CoNS were the most common isolates of CSF followed by *Acinetobacter* spp. and *E. coli* (Memon, Bano, Abbasi, & Tunio, 2022). It is pertinent that geographical location, temporal variations, and patient age are among the many factors that influence the etiology of BM (C. Li *et al.*, 2018; Okike *et al.*, 2014). In agreement with the findings of a previous study from Hyderabad in 2019 (Memon *et al.*, 2022), the data of the present study demonstrated that BM was more prevalent in the age group of less than one year age and up to five years.

Moreover, no BM cases were detected in age groups between 31 to 60 years of age in the present study.

In addition to the frequency of isolation of bacteria, their AMR patterns differ significantly, depending upon the locality and time (Tamma *et al.*, 2013). Consequently, rapid investigation and reporting of the AMR profiles and the BM causative agents are essential in assisting medical professionals in selecting the best empirical treatment. Colistin and meropenem were highly effective antibiotics against *K. pneumoniae* whereas Piperacillin / Tazobactam and Meropenem showed the highest efficacy against *E. coli* and Pseudomonas spp. Due to a lack of recent studies focusing on the prevalence of bacterial meningitis and its bacterial profiling in various age groups, the data of the present study would offer crucial insights into the antibiotic patterns of pathogenic bacteria isolated from CSF of patients with suspected bacterial meningitis for empirical therapy and better management of the infection.

CONCLUSION

Bacterial meningitis was more common in the age of less than 12 months. *K. pneumoniae* was a leading pathogen followed by Pseudomonas spp., and *E. coli* among Gram-negative isolates. Colistin, Piperacillin/Tazobactam and Meropenem were highly effective against *K. pneumoniae*, *E. coli* and Pseudomonas spp., respectively. In summary, culturing, and AST of clinical samples of CSF are crucial in proper diagnosis and empirical treatment of bacterial meningitis.

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