



Original Article

Bacteriological Profile of CSF Specimens Received at Rural Tertiary Care Hospital

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ABSTRACT

Introduction: Inflammation of meninges or infection of subarachnoid space called as meningitis^[1] There are two type of meningitis based on changes in leukocytes in CSF, Pyogenic meningitis shows elevated polymorphonuclear cells, bacterial agents are main causative agents for pyogenic meningitis. And Aseptic meningitis shows elevated lymphocytes in CSF, etiological agents includes, viral, parasitic, MTB, and fungal agents.^[1] Organisms such as *Streptococcus agalactiae* and *E.coli* followed by *Pseudomonas sps*, *S.aureus*, *CONS*, *Elizabethkingia*, *Citrobacter sps*. are commonly isolated from meningitis cases.^[2] **Aims:** To find out the bacterial profile in term of pathogens frequency in the CSF specimens received in Microbiology Laboratory. **Material Methods:** This is a Retrospective study of one year duration (June 2022- May 2023). CSF samples of suspected cases of meningitis received in Microbiology Laboratory were processed by automated culture system. **Result& Discussion:** Out of total 1227 CSF Samples 302 (24.61%) were found culture positive. Common isolates were *CONS*, *Acinetobacter sps*, *Enterococcus*, *Staphylococcus aureus*, *Klebsiella sps*, *Streptococcus sps*. **Conclusion:** CSF culture positivity showed both Gram positive and Gram Negative Organisms as a causative agents of meningitis and *CONS*, *Acinetobacter sps*, *Enterococcus* were the most common pathogens.

Keywords: Bacterial Meningitis, Gram Positive cocci, Antibiotic sensitivity pattern, CSF culture by automated culture system

INTRODUCTION:

Despite recent advances in diagnostic methods bacterial meningitis (BM) is responsible for high levels of both morbidity and mortality in children.^[3] The most effective way to confirm the diagnosis of bacterial meningitis is cerebrospinal fluid (CSF) culture. Bacterial meningitis is associated with considerable morbidity and mortality rates.^[4] Viral meningitis is shows good prognosis. In paediatric patients if bacterial meningitis not treated early may result in mental illness, hearing loss, learning disability and death.^[5]

Infectious agents enter into CNS by direct extension or through blood stream. Meningitis is common in certain age groups. Neonatal Meningitis mostly acquired from the mother.

Organisms responsible for bacterial meningitis vary with patient's age, like *H.influenzae Type B* common in the 6 months to 5-years age group, *N.Meningitidis* in all age groups.

S.pneumoniae meningitis decreased due to vaccination, but in adults it remains an important cause of bacterial meningitis.[2] Mortality in meningitis cases is 5-40% depending on pathogens isolated and including other factors like low immunity and age of the patients.[6] So periodic review is must to prevent the morbidity and mortality.

Based on the clinical history, Physical examination, and CSF examination we can diagnose a case of meningitis. Microbiological examination of CSF samples by Gram Staining can provide preliminary identification clue of pathogens. Conventional and automated culture methods are available for CSF culture. There might be a less chance of recovery of less number of organisms in CSF by conventional culture method. Now a day's use of automated blood culture system for the culture of normally sterile body fluids other than blood is widely accepted. [7,8]

Automated BacT/Alert system can detect the even presence of few numbers of organism or in fewer amount of specimens. It will detect the organism by presence and production of CO₂ with the help of CO₂-sensitive chemical sensor. [7,9]

For antimicrobial susceptibility testing commercial automated system have been available, like vitek 2 compact system which can detect 9-20 antibiotics.[10] Different bacterial pathogens and antibiotic resistance have been reported in several study.[11] Organisms most often isolated from meningitis cases are *Streptococcus agalactiae* and *E.coli* followed by *Pseudomonas* spp, *S.aureus*, *CONS*, *Elizabeth kingia*, *Citrobacter* spp. [2]

This study planned to find out the bacterial profile in term of pathogens frequency in the CSF specimens received in Microbiology laboratory.

MATERIAL & METHODS:

The present retrospective laboratory based study was carried out in the Department of Microbiology, Dr. BVP Rural Medical College, (PIMS-DU) Loni, Maharashtra, India, during June 2022 to May 2023. Approval reg. No by Institutional Ethical Committee (Reg. No. PIMS/ DR/ RMC/ 2024/711)

Inclusion criteria: Patients aged <14 years were included with both genders.

Exclusion criteria: Patients more than 14 years and repeat specimen were excluded.

Sample collection: All the CSF specimens of 0-14 year age group and from both genders were received in the Microbiology laboratory with duly filled requisition form with request to do culture and sensitivity.

Procedure

The received CSF samples were inoculated in Aerobic BacT/Alert PF plus bottle (approximately, 0.1 mL-2 mL). Inoculated bottles were incubated in BacT/Alert Machine (biomerirux) for five days. Flag positive bottles were taken out and sub-cultured on Blood agar, Chocolate agar and MacConkey agar and were incubated in incubator at 37 °C for 18-24 hours aerobically. Then isolates were subjected to biochemical reactions for identification and antibiotic susceptibility test done by Kirby Bauer Disc diffusion method. And some of the isolates were identified by vitek 2 automated system and Antibiotic sensitivity testing was done by vitek 2 automated system.

RESULT & DISCUSSION:

Present study shows out of 1227 specimens, from male 728 (59.33%) outnumbered the female specimens 499 (40.66%). [Figure 1]

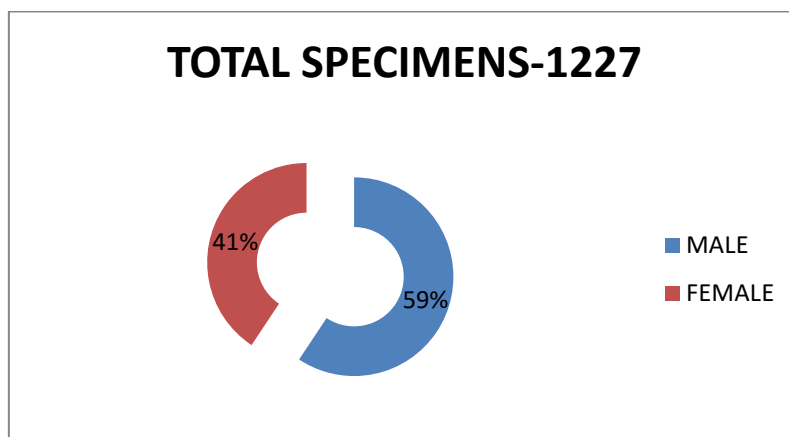


Figure1: gender wise distribution of study population

A total of 1227 specimens were processed during study period, out of which 302 (24.61%) specimens were shows a growth of various isolates.[Figure 2]

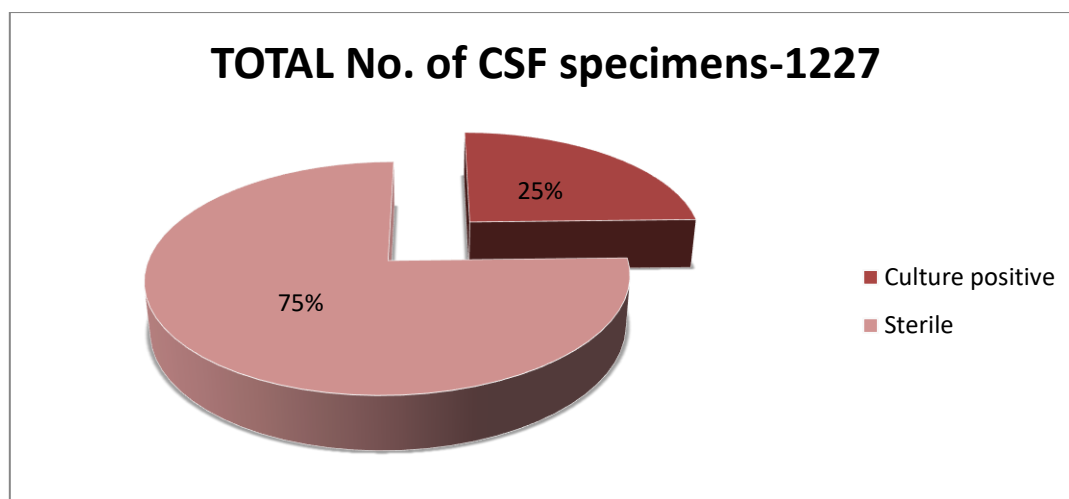


Figure 2: Distribution of CSF culture positivity in present study

Among 302 culture positive specimens 184 (60.92%) were male and remaining 118 (39.07%) were female most common age group was neonates 104 (34.43%) shown in Table 1.

Table 1: Age and Gender wise Distribution of positive culture

Age	Male (184)	Percentage (%)	Female (118)	Percentage (%)	Total (302)	Percentage%
0-1 Month (Neonates)	66	35.86%	38	32.20%	104	34.43%
1 months to 1-year (Infants)	49	26.6%	36	30.50%	85	28.14%
2-5 Year	29	15.76%	30	25.42%	59	19.53%
6-14 year	40	21.73%	14	11.86%	54	17.88%

Out of 302 isolates, Gram positive bacteria 199 (65.89%) were the most common as compared to Gram negative 103 (34.10%) and some rare isolates identified in present study. Out of 199 gram positive isolates, most common isolates were *CONS* 76 (23.17%), followed by *Enterococcus sps* 56 (17.07%), *Staphylococcus aureus* 53 (16.07%) and *Streptococcus pneumoniae* 14 (4.26%). Among 103 Gram negative isolates *Acinetobacter baumannii* 58 (19.20%) were common isolate followed by *Klebsiella pneumoniae* 19 (5.79%), *Escherichia coli* 7 (5.98%), *Sphingomonas sps* 7 (5.98%), *Pseudomonas aeruginosa* 3 (2.13%), *Enterobacter cloacae* 2 (0.60%) and some rare organisms were isolated like *Elizabeth kingie*, *Comomonas testoterani*, *Brivindimonas sps* 0.30% which are difficult to grow & identify by conventional culture technique shown in Figure 3.

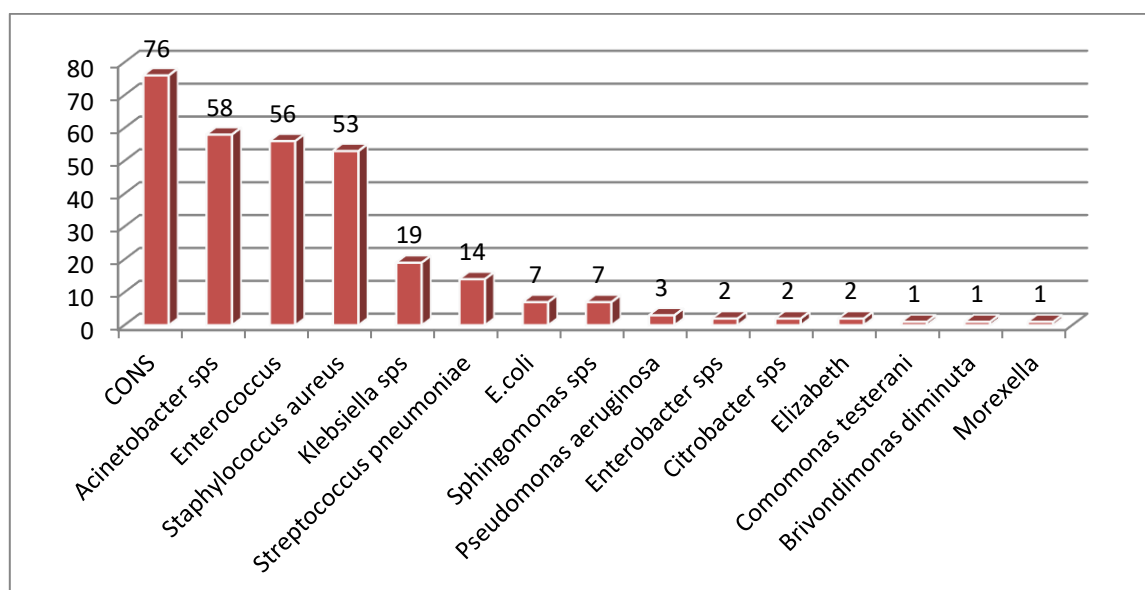


Figure 3: Distribution of CSF bacterial isolates in the present study

Type of antibiotic resistant identified by vitek 2 system in bacterial isolates shown in table 2.

Table 2: Type of antibiotics resistance in isolates

Organisms isolated	Type of resistant
<i>Staphylococcus aureus</i> (53)	MRSA 84.90%
<i>Enterococcus spp.</i> (56)	VRE 26.78%
<i>Gram negative isolates</i> (103)	MDRO 18.44%

MRSA- Methicillin resistant staphylococcus aureus, VRE- Vancomycin resistant Enterococci, MDRO- Multidrug resistant organism. According CLSI 2023 MDRO is defined as non-susceptibility to at least one agent in at least 3 antimicrobial classes of the following six antimicrobial classes Aminoglycoside, Carbapenems, Fluoroquinolone, β -lactam, Cephalosporin, Sulbactam.²¹

DISCUSSION:

Meningitis is a medical emergency with high morbidity and mortality, survival is depends on early diagnosis and prompt treatment. Like other vaccine preventable infections meningitis burden remains high despite of vaccines available against common organism causing meningitis like *Neisseria meningitidis* (meningococcus), *Streptococcus pneumoniae* (pneumococcus), *Haemophilus influenza*,^[13] and now a days these organism not isolated due to effective vaccination and difficult to isolate. Most of the cases and death occurring in childrens under 5 years of age.^[14]

The present study showed that out of 1227 specimens received Male 728(59.33%) outnumbered the female 499 (40.66%) [Figure:1] which is congruous to the study by Attia Bari et al[3] which comprised 63.8 % of male population and 36.2% of female and Savita et al[8] ,Kaul V et al[15] also shows the male dominance over female population, and some study did not shows any gender dominance.[16,17]

In our study CSF culture positivity in received specimens was 24.61% [Figure 2] which was quite similar to DEVI et al^[7], and incongruous to Attia Bari et al^[3] which shows culture positivity 12.6%, Muhammad Moaaz Ali et al^[4] shows 7.85%, Amresh Kumar Singh et al^[5] shows 9.01%, Orlando C. Mantese et al^[6] shows 16.63%.

This study shows majority of the patients 104(32.20%) were neonates followed by infants 85(28.14%), [table1] which was similar to the study done by Savita Baban Tajane et al^[8] and Fatima Khan et al ^[18]. Our study shows Male dominance over Female patients, in the age group 0-2 year shows 115 (38.507%) positivity in male patients and 76(24.50%) positivity in female patients and in age group 2-5 years shows 29(9.60%) in male patients, 30(9.93%) in female patients and in age group 6-14 years shows 40 (21.73%) in male patients and 14 (11.86%) in female patients which is quite similar to study done by Singh AK et al^[5]

In our study MRSA incidence was 84.9% and Vancomycin resistant Enterococcus (VRE) were isolated by vitek 2 automates system 26.78%.According to recent CLSI guidelines 2023^[21] MDRO in gram negative isolates was 18.44% shown in table 2. Other study like Devi et al^[11] also shows the VRE 50% and high resistant in gram negative isolates.

Among 302 isolates Gram positive organisms 199 (65.89%) were major causative agent as compared to Gram negative organisms 103 (34.10%) [Figure:3] Similar to many studies by Attia Bari et al^[3] shows Gram positive isolates 54.16% outnumbered the Gram negative isolates 45.83%, Sing AK et al^[5] shows 66.18% Gram positive organism and 28.86% Gram negative, F Khan et al^[18] shows 59.6%,Rajesh Bareja et al^[19] shows 67.39% gram positive isolates. Some studies by Devi U et al^[7] and Cohen-Wolkowicz M et al^[20] are incompatible to our study that shows the predominance of gram-negative organisms 48% over Gram positive organisms 39%.

In the present study, although significantly more CoNS 76 (23.17%) were isolated indicating that CONS as an emerging pathogen. We informed to clinician for clinical correlation. Various studies shows similar findings.[Table 3]

Table 3: Different studies shows the percentage of gram positive organism

Isolated Gram Positive Organism (199/302)	Percentage of present study	Attia Bar et al ^[3]	Singh AK et al. ^[5]	DEVI et al. ^[7]	Savita Baban Tajane et al. ^[8]	Fatima Khan et al. ^[18]	Rajesh Bareja et al ^[19]
CONS (76)	25.16%	6%	44.50%	16.9%	49.4%	-	0.32%
ENTEROCOCCUS SPS (56)	18.54%	-	-	17.5%	15.2%	4.5%	4.34%

STAPHYLOCOCCUS AUREUS(53)	17.54%	-	19.20%	1.72%	7.05%	37.7%	30.43%
STREPTOCOCCUS PNEUMONIAE (14)	4.63%	1.1%	36.12%	-	2.3%	8.7%	14.67%

Table 4: different studies shows the percentage of Gram negative organism

Gram negative isolates (103/302)	Percentage of isolates	Mod. Moaaz Ali et al ^[4]	Singh AK et al ^[5]	DEVI et al ^[7]	Savita Baban Tajane et al ^[8]	Fatima Khan et al. ^[18]	Rajesh Bareja et al ^[19]
Acinetobacter baumannii (58)	19.20%	18%	39.40%	18%	3.21%	2%	1.08%
Klebsiella Pneumonia(19)	6.29%	21%	20.20%	12%	3.01%	4.7%	4.34%
Escherichia coli(07)	2.31%	25%	19.20%	3.12%	0.75%	11.2%	11.41%
Sphingomonas sps (7)	2.31%	0	0	0	0	0	0
Pseudomonas aeruginosa (3)	0.99%	36%	5.5%	9%	0	12.2%	9.29%
Enterobacter cloacae (02)	0.66%	0	2.02%	0	0.37%	0	0
Citrobacter koseri (02)	0.66%	0	11.11%	0	0	2.2%	1.63%
Elizabeth kingie(2)	0.66%	0	0	0	0	0	0
Comomonas testoterani (1)	0.33 %	0	0	0	0	0	0
Brivindimonas sps (1)	0.33%	0	0	0	0	0	0
Morexella group (1)	0.33%	0	0	0	0	0	0

CONCLUSION:

In present study most common gram positive isolates was CONS, indication the common emerging etiological agent in all age group. This study may play important role in early diagnosis and accurate treatment of paediatric patients. It is necessary to do CSF culture for all suspected meningitis cases to know the etiological agents and antimicrobial resistance caused by these pathogens.

Declaration:

Conflicts of interests: The authors declare no conflicts of interest.

Author contribution: All authors have contributed in the manuscript.

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