**ORIGINAL ARTICLE**

# BACTERIOLOGICAL PROFILE OF PYOGENIC MENINGITIS IN TERTIARY CARE HOSPITAL, AHMEDABAD

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## ABSTRACT

**Aims:** Bacterial meningitis remains a major cause of mortality and long term neurological sequelae worldwide. The purpose of present study was to identify the pathogen in pyogenic meningitis and to determine its antibiotic susceptibility pattern.

**Material and Methods**: Present study was undertaken from July 2010 to December 2011 included 1470 CSF samples of clinically suspected pyogenic meningitis cases in all age groups. The samples were subjected to macroscopic examination, microscopic examination, Gram's stain and culture tests. The organisms isolated in the study were characterized by standard procedure and antibiotic susceptibility tests according to CLSI guidelines.

**Results**: Bacterial pathogens were isolated from 205 samples showing an isolation rate of 13.94%. Gram's stain positivity was 61.95%. Among the isolated organisms, 69.26% were gram negative bacilli and 30.74% were gram positive cocci. The most commonly isolated bacteria were K. pneumoniae (22.92%) & S. aureus in 19.02%. Pyogenic meningitis was more common in paediatric patients than adults. K. pneumoniae and Enterococci spp. were most common isolated in neonatal age group. Most common organisms isolated in neurosurgical patients were Pseudomonas aeruginosa and Staphylococci aureus. 09.10% gram negative organisms were ESBLs. Only 2 Gram positive isolates were MRSA.

**Conclusion:** The frequency of single as well as multiple drug resistance was very high among the bacterial isolates. Antibiogram study indicated that the third generation cephalosporins and aminoglycosides can be used as single or in combinations for the empirical treatment of bacterial meningitis till culture and sensitivity report is awaited.

**Key Words:** Pyogenic meningitis, Gram's stain, culture, antibiotic susceptibility testing, ESBL, MRSA.

## INTRODUCTION

Meningitis is a very serious infection of the meninges that surround the brain and the spinal cord[1]. It is usually caused by viral, bacterial or fungal pathogens. Bacterial meningitis can be quite severe and may result in brain damage, hearing loss, or learning disability and death if not treated early[2]. Despite advances in vaccine development and chemoprophylaxis, bacterial meningitis remains a common disease worldwide. The disease is more common in developing countries[3].Bacterial meningitis remains a major cause of mortality and long term neurological sequelae worldwide. Despite of availability of potent antibiotics the mortality rate due to acute bacterial meningitis remains significantly high in India and other developing countries.[4-7] There is a need for periodic review of bacterial meningitis worldwide, since the pathogens responsible for infection vary with time, geography and patient's age.[5] Increase in awareness, availability of vaccines may also reflect a change in the epidemiological pattern of these pathogens. The aetiological agents of community acquired meningitis may differ from hospital acquired meningitis. Delay in diagnosis and initiation of treatment can result in poor outcome of the disease.[8] The clinical signs and symptoms can't be always relied upon[7], laboratory support is imperative to achieve early diagnosis. As a result of emergence of antimicrobial resistance being reported, recommendations for therapy are changing. Laboratory surveillance of isolates is crucial to identify targets for formation of rational empirical treatment for potentially fatal bacterial meningitis. We reviewed the microbiological records of patients with bacterial meningitis to determine frequency of pathogens causing bacterial meningitis, to find out aetiological agents and their susceptibility pattern.

## METHODOLOGY

A retrospective analysis of 1470 clinically suspected cases of meningitis, admitted during a span of 1.5 years from July 2010 to December 2011 was undertaken. All the clinically suspected cases were included in this study. CSF samples were collected aseptically in a sterile test tubes.[9] CSF samples of varying quantity of 3 to 5 ml (3ml for neonates) were transported at ambient temperature to the laboratory without delay , since undue delay might result in poor recovery of some pathogens, such as meningococci, and also cause the disintegration of leukocytes.[10]

The macroscopic appearance of CSF samples was recorded. The specimens were examined for presence of turbidity and any sign of haemorrhage. Normal CSF samples were clear and colorless like water. A portion of CSF samples were centrifuged and deposits were subjected to Gram's stain, ZN stain, Negative staining with Nigrosin for Cryptococcus and culture.[10] All the samples were inoculated on Chocolate agar and MacConkey agar. Remaining portions of CSF was put in Brain-Heart Infusion broth for enrichment from which subcultures were done on Chocolate agar and MacConkey agar. All the culture plates were incubated in $CO_2$ incubator at 5% $CO_2$ at 37°C in incubator for 24-48 hours. The culture plates were observed daily for presence of growth. When growth appeared, secondary smear from colony was prepared to find out whether the growth is of Gram positive or Gram negative organisms. The isolates were identified by standard techniques and antibiotic susceptibility tests were done against locally available antibiotics by using disk diffusion method in accordance with CLSI, 2010 criteria, and interpreted accordingly[11]. Ampicillin (10 µg), Piperacillin (10 IU), Co-Trimoxazole (25 µg), Chloramphenicol (30 µg Gentamicin (10µg), Amikacin (30 µg), Ceftriaxone (30µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Ampicillin-Sulbactam (20 µg), Cetriaxone-Tazobactam (40 µg), Amoxycillin +Clavulanic acid (30 µg), Imipenem(10 µg ), Polymyxin B (300 unit),Vancomycin (30 µg) and Linezolid (30 µg) were used. For quality control -*Staphylococcus aureus* ATCC 25923, *Enteroccus feacalis* ATCC 25922, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used.[11]

## RESULTS

Total 205 bacteria were isolated from 1470 cases with isolation rate of 13.94%. The correlation between Gram's staining and culture was 61.95% (127 out of 205). Table 1 shows that out of the 205 confirmed meningitis cases, 123 (60.00%) were male and the remaining 82 (40.00%) were female. Male: Female ratio was 1.5:1. The age distribution patterns of the study subjects summarized in table-1. The age range was from 0 days to more than 60 years. Over half of the 205 patients, 122(59.50%) were children and the rest 83(40.50%) were adults.

**Table 1: Age, Sex & Ward wise Distribution of patient from which Organisms were Isolated**

| | Patients (n= 205) (%) |
|---|---|
| **Sex** | |
| Male | 123 (60.0) |
| Female | 82 (40.0) |
| **Age Group (In Year)** | |
| 0-1 Month | 46 (22.44) |
| 1 Month-1 Year | 29 (14.15) |
| 1-12 | 48 (23.42) |
| 12-25 | 36 (17.56) |
| 26-40 | 31 (15.12) |
| 40-60 | 03 (01.46) |
| >60 | 12 (05.85) |
| **Ward** | |
| Neonatal ICU | 46 (22.43) |
| Neurosurgical ward | 31 (15.12) |
| ICU | 10 (04.88) |
| Peadiatric ward | 77 (37.56) |
| Medical ward | 29 (14.15) |
| Surgical ward | 12 (05.85) |
| OPD | 00 |

Table-1 also showed that most bacteria pathogens isolated from paediatric ward 77 (37.56%), neonatal ICU 46(22.43%), neurosurgical ward 31 (15.12%) and medical ward 29 (14.15%). Table-2 showed that 142(69.26%) were gram negative bacilli and 63(30.74%) were gram positive cocci. The most commonly isolated bacteria were *K. pneumoniae* 47(22.92%) and *E. coli* 29(14.15%), followed by *Pseudomonas aeruginosa* 25 (12.19%), *Acinetobacter spp.* 23 (11.22%) and *Citrobacter spp.* 05 (02.44%).Among gram positive organisms, *S. aureus* in 39(19.02%) and *Enterococci spp.* 22(10.73%) isolated. *K. pneumonia, Acinetobacter spp.* and *Enterococci spp.* were most common isolated in neonatal age group. Most common organisms isolated in neurosurgical patients were *Pseudomonas aeruginosa Acinetobacter spp.* and *Staphylococci aureus*. According to table-3, Antibiotic susceptibility pattern showed that out of 25 strains of *Pseudomonas aeruginosa* isolated maximum 80% (20/25) were sensitive to Piperacillin- Tazobactam, 48% (12/25) to Piperacillin, 64% (16/25) to Ceftazidime, 52%(13/25) to Tobramycin and 76% (19/25) to Netilmicin. *Acinetobacter spp.* were showed maximum sensitivity to Ampicillin-Sulbactum 73.91% (17/23) followed by Amikacin 69.57% (16/23). *K. pneumoniae* were showed maximum sensitive to Ceftriaxone-Tazobactam 93.61% (44/47), Amoxycillin-Clavunic acid 80.85%(38/45) and Amikacin 68.09%(32/47). *E.coli* were showed maximum sensitive to Ceftriaxone-Tazobactam 96.55% (28/29), Amoxycillin-Clavunic acid 86.20% (25/29) and Amikacin 79.31%(23/29). 13 out of 143 (09.10%) gram negative organisms were ESBL-Extended Spectrum Beta Lactamase producer. All gram negative isolated were 100 % sensitive to

Imipenem and Polymyxin B .Amongst Gram positive isolates *Enterococcus spp.* and *Staphylococci aureus* were 100% sensitive to almost all the antibiotics tested, except 2 isolated which were Methicillin Resistant *Staphylococcus aureus* (MRSA).

## DISCUSSION

The successful management of patients suffering from bacterial illnesses depends upon the identification of the types of organisms that cause the diseases and the selection of an effective antibiotic against the organism in question[20]. Thus, the data presented in this study could provide information of immediate public health importance to clinicians in west India on the selection of antimicrobial agents for the treatment of patients suffering from bacterial meningitis.

**Table 2: Bacterial Isolated from CSF**

| Organisms | Number (n=205) (%) |
|---|---|
| K. pneumonia | 47 (22.92) |
| E. coli | 29 (14.15) |
| *Pseudomonas aeruginosa* | 25 (12.19) |
| Acinetobacter spp. | 23 (11.22) |
| *Citrobacter spp.* | 05 (02.44) |
| Enterococci spp. | 22 (10.73) |
| S. aureus | 39 (19.02) |
| ESBL | 13 (06.34) |
| MRSA | 02 (01.00) |

**Table 3: Antibiotic Susceptibility Pattern of Various Organisms Isolated from CSF**

| Antibiotics | Organisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | K. pneumoniae (47) | E. coli (29) | Pseudomonas aeruginosa (25) | Acinetobacter spp. (23) | Citrobacter spp (5). | Enterococci spp. (22) | S. aureus (39) | ESBL (13) | MRSA (02) |
| Ampicillin | - | - | - | - | - | - | 39 (100) | - | - |
| Piperacillin | - | - | 12 (48) | - | - | - | - | - | - |
| Co-Trimoxazole | 15 (31.91) | 11 (37.94) | - | 11 (47.83) | 00 | - | 39 (100) | 02 (15.38) | 02 (100) |
| Chloramphenicol | 24 (51.06) | 16 (55.17) | - | 15 (65.22) | 02 (40.00) | 22 (100) | 39 (100) | 08 (61.54) | 02 (100) |
| Gentamicin/ Tobramycin | 19 (40.43 | 12 (41.38) | 13 (52) | 08 (34.78) | 00 | - | - | 00 | - |
| Amikacin/ Netilmicin | 32 (68.09) | 23 (79.31) | 19 (76) | 16 (69.56) | 03 (60.00) | - | - | 09 (69.23) | - |
| Ceftriaxone | 26 (55.32) | 19 (65.52) | - | 10 (43.48) | 03 (60.00) | - | - | 00 | - |
| Ceftazidime | 26 (55.32) | 19 (65.52) | 16 (64) | 10 (43.48) | 03 (60.00) | - | - | 00 | - |
| Cefotaxime | 26 (55.32) | 19 (65.52) | - | 10 (43.48) | 03 (60.00) | - | - | 00 | - |
| Ampicillin-Sulbactam | - | - | - | 17 (73.91) | - | - | 39 (100) | - | - |
| Cetriaxone-Tazobactam | 44 (93.61) | 28 (96.55) | - | 12 (52.17) | 05 (100.00) | - | - | 13 (100) | - |
| Piperacillin-Tazobactam | - | - | 20 (80) | - | - | | | - | - |
| Imipenem | 47 (100) | 29 (100) | 25 (100) | 23 (100) | 5 (100) | - | - | 13 (100) | - |
| Polimyxin B | 47 (100) | 29 (100) | 25 (100) | 23 (100) | 5 (100) | - | - | 13 (100) | - |
| Clavulinate+Amoxycillin | 38 (80.85) | 25 (86.20) | - | - | 03 (60.00) | - | - | 13 (100) | - |
| Vancomycin | - | - | - | - | - | 22 (100) | 39 (100) | - | 02 (100) |
| Linezolid | | - | - | - | - | 22 (100) | 39 (100) | - | 02 (100) |

Figure in parenthesis indicate percentage

Meningitis is an inflammation of the membranes of the brain, spinal cord and ventricles often from an infective etiology. Broadly it can be divided into three general categories: pyogenic, granulomatous, and lymphocytic. Pyogenic (bacterial) meningitis is a potentially life-threatening disease that consists of inflammation of the meninges and the underlying subarachnoid CSF. Laboratory investigations of CSF specimens in suspected cases of bacterial meningitis are extremely important for prompt diagnosis and management of patients. Several studies have reported Gram's staining as the most useful single test for identifying bacterial meningitis, as it revealed more positive cases than cultures.[12,13,14,15] Some studies have reported a CSF Gram stain sensitivity of 60-90% and a high specificity of >97%, stressing its importance in the rapid and accurate diagnosis of the causative bacteria.[16,17.] In our study, Gram stain on CSF provided an evidence of the causative bacteria in 127 (61.95%) patients. Other workers have also reported low positivity on Gram's staining as compared to cultures.[18,19]

The yield of bacteria on a Gram stain depends on several factors like the number of organism present, prior use of antibiotics, technique used for smear preparation (centrifuged deposit, direct smear etc.), staining techniques and the observer's skill and experience.

CSF samples from only 205 (13.94%) patients were positive on culture in our study. Several studies from India report culture-negative cases of meningitis or a low CSF culture positivity, ranging from 6-50%.

Various reasons cited in the literature for a low yield of bacteria on culture are: [21]
* Improper technique of lumbar puncture
* Delay in transport of specimens to the laboratory
* Non-availability of special media for specific pathogens in the emergency setting
* Autolysis enzymes in CSF
* Fastidious nature of pathogen
* Antibiotic treatment prior to lumbar puncture.

Though common pathogens isolated were *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa*, *Acinetobacter spp*, and *Citrobacter spp.*, their relative frequency vary in different geographical area. As compared to western studies, the relative incidence of meningitis caused by *Haemophillus influenzae*, *Neisseria meningitidis* and *Listeria spp.* is less in South East Asia. On the contrary Gram negative bacilli such as *K. pneumoniae, Pseudomonas aeruginosa, Acinetobacter spp.* are increasingly being recognized as important pathogens. These Gram negative isolates are also reported as pathogens of bacterial meningitis in a recent study conducted at NIMHANS Bangalore in 2006.[22] Among Gram positive bacteria isolated in the present study commonest was *Enterococcus spp* and *Staphylococcus aureus.*

The high incidence of sterile CSF (86.06%) may be due to empirical antibiotic use as access to drugs, prescribed or otherwise is unrestricted[13]. This 'culture-negative CSF' debacle can be readily overcome if newer methods of diagnoses which do not require the growth of live pathogens such as latex agglutination and polymerase chain reaction are incorporated into medical laboratory practice in the region. This problem of antibiotic misuse may lend credence to the increasing resistance to the common anti-meningitic drugs i.e., ampicillin, penicillin and chloramphenicol, [23] Ceftriaxone, cefotaxime, had the most effective anti-bacterial activity across all the bacterial isolates tested in the study. Emergence of resistant bacterial strains to conventional antibiotics such as chloramphenicol and penicillin have also been reported in other studies in Nigeria, Mumbai, and Trinidad[24] and have raised concern for spread of resistance with resultant consequences i.e. in developing countries where the first line ceftriaxone is not readily available or affordable.

A rational use of antibiotics especially in this pediatric group cannot be over emphasized. The government programmes should comprehensively address the issue of proper distribution, dissemination and administration of conjugate vaccines and incorporation of the same in routine immunization schedule of children in our communities as practiced elsewhere in the world. Preventive antimicrobial treatment of pregnant women and a regular high vaginal swab for microbiologic study is advocated.

A limitation in the study includes loss of some fastidious bacteria leading to high culture negative CSF because of frequent inappropriate use of antibiotics by patients before presentation, delay in processing of CSF specimens and paucity of newer method of diagnoses which do not require the growth of live pathogens such as latex agglutination and polymerase chain reaction in the medical laboratory practice in the region.

## CONCLUSION

In recent years Gram negative bacilli are emerging as important pathogens causing acute bacterial meningitis in adults. Judicious use of antibiotics will prevent the emergence of drug resistance among Gram negative bacilli, so that morbidity and mortality can be reduced. Finally, in the absence of antibiotic susceptibility report, ceftriaxone should be considered as a first choice of reliable antibiotics for empirical treatment of meningitis.

## REFERENCES


1. M Cheesborough. District Laboratory Practice in Tropical Countries part-II. Cambridge Low Price Editions, Cambridge University Press; 2002; 116-124.
2. Richard E.B., et al. Nelson textbook of pediatrics, 16th ed. W.B. Saunders Company. New York USA,2000. P.707.
3. World Health Organization. Control of epidemic meningococcal diseases. WHO Practical Guidelines.2 ed. 1998. Geneva Switzerland.
4. Kabra SK, Praveen K, Verma IC, Mukherjee D, Chowdhary BH, Sengupta S, et al. Bacterial meningitis in India: An IJP survey. Ind J Pediatr 1991; 58: 505-11.
5. Tang LM, Chen ST, HSU WC, LYU RK. Acute bacterial meningitis in adults. A hospital based epidemiological study. QJM 1999; 92:719-25.
6. Ayaz C, Mehmet FG, Hosoglu S, Mustafa KC, Akalin S, Omer FK. Characteristics of acute bacterial meningitis in South East. Turkey 2004; 58: 327-33.
7. Chinchankar N, Mane N, Bhave S, Bapat S, Bardekar A, Pandit A, et al. Diagnosis and outcome of acute bacterial meningitis in early childhood. Ind Pediatric 2002; 39: 914-92.
8. Van de Beek D, de Gans J, Tunkel AR, Wijdicks EI. Community acquired bacterial meningitis in adults. N Eng J Med 2006; 354 : 44-53.
9. A Sonavane, VP Baradkar, M Mathur ; Bacteriological Profile of Pyogenic Meningitis in Adults. Bombay Hospital Journal 2008;50(3):452-455.
10. Forbes BA, Sahm DF, Weisfeld AS. Streptococcus Meningitis and other infections of the central nervous system. In: /laboratory manual of Bailey & Scotts diagnostic microbiology./ 12^th Ed. Mosby Elsevier publication 2007, pp 907-916.
11. Clinical Laboratory Standard Institute. Performance standard for antimicrobial disk susceptibility tests; Approved standard -20th Ed. Supplement/ M2 – A9 2006, 26(1).
12. Rao BN, Kashbur IM, Shembesh NM, et al. Etiology and occurrence of acute bacterial meningitis in children in Benghazi, Libyan Arab Jamahiriya. Eastern Mediterranean Health Journal 1998; 4 (1): 50-57.
13. Rosenstein NE, Perkins BA, Stephens DS, et al. Meningococcal disease. Medical progress 2001; 344 (18): 1378-88.
14. Panjarathinam R, Shah RK. Pyogenic meningitis in Ahmedabad. Indian Journal of Pediatrics 1993; 60 (2): 669- 73.

15. Dure-Samin A, Mubina A, Azra Y. An epidemic of meningococcal disease in Karachi (Pakistan): a study of children. Acta Paediatr Jpn 1991; 33 (3): 352-6.

16. Elmore JG, Horwitz RI, Quagliarello VJ. Acute meningitis with a negative Gram?s stain: Clinical and management outcomes in 171 episodes. Am J Med 1996;100:78-84.

17. Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS Jr, /et al/. Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med 1993;328:21-8.

18. Ogunlesi TA, Okeniyi JA, Oyelami OA. Pyogenic meningitis in Ilesa, Nigeria. Indian Pediatr 2005; 42 (10): 1019-1023.

19. Pusponegoro, Hardiono D, Oswari, et al. Epidemiological studies of bacterial meningitis in Jakarta and Tangerang: preliminary report. Pediatric Infectious Journal 1998; 17 (9): 176-78.

20. Jawetz, Melnick, Adelberg's Medical Microbiology.21 Ed. International Edition, Stanford, Connecticut, Prentice-Hall International Inc: 1998; Antimicrobial chemotherapy.145-176.

21. Madhumita P, Gupta N, Clinical and Bacteriological spectrum of community acquired bacterial meningitis in adults at Tertiary Care Hospital in Northen India, Int J Nutr Pharmacol Neurol Dis; 2011; 1; 194-200.

22. Mani R, Pradhans S, Nagarathna, Wasiulla R, Chandramukhi A. Bacteriological profile of community acquired acute bacterial meningitis: A ten year retrospective study in a tertiary neurocare center in South India. IJMM 2007; 25 (2) : 108-14.

23. Akuhwa RT, Alhaji MA, Bello MA, Okon KO. Susceptibility pattern of meningococcal meningitis outbreak in Nguru , Yobe state Nigeria. Internet j Trop med 2010;7(1):1-4.

24. Bacterial isolates from cerebrospinal fluid of suspected acute meningitis in nigerian children. Available at: http://www.emedpub.com/ bacterial- isolates- from-cerebrospinal- fluid- of- suspected- acute- meningitis- in-Nigerian- children. Accessed on June 3, 2011.