

Original Article

ETIOLOGICAL AGENTS AND THEIR ANTIBIOTIC RESISTANCE PATTERN ISOLATED FROM CEREBROSPINAL FLUID (CSF) SAMPLES OBTAINED FROM ADMITTED PATIENTS IN LAHORE GENERAL HOSPITAL (LGH), LAHORE.

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Objective: To determine the most common etiological agent and their antibiotic resistance pattern in CSF samples obtained from admitted patients in neurosurgical wards in LGH, Lahore

Methods: It was a descriptive cross sectional study performed in Microbiology section of Pathology Department of Post Graduate Medical Institute (PGMI), Lahore during October 2015 to April 2017. The CSF specimens were inoculated on Blood agar and Mac Conkey agar plates and were incubated at 35-37°C aerobically. After 24 hours of incubation, the plates were examined for the presence of bacterial colonies. Organisms were identified by standard microbiological methods. Antibiotic sensitivity test was conducted employing the modified Kirby-Bauer disc diffusion method according to CLSI 2016.

Results: Out of 511 CSF culture samples, 137 (26.81%) were culture positive and 374 (73.18%) were culture negative. Among culture positive samples, the meningitis due to *Acinetobacter* spp and *Pseudomonas* spp account for 39 (28%) and 32 (23.02%) of the cases. Non fermentor gram negative rods are most resistant to Doxycycline and most sensitive to Piperacillin Tazobactam. The meningitis caused by *E.coli* and *Klebsiella* spp are responsible for 12 (8.7%) and 16 (11.6%) cases of meningitis. The lactose fermentors are most resistant to Ampicillin and most sensitive to Imipenem. Staphylococci were 30(21.8%), most of the Staphylococci were Methicillin resistant Staphylococci (MRSA) and most were sensitive to Vancomycin and Linezolid. There are two cases of *Streptococcus pneumoniae* and *Ralstonia picketti* and one case each of *Ochrobactrum anthropi*, *Pasteurella* spp, *Vibrio metschnikovii*, *Aeromonas hydrophila* and *Chromobacterium violaceum*.

Conclusions: TBacterial meningitis caused by gram negative bacilli is most common in our setup. Gram positive bacteria are most resistant to Penicillins and most sensitive to Vancomycin and Linezolid. The lactose fermenter gram negative rods are most resistant to Ampicillin and most sensitive to Imipenem. Non fermenter gram negative rods are most resistant to Doxycycline and most sensitive to Piperacillin Tazobactam.

Keywords: etiology, CSF, antibiotic resistance pattern, LGH.

Introduction

Bacterial meningitis is a serious disease that causes inflammation of meninges of the brain, particularly the arachnoid and pia mater. It is also notorious for producing detrimental long-term clinical manifestations and life threatening consequences.¹ Meningitis may be due to medical causes or injury. Head injury is the surgical emergency that may be open or closed. In addition to contusions to the brain, hematoma, brain abscesses can cause meningitis, encephalitis, hydrocephalus and several other drastic complications and outcomes. Bacterial meningitis may appear even after years and decades after the initial injury. Besides urgent surgery, abrupt antimicrobial therapy against most common causative

microorganism is also initiated.^{2,3}

There are two stages of brain injury. The primary brain injury is due to mechanical impacts applied to the cranium and brain at the instance of smash, leads to either focal or dispersed damage, whereas the secondary brain injury is an outcome of complicating processes initiated by the primary injury. These induce neuroinflammation by activation of the innate immune reaction, such as complement activation, release of pro-inflammatory cytokines and oxidative burst of polymorphonuclear cells coupled with the discharge of proteolytic and neurotoxic enzymes.^{3,4}

Punjab Institute of Neurosciences is the first and foremost institute in the public sector where the patients with head injury from all over the Punjab, are brought

The etiology and most common isolated bacterial agent from the meningitis cases so that timely initiation of the appropriate antimicrobial therapy reduces the mortality and morbidity of the patients. Moreover it decreases the burden on the socioeconomic situation of the patients and health resources of the country. Therefore this study was planned to obtain data on commonly isolated etiological agent and their antimicrobial resistance pattern from the admitted patients in the neurosurgical wards.

Methods

The present study was a descriptive cross sectional study. A total of 511 specimen of cerebrospinal fluid were collected from the patients admitted in Lahore General Hospital (LGH) during October 2015 to April 2017. The samples were transported to Microbiology laboratory in Pathology Department of Post Graduate Medical Institute, Lahore for culture and sensitivity. The CSF samples from Neurosurgical wards, Neuro ICU were also included in the study.^{5,6} The gross appearance of CSF (clear, slightly turbid, cloudy, presence of blood or clots) was noted. The specimens were inoculated on Blood agar, Mac Conkey agar plate, and Chocolate agar (prepared as instructions given by the manufacturer) and were incubated at 35-37°C aerobically. After 24 hours of incubation, the plates were examined for the presence of bacterial colonies. Organisms were identified by standard microbiological methods, which include colony morphology, Gram's staining, and biochemical tests (catalase test, coagulase test oxidase test, citrate test, Triple sugar Iron test, Urease test, Indole Test and Motility test). Some of the non lactose fermenting gram negative rods have very unusual morphology and were identified by APINE strips.

Antibiotic sensitivity test was conducted on pure culture isolates employing the modified Kirby-Bauer disc diffusion method for determining the susceptibility of microorganism to different antimicrobials. The appropriate sensitivity discs were selected according to CLSI 2016 guidelines. The diameters of growth inhibition around the discs was measured and interpreted as sensitive, intermediate or resistant as per the guidelines set by CLSI.

Results

Out of 511 CSF culture samples, 137 (26.81%)

were culture positive and 374 (73.18%) were culture negative. Among culture positive sample, the meningitis due to *Acinetobacter* spp and *Pseudomonas* spp accounts for 39 (28%) and 32 (23.02%) of the cases. Non fermentor gram negative rods are most resistant to Doxycycline and most sensitive to Piperacillin Tazobactam. The meningitis caused by *E.coli* and *Klebsiella* spp are responsible for 12 (8.7%) and 16 (11.6%) cases of meningitis. The lactose fermentors are most resistant to Ampicillin and most sensitive to Imipenem. Staphylococci were 30(21.8%), most of the Staphylococci were Methicillin resistant Staphylococci (MRSA) and most were sensitive to Vancomycin and Linezolid. There are two cases of *Streptococcus pneumoniae* and *Ralstonia picketti* and one case each of *Ochrobactrum anthropi*, *Pasteurella* spp, *Vibrio metschnikovii*, *Aeromonas hydrophila* and *Chromobacterium violaceum*.

Discussion

Any type of brain insult is the pre-requisite for bacterial meningitis that require urgent diagnosis and aggressive therapy.⁹ The choice of initial antimicrobial therapy is based on the most common isolated pathogen, widespread in a specific ecological area and its antibiotic sensitivity pattern in that particular area.^{8,9}

Fig-1 Demonstrates that out of total 511 CSF

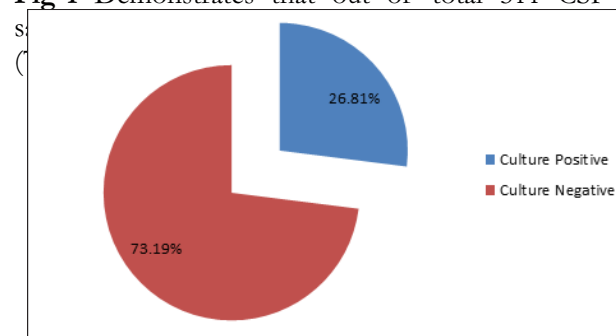


Fig-1: Number of cultures positive for bacterial growth (n= 511).

Various microorganisms isolated and their percentage is shown in **Table-1**. In our study, the meningitis due to *Acinetobacter* species and *Pseudomonas* species account for 28% and 23.02% cases. In a study conducted by Sonavane and Baradkar, the isolation rate of *Acinetobacter* and *Pseudomonas* is of the order of 20.93% and 23.25% respectively.¹⁵ In our study, the meningitis caused by fermenting gram negative rods such as *E.coli* and

and *Klebsiella*^{17,18} account for 4 (16.6%) and 3 (12.5%) cases respectively.¹⁹ In our study, Staphylococci caused 21.58% of the bacterial meningitis cases. In a study conducted by Gitali

Table-1: Various bacterial pathogens isolated from CSF samples (n= 137).

Pathogen Isolated	Frequency	Percentage
<i>Acinetobacter baumannii</i>	39	28.05%
<i>Pseudomonas aeruginosa</i>	32	23.02%
<i>Staphylococcus species</i>	30	21.58%
<i>Klebsiella spp</i>	16	11.51
<i>E.coli spp</i>	12	8.63%
<i>Ralstonia picketti</i>	2	1.43%
<i>Streptococcus Pneumoniae</i>	2	1.43%
<i>Ochrobactrum anthropi</i>	1	0.7%
<i>Aeromonas salmonicida</i>	1	0.7%
<i>Vibrio metschnikovii</i>	1	0.7%
<i>Chromobacterium</i>	1	0.7%
<i>Violaceum</i>	1	0.7%
<i>Pasteurella spp</i>	1	0.7%

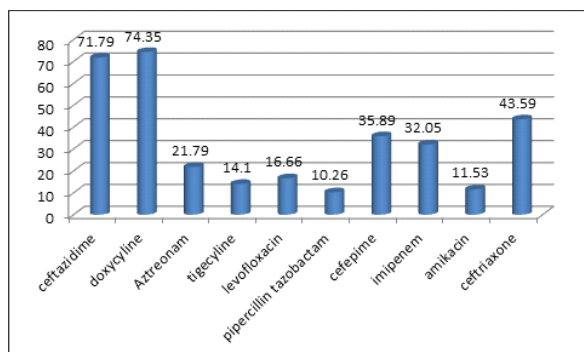


Fig-2: Antibiotic resistance pattern of non lactose fermentors (n=78)

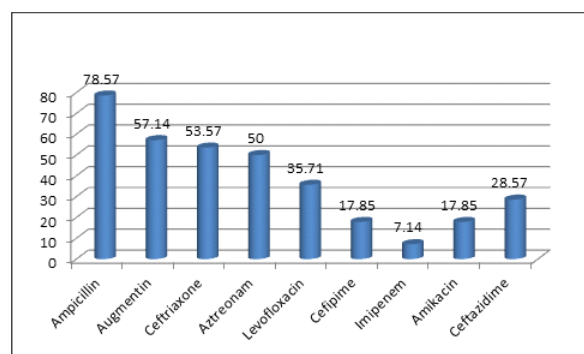


Fig-3: Antibiotic resistance pattern of lactose fermentors (n= 28)

Bhagawati and her colleagues in Guwahati Medical College and Hospital, Guwahati, Assam, India, staphylococci are responsible for 15 (29.41%) cases.¹⁰ There is 1.43 % case of meningitis caused by *Streptococcus pneumoniae* in our study. In literature, *Streptococcus pneumonia* is recognized as an proven cause of meningitis.^{11,12}

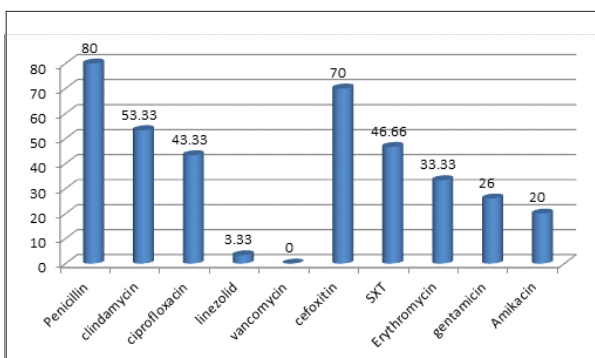


Fig-4: Antibiotic resistance pattern of Staphylococci (n= 30)

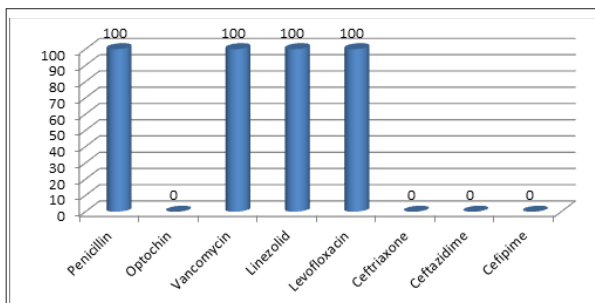


Fig-5: Antibiotic resistance pattern of Streptococci (n=2).

In our study, there is 0.7% case of meningitis caused by *Ochrobactrum anthropi*. In literature, Chang and his colleagues mentioned about three cases of meningitis caused by *Ochrobactrum anthropi*.²⁰ Meningitis caused by pasteurella account for 0.7% cases in our study. Pasteurella species is the cause of meningitis in literature as in a case report written by Minton published in post graduate medical journal in which a 38 year old man had developed meningitis by Pasteurella following dog bite.²¹ Another brief report was written by Christelle Guillet in 2007 in clinical infectious diseases about Pasteurella sepsis and meningitis.²² In our study, 0.7% case was caused by *Chromobacterium violaceum*. There were several cases reported in India in a study conducted by Karthik and his friends.²³ In our study, 1.43% cases of meningitis were caused by *Ralstonia picketti*. In literature, *Bonatti* wrote in his case report about *Ralstonia meningitis*, isolated in United States in 2009.²⁴ There is 0.7% case of meningitis caused by *Vibrio metschnikovii* in our study. Kim and Kim

performed a study in Korea, demonstrating *Vibrio vulnificus*, a cause of meningoenphlitis²⁵. There is 0.7% case of *Aeromonas salmonicida* meningitis in our study. In literature, recently a case of *Aeromonas hydrophila* meningitis has been reported in India²⁶. Antibiotic resistance pattern of non lactose fermenters is shown in **Fig-2**. Non fermenter gram negative rods are most resistant to doxycycline and most sensitive to piperacillin tazobactam. Antibiotic resistance pattern of lactose fermenters is shown in **Fig-3**. The lactose fermenters are most resistant to ampicillin and most sensitive to imipenem. Antibiotic resistance pattern of Staphylococci is shown in **Fig-4**. Most of the Staphylococci isolates were methicillin resistant Staphylococci (MRSA) and most were sensitive to vancomycin and

linezolid.

Conclusion

Bacterial meningitis caused by gram negative bacilli is most common in our setup. Gram positive bacteria are most resistant to Penicillins and most sensitive to Vancomycin and Linezolid. The lactose fermenter gram negative rods are most resistant to Ampicillin and most sensitive to Imipenem. Non fermenter gram negative rods are most resistant to Doxycycline and most sensitive to Piperacillin Tazobactam.

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