RESEARCH ARTICLE

BACTERIAL ISOLATES AND THEIR SENSITIVITY PROFILE FROM CSF SAMPLES – A 5 YEAR STUDY AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Objective: *The aim of the study was to evaluate the frequency of various bacteria isolated in cases of meningitis and their sensitivity profile.*

Study design: *Descriptive Cross sectional study*

Study place & duration: *Study place & duration: This study was carried out at Fauji Foundation Hospital Rawalpindi over a period of 5 years (January 2017 and December 2021).*

Methodology: *All CSF samples received in microbiology Lab during the study were centrifuged at 2500 r/min in the laboratory of Microbiology. The supernatant fluid was discarded. The sediment was inoculated on blood agar, Chocolate agar and MacConkey's agar and were incubated at 37^{0}C for 24-72 hours aerobically and in 5 % CO2. Cultures that yielded growth of any bacteria were further proceeded by standard microbiological methods of bacterial identification and sensitivity testing according to CLSI guide lines.*

Results: *A total of 2000 CSF samples were received during the study period examined. One hundred and fifty seven samples yielded growth of bacteria. Pseudomonas aeruginosa (30%) was the most frequent organism isolated from CSF from patients followed by methicillin resistant Staphylococcus aureus MRSA (13%).*

Conclusion: *Pseudomonas aeruginosa was the most frequent organism isolated from CSF of patients of meningitis followed by MRSA.*

Key words:

Cerebrospinal fluid, Hospital Acquired Infection.

INTRODUCTION

Medical emergencies like bacterial meningitis always have significant mortality rates. The most common and effective way to confirm the diagnosis of bacterial meningitis is cerebrospinal fluid (CSF) culture. The subarachnoid spaces of the cranium and spine, as well as the ventricles of the brain, are home to the ultra-filtrate of plasma known as cerebrospinal fluid (CSF). CSF helps to remove metabolic waste from the brain, including peroxidation products, glycated proteins, extra neurotransmitters and debris from the ventricular lining, including bacteria, viruses, and other pointless substances.[1] A prevalent clinical infectious disease that is mostly brought on by bacteria entering the brain and spinal cord is central nervous system infection (CNS). Different microorganisms can infect CSF and cause illness.[2] Bacterial meningitis is the one of the serious diseases associated with substantial morbidity and mortality rates. The most common organisms isolated

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from samples of bacterial meningitis are *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae*.[3] Microbiology laboratories commonly receive CSF or blood specimens from patients with meningitis or unexplained febrile illness. Presumptive identification of *Neisseria meningitidis, S.pneumoniae,* and *H.influenzae* is sometime possible on the basis of cytological/microscopic examination of CSF.[4] Different methods exists for the diagnosis of bacterial meningitis of which CSF culture is still considered the gold standard.[5] This treatment is often complicated by infection of shunt usually because of biofilm forming bacteria.[6] Catheters used as CSF shunts are foreign bodies that can become infected with bacteria. The most common organisms in this case is *Coagulase negative Staphylococci* followed by *Staphylococcus aureus*.[7] Gram negative bacteria and candida species are sometime also isolated. These organisms are usually thought to be introduced at the time of surgery. The incidence of shunt infections ranges from 10% -22% and its incidence is around 6% per procedure. Around 90% of these infections occur usually in first 30 days after the procedure. *Neisseria meningitidis, S. pneumoniae, Hemophilus influenzae, E. coli,* and *K. pneumoniae* were shown to be the most frequently isolated bacterial etiologic agents from CSF cultures, according to recent investigations. The introduction of vaccines in the Extended Program of Immunization (EPI) against *Pneumococci* and *H. influenzae* type b has significantly reduced the burden of bacterial meningitis caused by these agents. In past 20 years the agents of disease, epidemiology and strategies of treatment for bacterial meningitis have changed very significantly. The organisms like *Streptococcus pneumoinae, Hemophilus influenzae* that were considered important isolates for CSF infections but are now rarely isolated from CSF samples in our setup. Etiological pattern is changing so there is a need of finding out current pattern of isolates and their antimicrobial profile from CSF samples. The changing etiological pattern and changing antimicrobial profile needs serious attention, so as to give proper care to our patients.[8] In order to provide useful information for developing strategies for preventing pathogens and enhancing evidence-based treatment, we thoroughly investigated antimicrobial resistance patterns and the common pathogenic bacteria of positive cerebrospinal fluid cultures in tertiary care hospital Rawalpindi city, Punjab province over a period of five years.

MATERIAL AND METHODS

This study was carried out at Department of Microbiology at Fauji Foundation Hospital(FFH) Rawalpindi, Pakistan. This was a descriptive cross sectional study. Ethical approval was taken prior to starting the study from Institutional Ethical FFH Review Board. All CSF samples received in microbiology Lab during the duration of study (Jan 2017-Dec 2021) were included in the study. All duplicate samples and samples of patients already on antibiotics were excluded from study. A total of 2000 CSF samples were received during the study. Out of these samples around 18 duplicate samples were excluded from study.

CSF routine examination was done on all samples. The received CSF samples were centrifuged at 2500 r/min in the laboratory of Microbiology. The supernatant fluid was discarded. The sediment was inoculated on blood agar, Chocolate agar and MacConkey's agar and were incubated at 37^0C for 24-72 hours aerobically and in 5 % CO2.

Cultures that yielded growth of any bacterial agent were further processed by standard microbiological methods of bacterial identification like Gram Stain, catalase test, Coagulase test, API 20E and API 20NE (Biomeurix). All the isolated pathogens were identified to the species level. All microorganisms were subjected to the antimicrobial sensitivity testing by modified Kirby Bauer Disc diffusion method on Muller Hinton agar (Oxoid). The isolates were incubated with antimicrobials discs for 18-24 hours at 37^0C. The applied antimicrobials discs along with their strengths were as follows: Amikacin 30µg, Ceftazaidime 30 ug, Ceftriaxone 30µg, Ciprofloxicin 5µg, Gentamycin 10µg, Imipenem 10µg, Linezolid 20µg, Meropenem 10µg, Penicillin 10µg. The results of the antimicrobial sensitivity were interpreted according to the Clinical Laboratory Standards Institute Criteria (CLSI 2021).[9] Dependent variables like frequency of bacterial meningitis causing bacteria, pattern of their antimicrobial resistance and Independent variables like socio demographic data of patients were calculated using the SPSS ver 21.

RESULTS

A total of 2000 CSF samples were received during the study period. From these samples only 157 samples yielded the growth of pathogens. Over the period of 5 years the culture positive ratio has increased significantly as depicted in the Fig 1.

Figure 1: Number of positive cases of CSF culture in five years.

The ratio of positive cases in female neuro surgery ward was much higher from rest of the wards/ departments of the Hospital.(Fig 2.)

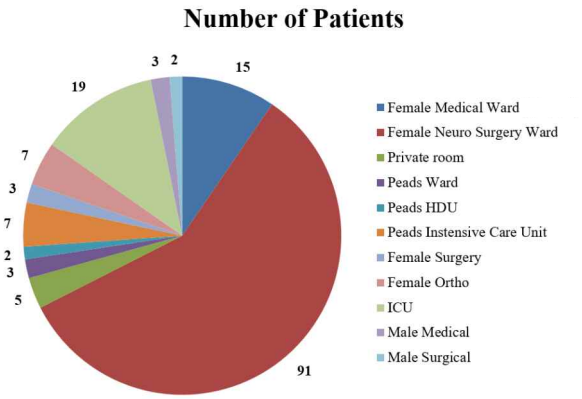

Figure 2: Number of positive cases from various wards.

Table I: Drug sensitivity Analysis of Gram Negative Pathogens isolated from CSF

Drugs	E.*Coli* (25%)	*Klebseila pneumoniae* (21%)	*Pseudomonas aeruginosa* (36%)	*Acinatobacter baumannii* (18%)
Amikacin30µg	28%	26%	53%	10.5%
Ceftazidime 30µg	-	-	69%	-
Ceftraiaxone 30µg	14%	-	14.7%	5.2%
Chlorempheniсol 30µg	28%	-	-	15.7%
Ciprofloxicin 5µg	8%	17.3%	76.4%	26%
Gentamycin 10µg	24%	17.3%	61%	15.2%
Imepenem10µg	76%	74.7%	100%)	46%
Meropenen µg	74%	74.7%	98%	44%
Polymixin B 300µg*	40%	39%	28(82.3%)	28.5%
Sulzone µg	18%	-	19(55.8%)	-

*MICs were not done.

In cerebrospinal fluid culture positive specimens, *Pseudomonas aruginosa* was the most prevalent organism (36%) followed by *Escherichia coli* (25%) among Gram negative organisms. Among Gram organisms *Methicillin resistant Staphylococcus aureus (MRSA)* was the most frequently isolated. Patients under 25 years old had the majority of positive cultures. G-ve bacteria differ significantly by gender, age, and season; *Escherichia coli* was more prevalent in people under the age of 15 and *Pseudomonas* species were more prevalent in those under the age of 25. Summer season marked the highest percentage of positive cultures.

Table II: Drug sensitivity Analysis of Gram Positive Pathogens in CSF

Drugs	*MRSA* 52%	*Staphylococusaureus* 13%	*Enterococcusfaecalis* 35%
Chloramphenicol 30µg	50%	80%	56.25%
Ciprofloxacin 5µg	25%	60%	25%
Gentamicin 10µg	75%	60%	6.25%
Penicillin 10µg	-	60%	
Vancomycin 30µg*	100%	100%	90%
Cotrimoxazle 25µg	40%	60%	-
Lineziolid 20µg	80%	80%	62.5%
Meropenum 10µg	-	-	31.25%
Erythromycin 15µg	-	-	12.5%

*disk diffusion method

DISCUSSION

One of the most fatal illnesses is bacterial meningitis. Even while effective antimicrobials and immunizations have decreased incidence and improved patient care, the mortality rate can still reach 34% in developing countries like ours. Meningitis causes severe side effects like brain damage, hearing loss, long-term consequences like epilepsy, and mental instability etc. All these complications are more common if bacterial meningitis is acquired during early child hood. Despite the availability of many broad spectrum antibiotics there is high level of resistance in pathogens causing CSF infections. The mortality associated with these CSF infections ranges from 16-32%.[10-16] The incidence of these infections after surgery i.e 8% still remains very high in developing world. The frequency of CSF culture positivity was constantly high in our setup. It is necessary to analyze the epidemiology and drug resistance of CSF microbial infection.[17] Despite improvements in the epidemiology of bacteremia in this population, the prevalence of group B Streptococcus remains the principal cause of meningitis.[18] In 90% of cases of meningitis, bacteria have been found to be the cause, 86% of cases were brought on by *H. influenzae*, 75% by *N. meningitidis*, and 50% by Gram-negative bacilli.[19] Due to misuse of antibiotics in our country we analyze that the documented pathogens which were present in positive cultures of our setup were not seen in our hospital. On the other hand the resistant pattern of antibiotics in our setup were little different from our neighboring countries as indicated by studies come out from India and Iran[20] Our study identified *Pseudomonas aeruginosa*, as the most prevalent microorganism and it was identified in 36% of culture in this study. *Klebsiella pneumoniae* is also seen very commonly isolated from CSF samples in patients with critical medical conditions, leading to high mortality rates (33-48%).[21-22] Timely detection of such pathogens and having a consideration for them in empirical therapy may improve critical outcomes.[23] More than half of the patients in the current study have a long duration of illness and empirical antibiotic treatment before hospital presentation, resulting in a poor outcome of patients with bacterial meningitis which depends on a timely selection of appropriate antibiotics. This result was different from studies conducted in Ethiopia, Denmark and review study from which showed a positive relation of short duration illness and timely selection of antibiotics. This difference might be the difference in the use of standard guidelines for clinical presentation and lack of culture for etiologic identification and antibiotics susceptibility testing at all levels.The main issue in Klebsiella isolates is the emerging resistance against beta lactams and even carbapanems.[24] A meta-analysis study carried out in Pakistan revealed that pathogenic E.coli and Klebsiella species isolated from CSF samples and other important samples are highly resistant organisms and they were resistant to Penicillins 100%, ampicillin 91%, amoxicillin 85%, cefotaxime 82% and cefaclor 100%.[25] We found MRSA and *S. aureus* to be highly resistant to most of antibiotics. Similarly, in various reports from Pakistan reveal high resistance of *Staphylococcus aureus* to Penicillins (98%), Cefoxitin and other antimicrobials.[24] Therefore, evidence-based management of bacterial meningitis by using culture and antimicrobial susceptibility tests should be strengthened before empirical treatment. Moreover, all the bacterial isolates in this study had decreased susceptibility to the routinely prescribed drugs like ciprofloxacin and ceftriaxone. And further study with a better design, large sample size and survey of antimicrobial susceptibility at large scale should be done to draw important information.

The high resistance level was due to easy availability of antimicrobials, self-medications and taking incomplete dosage of antimicrobials.[25]

CONCLUSIONS

Pseudomonas aeruginosa was the most frequent organism isolated from CSF of patients of meningitis followed by MRSA.

RECOMMENDATIONS

In our country, additional studies are recommended for enhancing the surveillance and timely reporting of antibiotic resistance.

LIMITATIONS

Our research has potential limits. This hospital is for retired military person's families and their children. So the ratio of female and children patients was higher than men.

Conflict of interest: The authors declared no conflict of interest.

Authors' contributions:

Muhammad Moaaz Ali: Conception of study / Designing / Planning, Experimentation / Study Conduction, Analysis / Interpretation / Discussion, Manuscript Writing, Material Analysis

Umme Farwa: Analysis / Interpretation / Discussion, Manuscript Writing, Critical Review

Haider Ali: Analysis / Interpretation / Discussion, Material Analysis

Saima Ishtiaq: Experimentation / Study Conduction, Analysis / Interpretation / Discussion, Critical Review, Material Analysis

Saima Syed: Experimentation / Study Conduction, Analysis / Interpretation / Discussion, Critical Review, Material Analysis

Samina Javed: Analysis / Interpretation / Discussion, Critical Review, Material Analysis

Shahid Ahmed Abbasi: Conception of study / Designing / Planning, Analysis / Interpretation / Discussion, Critical Review

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