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BACTERIAL PROFILING AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN ACUTE BACTERIAL MENINGITIS PATIENTS AT A TERTIARY HOSPITAL IN PAKISTAN.

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Abstract

Background: Bacterial meningitis is a life-threatening type of inflammation of the membranes (meninges), which surround and shield your brain and spinal cord. Bacterial meningitis can be fatal within hours, but most patients recover. However, lifelong impairments like learning difficulties, hearing loss, or brain damage may persist. Bacterial meningitis is particularly dangerous, with 1 in 6 cases resulting in death and 1 in 5 having serious side effects. Safe, affordable vaccinations are the best way to provide long-lasting protection.

Objectives: Study objectives are:
To isolate and characterise bacterial pathogens from CSF and determine the antimicrobial susceptibility profiling of the isolated bacteria.

Study Design and Setting: A cross-sectional study was conducted at the Paediatric Department of Chandka Medical College (CMC) Shaheed Mohatrma Benazir Bhutto Medical University (SMBBMU) Larkana during the period January 2019 to December 2021.

Materials and Methods: Cerebrospinal fluid (CSF) samples from 78 clinically suspected cases of acute bacterial meningitis were processed for isolation, identification, culture, and sensitivity. The data was analysed using SPSS version 20.

Results: A total of 78 patients suspected of having meningitis were included. Of these mean ages of 3.06±0.402 years (range 2 months–15 years), according to age group 2–12 months, children seemed more affected, while gender-wise, 46 (59%) were males and 32 (41%) were females. According to the EPI schedule, only 42 (53.8%) children were fully vaccinated.
The common clinical presentations were fever 72 (92.3%), fits 46 (58.97%), lethargy 39 (50%), headache 26 (33.3%), disorientation 17 (21.79%), and unconsciousness 15 (19.23%). Of the 78 CSF specimens, 17 (21.79%) had a positive bacterial culture, while the remaining samples exhibited no growth. On Gram stain, there were 13 (76.47%) cases of Gram positivity and 4 (23.52%) cases of Gram negativity. The common pathogens identified on CSF culture were *Streptococcus pneumonia* in 5 (29.41%), followed by *Coagulase-negative Staphylococci* (CoNS) in 4 (23.52%), *Staphylococcus aureus* in 4 (23.52%), *E. coli* in 3 (17.64%), and *H. influenzae* in 1 (5.88%). *Coagulase-negative Staphylococcus*, *Staphylococcus aureus*, and *Escherichia coli* show higher resistance rates, while *Streptococcus pneumoniae* and *H. influenza* exhibit predominant sensitivity.

Conclusion: Acute bacterial meningitis primarily affects children under 1 year of age. CSF culture revealed both Gram-positive and Gam-bacteria, i.e., *Streptococcus pneumoniae*, the most common pathogen in CSF culture. *Coagulase-negative Staphylococcus, Staphylococcus negative s aureus and E. coli* show higher resistance rates, while *Streptococcus pneumoniae and H. influenza* exhibit predominant sensitivity.

Keywords: Meningitis, Bacterial, EPI, Immunized, Non-immunized.

INTRODUCTION

Acute bacterial meningitis (ABM) is a dangerous disease occurring throughout the world. ABM is defined as an acute inflammatory process affecting the layers that cover and protect the brain and spinal cord, known as the meninges (Motamedifar et al., 2015).It is a potentially fatal condition that requires immediate diagnosis and treatment. Bacterial meningitis continues to be a serious infection with an elevated mortality rate and over time neurological disabilities, such as mental retardation, developmental delay, and cerebral palsy (CP), despite advancements in treatment and vaccination. The yearly incidence of pneumococcal and meningococcal meningitis is 4 to 5 cases and 2.5 cases, respectively, per 100,000 children under the age of five. Thus, to guarantee proper management, a precise diagnosis concerning the key etiological agents is required.(Mann, and Jackson, 2008; Namani, et al., 2013).

Patients suffering from ABM usually present to the clinicians with fever, headache, vomiting, lethargy, loss of consciousness, fits, skin rash and neck stiffness, especially in older children. However, refusal to feed, photophobia, bulging anterior fontanel, poor activity, and abnormal movements are presenting features in infants and small children (CDC, 2014; Attia Bari et al., 2017). Meningitis is categorized into different types, i.e., bacterial, also called pyogenic, and aseptic meningitis. Viral, fungal, malignant diseases, systemic autoimmune infections, and certain drugs are the main causatives for aseptic meningitis (Mace, 2008).

In children, the infectious agents responsible for meningitis in the underdeveloped world are *Streptococcus pneumoniae* and *Haemophilus influenza type b* (Singhi, et al., 2007; Nhantumbo, et al., 2016). Other common organisms are *Neisseria meningitides (N. meningitides), Streptococci group B,* and *Listeria monocytogenes* (Davison and Ramsay, 2003). To minimize complications and adverse outcomes, rapid clinical assessment and meticulous measures are necessary for appropriate antimicrobial therapy (Macaluso, et al., 1996). In a large survey conducted on ABM with the collaboration of the World Health Organization (WHO) and the Centre for Disease Control (CDC) from 2002 to 2008, 74,515 cases were collected. Out of these 4,674 (7%) cases, culture-proven for three common culprits, i.e., *S. pneumoniae moniae, H. influenza type b,* and *N. meningitides* (CDC, 2009). A prevalence of 3-5% was observed regarding ABM conducted in the year 2000 at the Children Department of Lady Reading Hospital Peshawar in Pakistan (Ahmad and Khan, 2011).

The predisposing factors that lead to the development of ABM in individuals are grouped mainly into age, quality of life standards, immunization status, frequent infections, otitis media, and poor immunity (Mace, 2008).To rationalize treatment, we have to perform in vitro antimicrobial susceptibility testing for the microorganisms. Bacterial cerebrospinal fluid (CSF) analysis is a main diagnostic tool, and CSF culturing is the gold standard test for ABM (Brouwer, et al., 2010).In 50–80% of cases, bacteria can be isolated using Gram staining, and 80% is the maximum CSF culture positivity.(Neuman, et al., 2008). Patientsalready on antibiotics therapy will affect the sensitivity of both Grams staining and culture to less than 50%; in such a situation, CSF chemical and microbiological examinations will be less reliable (Nigrovic, et al., 2008). In one of the studies from Pakistan regarding bacterial isolation and antimicrobial susceptibility in ABM cases, they found 17 different types of bacterial isolates. Almost all the bacterial isolates were susceptibility to vancomycin, combination of sulbactam plus cefoperazone and meropenem, however, cefotaxime and ceftriaxone were less susceptibility; more than 55% of S. pneumoniae were not responding to penicillin (Rezaeizadeh, et al., 2012; Tajdin, et al., 2013).

Rationale of study:
The goal of the current investigation is to identify a novel trend or profile of bacterial isolates. their culture sensitivity, drug-resistant strains, and possible associated risk factors in childhood ABM, especially in the Larkana region. Therefore, this study will also enable us to compare the occurrence of ABM between immunised and unimmunised children in the Larkana region and the antimicrobial susceptibility of bacteria responsible for ABM.

ORIGINAL STUDY

Objectives:
To isolate and characterize bacterial pathogens from CSF and determine the antimicrobial susceptibility profiling of the isolated bacteria.

Operational Definition:
Acute Bacterial Meningitis Cases were considered if the patient was febrile with a temperature > 38 °C plus one or more of the following symptoms or signs:**such as**neck stiffness, convulsions, lethargy, bulging fontanels, poor sucking, unconsciousness, coma, rash, and further laboratory evidence of CSF, i.e., decreased sugar, increased proteins, and elevated differential Leukocyte count (Moïsi et al., 2009).

MATERIALS AND METHODS

Study Design: A descriptive cross-sectional study

Study Setting and Duration
The study was conducted at the Paediatric Department of Chandka Medical College (CMC) Shaheed Mohatrma Benazir Bhutto Medical University (SMBBMU) Larkana during the period January 2019 to December 2021.

Sampling technique:
Non-probability convenience sampling

SAMPLE SELECTION CRITERIA

All patients from the age of one month up to fifteen years with ABM of either sex, belonging to the Larkana Region only, having no history of I.V. antibiotics in the last 72 hours, and willing to participate in this study were included.
Patients with congenital defects of the central nervous system and spinal cord or a recent history of trauma and fits were excluded.

DATA COLLECTION

A total of 78 CSF samples were gathered from the Paediatric department of Chandka Medical College Larkana and private Paediatric clinics in the Larkana. All information is kept confidential, and patient/guardians are updated regarding relevant information for management purposes. A pre-described questionnaire was used to collect data such as age, sex, residence, clinical symptoms, signs, and risk factors in cases of ABM. The following actions were taken throughout the entirety of the study:
After lumber puncture, CSF specimens were collected in a clean tube using aseptic procedures, and they were quickly transferred within an hour to the CMC Pathology Laboratory in Larkana for analysis.

Physical (colour, pressure, volume), chemical (protein and sugar), and microbiological (TLC, DLC, Gram staining) analyses were performed on the CSF samples obtained from ABM patients (Vandepitte et al., 2003; CLSI, 2012).
In microbiological examination, inoculation was done on Mac Conkey agar, blood agar, eosin Methylene Blue (EMB) agar, nutrient agar, and chocolate agar at 37°C for 18 to 24 h. (Vandepitte et al., 2003; CLSI, 2012).
Identification of bacterial isolates was carried out by applying different biochemical reactions, e.g., coagulase tests, catalase tests, bile solubility tests, indole production tests, and oxidase tests.
The antimicrobial drug susceptibility of the isolates was tested by the Kirby-Bauer technique, and the results were interpreted according to the Clinical Laboratory Standards Institute guidelines. (Vandepitte et al., 2003; CLSI, 2012).

Result:
During the two-year study period, a total of 78 patients were selected according to ABM criteria. Gender-wise, male 46 (59%) patients participated more as compared to female 32 (41%). Their mean age was 3.06±0.402 years (range: 2 month–15 years), while according to age-wise group distribution, the highest numbers of children were in the two-month to one-year age group 37 (47.4%), after which 22 (28.2%) seemed among the 1–5 year age group, 16 (20.5%), and 3 (3.8%) seemed among the 6–10 year and 11–15 year age groups, respectively. (Table-I) (Fig-I). The most common presenting symptom was fever; 72 (92.3%); fit 46 (58.9%); 10 (12.8%), 39 (50%), 17 (21.79%), and 15 (19.23%) had presented with refusal to feed, lethargic, disorientation, and unconsciousness, respectively. Such as the other clinical presentation, excessive crying (16 (20.21%), frontal budging 13 (16.66%), headache, and SOMI both were 26 (33.33%). Only one case presented with a rash (1.28%) (Table II). Furthermore, at other frequencies, only 42 (53.8%) of children were fully vaccinated, while the distribution of socioeconomic conditions was 52 (66.67%) poor and 26 (33.33%) belonged to middle families, whereas no economically high-status patients were observed in the study.
In the study on parental education, 50 (64.11%) were educated, while only 4% and 5% of parents were graduates and intermediate, respectively. (Table I). Of the 78 CSF specimens, 17 (21.79%) had a positive bacterial culture, while the remaining samples exhibited no growth. (Figure-II) On Gram stain, there were 13 (76.47%) cases of Gram positivity and 4 (23.53%) cases of Gram negativity. The common pathogens identified on CSF culture were *S. pneumoniae* in 5 (29.41%), followed by *Coagulase-negative staphylococci (CoNS)* in 4 (23.52%), *S. aureus* in 4 (23.52%), *E. coli* in 3 (17.64%), and *H. influenzae* 1 (5.88%). (Fig-III)
Antibiotic sensitivity testing was conducted using the Kirby-Burr method on Müller Hinton agar, and the respective antibiotic profiles are detailed in Table III. The following 10 antibiotics were applied: Gentamicin, Penicillin, Ampicillin, Ceftriaxone, Cefuroxime, Vancomycin, Cefotaxime, Meropenem, Chloramphenicol, and Novobiocin
The study reveals varying antibiotic susceptibility and resistance patterns among different pathogens. *Coagulase-negative Staphylococcus*, *Staphylococcus aureus,* and *E. coli* show higher resistance rates, while *Streptococcus pneumoniae and H. influenza* exhibit predominant sensitivity, as shown in Table III.
One of the *coagulase-negative staphylococcus* isolates demonstrated resistance to all antibiotics. The second was only susceptibility to gentamicin but resistance to the other nine drugs. The third isolate resisted Novobiocin and cefotaxime but was susceptible to all, and the fourth one resisted ampicillin, cefuroxime, vancomycin, chloramphenicol, gentamicin, and Novobiocin while being susceptible to the remaining.
The first culture of *E. coli* was resistant to all of the antibiotics. The second one was resistant to all antibiotics except gentamicin alone. The third was susceptibility to ampicillin and gentamicin only.
For *Staphylococcus aureus* isolates:
The first was susceptibility to cefuroxime, meropenem, and Novobiocin but resistance to all others. The second isolate was susceptible to ampicillin, vancomycin, gentamicin, and Novobiocin but

resistant to the others. The third isolate was resistant to penicillin, ceftriaxone, cefotaxime, and meropenem and resistant to the remaining antibiotics. The last isolate was sensitive to vancomucin, ceftriaxone, cefotaxime, and meropenem while being resistant to others.
Out of five isolates of *S. pneumoniae, two* were susceptible to all antibiotics; one isolate was resistant to ampicillin only,whereas the other two were resistant to ampicillin and pencillin.
Only one *H. influenza* was isolated which was susceptibleto all antibiotics except ampicillin and cefuroxime.

DISCUSSION

This is a result of the constant evolution of bacterial resistance to antibiotics. This means that drug-resistant infections, like meningitis, could kill ten million individuals annually worldwide by the time the current generation of first-time parents becomes first-time grandparents. [23] The demographic analysis reveals a significant percentage of male patients. Researchers Attia Bari et al. from Pakistan observed a preponderance of males, and Nguyen-Huu, Chau Duc, et al. from Vietnam found that male children had higher positivity rates (60%) than female children. [24,25,34]
Bacterial meningitis is more common in infants under 1 year of age and young people ages 16 to 21 years. [26] In the present study, the mean age of the study patients was 3.06±0.402 years, with a predominant population below 1 year of age, as was seen in many studies. [27,34] In the Pakistani study by Ayud, A., et al., 47.8% of the children were 1 month to 12 months old. [28] It is suggesting that the younger the age, the greater the risk of getting infection universally. This may be because, in infancy, the ability to resist meningeal infection is less considering the virulent nature of the etiological organism, thus underscoring the severity of the disease.[29]
Immunisation safeguards future generations by reducing and, in some instances, eliminating many diseases that killed or severely disabled people just a few generations ago. Like many other studies, the majority of children in our study—about 53%—were vaccinated. A similar study showed that 51.1% of the children in the Mustafa, Anum, et al. study had received all recommended vaccinations. [30] Meningitis deaths in children in Brazil have decreased by 50% since the introduction of pneumococcal and meningococcal vaccinations into the childhood immunisation programme. [31]The patients presented various clinical characteristics. The most common symptoms were fever, headaches, and fits. Videholm, Samuel, et al.'s findings were comparable to this.[32] Additionally, in our study, low parental education and socioeconomic factors were significantly associated with a higher risk of ABM among children; similar risk factors were observed in many studies. [32,33] In a neighbouring study conducted in India, 25% of the cases were ulture-positive, with an almost similar pathogen pattern to our study. [35] Another Indian study shows similar culture positivity with the organism pattern of close resemblance, in which coagulase-negative staphylococcus (22.2%) is most common, followed by *E. coli (18.5%)* and S. aureus (18.5%). [36]
As far as the susceptibility of isolates is concerned, we found that staphylococcal isolates have shown increased resistance to most of the common drugs: 100% to chloramphenicol and 75% to ampicillin, pencillin G, cefuroxime, and gentamicin, resembling the study conducted in Quetta, Pakistan. 37 In the Hongchao et al. study conducted in China,the susceptible rates of *Streptococcus pneumoniae* isolates to penicillin G, chloramphenicol, and ceftriaxone were 68.8%, 87.5%, and 81.3%, respectively. Gentamycin and vancomycin were identified as the most effective antibiotics for *Streptococcus pneumoniae*, each with susceptible rates of 100% resembling to our study.[38] *E.coli* in our study is highly resistant to all drugs except vancomycin (100% susceptible) and gentamicin (67%) which is resembling with Chineese study showing > 93.0% isolates were susceptible to, amikacin and carbapenems, and resistant to ceftriaxone, cefotaxime and ceftazidime.Ali SA et all in their study has shown similar results. [37,39]Like our study, Hongchao et al. also found that *Haemophilus influenzae* type b had high susceptible rate to most drugs except ampicillin, with a susceptible rate of 5.6%. [38]

CONCLUSION:

Acute bacterial meningitis primarily affects children under 1 year of age. CSF culture revealed both Gram-positive and Gram-negative bacteria, i.e., S. pneumoniae, the most common pathogen in CSF culture. *Coagulase-negative Staphylococcus*, *Staphylococcus aureus,* and *E. coli* show higher resistance rates, while *Streptococcus pneumoniae* and *H. influenza* exhibit predominant sensitivity. This study may play an important role in the diagnosis and more accurate treatment of patients suffering from acute bacterial meningitis, especially in our area.

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Table I: Variable of the Study

VARIABLE		N	%
Mean Age	3.06±0.402		
Age group	2-12 m	37	47.4
	1-5 y	22	28.2
	6-10 y	16	20.5
	11-15 y	3	3.84
Gender	Male	46	59.0
	Female	32	410
Vaccination	Yes	42	53.8
	No	36	46.2
Socioeconomic Status	Poor	52	66.67
	Middle	26	33.33
	High	0	00
Maternal education	Yes	50	64.11
	No	28	35.89

Table II Clinical presentation of ABM cases

CLINICAL PRESENTATION OF PARTICIPATANT OF STUDY		
CLINICAL PRESENTATION	NO	%
FEVER	72	92.30
REFUSED TO FEED	10	12.82
FITS	46	58.97
LETHARGIC	39	50.0
DISORIENTATION	17	21.79
UNCONSCIOUSNESS	15	19.23
EXCESSIVE CRY	16	20.21
BULGE FONTANEL	13	16.66
RASH	1	1.28
HEADACHE	26	33.33
SOMI	26	33.33

Table III: Drug resistant profile of the isolated bacterial strains from CSF specimen.

Antibiotics	*S. pneumoniae*		*S. aureus*		*CoNS*		*E.coli*		*H.influezae b*	
	S %	R%	S%	R%	S%	R%	S%	R%	S%	R%
Ampicilin	40	60	25	75	25	75	33.33	66.67	0	100
Pencilin G	60	40	25	75	50	50	0	100	100	0
Ceftriaxone	100	0	50	50	50	50	0	100	100	0
Cefuroxime	100	0	25	75	25	75	0	100	0	100
Vancomycin	100	0	50	50	25	75	100	0	100	0
Cefotaxime	100	0	50	50	25	75	0	100	100	0
Meropenem	100	0	75	25	50	50	0	100	100	0
Cholramphenicol	100	0	0	100	25	75	0	100	100	0
Gentamicin	100	0	25	75	50	50	66.67	33.33	100	0
Novobiocin	100	0	50	50	0	100	0	100	100	0

S= susceptibility, R=resistant
CoNS= coagulase-negative Staphylococci

Figure I

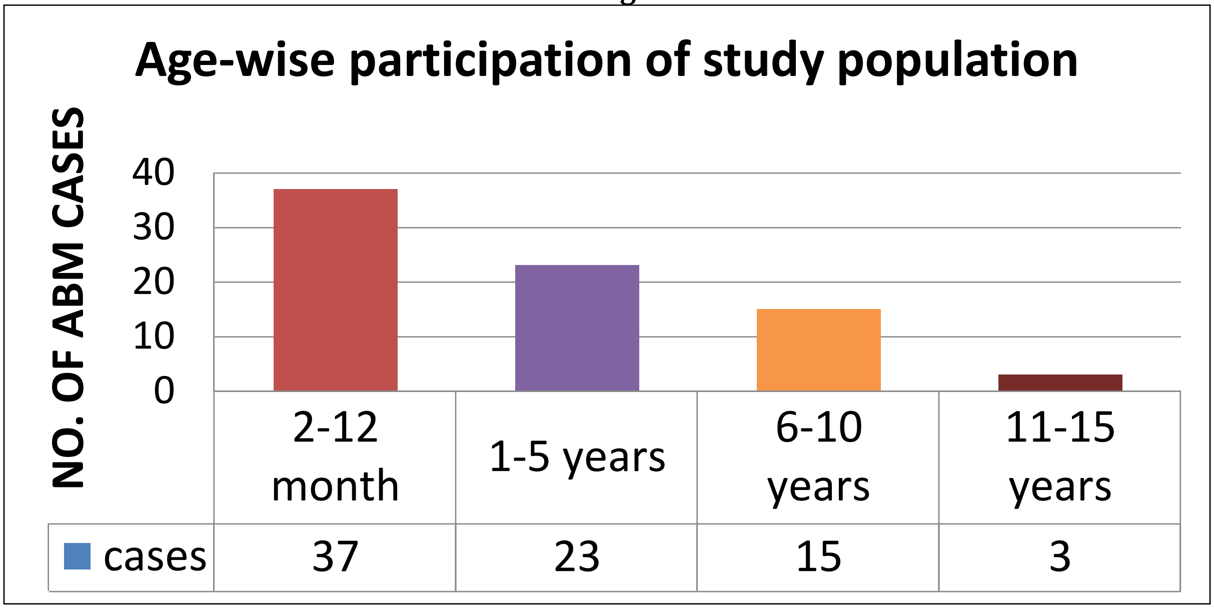

Figure II

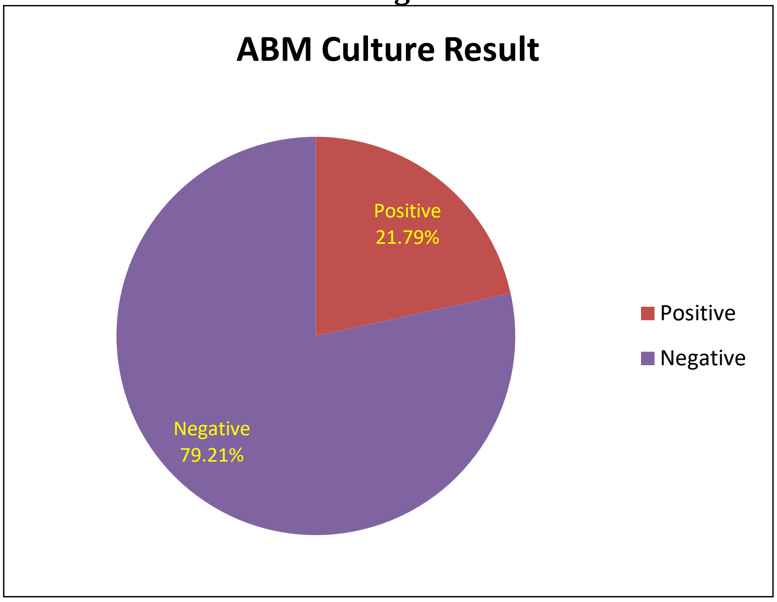

Figure III