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Original Research Article.

Microbiological Profile of Cerebrospinal Fluid (CSF) in Pyogenic Meningitis Patients at Tertiary Care Hospital

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ABSTRACT

Introduction: Acute bacterial meningitis is a life threatening illness that is prevalent worldwide. It is a medical emergency that needs early diagnosis and treatment.

Aims and objective: The study includes microbiological profile and antibiotic susceptibility pattern of the bacteria isolated in CSF in clinically suspected cases of pyogenic meningitis in children.

Methods and Materials: A total of 112 clinically suspected cases of meningitis in paediatric age group were included. It was a hospital based Descriptive cross sectional study from January 2017 to November 2017. All the specimens were processed by standard bacteriological techniques that include microscopy (Gram staining, wet mount, India ink), culture in blood agar, Mac Conkey agar, Chocolate Agar plates, Automated culture (BACTEC) method and etc.

Results: A total of 112 CSF samples were collected from suspected pyogenic meningitis patients. 27 cases were culture positive. Among suspected case there was male predominance (54.5%). Highest rate of culture positive cases were found in the age group of less than 01 months (33.3%). The most common organism isolated was *CONS* (22.2%) followed by *Escherichia coli* (18.5%).

Conclusions: Acute bacterial meningitis is a medical emergency and making an early diagnosis and providing early and accurate treatment, are lifesaving to reduce morbidity. This study may play an important role in the diagnosis and more accurate treatment for the ABM patients.

Keywords: Pyogenic Meningitis, BACTEC, Group B Streptococci, Kernig's Sign, Brudzinski's Sign.

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INTRODUCTION

Meningitis is an inflammation of the meninges, the membrane covering the brain and spinal cord. When a microorganism (usually a bacterium or virus) enters the subarachnoid space, there is inflammatory response in the meninges.[1] There is intense inflammation of meninges due to bacterial products such as to gram-negative lipopolysaccharide or gram positive peptidoglycan even after destruction of bacteria by antibiotic therapy.

The microbiological causes of meningitis include bacteria, viruses, fungi and parasites. Among these, bacterial meningitis include pyogenic meningitis and tubercular meningitis.

The most common etiological agents in neonates are group B Streptococci *(Streptococci agalactiae), Escherichia coli* and *Listeria monocytogenes*. *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* type–b are the common cause of pyogenic meningitis in infant and young children worldwide. Patients with ventriculo-peritoneal (VP) shunt are at risk of meningitis caused by coagulase negative Staphylococcus and Pseudomonas species. These bacteria reach the CNS either by haematogenous spread or by direct extension. In neonates the infection is acquired from the maternal vagina during normal delivery. The organisms that colonise the upper respiratory tract of infant and young children can also cause pyogenic meningitis.

Manifestation of pyogenic meningitis depends on the age of the patients. Headache, fever and altered sensorium are the important symptoms of pyogenic meningitis. Signs of meningeal irritation such as neck stiffness, Brudzinsky's sign and Kernig's sign or the Tripod phenomenon, in children are not specific for pyogenic meningitis. In one study pyogenic meningitis was present in only about 1/3rd of children with signs of meningeal irritation.[2] Older children and adults develop a stiff neck usually with fever and headache. Infants and young children may have a high or low body temperature, be irritable or drowsy, or have a poor appetite. Seizure occurs in 1/3rd of children suffering from pyogenic meningitis (caused by *S. pneumoniae* and *H. influenzae* type b).

Petechiae and purpura is common in patients with meningococcal meningitis. Signs in infants and neonates include fever, apnoea, seizure, a bulging fontanel and rash.

Epidemiology

Bacterial meningitis is the most common form of suppurative CNS infection, with an annual incidence in United States of >2.5 cases per 100,000 population.[3]

The use of (PCV7) heptavalent pneumococcal protein polysaccharide conjugate vaccine and Hib vaccine to infant causes dramatic decrease in rate of pyogenic meningitis caused by invasive pneumococcal disease and *Haemophilus influenzae* type-b. The incidence rate of *Streptococcus pneumoniae* meningitis are 228 per 100,000 in children at age of 6-12 month.[4]

The major risk factor for pyogenic meningitis is due to lack of immunity to specific pathogens, other risk factor include colonization of pathogenic bacteria, close contact of individual having invasive disease caused *by N. meningitis, H. influenza* type b. The mode of transmission is person to person.[4]

MATERIAL AND METHODS

It was a hospital based Descriptive cross sectional study conducted in Department of Microbiology, Rajendra Institute of Medical Sciences (RIMS), Ranchi. It included patients of all paediatric age group, from January 2017 to November 2017.

Inclusion Criteria

- Children up to 14 years age admitted to Department of Paediatrics, RIMS
- Clinical diagnosis made by paediatrician with symptoms and signs; history of high fever, intense headache, neck rigidity, refusal to feed, vomiting, drowsiness, convulsion, signs of meningeal irritation: Kernig's sign (with the hip joint flexed, extension at the knee causes spasm in the hamstring muscles) and Brudzinski's sign (passive flexion of the neck causes flexion of the thighs and knees) and altered sensorium was included in the study.

Exclusion Criteria

- Cases who were treated with antibiotics prior to admission in RIMS, Ranchi
- Patients on antipsychotic drugs and on any other medication
- Other systemic diseases like diabetes mellitus, nephritic syndrome, renal failure, heart failure and following head trauma were excluded from this study.

Sample Size

A total of 112 clinically suspected cases of meningitis in paediatric age group admitted to Department of Paediatrics, RIMS, Ranchi were recruited for the study.

Sample Collection and Laboratory Testing

Three vials of CSF samples were collected from the cases with clinical suspicion of meningitis. One vial was used for cytological examination, the other for bacteriological examination and the biochemical examination was done on the sample of third vial.[5]

The CSF bottles were transported without delay to the Microbiology Laboratory of the college and processed without any delay; whenever delay was anticipated the CSF samples were kept at room temperature.[6]

Physical Examination of CSF

CSF was classified as clear, opalescent, turbid, purulent, haemorrhagic and xanthochromic.

Processing of Sample and Identification

All the specimens were processed by standard bacteriological techniques that include microscopy (Gram staining, wet mount, India ink), culture in blood agar, Mac Conkey agar, Chocolate Agar plates, Automated culture (BACTEC) method and etc.

CSF is Divided Into Three Portions

One portion is centrifuged and Gram Stained smears was prepared from deposit. Second portion of CSF was inoculated either on blood agar, Mac Conkey agar plate, Chocolate agar plate or BD BACTEC Peds Plus culture vial. Third portion of CSF will be kept at room temperature.

Direct Microscopy

Includes Wet mount preparation, India ink preparation and Gram-stained smears preparation of CSF.

Culture

If bacteria were seen in the Gram-stained smear, the appropriate culture media was inoculated. If no organisms were seen, or if the interpretation of the Gram smear was unclear, it was desirable to inoculate a full range of media, including blood agar with a streak of *Staphylococcus aureus* to promote growth of *H. influenzae*.

The second portion of CSF was inoculated either on to the following media or in BD BACTEC Peds Plus culture vial.

- Blood agar (BA) plate incubated at 37^0C in 5-10% CO_2.
- Chocolate agar plate incubated at 37^0C in 5-10% CO_2.
- MacConkey agar incubated aerobically at 37^0C.

BHI broth incubated aerobically at 37^0 C. Inoculated primary plates were incubated for 48 to 72 hours. The plates were examined daily for 72 hours before reporting as negative.

BHI broth was incubated for 7 days and examined daily for presence of growth or turbidity and was considered negative at the end of 7 days of incubation.

BD BACTEC Peds plus culture vial were put in automated BD BACTEC FX 200™ System and was declared negative only if not indicated by the system at the end of 5 days of aerobic incubation.

OBSERVATIONS AND RESULTS

The present study was conducted in Department of Microbiology, Rajendra Institute of Medical Science (RIMS) Ranchi. During this period all patients who were admitted in the paediatric ward with signs and symptoms suspected of meningitis were included in the study. A total of 112 CSF samples were collected. 27 cases were culture positive. Among suspected case there was male 54.5% and female 44.5%. Among 27 culture positive cases there were 16 (59.25 %) males and 11 (40.75%) females.

Table 1: CSF Gram's stain smear study among the culture positive cases.

CSF Gram stain	Number of culture positive cases	Percentage
Gram positive cocci	14	52%
Gram negative bacilli	13	48%
Total	27	100

Table 2: Organism isolated in culture positive cases

Etiological agent	0-1month	1-12months.	1yrs. – 5 yrs.	5 yrs. – 14 yrs.	Total N(%)
Escherichia coli	03	02	00	00	5 (18.5%)
CONS	02	01	00	00	6 (22.2%)
Streptococcus agalactiae	01	00	00	00	1 (3.7%)
Streptococcus pneumoniae	00	01	01	00	2 (7.4%)
Klebsiella pneumoniae	02	01	00	00	3 (11.1%)
Staphylococcus aureus	00	01	01	02	4 (14.8%)
Pseudomonas aeroginosa	00	00	02	01	3 (11.1%)
Acinetobacter spp	00	01	01	00	2 (7.4%)
Enterococcus	01	00	00	00	1 (3.7%)
Total	09	07	06	05	27

Table 3: Antibiotic sensitivity pattern of gram positive bacteria (% sensitivity)

Antibiotic	CONS (06)	*Streptococcus agalactiae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Entetococcus*
Amoxyclav	64%	0%	50%	50%	100%
Ciprofloxacin	33.3%	0%	25%	50%	-
Chloramphenicol	50%	0%	50%	50%	100%
Clindamycin	64%	0%	75%	50%	0%
Erythromycin	0%	0%	50%	50%	0%
Gentamicin	50%	100%	75%	50%	0%
Linezolid	100%	100%	100%	100%	100%
Vancomycin	100%	100%	100%	100%	100%
Tetracycline	66.6%	0%	-	100%	0%
Netlimicin	64%	0%	75%	50%	0%
Co-trimoxazole	64%	0%	75%	50%	-
Daptomycin					100%

Table 4: Antibiotic sensitivity pattern of gram negative bacteria (% sensitivity)

Antibiotic	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas*	*Acinetobacter*
Amikacin	60%	66%	66%	50%
Imipenem	100%	100%	100%	100%
Piperacillin + Tazobactam	100%	66%	100%	-
Ceftazidime	40%	33%	33%	50%
Levofloxacin	80%	0%	100%	50%
Ciprofloxacin	80%	0%	100%	50%
Tetracycline	40%	100%	33%	50%
Ampicillin	40%	0%	0%	0%
Ceftriaxone	60%	33%	33%	50%
Colistin	100%	66%	100%	100%
Tobramycin	-	-	-	100%
Chloramphenicol	80%	33%	33%	50%
Co-Trimoxazole	60%	33%	66%	50%

Highest number of suspected cases were found in the age group of 05 yrs. to 14 yrs. (33.9%), followed by 25% in age group of less than 01 months. Similarly the highest rate of culture positive cases were found in the age group of less than 01 months (33.3%). Fever was the most common presentation (91%), followed by seizure (81.25%). The most common organism isolated was *CONS* (22.2%) followed by *Escherichia coli* (18.5%)

Fig 1: Distribution of cases according to age

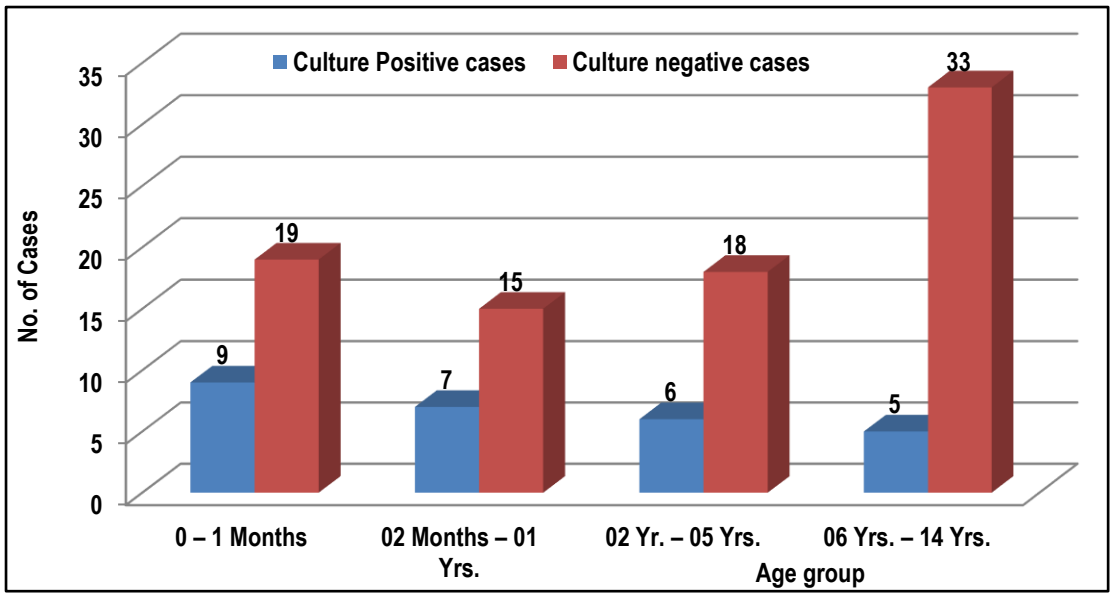


Fig 2: Sex wise distribution of cases

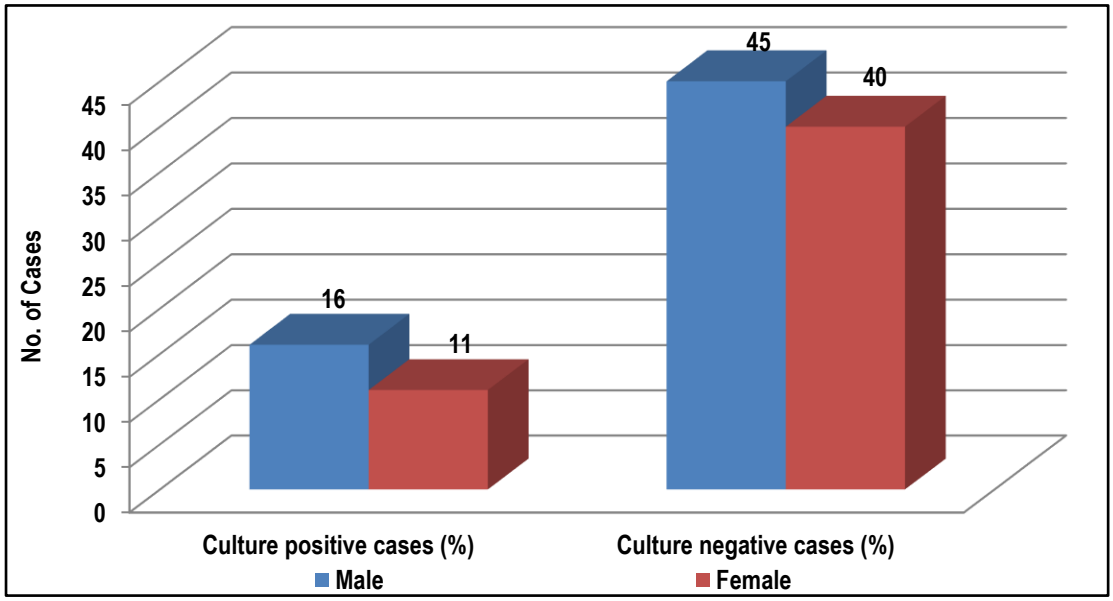


Fig 3: Distribution of cases according to clinical features (sign and symptoms)

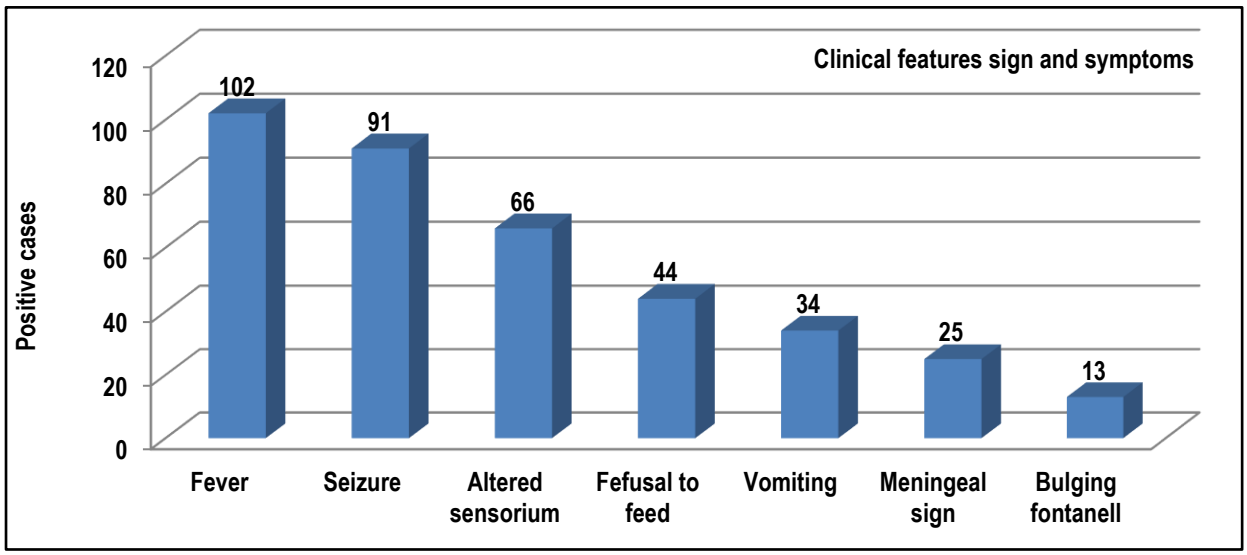


Fig 4: Culture positive cases isolated in suspected pyogenic meningitis

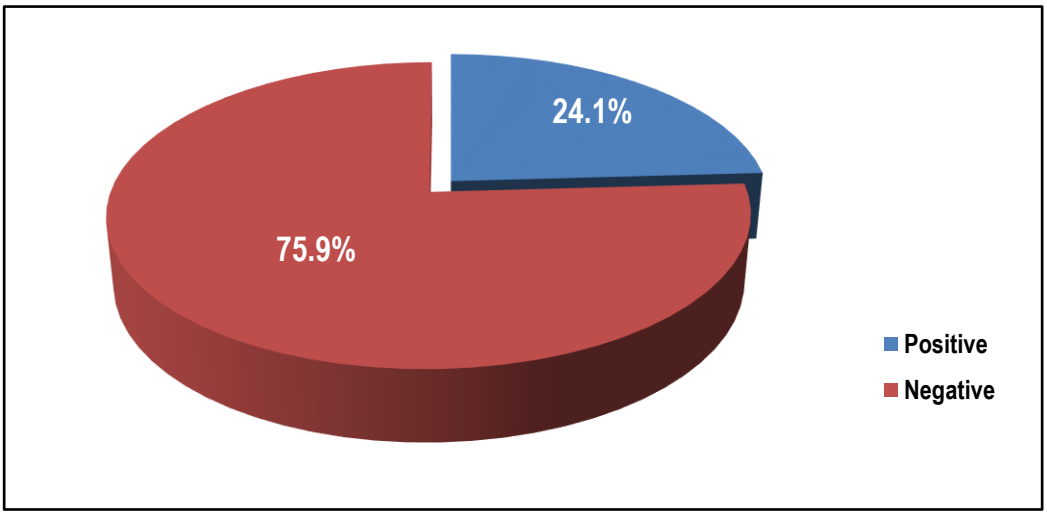
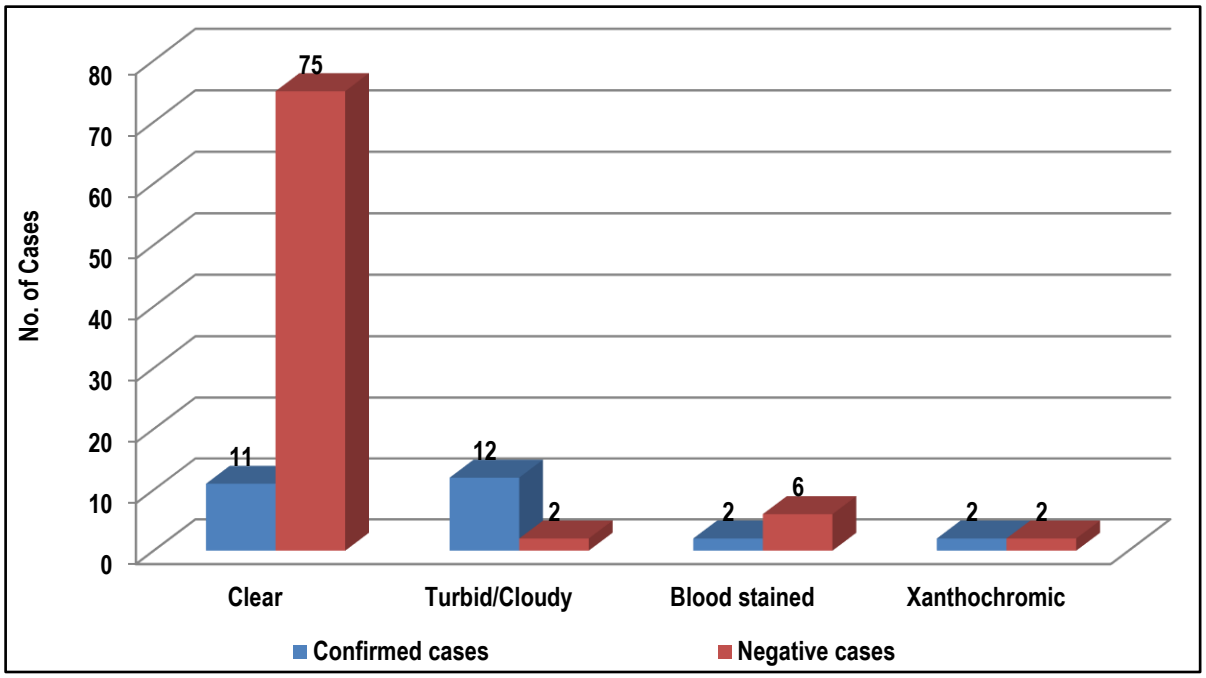


Fig 5: Gross appearance of CSF



DISCUSSION

In the present study, 59% of cases were below one year of age. Similarly Shrestha et al[7] in their study noted 55.6% of cases, Modi Gaurav B et al[8] found 36.5% of cases and Kabra SK et al[9] found 52% of cases below one year of age.

More than two third of cases of meningitis occur in first two years of life this is due to under developed immune system and high vascularity of brain.

In the present study out of 27 confirmed cases of acute bacterial meningitis male to female ratio was 1.47:1 in which male were 16 (59.25%) and female were 11 (40.75%) which correlate with study of Modi Gaurav B et al[8] and Bijay Mirdha et al[10] who also reported ratio of 1.5:1 in their studies. All the studies showed male preponderance.

The present study showed that fever was the most common clinical presentation (91%) followed by seizure (81.25%), altered sensorium (59.8%) and meningeal signs (22.3%) of cases. Similar observation were made by studies of Chinchankar N et al[11], Attia Bari et al[12], Fatima Zeeshan et al and Khan F et al.[13]

In the present study, among the 27 culture confirmed cases CSF appeared turbid in 12 (44.5%), clear in (40.7%), 7.4%have blood stained and 7.4% Xanthochromic. None of the samples showed cobweb formation.

In the present study out of 112 CSF samples collected 27 (24.1%) showed positive growth. Similar finding were observed by Rajesh Breja et al[14], Dr. Sudharshan Raj C et al[15], IMAD S. Mahmoud et al[16] and Ramesh S. T et al[17] who reported culture positivity in 23.58%, 19.1%, 34.5% and 25.1% of cases respectively.

In present study out of total culture positive cases gram positive cocci were 52% and gram negative bacilli were 48%. Similar observations were made by Fatima Khan et al, Rajesh Breja et al and Dr. Sudharshan Raj C et al.

In the present study coagulase negative staphylococcus (CONS) was the most common organism isolated in 22.2% cases of acute bacterial meningitis. Similar results were observed by R. Basri et al, Attia Bari et al and Amresh Kumar Singh et al[18] who reported CONS to be 21.6%, 45% and 44.5% respectively. Utpola Devi et al[19] 16% CONS was detected.

E.coli was the etiological agent in 18.5% cases followed by 14.8% cases of *Staphylococcus aureus* of acute bacterial meningitis in the present study. Similar finding were observed by IMAD S. Mahmoud et al, Amresh Kumar Singh et al and Modi Gaurav B et al who reported *Escherichia coli* to be 16.2%, 19.2% and 14.15% respectively. R. Basri et al, Modi Gaurav B et al, Dr. Sudharshan Raj C et al and Amresh Kumar Singh et al who reported 13.6%, 19%, 11.4% and 19.83% was caused by *staphylococcus aureus*.

In the present study *Klebsiella* spp. 11.1%, *Pseudomonas* spp. 11.1% and *Acinetobacter* 7.4% was isolated in acute bacterial meningitis cases. Similar observation was observed by studied of Utopala Devi et al, Dr. Sudharshan Raj C et al and Modi Gaurav B et al who observed that 12%, 18.1% and 22.92% of *Klebsiella* spp. were isolated, 9%, 6.6% and 12.19% *Pseudomonas* spp. was isolated,18%, 1.4% and 11.2% *acinetobacter* spp. was isolated in acute bacterial meningitis cases.

In the present study all the gram positive cocci were highly sensitive to Linezolid and Vancomycin. They were highly resistant to Ciprofloxacin and Erythromycin. Mild resistance was seen with Choramphenicol and Clindamycin.This was similar to studies done by Amresh Kumar Singh et al and Dr. Sudharshan Raj C et al who also reported that gram positive cocci were highly sensitive to Vancomycin and Linezolid and highly resistant to Ciprofloxacin and Erythromycin.

In the present study all the gram negative bacilli were highly sensitive to Imipenem, and Colistin and highly resistant to Ampicillin, Ceftazidime and Co-Trimoxazole. Similar studies were done by Dr. Sudharshan Raj C et al, Amresh Kumar Singh et al and Modi Gaurav B et al, who observed that gram negative bacilli were completely sensitive to Imipenem and colistin and highly resistant to Co-Trimoxazole, ceftazidime and Ampicillin.

CONCLUSION

This study may play an important role in the diagnosis and more accurate treatment to the patients suffering with acute bacterial meningitis. Regular prevalence and antibiotic susceptibility studies will help to enhance antimicrobial stewardship thus minimizing the emergence and spread of antimicrobial resistance and it would also be helpful for clinicians choosing an appropriate empirical antimicrobial. Hence, continued surveillance with more detailed studies is warranted to know the actual magnitude of problem and the spectrum of diseases and antimicrobial resistance caused by these pathogens.

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