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The Bacteriological and Antimicrobial Susceptibility Profile of Cerebrospinal Fluid Isolates in a Tertiary Hospital, Northeast India

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CNS infections are life-threatening and significant causes of morbidity and mortality, especially in developing countries. To ensure appropriate therapy, current knowledge of the organisms that cause CNS infection and their antibiotic susceptibility pattern in a particular setting or region is of utmost importance. This study was therefore undertaken to determine bacterial isolates and their antibiogram in the cerebrospinal fluids of patients as seen at Tertiary Hospital. The study was carried out at a tertiary care hospital from June 2016 to December 2016 irrespective of age group. Cerebrospinal fluid (CSF) samples were subjected for bacteriological analysis. Total samples received were 639 out of which 57 samples showed growth. Only 40 pathogens were isolated in culture, which amounts to isolation rate of 7.67%. Gram negative isolation rate (70%) was more than gram positive organism (30%) but the most common organism that was isolated was *Enterococcus spp*. Most common Gram negative isolate was *Acinetobacter baumanii* (11.8%), followed by *Escherichia coli* (10.17%). Gram negative organisms showed higher resistance to beta lactam antibiotics like piperacillin (61.54%), ampicillin (80%). cephalosporins like cefotaxime (81.25%), cefoperazone (63.64%) and ceftriaxone (50%) resistance rates were also on the higher side. Cotrimoxazole resistance was also bit high 57.89%. Out of 9 *Enterococcus species* we isolated, 5 were HLAR (High level Aminoglycoside resistant). Though HLAR *Enterococcus species* showed 100% resistance to Penicillin, Ampicillin, Ciprofloxacin but were susceptible to drugs like Vancomycin, Linezolid and Teicoplanin. CNS infections are life-threatening and significant causes of morbidity and mortality, so early management is essential. Since we are encountering resistance to commonly used antibiotics, so microbiological surveillance is important to identify the common pathogens and their antimicrobial susceptibility patterns in order to select the rational empirical antimicrobial therapy based on regional and national data to reduce the emergence of resistant organisms

Introduction

CNS infections are life-threatening and significant causes of morbidity and mortality, especially in developing countries (Ho Dang Trung *et al.,* 2012; Marchiori *et al.,* 2011).

The cause of CNS infection may vary over time, by geographic region, with age, comorbidities, vaccination programs and the routes by which the pathogens are acquired (Ho Dang Trung *et al.,* 2012). Bacterial infections have been known as the most

common cause of the CNS infections, meanwhile fungi and mycobacteria are also frequently reported (Vengamma *et al.,* 2014). The pattern of pathogens recovered from CSF culture varies from Gram-positive cocci to multidrug-resistant Gram-negative bacteria depending on geographic region, age, co-morbidities, type of neurosurgical procedures, and site of infection (Cucunawangsih *et al.,*). Antibiotics are usually administered before the laboratory results of CSF culture and sensitivity are available.

To ensure appropriate therapy, current knowledge of the organisms that cause CNS infection and their antibiotic susceptibility pattern in a particular setting or region is of utmost importance. Information obtained from laboratory based surveillance is important in determining the most common aetiology of pathogens. This information is also necessary for improving the clinical management of cases, guiding therapeutic decisions and for designing preventive strategies (Lucy and Temitayo, 2015).

This study was therefore undertaken to determine bacterial isolates and their antibiogram in the cerebrospinal fluids of patients as seen at Tertiary Hospital.

Materials and Methods

Site of Study: Microbiology department

Duration: 1 year (Jan 2016 – Dec 2016)

Type of Study: Prospective observational study

Methods

All CSF samples received for aerobic culture and sensitivity from different IPDs in Microbiology department, NEIGRIHMS, Shillong.

Processing of sample

Directly from the sample, wet mount, India ink preparation and Gram staining was done. Then all these samples were processed for culture and sensitivity by standard method (Mackie and Mccartney, 1996). Media used for culture were 5% Sheep Blood agar, Chocolate agar, Mac-Conkey agar, Brain Heart Infusion broth (Himedia, Mumbai, India).

All significant isolates were identified by standard procedures and their antimicrobial susceptibility was tested by Kirby Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI, 2015) recommendations (M100-S25 Performance Standards for Antimicrobial Susceptibility Testing).

The routine antimicrobial sensitivity tests were put for the following antibiotics:

Drugs for GPC pathogen

Penicillin (10 units), Ampicillin (10µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Cefotaxime (30µg), Chloramphenicol (30µg), Gentamicin (120µg), Erythromycin (15µg), Clindamycin (2µg), Vancomycin (30µg), Tetracycline (30µg), Linezolid (10µg), Teicoplanin (30µg), Ofloxacin (5µg) (Himedia, Mumbai, India).

Drugs for GNB pathogen

Piperacillin (100µg), Ampicillin (10µg), Ciprofloxacin (5µg), Amikacin (30µg), Gentamicin (10µg), Cefotaxime (30µg), Cefoperazone (75µg), Chloramphenicol (30µg), Cotrimoxazole 25µg (1.25/23.75µg), Imipenem (10µg), Meropenem (10µg), Cefop + Sulbacta (75µg/10µg), Piperacillin + Tazobactam (100 µg /10µg), Ofloxacin (5µg), Ceftazidime (30 µg) (Himedia, Mumbai, India).

Results and Discussion

Total of 692 CSF Samples was collected from 401 males and 291 female patient admitted in different wards and ICU of our hospital. Majority of patients were in the age group of 1-20 years and maximum growth was obtained in this group (Fig. 1).

The total number of organisms isolated was 57.

Out of these 57 organisms 17 organism was micrococci which is a normal skin flora and remaining 40 organism was pathogenic organism (Table 1).

Total isolation rate of organism was 8.23% but isolation rate of pathogenic isolate was just 5.78%. Majority of pathogenic organism that was isolated was gram negative (70%) while gram positive isolation rate was just 30% (Table 2).

Among gram negative isolates gram negative bacilli accounted for maximum percentage whereas only one isolate was gram negative cocci.

Most common isolate was *Acinetobacter baumanii* followed by *Escherichia coli*, *Enterobacter spp*, *Klebsiella pneumoniae*.

There were few Gram negative bacilli that could not be identified by routine biochemicals and these isolates were more in number compared to *Pseudomonas aeruginosa* and *Haemophilus influenzae*.

The only Gram negative cocci isolated was *Neisseria meningitidis* (Fig. 2).

Enterococcus species was the most common gram positive cocci to be isolated of which *HLAR Enterococcus species* isolation rate (8.47%) was more than Enterococcus species

(6.78%). *Streptococcus pneumoniae* (3.39%) and *Streptococcus* species (1.69%) was isolated in 3 cases (Fig. 3).

In our study total samples received were 639 out of which 57 samples showed growth. Only 40 pathogens were isolated in culture, which amounts to isolation rate of 7.67%. Few studies too had similar low isolation rate like 9.6% (Mengistu *et al.,* 2013), 9.01% (Singh *et al.,* 2016) and 1.83% (Lucy and Temitayo, 2015).

Some studies showed slightly high isolation rate of 13.91% (Gitali *et al.,*), 15% (Cucunawangsih *et al.,*). While very high isolation rate 22.11% (Devi *et al.,* 2017), 25% (T and J, 2016) was reported in few studies.

Various reasons cited in the literature for a low yield of bacteria on culture are 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 and antibiotic treatment prior to lumbar puncture (Singh *et al.,* 2016).

This might be the reason for low isolation rate in our settings and also we did not access to automated culture system which can significantly increase the sensitivity of culture for diagnosis of infection of sterile body fluids, especially in patients with previous antibiotic therapy (Rezaeizadeh *et al.,* 2012).

Demographic data analysis of patients revealed that they were mainly males (57.95% versus 42.05% for females).

This finding agreed with most of the studies (Lucy and Temitayo, 2015; Singh *et al.,* 2016). Reason for male preponderance for CNS infection is not mentioned in any of the studies.

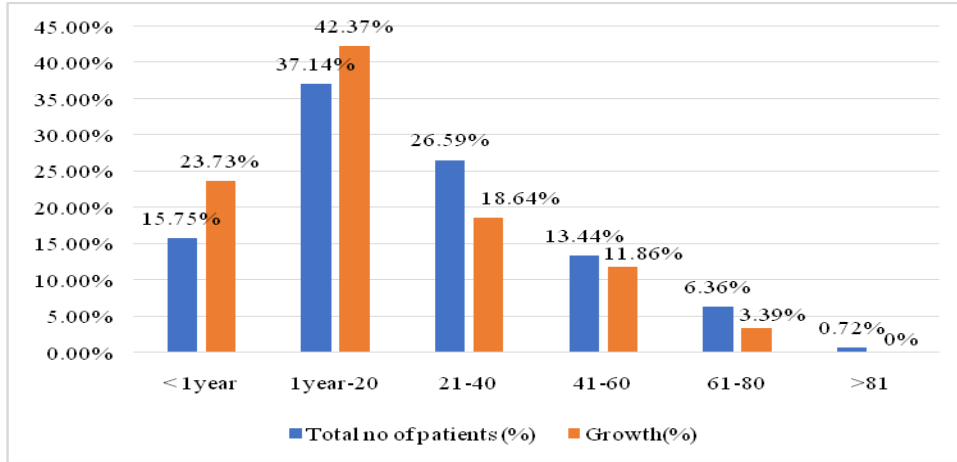**Fig.1** Bar diagram showing age distribution and percentage of growth

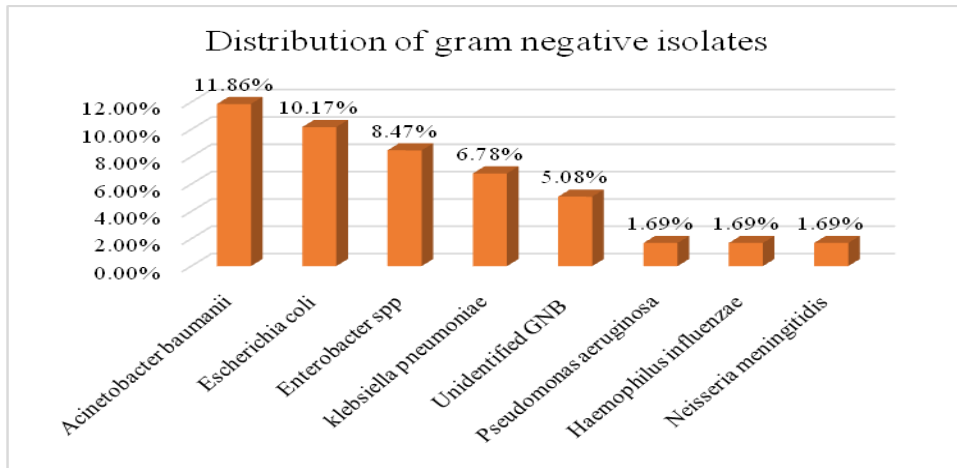**Fig.2** Percentage distribution of gram negative isolates

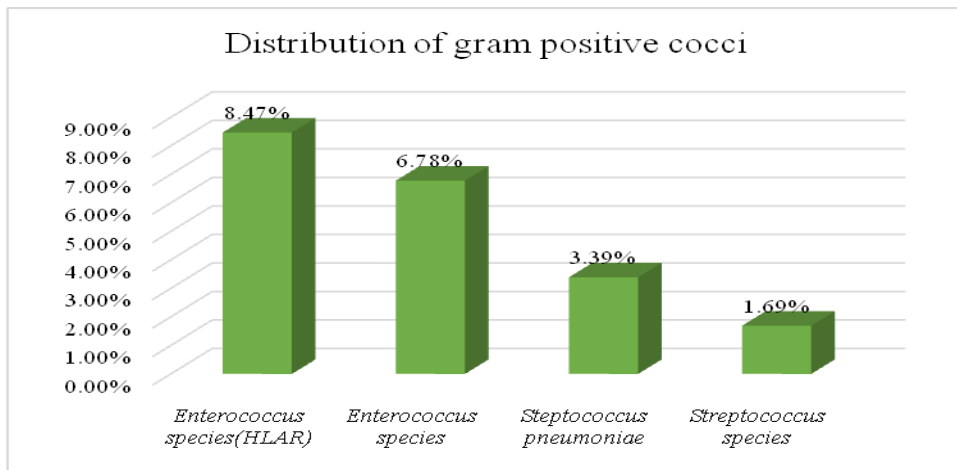**Fig.3** Percentage distribution of gram positive isolates

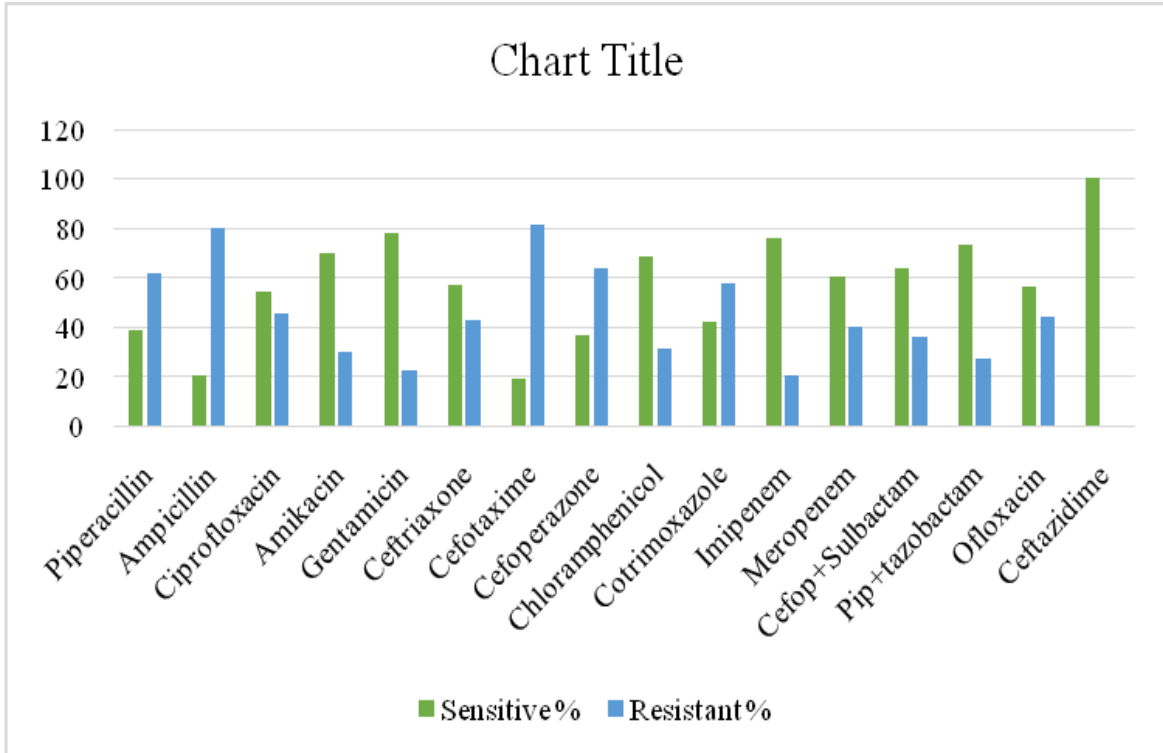**Fig.4** Anti-biogram of gram negative isolates
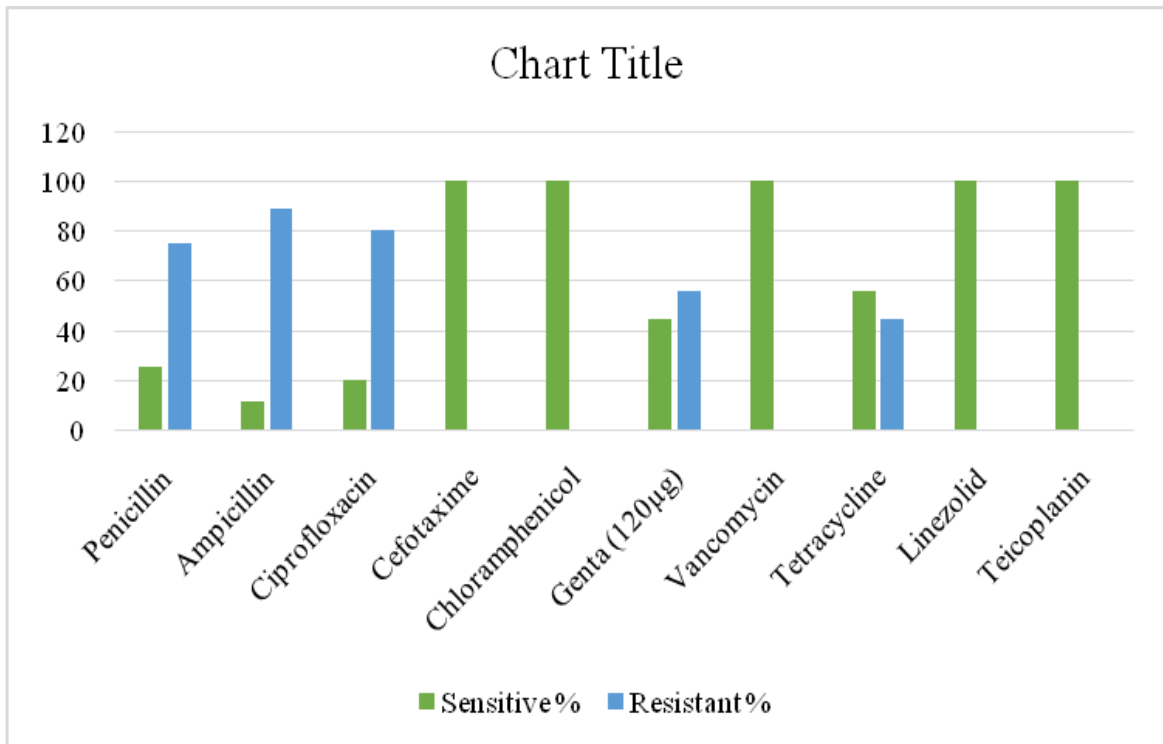

Fig.5 Anti-biogram of gram positive isolates

Table.1 Percentage distribution of isolated organism

Organism	Count of Organism	Percentage
Micrococci spp	17	29.83%
Acinetobacter baumanii	7	12.28%
Escherichia coli	6	10.53%
Enterobacter spp	5	8.77%
Enterococcus species (HLAR)	5	8.77%
Klebsiella pneumoniae	4	7.02%
Enterococcus species	4	7.02%
Unidentified GNB	3	5.26%
Streptococcus pneumoniae	2	3.51%
Streptococcus species	1	1.75%
Neisseria meningitidis	1	1.75%
Pseudomonas aeruginosa	1	1.75%
Haemophilus influenzae	1	1.75%
Grand Total	**57**	**100.00%**

Table.2 Percentage distribution of pathogenic gram negative and gram positive isolates

Organism	Number	Percentage
Gram Negative isolates (28) (70%)		
Acinetobacter baumanii	7	11.86%
Escherichia coli	6	10.17%
Enterobacter spp	5	8.47%
Klebsiella pneumoniae	4	6.78%
GNB under identification	3	5.08%
Pseudomonas aeruginosa	1	1.69%
Haemophilus influenzae	1	1.69%
Neisseria meningitidis	1	1.69%
Gram positive isolates (12) (30%)		
Enterococcus species (HLAR)	5	8.47%
Enterococcus species	4	6.78%
Streptococcus pneumoniae	2	3.39%
Streptococcus species	1	1.69%

Table.3 Antibiotic resistance pattern of Gram positive isolates

Agent	*Enterococcus spp (%)*	*HLAR Enterococcus spp (%)*	*Streptococcus pneumoniae (%)*	*Streptococcus spp (%)*
Penicillin	100	100	0	0
Ampicillin	75	100	ND	ND
Ciprofloxacin	100	100	0	ND
Cefotaxime	ND	ND	0	0
Chloramphenicol	ND	ND	ND	0
Genta (120)	0	100	ND	ND
Vancomycin	0	0	0	ND
Tetracycline	75	20	ND	ND
Linezolid	0	0	ND	ND
Teicoplanin	0	0	ND	ND

Table.4 Antibiotic resistance pattern of Gram negative isolates

Agent	*Enterobacter spp (%)*	*Escherichia coli (%)*	*Unidentified GNB (%)*	*Haemophilus influenzae (%)*	*Klebsiella pneumoniae (%)*	*Neisseria meningitidis (%)*
Piperacillin	80	83.33	33.33	ND	75	ND
Ampicillin	ND	100	66.67	0	ND	ND
Ciprofloxacin	50	60	0	0	75	ND
Amikacin	66.67	50	0	0	0	ND
Gentamicin	0	50	33.33	ND	0	ND
Ceftriaxone	100	ND	ND	0	100	0
Cefotaxime	100	80	66.67	ND	100	ND
Cefoperazone	50	66.67	66.67	ND	75	ND
Chloramphenicol	50	33.33	66.67	0	0	0
Cotrimoxazole	75	80	33.33	0	75	ND
Imipenem	0	16.67	0	ND	25	ND
Meropenem	20	50	0	ND	75	ND
Cefop+Sulbactam	40	16.67	0	ND	75	ND
Pip+tazobactam	0	16.67	0	0	75	ND
Ofloxacin	40	50	0	ND	75	ND

Table.5 Antibiotic resistance pattern of non-fermenters

Agent	*Acinetobacter baumanii*	*Pseudomonas aeruginosa*
Piperacillin	42.86	0
Ciprofloxacin	40	ND
Amikacin	33.33	0
Gentamicin	0	ND
Ceftriaxone	33.33	ND
Cefotaxime	66.67	ND
Cefoperazone	60	ND
Cotrimoxazole	0	ND
Imipenem	57.14	0
Meropenem	42.86	0
Cefop+Sulbactam	42.86	ND
Pip+tazobactam	42.86	0
Ofloxacin	42.86	ND
Ceftazidime	ND	0

The best test to confirm CNS infection is the detection of pathogens in CSF culture (Cucunawangsih *et al.,*). In our study after CSF culture both Gram positive and Gram negative organisms was isolated. Though Gram negative isolation rate (70%) was more

than Gram positive organisms (30%) but the most common organism that was isolated was *Enterococcus spp* (Fig. 4 and 5). Once thought to be relatively harmless commensals, enterococci have emerged in recent years as significant pathogens causing infections in both nosocomial and community settings. Though it is very uncommon cause of CNS infection but antimicrobial resistance in *Enterococci* has been reported increasingly (Wang *et al.,* 2014). So, we cannot ignore these pathogens. Out of 9 *Enterococcus species* we isolated, 5 was HLAR (High level Aminoglycoside resistant). Though HLAR *Enterococcus species* showed 100% resistance to Penicillin, Ampicillin, Ciprofloxacin but showed no any resistance to higher drugs like Vancomycin, Linezolid and Teicoplanin. Similar findings was seen in few studies as well (Rezaeizadeh *et al.,* 2012). *Enterococcus species* too exhibited similar resistance profile. Apart from *Enterococcus species* the other Grampositive isolate was *Streptococcus pneumoniae*, *Streptococcus species* and these organism did not encounter any resistance. In our study no *Staphylococcus aureus* and *CONS* was isolated which is in contrast to some studies which showed *Staphylococcus aureus* and *CONS* predominance (Cucunawangsih *et al.,* Gitali *et al.,* Rezaeizadeh *et al.,* 2012). In most of the studies it was gram positive whose isolation rate was more compared to gram negative (Lucy and Temitayo, 2015; Singh *et al.,* 2016; Modi and Anand, 2013) whereas some studies found out that there is a trend towards an increase number of GNB isolates over the period of time (Cucunawangsih *et al.,* Yadegarynia *et al.,* 2014) (Table 3–5).

Most common Gram negative isolate was *Acinetobacter baumanii* (11.8%) which is similar to other studies (Cucunawangsih *et al.,* Singh *et al.,* 2016; Yadegarynia *et al.,* 2014), followed by *Escherichia coli*

(10.17%). E.coli has also been mentioned as predominant organism in some studies (T and J, 2016; Ravikumar *et al.,* 2012).

Gram negative organisms showed higher resistance to beta lactam antibiotics like Piperacillin (61.54%), Ampicillin (80%). Cephalosporins like cefotaxime (81.25%), cefoperazone (63.64%) and Ceftriaxone (50%) resistance rates were also on the higher side. Cotrimoxazole resistance was also bit high 57.89%. High resistance rates to cefotaxime, cefoperazone and ceftriaxone among these organisms are suggestive of presence of extended spectrum β-lactamases (ESBLs) (Rezaeizadeh *et al.,* 2012). Organisms that express ESBLs are frequently resistant to other antimicrobial agents and treatment of infections caused by these organisms is difficult (Rezaeizadeh *et al.,* 2012). In this study we did not test the presence of ESBL enzymes genetically.

In our study, antibiotic resistance among *E. coli* isolates was frequently identified. High resistance rates to beta lactam antibiotics like Piperacillin, Ampicillin, Cephalosporins like cefotaxime and cefoperazone, Fluoroquinolones like Ciprofloxacin and Ofloxacin were seen. But *E.coli* showed increased sensitivity to higher antibiotics like carbapenem and combination antibiotics. This resistance problems have been mentioned in few studies as well (Jiang *et al.,* 2017). The reason for this may be due to increased use of all these drugs. Therefore, the use of piperacillin/tazobactam for the treatment of CNS infection caused by *E. coli* may be helpful. *Acinetobacter baumanii* showed resistance to most of the antibiotics. Highest resistance was seen against cefotaxime (66.67%) followed by cefoperazone (60%). Though this resistance rate is bit lower then *E.coli* but Carbapenem, Imipenem (57.14%) and combination antibiotic, Ceforperazone + Sulbactam (42.86%) and Piperacillin +

Tazobactam (42.86%) resistance rate was on the higher side. *E.coli* and *Acinetobacter baumanii* are the two major pathogens in our study. Similar to this other studies also observes an increasing resistance pattern among the major pathogens and is an alarming sign (T and J, 2016). The reason could be due to the fact that our hospital is a tertiary care centre to which majority of cases referred are pre-treated with antibiotics. The résistance to different group of drugs can be attributed to the rampant and indiscriminate use of antibiotics leading to the development of large scale drug resistance (T and J, 2016). Other Gram negative bacteria which showed higher resistance pattern was *Klebsiella pneumoniae* whose isolation rate was 6.78%. Though it showed high resistance rate to almost all the antibiotics but it showed 0% resistance rate to Aminoglycoside.

CNS infections are life-threatening and significant causes of morbidity and mortality, so early management is essential. Since we are encountering resistance to commonly used antibiotics, so microbiological surveillance is important to identify the common pathogens and their antimicrobial susceptibility patterns in order to select the rational empirical antimicrobial therapy based on regional and national data to reduce the emergence of resistant organisms.

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