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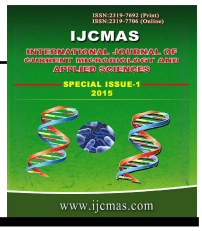

Original Research Article

Trends of Bacterial Meningitis and Sensitivity Profile of Isolates: A Five Year Study

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Keywords

Meningitis, Antimicrobial resistance, MRSA, ESBL, HLAR

A B S T R A C T

Acute bacterial meningitis (ABM) is a significant worldwide cause of death in adults. It is important to know the regional bacterial etiology along with their sensitivity profile to allow optimum management of patients. This study was undertaken to evaluate the trends in etiology of bacterial meningitis and antimicrobial resistance pattern of pathogens over a period of 5 years. The study was performed from Oct 2009 to Oct 2014. CSF samples were collected from all patients suspected of meningitis and cultured on chocolate agar, blood agar and MacConkey agar. Antimicrobial susceptibility testing was done using Kirby Bauer disc diffusion method. Detection of MRSA, HLAR, ESBL, AmpC and MBL was done. Total 2,313 CSF samples were received, of these 262(11.32%) were culture positive. Majority of the patients were from paediatric age group. Female to male ratio was 1:1.2. Gram positive bacteria 152(58.0%) were the predominant pathogen followed by 75(28.6%) Gram negative bacilli. The most common bacteria isolated was*Staphylococcus aureus*88 (33.6%) followed by *Citrobacter* 38(14.5%) and 32(12.2%) *Acinetobacter species*. During five year study period there is decrease in meningitis cases [2010(27.9%), 2011(27.5%), 2012(21.8%), 2013(4.9%), 2014(10.7%)] and constant decrease in number of *S. pneumonae*. Vancomycin and amikacin was the most sensitive antibiotic for gram positive bacteria. Gram negative bacilli showed good sensitivity for amikacin. Methicillin resistance in *S. aureus* was 48.4%, HLAR among the *Enterococci* was 70% while among the *Enterobacteriaceae* ESBL and AmpC production was 21.67% and 52% respectively. The high prevalence of drug resistant pathogens should be dealt with by rational use of antimicrobials. Measures should be taken to control the spread of these resistant strains before they reach the alarming levels.

Introduction

Acute bacterial meningitis (ABM) remains a major cause of mortality and long-term neurological sequelae worldwide. Despite the availability of potent newer antibiotics, the mortality rate due to ABM remains significantly high in India and other developing countries, ranging from 16–32% (Mani *et al.,* 2007). Optimum management

of patients requires determination of regional bacterial etiology along with their respective sensitivity profiles. According to reports released by the World Health Organization (WHO), *Neisseria meningitides, Streptococcus pneumoniae and Haemophilus influenzae* Type b represent the triad responsible for over 80% of all cases of bacterial meningitis worldwide (WHO, 2005).

There have been several published studies regarding meningitis conducted in hospitals in the developed countries but there is paucity of data collected from developing countries, particularly the Indian subcontinent. Regional information regarding trends in terms of etiology and antimicrobial susceptibility are essential for correct and timely management of meningitis. Keeping the current dismal scenario in mind, the following study was undertaken to evaluate the changing trends in etiology and antimicrobial resistance pattern of pathogens over a period of five years (2009–2014) in a tertiary care hospital of north India with emphasis on the prevalence of methicillin resistant *Staphylococcus aureus* (MRSA), high level aminoglycoside resistance in *Enterococcus species* (HLAR), extended spectrum β lactamases (ESBL), Amp C and metallo-betalactamases (MBL).

Material and Methods

This retrospective study was performed in the Department of Microbiology, JNMCH between January 2010 and December 2014. Cerebrospinal fluid (CSF) samples were collected from all patients suspected for meningitis. 3-5 ml of CSF was collected by lumbar puncture taking all the aseptic precautions (Collee, 2006a). The specimens were processed immediately and in case of delay, they were kept in the incubator at 37^{o}C.

After the naked eye examination for the presence of turbidity, microscopic examination was done by Gram's staining of the centrifuged deposit of CSF. Immediately after centrifugation of CSF, culture was done on a plate of chocolate agar, 5% sheep blood agar, Mac Conkeyagarand a tube of brain heart infusion broth. These plates were incubated for 24-48 hours in humid air plus 5-10% CO_2 at 37^{o}C. Cultures showing growth were identified byusing standard biochemical test (Collee 2006b).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed by Kirby Bauer disc diffusion method as per CLSI guidelines on Mueller Hinton agar (CLSI 2008). Gram positive isolates were tested against amikacin (30µg), gentamicin (10µg), levofloxacin (5µg), sparfloxacin (5µg), erythromycin (15µg), vancomycin (30µg), oxacillin (1µg), tobramycin (10µg), amoxycillin (30µg).

Gram negative isolates were tested against, amikacin (30µg), gentamicin (10µg), levofloxacin (5µg), sparfloxacin (5µg), ceftriaxone (30µg), cefoperazone (75µg), cefoperazone-sulbactam (75µg, 1:1), cefixime (5µg) ceftriaxone-salbactam (30/15µg), piperacillin (100µg), piperacillin-tazobactum (100:10µg), cefotaxime (30µg) and tobramycin (10µg), cefoxitin (30µg), imipenem (10µg). All discs were obtained from HiMedia, India.

Detection of extended spectrum and AmpC beta lactamases

Screening of possible ESBL production was done by using ceftriaxone (30µg) and cefoparazone (75 g). Isolates showing zone diameter less than 25 mm for ceftriaxone and less than 19 mm for cefoparazone were subsequently confirmed by disc potentiation

test using cefoparazone and cefoparazone-salbactam combination (Rizvi *et al.* 2009). Organisms sensitive to cefoxitin and resistant to cefoperazone-salbactam and piperacillin-tazobactam combination were considered to be Amp C producers (CLSI 2008).

Detection of metallo-betalactamases

Imipenem resistant isolates were tested for metallo-beta-lactamases (MBL) production by modified Hodge test and Double Disc synergy test using EDTA (Lee *et al.* 2007)

Screening for methicillin resistance using oxacillin disc test

Test was performed on Muller Hilton agar with 4% NaCl using Oxacillin 1µg disc. Any decrease in sensitivity zone was considered as resistant.

HLAR resistance

In case of *Enterococcus*, HLAR was detected using High content gentamycin (120µg) and streptomycin (300µg) (Murray 2003).

Result and Discussion

Acute bacterial meningitis continues to be a formidable illness with high morbidity and mortality among children and adults in India which warrants early diagnosis and aggressive therapy. The main pathogens of bacterial meningitis are known to be different in different age groups, eras, and geographic areas. The choice of antimicrobial therapy is based on the most common pathogen prevalent in a particular geographical area and age group and their antibiotic susceptibility pattern. Though the common pathogens associated with bacterial meningitis in the west are *H. influenzae, N. meningitidis, S. pneumoniae* (Schlech *et al.*, 1985) and *Listeria monocytogenes* (Schuchat *et al.*, 1997), the relative incidence of meningitis caused by these agents is less in South East Asia (Bhat *et al.*, 1991).

During the 5 year study period, total 2,313 CSF samples were received from patients suspected of meningitis. Out of these 274(11.8%) were culture positive. Culture positive cases have increased (11.8%) as compared to our previous reports (Khan *et al.* 2015). Although there was a constant decrease in culture positive meningitis cases over the five year study period [2010: 76(27.7%), 2011: 68(24.8%), 2012: 61(22.3%), 2013: 36(13.1%), and 2014: 33 (12.0%)] (Graph 1). The female-to-male ratio in our patients was 1:1.2. This indicates that males are prone to have bacterial meningitis. Similar gender discrepancy in meningitis is found in other countries (McCormick *et al.*, 2013).

Majority of the patients were from paediatric age group (<12years). Prevalence was predominantly in the 1–5 year age group with 77(28.1%) cases followed by 5–15year 53(19.3%) cases. Among the infant group, majority of cases 46(16.8%) were between 0-1month of age, while between 1 and 3 month 28(10.2%) cases, 3–6month 21(7.7%) cases, 6–12month 34(12.4%) cases were positive. Among adults the distribution was as follows: 35–45year 5(1.8%), 15–25 year 4(1.5%), 25–35year 4(1.5%) and >45year 2(0.7%). (Graph 2 describes aetiology with age).

Etiology

Gram positive bacteria were the predominant isolates in majority of cases of meningitis 164(59.9%) while 110(40.1%) were gram negative bacilli. In our study *S.*

aureus has emerged as the most common pathogen causing acute bacterial meningitis in all the five years accounting for a total of almost 35.8% of all isolates followed by *Citrobacter species* which were way behind at 13.9% and *Pseudomonas aeruginosa* at 13.1%. Frequency of *S. pneumoniae* isolation has increased 13(4.7%) as compared to our previous reports (Khan *et al.* 2011) but prevalence is low as reported in another Indian study (Bareja *et al.* 2013). There was constant decrease in the prevalence of *S. pneumoniae* during the consecutive years [2010, 10(0.36%), 2011, 2(0.73%), 2012, 1(0.36%), 2013, 0(0%), 2014, 0(0%)].

Studies have shown a similar increase in the incidence of staphylococcal infection and a decrease in the incidence of *Streptococcus pneumoniae* (Huang *et al.*, 2005, Pederson *et al.*, 2006). *H. influenzae, N. meningitidis* and *S. agalactiae* were not isolated in our study. Other Indian studies have also quoted a low isolation rate of these pathogens (Bhat *et al.*, 1991, Kabra *et al.*, 1991). However, in this study *Listeria monocytogenes* was isolated in 3(1.1%) of cases and 1 Coryneform species was isolated.

The other important emerging pathogens were *CONS* and *Enterococcus species* accounting for 9.9% and 8.0% cases of meningitis respectively. Recent reports from India have also shown similar frequency of CONS in bacterial meningitis cases (Bareja *et al.*, 2013). An increase in number of *CONS* and *Enterococcus species* in the earlier part of study pointed their recent emergence but incidence was decreased in later years.

Across all age groups *S. aureus, Citrobacter species, E. coli, P. aeruginosa,* and *E. faecalis* were most commonly isolated. *Citrobacter species* has emerged as most common pathogen in neonates replacing *Klebsiella* and *S. aureus*. *CONS* was seen mostly in the 1–5years age groups. Most common pathogen between 1-5 years of age was *Citrobacter species. S. pneumoniae* cases were clustered between 0-5 years age group. All three cases of *Listeria monocytogenes* occurred between 0 and 1 month of age. Among 5–15 years *S. aureus* was the most common isolate.

The difference in etiology from the temperate west may be due to the fact that India is a semitropical country where hardy bacteria like *S. aureus, CONS* and gram negative bacilli flourish and the relatively more fragile bacteria like *H. influenzae, N. meningitidis* and *S. agalactiae* in comparison do not have a survival advantage (Khan *et al.*, 2011). These results highlight the very different etiological profile in India in comparison to that of the west.

An increasing drug resistance was seen among the *Staphylococcal species* and the *Enterococcus* while *Streptococcus species* maintained a uniform sensitivity throughout the study period. A constant decline was noticed in isolates of *S. aureus* against fluoroquinolones from 20(83.3%) in 2010 to 6(60%) in 2014. Prevalence of MRSA was nearly 30–35% in the study period except in 2011 when 15.3% isolates were methicillin resistant. Frequency of MRSA and HLAR has decreased as compared to our previous reports (Khan *et al.* 2015). However, this incidence of MRSA and HLAR is high as compared to study by Bareja *et al.* (2013). Fortunately, no vancomycin resistance was detected in *S. aureus* or *Enterococcus species.* Certain studies have reported low level resistance to vancomycin in *Staphylococcal* isolates (Gad *et al.*, 2010) which indicate an upcoming resistance to even this reserve drug. Around 80%–90% isolates were sensitive to aminoglycosides.

Table.1 Antimicrobial susceptibility pattern among Gram Positive cocci

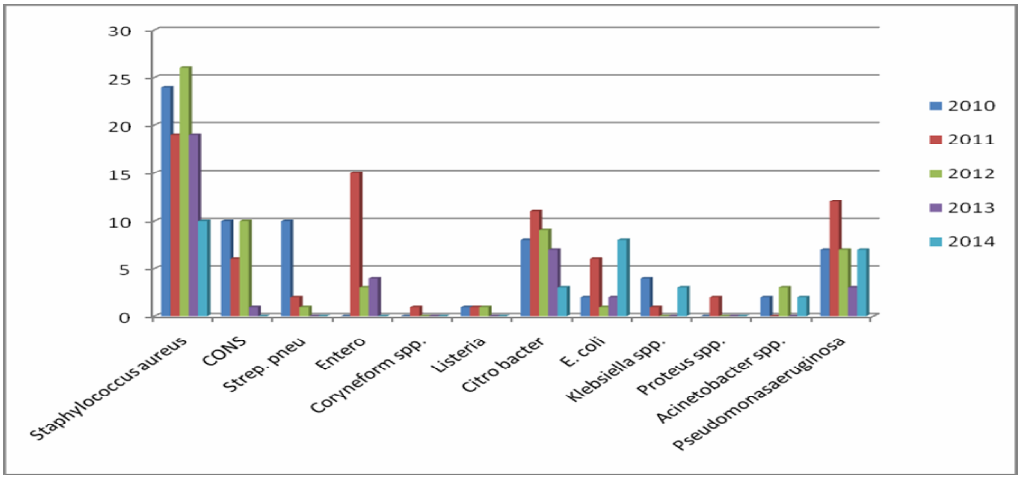
Organism	Erythromycin	Cephalosporins	Vancomycin	Oxacillin	Flouroquinolones	Aminoglycosides
S. aureus						
2010	13(54)	15(62.5)	24(100)	16(66.6)	20(83.3)	20(83.3)
2011	18(94.7)	15(78.9)	19(100)	16(84.7)	16(84.7)	19(100)
2012	22(84.6)	16(61.6)	26(100)	17(65.3)	20(76.9)	22(84.6)
2013	13(73.6)	11(57.8)	19(100)	13(68.4)	13(68.4)	17(89.4)
2014	4(40)	6(60)	10(100)	6(60)	6(60)	7(70)
CONS						
2010	4(40)	8(80)	10(100)	8(80)	8(80)	8(80)
2011	4(66.6)	6(100)	6(100)	5(83.3)	5(83.3)	6(100)
2012	7(70)	8(80)	10(100)	8(80)	8(80)	10(100)
2013	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
2014	-	-	-	-	-	-
S. pneumoniae						
2010	-	10(100)	10(100)	-	10(100)	8(80)
2011	-	2(100)	2(100)	-	2(100)	1(50)
2012	-	1(100)	1(100)	-	1(100)	1(100)
2013	-	-	-	-	-	-
2014	-	-	-	-	-	-
E. faecalis						
2010	-	-	-	-	-	-
2011	-	5(33.3)	13(86.6)	-	8(53.3)	7(46.6)*
2012	-	0(0)	3(100)	-	2(66.6)	1(33.3)*
2013	-	1(25)	3(75)	-	2(50)	2(50)*
2014	-	-	-	-	-	-

*High content gentamycin and streptomycin to detect HLAR

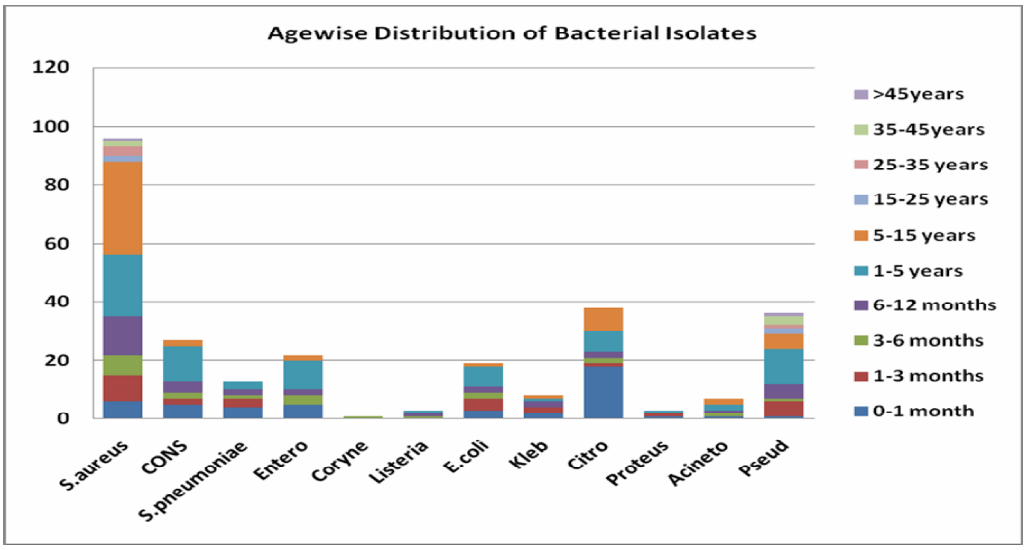
Table.2 Trend of antimicrobial susceptibility among Gram negative isolates

Organism		*E. coli*					*Klebseilla*					*Citrobacter*					*Pseudomonas sp*				
		2010	**2011**	**2012**	**2013**	**2014**	**2010**	**2011**	**2012**	**2013**	**2014**	**2010**	**2011**	**2012**	**2013**	**2014**	**2010**	**2011**	**2012**	**2013**	**2014**
Aminoglycosides		1(50)	4(66.6)	1(100)	1(50)	5(62.5)	3(75)	1(100)	-	-	1(33.3)	3(37.5)	8(72.7)	3(33.3)	3(42.8)	0(0)	3(42.8)	6(50)	6(85.7)	2(66.6)	3(42.8)
Flouroquinolones		1(50)	3(50)	0(0)	0(0)	3(37.5)	0(0)	1(100)	-	-	1(33.3)	4(50)	5(45.5)	3(33.3)	-	0(0)	1(14.2)	7(58.3)	5(71.4)	3(100)	6(85.7)
Cephalosporins		0(0)	3(50)	0(0)	1(50)	3(37.5)	2(50)	1(100)	-	-	0(0)	3(37.5)	1(9)	4(44.4)	0(0)	0(0)	2(28.5)	4(33.3)	5(71.4)	3(100)	3(42.9)
Cephalosporins+ inhibitor combination		0(0)	4(66.7)	0(0)	1(50)	4(50)	4(100)	1(100)	-	-	2(66.6)	6(75)	1(9)	5(55.5)	0(0)	0(0)	4(57.1)	10(83.3)	3(100)	3(100)	4(57.1)
Carbepenems		2(100)	6(100)	1(100)	2(100)	8(100)	4(100)	1(100)	-	-	3(100)	8 (100)	11(100)	9(100)	7(100)	3(100)	7(100)	12(100)	0(0)	3(100)	7(100)

Graph.1 Year wise distribution of bacterial isolate



Graph.2 Age wise distribution of bacterial isolates



Thus aminoglycosides are still the most effective group of antimicrobials barring glycopeptides (vancomycin) against GPCs.

Among GNRs *Citrobacter* species were the most resistant pathogens with 44.4% ESBL production. There was an increase in ESBL from 37.5% in 2010 to 44.4% in 2014 and AmpC producers from 25% in 2010 to 100% in 2014 respectively. Resistance to aminoglycosides and fluoroquinolones was almost constant with slight decrease (27.3%) in 2011. Frequency of ESBL production has increased as compared to our previous report (Khan *et al.* 2011). Another interesting finding is the alarming rise in AmpC producers among all GNRs which was not there in our previous report (Khan *et al.*, 2011). In E. coli, resistance was maximum in 2010 and 2012 when all the isolates were AmpC producers. Strains of Proteus were resistant to all the drugs tested except carbepenems. Unlike GPCs aminoglycosides resistance has emerged significantly among GNRs.

Amongst the nil-fermenters, poor sensitivity was shown against cephalosporins (28.5%) in 2010 which improved to 71.4% in 2012 whereas cephalosporin+ inhibitor combination showed sensitivity greater than 80% except during 2010 and 2014 where a dip in the sensitivity was noted (57.1%). An interesting finding was improvement in fluoroquinolone sensitivity from 14.2% in 2010 to 85.7% in 2014. This could be due to less use of fluoroquinolones in treatment of meningititis in our setting. *Acinetobacter species* were sensitive to all the antibiotics tested. MBL production was not detected in any of the strains isolated.

The epidemiologic trends of ABM should be examined frequently because any change may influence the choice of initial empiric antibiotic therapy greatly. These results revealed the altered trend in etiology of meningitis cases and also signify the upcoming levels of drug resistance amongst the gram positive and the gram negative microbes. Stress should be given to control the spread of these resistant strains before they reach the alarming levels in this region and on the restrained & rationale use of antimicrobials both in and outside the hospital.

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