Original Article

DOI: 10.7860/JCDR/2022/52513.15842

Microbiology Section

Culture Positivity of Cerebrospinal Fluid by Automated Blood Culture System in Neonates: A Cross-sectional Study from Loni, Maharashtra, India

SAVITA BABAN TAJANE[1], ANAGHA SUBHASHCHANDRA VAIDYA[2], DEEPIKA SHIVAJI BHALERAO[3], SHAHRIAR BAHMAN ROUSHANI[4], SANJEEV GOPALRAO KULKARNI[5], VAIBHAV VITTHALRAO RAJHANS[6], ANITA BALAKRISHNAN NAIR[7]

ABSTRACT

Introduction: Bacterial meningitis especially in neonates remains a major cause of mortality and long term health sequelae. There is a need for periodic review, since pathogens responsible for the meningitis vary with time and geography.

Aim: To find the culture positivity of Cerebrospinal Fluid (CSF) specimens before and after installation of automated blood culture and identification system in suspected neonatal meningitis cases.

Materials and Methods: The present descriptive cross-sectional study was carried out during July 2020 to December 2020, for six months duration in Department of Microbiology, Rural medical college, (Pravara Institute of Medical Sciences-Deemed University), Loni, Maharashtra, India. All CSF specimens from neonates <28 days received in Department of Microbiology were included in the study. All CSF specimens collected in BacT/Alert bottle were incubated and further subjected to identification by Vitek 2 system. All CSF specimen smears, received were subjected to Gram staining and Ziehl Neelsen Staining. Descriptive statistics was used for result analysis.

Results: A total of 265 CSF specimens were received during the study period. Males (59%) outnumbered females (41%) in the present study. Bacterial growth by automated blood culture system (BacT/Alert 3D) was detected in 85 CSF specimen giving the culture positivity as 32.08%. The most common bacterial isolate was found to be *Staphylococcus haemolyticus* followed by *Enterococcus* species and *Acinetobacter* species. Also, an attempt was made to compare culture positivity results with results by conventional culture method before installation of automation which showed heightened results for culture positivity and diversity of clinical isolates.

Conclusion: Bacterial neonatal meningitis is a common entity and aetiological diagnosis is crucial in every healthcare setting. The present study describes the various aetiological agents isolated by automated blood culture system in neonatal CSF specimens. Retrospective comparison with conventional culture has shown promising results for automated system. Early isolation and definitive identification with drug sensitivity, has got massive impact in management of neonate, further in timely progression of child's developmental milestones.

Keywords: Cerebrospinal fluid culture, Gram positive cocci, Neonatal meningitis

INTRODUCTION

Bacterial meningitis is one of the Central Nervous System (CNS) disorders, which is an infection of the membranes (meninges) and CSF surrounding the brain and spinal Cord. It is one of the major causes of disability worldwide. Bacterial meningitis remains a severe infection with high rate of mortality. Earlier clinical suspicion and implementation of appropriate antimicrobial therapy were critical to minimise adverse outcomes therefore, accurate diagnosis is necessary regarding the important aetiological agents to ensure appropriate management [1,2].

The CSF is normally water clear has no more than five lymphocytes per mm, has glucose concentration between 45 and 100 mg/dL, protein concentration between 14 to 45 mg/dL and it is sterile. Infectious agents can get to the CNS through the blood stream or by direct extension from adjacent structures. The neonatal sepsis is prompting factor for meningitis [3-5].

Most cases of bacterial meningitis occur in early childhood. Pathogens which infect CNS system are different in different child age groups. *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influnezae* type-b are among the prevalent bacterial pathogens of this disease. There has been a decrease in the incidence of *H. influenzae* type b (Hib) and *S. pneumoniae* meningitis in countries where vaccination plan is generally performed against the two bacteria. Recently, Universal Immunisation Program by Government of India has included the *H. influenzae* (Hib) vaccine for children [5,6].

Meningitis in neonates most commonly results from the infection that is acquired from mother in utero or during vaginal delivery. In neonates, clinical signs and symptoms of CNS infections are often non specific and include fever, hypothermia, food retention, skin lesions, irritability or general malaise. Meningitis is mainly diagnosed on the basis of history, clinical examination, and CSF examination by conventional culture methods and automated culture methods. Gram stain of CSF can provide a rapid preliminary identification of the infective organism [7,8].

Growth of the organism is frequently hampered by less number of organisms and their slow growth in CSF specimen by conventional method. The use of blood culture system for the culture of normally sterile body fluids other than blood is widely accepted now [7-9].

The BacT/Alert PF plus bottles provides detection of microorganisms when a small volume of blood/body fluid is available. An inoculated bottle is placed into the instrument where it is incubated and continuously monitored for the presence of microorganisms. The BacT/Alert Microbial detection system utilises a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide (CO_2) dissolved in the culture medium. If microorganisms are present in the test sample, CO_2 is produced as the organisms metabolise the substrate in the culture medium. When growth of the organisms produces CO_2, the colour of the gas permeable sensor installed in the bottom of each culture bottle changes from blue-green to yellow and signals the growth positive bottle [8-12].

The Vitek 2 compact system uses a fluorogenic methodology for organism identification and a Turbidimetric method for susceptibility testing using a 64 well card that is barcoded with information [9-11].

The present study was planned to find the culture positivity of neonatal bacterial meningitis in and around the Pravara Rural hospital, Loni, Maharashtra, India. Also, an attempt was made to compare observations with culture positivity for six months before installation of automated system by conventional technique retrospectively.

MATERIALS AND METHODS

The present descriptive cross-sectional laboratory based study was carried out in the Department of Microbiology, Rural Medical College, (PIMS-DU) Loni, Maharashtra, India, during July 2020 to December 2020. The study was approved by Institutional Ethical Committee (Reg.No.DR/RMC/UG-PG/2020/102).

Inclusion criteria: Patients aged <28 days were included with both genders.

Exclusion criteria: Patients more than 28 days and repeat specimen from the neonate were excluded.

Sample collection: All the CSF specimens from both genders, age less than or 28 days, were received in the Department of Microbiology with duly filled requisition form with request to do culture and sensitivity. Informed consent was taken by clinician before collecting specimen.

Procedure

Aerobic BacT/Alert PF plus bottle which was inoculated with CSF specimen (approximately, 0.1 mL-2 mL) and two glass slides with CSF smears from the suspected cases of meningitis from the ward/Neonatal Intensive Care Unit/Paediatric Intensive Care Unit were received in the Department of Microbiology. Two glass slides with CSF smear were heat fixed and stained by Gram staining and Ziehl Neelsen and further subjected to direct microscopy. CSF inoculated aerobic BacT/Alert PF plus bottles were incubated in BacT/Alert incubator for a maximum period of five days or until designated positive for growth. The automated BacT/Alert Microbial Detection System gave signals the bottle with growth of organism. Smear and subculture of all positive bottles was done on Mac-Conkey agar, blood agar and chocolate agar. Identification of organism was done by automated identification system. Antibiotic Sensitivity Testing (AST) was done by automated Vitek-2 system [10]. Routine conventional culture technique included the direct inoculation of CSF specimens on Mac-Conkey, blood agar and chocolate agar. After overnight incubation the isolates were identified by conventional biochemicals reactions. An antibiotic susceptibility test was performed by Kirby Bauer Disc diffusion method with standard technique [3,10].

STATISTICAL ANALYSIS

Since the present study was descriptive type, descriptive statistics was used for data analysis.

RESULTS

A total of 265 CSF specimens were received during the study period. In the present study, mean neonatal age was found to be 5.3 days. Male neonates 157 (59%) population was higher than the female 108 (41%) neonates. Smear positivity and culture positivity were fairly associated in the study [Table/Fig-1]. [Table/Fig-2] shows CSF culture positivity in neonate study population by automated culture system as 85 (32.07%) out of 265. Culture positivity in male and female population was much similar. Day wise BacT/Alert flag positivity by automated culture system was maximally seen on 1st and 2nd day (35, 41.18 % and 27, 31.76%) of incubation [Table/Fig-3]. Culture positivity and organism wise distribution of CSF isolates by automated culture system and conventional culture (before installation of automated system) showed various gram positive and gram negative organisms where most common gram positive isolate found was *Staphylococcus* species (43 out of 63) by automated culture [Table/Fig-4]. An attempt was made to compare the culture positivity of automated culture system and conventional culture technique before installation of automated system which showed very encouraging observations for automated system. Organism wise distribution by conventional culture method showed dominance of gram negative isolates retrospectively [Table/Fig-4]. Drug resistance for gram positive and gram negative organism by Vitek 2 system is shown in [Table/Fig-5].

Culture / Gram stain	Culture (+)	Culture (-)	Total (n=265)
Gram smear (+)	52	08	60 (22.64%)
Gram smear (-)	33	172	205 (77.36%)
Total	85 (32.08%)	180 (67.92%)	

[Table/Fig-1]: Concordance between gram smear and culture positivity by automated system.
Ziehl Neelsen (ZN) stain of all CSF specimen smears received were negative for acid fast bacilli

Month	Male positive/ Total	Female positive/ Total	Culture positive/ Total
July 2020	05/28	02/08	07/36
August 2020	05/17	05/17	10/34
September 2020	13/28	02/11	15/39
October 2020	05/16	03/12	08/28
November 2020	11/39	10/23	21/62
December 2020	13/29	11/37	24/66
Total	52/157 (33.12%)	33/108 (30.5%)	85/265 (32.08%)

[Table/Fig-2]: Six month data of CSF culture positivity by automated culture system.

Month	Flag positive bottle (n=85)	1st Day	2nd Day	3rd Day	4th Day	5th day
July 2020	07	03	02	01	01	00
August 2020	10	04	04	00	02	00
September 2020	15	08	04	02	01	00
October 2020	08	05	02	01	00	00
November 2020	21	13	02	03	03	00
December 2020	24	02	13	07	01	01
Total		35 (41.18%)	27 (31.76%)	14 (16.47%)	8 (9.41%)	1 (1.18%)

[Table/Fig-3]: Day wise Bact/Alert flag positivity by automated culture system.

Conventional culture (N=191)		Automated culture (N=265)	
Culture positivity 5.75% (n=11)		Culture positive 32.08% (n=85)	
Gram positive (01)	Gram negative (10)	Gram positive (63)	Gram negative (22)
CONS*(01)	*Klebsiella* spp. (03)	*Staphylococcus haemolyticus* (16)	*Klebsiella pneumoniae* (08)
	Acinetobacter spp. (03)	*Enterococcus faecium* (10)	*Acinetobacter baumannii* (06)
	Escherichia coli (02)	*Staphylococcus epidermidis* (10)	*Acinetobacter lwoffii* (02)
	Serratia marcescens (01)	*Staphylococcus homminis* (07)	*Escherichia coli* (02)
	Enterobacter spp. (01)	*Staphylococcus aureus* (06)	*Serratia marcescens* (01)
		Enterococcus faecalis (03)	*Enterobacter cloacae* (01)
		Streptococcus pyogens (02)	*Burkhoderia cepacia* (01)
		Micrococcus luteus (02)	*Stenotrophomonas maltophilia* (01)
		Kocuria rhizophilia (02)	
		Staphylococcus sciuri (01)	
		Staphylococcus arlette (01)	

		Kocuria kristinae (01)	
		Staphylococcus capitis (01)	
		Staphylococcus gallinarum (01)	

[Table/Fig-4]: Culture positivity and organism wise distribution of CSF bacterial isolates by conventional culture method (before installation of automation) and automated culture system.
*CONS: Coagulase negative *Staphylococcus*

CSF isolates	Type of drug resistance	%
Staphylococcus aureus (06)	MRSA* (05)	83.33
Enterococcus spp. (13)	VRE+ (03)	23.07
Gram negative isolates (22)	MDR‡ Gram Negative isolates (02)	9.09

[Table/Fig-5]: Type of drug resistance seen in CSF isolates by Vitek-2 compact system.
*MRSA: Methicillin resistant *Staphylococcus aureus*; +VRE: Vancomycin resistant *Enterococcus;*
‡MDR: Multidrug resistance

DISCUSSION

Present study was rare, as authors selected neonate population and intended to see the result of CSF culture positivity before and after installation of automated culture system. Before installation of automated system, authors were routinely using conventional method for CSF culture and sensitivity. Department of Microbiology do compile culture positivity and sensitivity data periodically which actually lead to do such study in department. To some extent, authors compared the result and found augmented culture yield by automated culture system. Other than culture positivity, authors could identify the isolates till species level with standard antibiotic susceptibility test results within a short time frame. Also, it was advantageous to identify few bacterial isolates which could not be identified with conventional method easily.

The present study showed the dominance of male neonates (59%) over female neonates (41%) which was quite similar to the study by Umate S et al., in Mumbai which comprised (62.0%) of male neonate population in their study [13] and Kaul V et al., also noted the male predominance while some studies did not noted any gender dominance [14-16]. CSF culture positivity by automated culture system in present study was found to be (32.08%) which was comparable to the neonatal study by Boskabadi H et al., in Tonekabon (Iran) which reports it to be (36.5%) [17].

Direct microscopy using Gram stain and Zeihl Neelsen stain was done on all (265) received CSF specimen smears. The bacterial pathogen could be demonstrated by gram stain in 60 neonatal CSF specimens (22.64%), while in total 85 (32.08%) specimen's yielded growth. This signifies necessity of gram staining method as rapid affordable method for provisional diagnosis of bacterial meningitis in Indian scenario. Gram staining depends on several factors like the number of organism present, prior use of antibiotics and techniques used. Low yield of microscopy result may be due to scanty smear of CSF specimen on slides. But, the use of automated system definitely increased the culture yield. No acid fast bacilli was found in any of the specimen indicating very low probability of mycobacterial aetiology in neonatal meningitis. Observations of gram stain were comparable to observations made by Mani R et al., in there bacterial meningitis study done at Bangalore [18].

Among causative agents, authors found the predominance of gram positive organism (74.0%) than gram negative organism (26.0%) by automated culture system. Studies by Cohen-Wolkowiez M et al., and Devi U et al., showed the predominant gram-negative organisms in neonates [19,20]. Paediatric study by Attia Bari FZ et al., [21] in Lahore, showed the gram positive organisms as major causative agents similar to Yoo IY et al., who used automated culture technique [7]. Keniyan Neonatal meningitis study by Laving AM et al., reported *E.coli* as most common (46.7%) causative agent by Latex Particle Agglutination (LPA) assay Antigen test [22], while Dirkje de Blauw AH et al., reported *E.coli* as most common bacterial agent in their neonatal study of CNS infections [4].

As neonatal CSF specimen is a very precious sample, author could not ask it for both conventional and automated culture simultaneously. An attempt was made to compare the results of six months prospective data with the six months retrospective CSF culture data by conventional method only before installation of BacT ALERT automated system. Out of 191 specimens received for conventional culture, only 11 showed the growth of bacteria giving culture positivity (5.75%). In which gram positive organism were found to be fewer (1 out of 11) and dominance of gram negative (10 out 11) organism was observed. Most of the time identification was made till genus level only. In the neonatal study by Laving AM et al., the CSF culture positivity by conventional method was reported to be 17.9% and gram negative organism as major aetiological agent [22].

Advantage of automated culture was its flag positivity which can be as early as day 1st of incubation further reducing the time for final culture report. Out of 265 specimens, 41.18% and 31.76% specimens were flagged positive on day 1 and day 2, respectively. Authors could also report antibiotic susceptibility results at earliest which is very important and relevant for decisions by clinicians to switch over the antibiotics.

MRSA incidence in present neonatal study was found to be 83.33% and incidence of Vancomycin Resistant Enterococcus (VRE) was 23.07% by automated Vitek 2 system. In gram negative bacterial isolates, incidence of Multidrug Resistance (MDR) was found to be 9.09%. This can be compared to study by Devi U et al., who reported VRE as 50% and high incidence of resistance in gram negative isolates [20].

In present study, most common gram positive isolate found as *Staphylococcus* species. *Staphylococcus haemolyticus, Staphylococcus epidermidis, Staphylococcus homminis, Micrococcous luteus, Kocuria rhizophilia, Staphylococcus sciuri, Staphylococcus arlette, Kocuria kristinae, Staphylococcus capitis, Staphylococcus gallinarum* belong to group Coagulase Negative Staphylococci (CONS) which were identified by Vitek 2 Compact system to species level.

A total of 42 (49.4%) CONS were among the 85 total isolates indicating the labelling of CONS as emerging pathogen. The Korean paediatric BacT/alert CSF culture study by Yoo IY et al., also found CONS as most common isolate [7]. Amongst CONS *Staph. haemolyticus* was most common isolate followed by *Staphylococcus epidermidis* and *Staphylococcus hominis* by the Vitek 2 system. Among Streptococci, authors found *Enterococcus* species as most common and *Streptococcus* pyogenes as of two isolates only. Among the *Enterococcus* species *E.faecium* was most common (10 out of 13) than *E.faecalis* as noted by Devi U et al., in her study done in Dibrugrh (Assam) [20].

Staphylococcus capitis, Staphylococcus arlette, Staphylococcus sciuri, Staphylococcus gallinarum and Kocuria rhizophila, Kocuria kristinae were the isolates which could be identified by automated system only. Similar association of these Staphylococcal species with bacterial meningitis was mentioned by Gheibi S et al, and Azimi T et al., in their study articles [16,23].

In present study, most common gram negative isolate as *Klebsiella pneumoniae* followed by *Acinetobacter baumannii.* Modi GB et al., also noted dominance of gram negative bacilli in his CSF culture study [24]. Study by Barnawal RK et al., in Ranchi noted *E.coli* as most common gram negative isolate in neonate population [25]. In present study, some nil fermenter pathogens like *Burkholderia cepacia* and *Stenotrophomonas maltophilia* were also found which are difficult to grow and to identify by conventional culture technique. MDR, non fermenting gram negative Acinetobacter isolates (50 %) were isolated by Viswanathan R et al., in their neonatal study [26]. One Serratia *marcescens* isolate was also observed in present study.

Though recent CSF studies are showing better sensitivity of Multiplex Polymerase Chain Reaction (PCR) for detection of *H. influenzae, Streptococcus pneumoniae, Neisseria meningitidis* agents, its practicality in routine diagnosis is questionable due to its cost and expertise support issues [27]. Role of CONS as pathogen is a debatable and many authors reported it as major CSF isolate which gives it growing significance [21,28,29].

In the present study, *Haemophilus influenzae* and *Neisseria meningitidis* were not isolated similar to finding by Debnath DJ et al., a study from Maharashtra [30]. In contrast NIMHANS, Bangalore study accounted for *Haemophilus influenzae* and *Neisseria meningitidis*, though less in number [18]. Except few cities of North India, low incidence of endemic meningococcal disease is reported in India [31]. Geographically, study place is situated in western part of India where people are much aware of child immunisation leading to very low presence of these agent in community.

Limitation(s)

The study could not do the CSF culture by conventional and automated culture system in parallel due to very small quantity of CSF available for culture from the neonates.

CONCLUSION(S)

The present study demonstrates the advantage of automated culture technique for better recovery of organism from CSF in neonates and timely availability of results. Comparison of BacT/Alert results with earlier conventional culture outcomes showed promising results. The present research recommends the routine CSF culture by automated culture system especially in paediatric population.

REFERENCES

[1] Winn, Wasington C, Gary P, Koneman EW, Allen SD, Janda WM, Schreckenbergu PC. Color atlas and textbook of diagnostic microbiology, 7th edition. lippincott Williams and Wikins. 2006:90-93.
[2] Mackie TJ, Collee JG, McCartney JE. Mackie and McCartnry Practical Medical Microbiology Book 14 edition. New York: Churchill Livingstone, 1996:77-80.
[3] Cheesebrough M. District laboratory practice in tropical countries. 2nd ed; 2005:116-24.
[4] Dirkje de Blauw AH, Vijn LJ, Wildenbeest JG, Wolthers KC, Biezeveld MH, van Wermeskerken AM, et al. Blood and cerebrospinal fluid characteristics in neonates with a suspected central nervous system infection. Medicine. 2019;98(25):e16079. Doi: 10.1097/MD.0000000000016079.
[5] Tille, Patricia M. author. Bailey and Scott's Diagnostic Microbilogy. St. Louis, Missouri: Elsevier, 2014:966-75.
[6] Muhammad J, Rehman Y, Amir S. Etiological spectrum and antibiotic sensitivity in children with acute pyogenic meningitis. J Med Sci. 2018;26(3)234-36.
[7] Yoo IY, Chun S, Song DJ, Huh HJ, Lee NY. Comparison of BacT/Alert FAN and FAN plus bottles with conventional medium for culturing cerebrospinal fluid. J Clin Microbiol. 2016;54(11):2837-40.
[8] Simor AE, Scythes K, Meaney H, Louie M. Evaluation of the BacT/Alert® microbial detection system with FAN aerobic and FAN anaerobic bottles for culturing normally sterile body fluids other than blood. Diagnostic Microbiology and Infectious Disease. 2000;37(1):05-09.
[9] Pincus D. Microbial identification using the biomerieus vitek's system. Encyclopedia of rapid microbiology. 01-09.
[10] Clinical and laboratory standards institute, performas standards for antimicrobial suspectibility testing, M100-S25,vol 37 No.1 Jan 2017, CLSI, Wayne, PA 19087 USA.
[11] Flayhart D, Borek AP, Wakefield T, Dick J, Carroll KC. Comparison of BACTEC PLUS blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J Clin Microbiol. 2007;45:816-21.
[12] Miller R, Houlberg K. Meningitis service age personnel. Journal of the Royal Naval Medical Service. 2015;(101):63-68.
[13] Umate S, Garg BD, Kabra NS. Incidence of meningitis in neonates with late-onset sepsis at a Tertiary Care Center in Western India: An observational study. J Clin Neonatol. 2019;8:67-70.
[14] Kaul V, Harish R, Ganjoo S, Mahajan B, Raina SK, Koul D, et al. Importance of obtaining lumbar puncture in neonates with late onset septicemia a hospital based observational study from North-West India. J Clin Neonatol. 2013;2:83-87.
[15] Jiang JH, Chiu NC, Huang FY, Kao HA, Hsu CH, Hung HY, et al. Neonatal sepsis in the neonatal intensive care unit: Characteristics of early versus late onset. J Microbiol Immunol Infect. 2004;37:301-06.
[16] Gheibi S, Fakoor Z, Karamyyar M, Khashabi J, Ilkhanizadeh B, Asghari-Sana F, et al. Coagulase negative *Staphylococcus*; the most common cause of neonatal septicemia in Urmia, Iran. Iran J Pediatr. 2008;18:237-43.
[17] Boskabadi H, Heidari E, Zakerihamidi M. Etiology, clinical findings and laboratory parameters in neonates with acute bacterial meningitis. Iran J Microbiol. 2020;12(2):89-97.
[18] Mani R, Pradhan S, Nagarathna S, Wasiulla R, Chandramuki A. Bacteriological profile of community acquired acute bacterial meningitis: A ten-year retrospective study in a tertiary neurocare centre in South India. Indian J Med Microbiol. 2007;25:108-14.
[19] Cohen-Wolkowiez M, Moran C, Benjamin DK, Cotten CM, Clark RH, Benjamin Jr DK, et al. Early and late onset sepsis in late preterm infants. The Pediatric Infectious Disease Journal. 2009;28(12):1052.
[20] Devi U, Bora R, Malik V, Deori R, Gogoi B, Das JK, et al. Bacterial aetiology of neonatal meningitis: A study from north-east India. The Indian Journal of Medical Research. 2017;145(1):138.
[21] Attia Bari FZ, Zafar A, Ejaz H, Iftikhar A, Rathore AW. Childhood acute bacterial meningitis: Clinical spectrum, bacteriological profile and outcome. Journal of the College of Physicians and Surgeons Pakistan. 2016;26(10):822-26.
[22] Laving AM, Musoke RN, Wasunna AO, Revathi G. Neonatal bacterial meningitis at the newborn unit of Kenyatta National Hospital. East African Medical Journal. 2003;80(9):456-62.
[23] Azimi T, Mirzadeh M, Sabour S, Nasser A, Fallah F, Pourmand MR. Coagulase-negative staphylococci (CoNS) meningitis; A narrative review of the literature from 2000 to 2020. New Microbes and New Infections. 2020;2020:100755.
[24] Modi GB, Patel KD, Soni ST, Patel KJ, Mangukiya JD, Jain PS. Bacteriological profile of pyogenic meningitis in tertiary care hospital, Ahmedabad. Natl J Med Res. 2012;2(3):313-17.
[25] Barnawal RK, Kumar M, Sharma AK, Prasad A, Kumari S, Priydarshini V. Microbiological Profile of Cerebrospinal Fluid (CSF) in pyogenic meningitis patients at tertiary care hospital. Int J Med Res Prof. 2018;4(3):113-18. Doi: 10.21276/ijmrp.2018.4.3.023.
[26] Viswanathan R, Singh AK, Basu S, Chatterjee S, Roy S, Isaacs D. Multi-drug-resistant, non-fermenting, gram-negative bacilli in neonatal sepsis in Kolkata, India: A 4-year study. Paediatr Int Child Health. 2014;34:56-59. [PubMed] [Google Scholar].
[27] Seth R, Murthy PSR, Sistla S, Subramanian M, Tamilarasu K. Rapid and accurate diagnosis of acute pyogenic meningitis due to *Streptococcus pneumoniae, Haemophilus influenzae* type b and *Neisseria meningitidis* using a multiplex PCR assay. J Clin Diagn Res. 2017;11(9):FC01-04. Doi: 10.7860/JCDR/2017/28114.10532.
[28] Basri R, Zueter AR, Mohamed Z, Alam MK, Norsa'adah B, Hasan SA, et al. Burden of bacterial meningitis: A retrospective review on laboratory parameters and factors associated with death in meningitis, Kelantan Malaysia. Nagoya J Med Sci. 2015;77(1-2):59-68. PMID: 25797971; PMCID: PMC4361508.
[29] Singh A, Kumar A, Gaur V, Jasuja J, Pandey J, Mishra R. Bacteriological profile of acute bacterial meningitis at a tertiary care hospital of North India. Int J Res Med Sci. 2016;4(10):4387-93.
[30] Debnath DJ, Wanjpe A, Kakrani V, Singru S. Epidemiological study of acute bacterial meningitis in admitted children below twelve years of age in a tertiary care teaching Hospital in Pune, India. Med J D Y Patil Univ. 2012;5:28-30.
[31] Sinclair D, Preziosi MP, Jacob John T, Greenwood B. The epidemiology of meningococcal disease in India: Epidemiology of meningococcal disease in India. Trop Med Int Health. 2010;15:1421.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.
2. Professor, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.
3. Professor, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.
4. Professor and Head, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.
5. Associate Professor, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.
6. Associate Professor, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.
7. Assistant Professor, Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Anagha Subhashchandra Vaidya,
Department of Microbiology, Rural Medical College, (PIMS-DU), Loni, Tal. Rahata,
Dist. Ahmednagar-413736, Maharashtra, India.
E-mail: anagha.kinikar@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Sep 23, 2021
- Manual Googling: Nov 05, 2021
- iThenticate Software: Dec 08, 2021 (12%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: Authors are thankful to PIMS, Loni (Deemed to be University) for providing institutional support.
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Sep 22, 2021**
Date of Peer Review: **Nov 03, 2021**
Date of Acceptance: **Dec 11, 2021**
Date of Publishing: **Jan 01, 2022**