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Diagnosis of Acute Bacterial Meningitis by Conventional Culture Method

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Abstract: ***Background: Bacterial Meningitis is one of the leading causes of mortality and morbidity worldwide requiring immediate diagnosis followed by medical intervention to prevent sequeale. The clinical presentation is not subtle in all cases, and hence confirmation by laboratory methods is necessary to know the exact etiology. Empiric antimicrobial therapy can be misleading; giving rise to resistant strains or it may further worsen the clinical prognosis, if ineffective. Therefore, in this study, an attempt has been made to diagnose the causative pathogen on conventional culture. Methods: 100 CSF and blood samples were collected from suspected /clinically diagnosed cases of meningitis over a period of 1year. CSF samples were processed by gram stain and conventional Culture. This was followed by Anti-microbial susceptibility testing by Kirby Bauer's disk diffusion method. The blood samples were collected in BACTEC bottles and subjected to automated system. The objectives of the study were: To identify the common bacterial etiological agent and to know the antimicrobial susceptibility pattern. Results: Out of 100 samples, maximum cases presented with frank meningeal signs (45%) followed by clinically suspected cases (42%). Microscopic examination and CSF culture 7% and 24% positive cases respectively. 13% were detected positive by blood culture. The positivity on CSF culture was comparable to the study done by R Mani et al (40.5%). The predominant pathogens isolated were K.pneumoniae (37.5%) followed by Streptococcus pneumoniae and H.influenzae (each 16.67%). Discussion: Culture is considered as the gold standard. However, it is not without limitations. It fails to give urgent results. Therefore, preliminary diagnosis was made on gram stain. The kappa coefficient-0.24 showed a fair agreement, and diagnostic accuracy was 79% considering the modified gold standard. Conclusion: Gram negative enteric bacteria were the most common etiology reported by Conventional Culture. Apart from microbiological parameters, clinical and other laboratory parameters should be considered to achieve a precise diagnosis.***

Keywords: Acute bacterial meningitis; Conventional Culture

1. Introduction

Acute Bacterial Meningitis is a fatal illness demanding prompt medical intervention owing to its high potential to cause mortality and life-long morbidity. [1] However, the clinical presentation is not demarcated in all cases, and hence confirmation by laboratory methods is necessary to know the exact etiology. Also, laboratory diagnosis proves to be an effective guide, thereby avoiding haphazard anti-microbial treatment and its complications. [2] Various studies on this have thrown light on the multi-factorial etiology of pyogenic meningitis. The burden of the disease varies with host factors like age; gender; nutrition of the patient and immune status of the patient; agent factors like antigenic structure; virulence factors and resistance shown to anti-microbial drugs and environmental factors like overcrowding; climate and socio-economic status. [2, 3]

In the present study, the clinical spectrum of cases of meningitis was studied in detail and the CSF samples were analyzed in the laboratory to diagnose the causative pathogen. This study was conducted to correctly guide the clinician's efforts in preventing the otherwise fatal outcome of bacterial meningitis.

2. Aims and Objectives

1. To identify the bacterial etiology causing meningitis with antimicrobial susceptibility pattern.
2. To know the common bacterial pathogen responsible in the causation of meningitis.
3. To know the utility of Conventional Methods in the diagnosis of meningitis.

3. Materials and Methods

The present study was conducted in Sassoon General Hospital; Pune over a period of 1year (January 2016-December 2016). Clinically suspected cases of meningitis belonging to all age-groups were studied. This included cases presenting with frank meningitis (showing fever or meningeal signs) and sepsis. HIV positive and post-operative cases of meningitis were excluded. [4]

100 CSF samples were collected. Additionally, blood samples were collected in BACTEC bottles [BD Diagnostics], from the same patients. [5]

The patients were selected after noting the detailed clinical history and informed consent was taken. Lumbar puncture was performed using 70% isopropyl alcohol and povidine iodine to avoid the growth of commensal flora on the culture media. The first few drops of CSF were inoculated on the transport media (A set of 3 media was used for each sample- Amie's [6]; Stuart's and Levinthal's medium [7]). The samples were processed as per the standard guidelines [8]. The diagnosis was made by microscopic method followed by Conventional Culture.

Microscopy by Gram stain was performed using the Hucker's modification [9]. It provided guidance to choose the selective media. Accordingly, Haemophilus Test Medium; New York City Medium were streaked in addition to 5% Sheep Blood Agar; Chocolate Agar and McConkey Agar. [10, 11] The colony characteristics were noted and further identification was done by biochemical tests- as per standard guidelines. This was followed by Anti-Microbial Susceptibility Testing by Kirby Bauer's disk diffusion method according to CLSI guidelines. [8]

The blood samples collected in BACTEC bottles [BD Diagnostics] were subjected to automated system and processed as per the laboratory protocol. [5]

Case definitions:

1] Frank meningitis is defined as a patient having 2 out of the following 4 symptoms: fever, headache, stiff neck, and altered mental status regardless of laboratory findings on CSF biochemistry. [12]

2] Clinically suspected cases are defined as patient showing vague manifestations like (continuous crying; bulging fontanelle; irritability; refusal to feed; blurred vision esp in pediatric age-groups) in addition to CSF biochemistry/ CSF gram stain. [12]

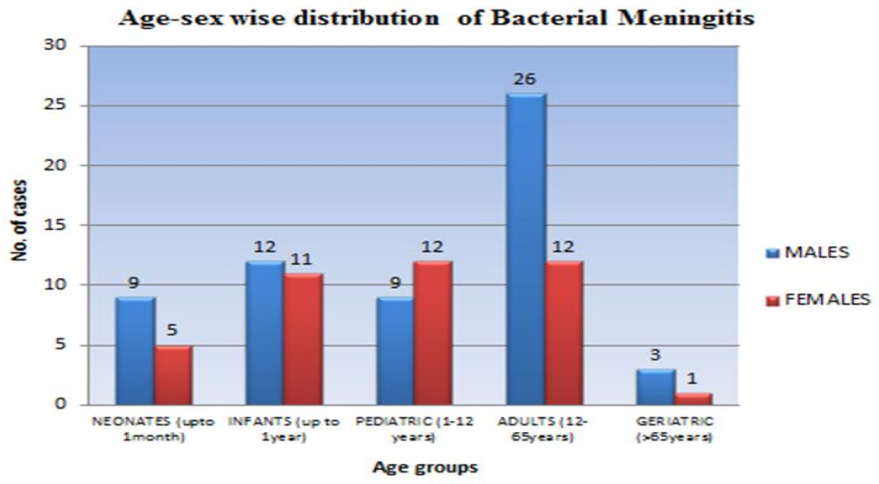
3] Septicemia- Clinical presentation with fever with/without meningeal signs in addition to growth on blood culture [12]

4. Results and Observations

I] Overview of positivity by laboratory methods:

Gram stain	CSF Culture	Blood Culture
07	24	13

2] Age and Gender-wise distribution of cases of bacterial meningitis:



3] Distribution of cases according to Clinical Presentation:

	Frank meningitis	Septicemia	Clinically Suspected Cases
Growth on CSF Culture	13	1	11
Growth on Blood Culture	4	1	7
Total Cases (n=100)	45	13	42

4] Microscopy vis-à-vis Conventional Culture:

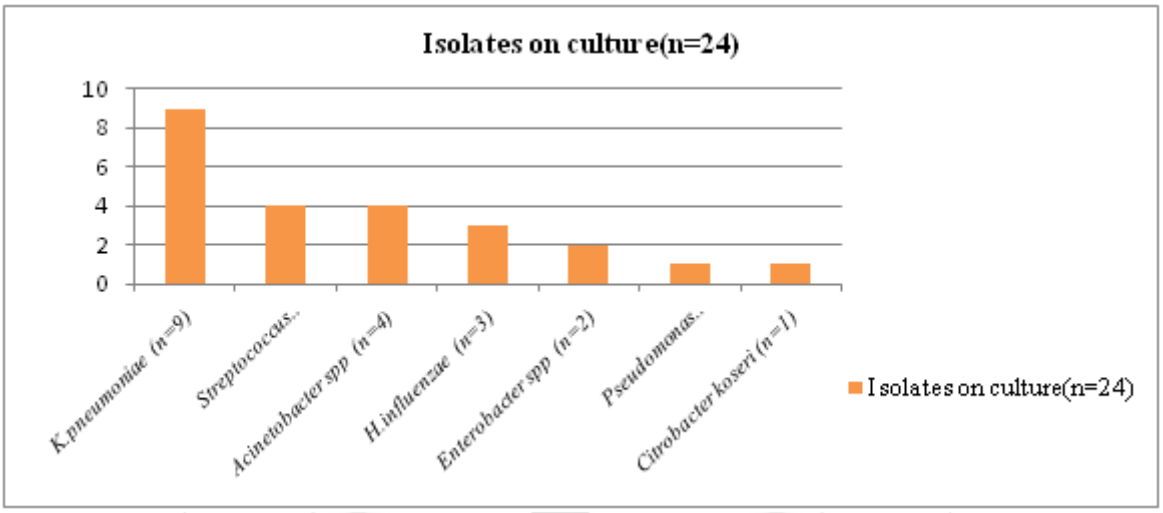
Microscopy	Culture Positive	Culture Negative	Total
Positive	5	2	7
Negative	19	74	93
Total	24	76	100

Sensitivity- 20.83% Diagnostic Accuracy- 79% PPV- 71.42%

Specificity- 97.36% Kappa co-efficient- 0.24 NPV- 79.56%

5] Pathogens isolated on Culture:

Isolates on CSF culture	% positivity
K.pneumoniae (n=9)	37.5%
Streptococcus pneumoniae (n=4)	16.67%
Acinetobacter spp (n=4)	16.67%
H.influenzae (n=3)	12.5%
Enterobacter spp (n=2)	8.34%
Pseudomonas aeruginosa (n=1)	8.34%
Citrobacter koseri (n=1)	4.16%



6] Anti-microbial susceptibility pattern of the isolated pathogens:

Sr.no	Sensitive Strains	Sensitive to 2nd line	MDR strains	Total
1	K.pneumoniae (2)	K.pneumoniae (5)	K.pneumoniae (2)	9
2	---	Acinetobacter spp (2)	Acinetobacter spp (2)	4
3	Streptococcus pneumoniae (n=1)	Streptococcus pneumoniae (3)	----	4
4	---	Enterobacter spp (1)	Enterobacter spp (1)	2
5	H.influenzae (1)	H.influenzae (1)	---	2
6	---	Ps.aeruginosa (1)	Ps.aeruginosa (1)	2
7	Citrobacter koseri (1)	---	---	1

MDR strains amongst the isolated pathogens were: K.pneumoniae (8.34%); Acinetobacter spp (50%); Enterobacter spp (50%) and Ps.aeruginosa (50%).

5. Discussion

Acute bacterial meningitis has notoriously contributed to significant mortality and morbidity worldwide. [13] Clinical misdiagnosis happens frequently owing to its atypical clinical presentation. The routine practice of instituting empirical anti-microbial treatment may take a haphazard course, prolonging the disease thereby, culminating in undesirable outcome. Case-fatality rates vary with age at the time of illness and the species of bacterium causing infection, but typically range from 3 to 19% in developed countries. Higher case-fatality rates (37-60%) have been reported in developing countries. Up to 54% of survivors are left with disability due to bacterial meningitis. [14, 15] Therefore, reporting of specific antimicrobial drugs that are effective to sustain the isolated pathogens has served to resolve this calamity.

In a study conducted by Nandita Chinchankar et al [16] gram stain was positive in 67% and culture was positive in 50%. R Mani et al [17] observed the positivity rate on Gram stain -65.7% and on culture-40.8%. Various studies have reported culture negative cases of meningitis or a low CSF culture positivity, ranging from 6 to 50%. [17, 18, 19] In the present study, the positivity rate on gram stain was 7%; CSF culture -24%; Blood culture-13%.

In the present study, higher rate was observed in males in almost all age-groups (except the pediatric population). [Table no.2] Similar results have been found in a study done by Maria Karanika et al. [12] Owing to the changing epidemiological trend, the affected age group has shifted from pediatric to adults due to immunoscenescence with age [20] and the availability of conjugate vaccines for pediatric age-group. [21] In the present study, highest affected was the adult age-group (13-65years). Similar findings were observed in a study done by Emma C et al [22] and Thomas T. Yoshikawa et al [23] According to them, the reason for higher incidence of meningitis in adults was hampered immune status due to diabetes; alcoholism; neuromuscular disorders; secondary to chronic illnesses.

Etsegenet Gedlu et.al. [24] found that "boys accounted for 64% of the patients, giving a male: female ratio of 1.7:1. The present study also showed slight male preponderance in pediatric age group.

The neonatal age-group is very critical and highly susceptible to serious illnesses. However, it lacks typical manifestations. Meningitis should be suspected in irritable or lethargic febrile children despite absence of neck rigidity or fever. [13]

Van de Beek et al (2007) [13] defined a case of frank meningitis as a patient having 2 out of the following 4 symptoms: fever, headache, stiff neck, and altered mental status. This formed the first patient group in the present study. The indefinite features in the extreme age-groups and the prolonged immunocompromised states due to non-infective etiology prompted us to consider the 2nd population group-"clinically suspected cases" [12] In the present study, extremes of age (17%); alcoholism (30.5%); diabetes (32.%); smoking (16.94%); [26] were the non-infective etiologies that raised suspicion. Similar findings were also observed in the studies done by Maria Karanika et al. [12] As recommended by WHO, blood cultures are often positive and valuable to detect the causative organism and establish the susceptibility patterns if CSF cultures are negative. Hence, in the present study the third group included septicemic patients. [12]

In the present study, the patients were divided into 3 groups: Frank meningitis; clinically suspected cases and Sepsis.

In the present study, Gram stain had sensitivity of 20.83% and specificity of 97.36%.Henry M Wu et. al [26] stated the sensitivity and specificity of gram stain as 97.5% and 94.1% respectively. Mohammadi Syeda Fasiha et al. [27] observed that Gram-stain showed a sensitivity of 53.33% and specificity of 83.52%. Neuman MI [28] reported the sensitivity as 67% and specificity as 99.9%.Yahia MA et al. [29] gave the sensitivity and specificity as 100% and 97.1% respectively. However, the sensitivity of gram stain given by P. Chakrabarti et al. [30] was 24.5%, comparable to our study.

The above references showed high sensitivity of gram stain. The specificity of the present study matches with those mentioned above indicating utility of gram stain to detect true negative cases.

In the present study, microscopy showed low sensitivity, which had also been documented in other studies: Lindiya Chaidir et.al; Larry et al; M. E. Török et al; Rajani Ghaju Shrestha et al.

The possible reasons for low sensitivity, in the present study, could be-less volume of sample received and low bacterial load in the received samples. [31] Leonard J et al [5] noted that the bacterial concentration had a profound effect on the sensitivity of microscopy. In their study, they noted a considerable rise in the sensitivity on microscopy from 25% to 97% when the bacterial load was increased from $<10^3$ to $>10^5$CFU/ml.

Yahia et al [29] observed that the sensitivity of gram stain was more for gram positive bacteria: Streptococcus pneumoniae (90%); H.influenzae (86%); while it was ~50% for gram negative pathogens. It varies with the type of pathogen involved. In the present study, gram negative bacteria being in majority, the sensitivity of gram stain may be considerably low. In the present study, microscopy was done by gram stain only. However, Larry et al [2] stated that the sensitivity can be improved by using special staining techniques and methods for individual pathogens. For example, quelling reaction for Streptococcus pneumonia and modified Gram stain for N.meningitidis. [3]

In the present study, positive predictive value and negative predictive value were 71.42% and 79.56% respectively. Mohammadi Syeda Fasiha et al. [27] observed the PPV and NPV as 36.36% and 91.02% respectively. Neuman MI [28] reported the PPV and NPV as 60% and 99.9% respectively. Diagnostic accuracy in the present study was 79%. It refers to the discriminatory power of both the tests together to identify the correct diagnosis. This combination of tests can successfully provide laboratory support to the clinical diagnosis [32]Kappa coefficient shows poor concordance.

In the present study, 3 samples (3%) were positive by both these tests suggesting 42% concordance. Rajani Ghaju Shrestha et al. [33] observed the positivity rate both on gram stain and culture as 7.2% i.e 100% in concordance when a large sample size was studied. However, Kristyn S. Beam et al [34] reported the concordance as 39%. However, it can be said that gram stain serves as a preliminary tool for prompt initiation of therapy till the pathogen is isolated on culture.

In the present study, the predominant pathogen isolated on culture was K.pneumoniae- 37.5% followed by Acinetobacter spp-16.64% and Streptococcus pneumoniae-16.64%.

The predominant organisms causing meningitis that have been documented till date are Streptococcus pneumoniae; N.meningitidis and H.influenzae. [35] Among these three, Streptococcus pneumoniae (16.64%) and H.influenzae (8.34%) have been isolated in the present study. N.meningitidis was not isolated. Moumita Adhikary et al [36]; et al also did not document any isolation of N.meningitidis. "N. meningitidis is known to occur in epidemics and isolation rates during inter-epidemic periods are generally low." [36] In the present study, out of 24 isolates obtained, 20 were gram negative bacilli (83.34%). In the Indian scenario, predominance of gram negative bacteria is explained by Shukla I et al [37]; Utpala Devi, et al [34]; R. Panjarathinamet al [36]. They stated that, lack of need for stringent growth conditions; ease of growth on ordinary media and presence in large numbers along the mucosal surfaces of the body make them predominantly detected pathogens. In the present study, the inclusion and exclusion criteria were strictly followed and the sample collection and transport were according to the laboratory protocol. Sensitive strains were noted majorly in this study contradicting their nosocomial origin.

K.pneumoniae was the dominant organism in the present study. R. Panjarathinam et al [38]; Huang CR et al [39] also observed similarly. The reason for the sudden rise of K.pneumoniae as a predominant pathogen is attributed to the production of hypermucoviscous strains with capsular serotypes K1 or K2 imparting invasiveness to the organism. [40]The pathogenicity is governed by cps; magA and rmpA genes. [41] Acquisition of c-rmpA, kfu and all S genes makes it a multi-drug resistant strain. K.pneumoniae has the ability to transfer genes horizontally [42] and being a nosocomial pathogen, its presence in CSF with susceptibility pattern needs to be monitored regularly. In the present study, 2 isolates (22.23%) were multi-drug resistant.

In the present study, other organisms with a nosocomial potential like Acinetobacter spp (16.64%) and Pseudomonas aeruginosa (8.34%) and Enterobacter spp (8.34%) were isolated. Similar findings were also documented by Moumita Adhikary et al [36].

Citobacter koseri (1%) isolated in the present study is a known pathogen of neonatal age. It has a tendency of causing cerebral abscess and sepsis and shows tropism for CNS. [43] In the present study, Citrobacter koseri was isolated from a patient of neonatal age-group that presented with sepsis. Other studies done by Clara Vaz Marecos et al [43]; Kari Saraswathi et al [45] also showed the isolation of Citrobacter koseri from neonates having cerebral abscess or sepsis.

20 isolates (83.34%) out of 24, were gram negative bacilli. MDR strains out of 20 were: K.pneumoniae (22.23%); Streptococcus pneumoniae (75%); Acinetobacter spp (33.34%); 50% each for Pseudomonas aeruginosa and Enterobacter spp.
Yohei Doi et al [42] noted that the reason for evolution of MDR strains was the acquisition of resistance by horizontal gene transfer. Susanne Schjørring et al [46] studied the evolution of antibiotic resistant strains in the gut which is the normal habitat for most gram negative pathogens. One of the complications could be meningitis due to resistant strains mentioned in the study.

6. Conclusion

Acute bacterial meningitis universally affects all age groups. However, in the present study, the most affected age group was 12-65 years with a higher incidence among males except in the pediatric age-group. The higher number of cases in adult age group was largely attributed to their frequent association with pathological factors like smoking (16.94%); alcoholism (30.5%); chronic illnesses (32.2%). Neonatal meningitis was also governed by risk factors like low birth weight and preterm labour that consisted 8 cases (53.34%). Besides, maternal morbidity also contributed to 7 cases (46.67%). Depending on the clinical manifestations, the cases of meningitis were divided into 3 groups:

1] Frank meningitis (45%); 2] Clinically suspected cases (42%); 3] Sepsis (13%)

The highest reported cases were of frank meningitis. The most predominant pathogen isolated was K.pneumoniae. The anti-microbial susceptility test was done for all the pathogens isolated on culture.

MDR strains amongst the isolated pathogens were: K.pneumoniae (2 out of 9 isolates); Acinetobacter spp (1 out of 2 isolates); Enterobacter spp (1 out of 2 isolates) and Ps.aeruginosa (1 out of 2 isolates).

Apart from microbiological parameters, clinical and other laboratory parameters should be considered to achieve a precise diagnosis.

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