

# Clinical Profile of Bacterial Meningitis in Children and Comparative Inter-Alia Analysis of Various Microbiological Tests

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## Abstract

Acute bacterial meningitis (ABM) is a life-threatening and neurologically debilitating infectious disease. We studied the clinical profile, organisms involved in bacterial meningitis in children, and compared the tests on cerebrospinal fluid (CSF), latex agglutination test (LAT), polymerase chain reaction (PCR), Gram stain (conventional) and Cyto-Tek cytospin centrifuge Gram stain to culture which is the gold standard. This was an observational cross-sectional study (age range 3 to 12 months) conducted in a tertiary care hospital, New Delhi, India over 1 year. A total of 101 patients were enrolled and divided into three age groups, namely, < 1 year, 1 to 5 years, and > 5 years. Fever was the most common presenting symptom in all groups (84.2%). Refusal to feed, headache, altered sensorium, vomiting, and blurring of vision were significantly associated with bacterial meningitis in all age groups. Cranial nerve palsies and neck rigidity were significantly higher in older children. Age < 5 years, low-socioeconomic status, overcrowding, and smoke exposure were identified as risk factors for meningitis. Eight children died within 48 hours of admission and the rest ( $n = 93$ ) recovered without complications. CSF culture was positive in 35.6% cases, with *streptococcus pneumoniae* being the most common organism. PCR was most sensitive (86.1%) and cytospin gram stain showed positivity in 65% cases which was statistically higher compared with conventional gram stain. Cytospin-prepared Gram stain was a viable low-cost alternative for early diagnosis of meningitis in low-income countries like India.

## Keywords

- ▶ clinical features
- ▶ culture
- ▶ PCR
- ▶ cytospin-prepared Gram stain

## Introduction

Acute bacterial meningitis (ABM) is a neurologically debilitating infectious disease, which is a major cause of childhood morbidity and mortality.<sup>1,2</sup> Mortality rates of approximately 5% have been recorded in the developed world, but these numbers may be as high as 30% in the developing world.<sup>1,2</sup> In India, bacterial meningitis constitutes approximately 1.5 to 2.6% of pediatric hospital admissions, and the mean case fatality rate is approximately 16%.<sup>3,4</sup>

Worldwide, *Hemophilus influenzae* type B (HiB) is the most common causative agent for meningitis in children.<sup>5</sup> This led to the introduction of vaccination against this organism to address pediatric meningitis. However, there have been conflicting data regarding the most common organism(s) that cause meningitis in Indian children.<sup>3,4,6–8</sup> Interestingly, widespread vaccination against *Hemophilus influenzae* type B (HiB), *Streptococcus pneumoniae*, and *Neisseria meningitidis* under the national immunization program in India is absent. Most of these infections have been

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reported retrospectively in case of an outbreak or as anecdotal reports.<sup>9</sup>

Cerebrospinal fluid (CSF) culture is considered the gold standard (due to high-specificity) for diagnosis of ABM, but it poses certain technical difficulties.<sup>10</sup> Many children report to hospital after being treated with inadequate doses of antibiotics. This prior administration of antibiotics may lead to a negative CSF culture.<sup>11,12</sup> Also, culture results are not available earlier than 3 to 4 days, and organisms causing meningitis may have fastidious requirements.<sup>10</sup> Gram staining and bacterial antigen tests also permit a rapid diagnosis but have limited sensitivity, resulting in many false-negative cases.<sup>13</sup> Molecular methods like polymerase chain reaction (PCR) are pathogen-specific but are not available widely in some urban and many rural hospitals.<sup>10</sup> Therefore, a rapid and sensitive tool is needed to diagnose ABM and improve the management of patients by early initiation of specific antibiotics. In a previous study it was seen that cytospin slide centrifuge could concentrate CSF samples, improving the sensitivity of CSF Gram stain by 2 to 3 logs compared with unconcentrated and conventional centrifuge smears.<sup>14</sup> This can expedite the diagnosis by identifying the organism and improve the management of patients with meningitis.

We studied the clinical profile, causative organisms of meningitis cases in children, over 1-year period and also did inter-alia analysis of CSF cytospin centrifuge (Gram stain) to Gram stain (conventional), culture, PCR, and latex agglutination test (LAT).

## Materials and Methods

This was a cross-sectional observational study at a tertiary care center, Safdarjung Hospital, New Delhi, India.

The inclusion criteria of the study are as follows: children in the age group of 3 months to 12 years presenting to the emergency department with signs and symptoms of pyogenic meningitis, that is, fever ( $> 38^{\circ}\text{C}$ ), altered sensorium, neck rigidity, headache, vomiting, blurring of vision, focal neurological deficits, refusal to feed, seizures along with CSF findings of  $> 5$  neutrophils/HPF, glucose  $< 40$  mg/dl or CSF glucose  $< 2/3$ rd of random blood glucose, and proteins  $> 45$  mg/dl.

### Exclusion Criteria

- Patients with alternative diagnosis and a normal CSF picture or mild pleocytosis.
- Patients with tubercular meningitis, viral meningitis, or fungal meningitis.
- Patients with a prolonged history or cerebral malaria were excluded.

Complete blood count, electrolyte tests, renal function tests, liver function tests, and urine examination were performed for all patients. Consent for lumbar puncture was taken from parents. CSF was collected under strict aseptic precautions and sent for cytology, biochemical analysis for sugar and protein estimation, CSF Gram stain by conventional and cytospin cytocentrifuge technique, and LAT for capsular organisms, CSF PCR and culture.

CSF culture (0.5 mL) to detect bacterial growth was done by BacT/ALERT 3D (BacT) (bioMérieux, Inc.; Durham, N.C., USA) automated culture system. Positive growth was confirmed by bacteria demonstration by subculture into chocolate agar, blood agar, and Macconkey agar. LAT was performed using Wellcogen bacterial antigen kit (Murex Diagnostics Ltd) for detection of specific antigens of certain organisms. CSF culture was taken as gold standard for diagnosis of ABM. CSF PCR was done using Rotor-Gene 2000/3000 (Qiagen N.V, Germany) to detect *Streptococcus pneumoniae*, *Nisseria meningitidis*, and *Hemophilus influenzae type b*.

### Cytospin Cyto-Centrifuge

Cyto-Tek (cytospin) centrifuge (Sakura Finetek; Torrance, USA) is a self-contained instrument engineered to transfer cells from suspension onto a glass slide through the process of cytocentrifugation. The Cyto-Tek centrifuge has a 12-specimen holder capacity and cell dispersion on the glass slide in a specific, square area. All the cells from the specimen were deposited evenly on the glass slide; therefore, screening of the slide is simplified.

1.0 mL of CSF was transferred in autoclavable screw-capped 15 mL centrifugation plastic tubes, and equal volumes of 5% sodium hypochlorite was added within the biosafety class II cabinet. The tube caps were tightly closed and shaken for 1 minute on a vortex mixer. The mixture was allowed to stand for 5 minutes to react with the decontaminating agent and then 1.0 mL of the mixture was dispensed each into two assembled Cyto-Tek chambers (1.0 mL disposable units) for preparation of duplicate smears. All samples were centrifuged in the Cyto-Tek centrifuge (cytospin) at 2500 rpm for 10 minutes, as specified by the manufacturer. After centrifugation, the slides were carefully removed from the Cyto-Tek chamber, air dried for 2 minutes, and heat fixed for 1 minute by passing through flame. The smears were stained by the Gram staining method.

The patients were treated as per standard protocol.

### Data Analysis

Analysis was performed by using SPSS software version 16.0. Accrement analysis with kappa coefficient will be done to evaluate without gold standard. If we have to analyze the test with respect to the gold standard, then standard tests like sensitivity, specificity, positive predictive value, negative value and accuracy were used.

## Results

Out of 9216 admissions in the pediatric department of the hospital, 101 were identified as ABM, which constituted 1.09% of children admitted to the pediatric department.

The children were divided into three groups for analysis, as follows:  $< 1$  year, 1 to 5 years, and  $> 5$  years. The majority number of cases were in the age group  $< 1$  year, with 42/101 (41.6%). A significant percentage (32.7%) were in the age group 1 to 5 years, and 25.7% in the age group  $> 5$  years. There was no significant difference between the three age groups. Sex ratio in the three groups was comparable.

**Table 1** Analysis of symptoms among three age groups

Complaints	< 1 y	1–5 y	> 5 y
Fever < 7 d	33 (76.8%)	27 (81.8%)	25 (96.2%)
Fever > 7 d	0	1 (3%)	1 (3.8%)
Refusal to feed	21 (50%)	4 (12.1%)	2 (7.7%)
Headache	1 (2.4%)	9 (27.3%)	20 (76.9%)
Altered sensorium	4 (9.5%)	19 (57.6%)	18 (69.2%)
Seizure	33 (78.6%)	28 (84.8%)	16 (61.5%)
Vomiting	8 (19%)	11 (33.3%)	19 (73.1%)
Blurring of vision	2 (4.8%)	0	6 (23.1%)
Focal neurological deficit	2 (4.8%)	0	2 (7.7%)
Other complaints	5 (11.9%)	2 (6.1%)	0

Presenting complaints analyzed showed that fever (median duration–3 days; range 1 to 9 days) was a universal complaint among all the age groups, amounting to 84.2% of all the cases (►Table 1). Refusal to feed, headache, altered sensorium, and vomiting were significantly associated with bacterial meningitis in infants (< 1 year) ( $p < 0.05$ ). Symptoms of altered sensorium, vomiting, blurring of vision, and headache were significant for meningitis in older children (> 5 years) when compared with infants. Focal neurological deficits and blurring of vision were not seen in children in the age group 1 to 5 years. Listlessness/malaise/feed refusal, seizures, and altered sensorium were commonly seen in younger children (< 5 years) with meningitis. ►Table 2 shows the risk factors that were predisposed to meningitis– among these, age less than 5 years, overcrowding, low-socioeconomic status, and exposure to smoke at home were most prominent. About 75% children were incompletely immunized and none had been vaccinated for either *Nisseria meningitidis*, *Streptococcus pneumoniae*, or *Hemophilus influenzae*.

On examination, most of the children had a normal nutritional status (75.2%). Severe malnutrition (grade 3 and 4) was reported in 10/101 (10%). Although age specific distribution of weight for age shows no statistically significant difference ( $p = 0.4$ ). When the three groups ►Table 3 were compared, they did not differ with regard to physical signs of malnutrition (vitamin deficiencies), change in level of consciousness, and motor system changes (muscle strength, deep tendon reflexes and muscle tone). Hypertension, abnormal fundus examination and cranial nerve palsies were predominantly seen in children older than 5 years. Neck stiffness and neck signs were significantly higher in older children (80.6% and 65%). Sensory deficits were not seen in any of the children.

### CSF Parameters

CSF opening pressure was raised in all of the cases. On gross examination, CSF was opaque/turbid in 6.9% cases. Comparison of CSF parameters among the cases showed the mean CSF neutrophils were nonsignificantly raised in the age group > 5 years when compared with the other groups.

**Table 2** Various risk factors for meningitis that were evaluated in the cohort

Risk factors	Number
Age < 5 y	75 (74.2%)
Male	52 (51.5%)
Low-socioeconomic status	81 (80.1%)
Overcrowding	79 (78.2%)
Household contact	0
Contiguous infection (sinusitis, mastoiditis, otitis media)	0
Bacterial endocarditis	0
IV drug abuse	0
Dural defects: post neurosurgery, CNS trauma	0
VP shunt and other devices/cochlear implants	0
Winter season	51 (50.5%)
Exposure to smoke (kitchen fuel/smoker in family)	76 (75.2%)
Post splenectomy status	0
Sickle cell anemia/thalassemia	0
Malignancy	0
Diabetes	0
Immunodeficiency/immunosuppression	0
HIV	0

Abbreviations: CNS, central nervous system; VP, ventriculoperitoneal.

CSF protein and sugar values were however comparable between the three groups ►Table 4.

### Organisms Isolated

Of all the meningitis cases, 36/101(35.6%) cases showed CSF culture positivity by Bact Alert. Among 32/36 (88%), *Streptococcus pneumoniae* were isolated, and one case each of *Escherichia coli*, *Nisseria meningitidis*, *Staphylococcus aureus* and *a hemolytic streptococcus*. *H. influenzae* was not isolated in any of the cases (►Fig. 1).

Of all the cases, PCR was positive in 39/101 (38.6%). LAT was positive in 39/101(38.6%) of cases. Conventional Gram stain was positive in only 7/101(6.9%) cases. Cytospin cyto-centrifuge Gram stain was positive in most number of 56/101 (55.4%) cases ►Table 5.

Each of these tests was compared with each other and to the gold standard (CSF culture). In comparison with the gold standard, cytospin cyto-centrifuge Gram stain showed a sensitivity of 65.65% which was significantly higher than conventional Gram stain (sensitivity– 11.1%). The sensitivity and specificity for each of the tests has been shown in (►Table 6). Comparing cytospin cyto-centrifuge Gram stain with LAT, there is 61.4% concordance in the two tests in the diagnosis of bacterial meningitis, and 38.6% discordance in the two tests, and the kappa value is significant (0.009).

**Table 3** Analysis of clinical signs in the three age groups

Clinical signs	< 1 y	1–5 y	> 5 y
Signs of vitamin deficiency	1 (2.4%)	1 (3%)	1 (3.8%)
Hypertension	0	1 (3%)	4 (15.4%)
Abnormal fundus	0	0	3 (11.5%)
Decrease in consciousness	6	12	16
Cranial nerve palsy	0	0	6 (23.1%)
Abnormal tone	10	10	11
Loss of power	0	0	0
Abnormal DTR	4- hypo 4 brisk	2- hypo 11-brisk	3- hypo 7- brisk
Positive Babinski	28 (66.7%)	25 (75.8%)	20 (76.9%)
Neck rigidity	0	15 (45.5%)	21 (80.8%)
Neck signs (Kernig, Bruzinski)	0	12 (36.4%)	17 (65.4%)
Other systems	2 (4.8%)	1 (3%)	1 (3.8%)

Abbreviation: DTR, deep tendon reflexes.

**Table 4** Comparison of CSF parameters

	< 1 y (mean ± 1SD)	1–5 y (mean ± 1SD)	5 y (mean ± 1SD)
Cytology (cells/mm <sup>3</sup> )	147 ± 190	120 ± 177	266 ± 592
Protein (mg/dl)	117 ± 100	96 ± 72	115 ± 81
Sugar (mg/dl)	34 ± 12	37 ± 15	30 ± 14

Abbreviation: SD, standard deviation.

When cytospin cyto-centrifuge Gram stain was compared with PCR, there was 53.5% concordance between the two tests, and 46.6% discordance between the two tests. Here too, kappa value was significant (0.082).

**Table 5** Cases that were positive or negative by each of the tests

Test (n = a + b); n = 101	a = positive	b = negative
CSF culture	36 (35.6%)	65
CSF gram staining (conventional)	7 (6.9%)	94
LAT	39 (38.6%)	62
CSF PCR	39 (38.6%)	62
Cytospin Cyto-Tek gram staining	56 (55.4%)	45

Abbreviations: CSF, cerebrospinal fluid; LAT, latex agglutination test; PCR, polymerase chain reaction.

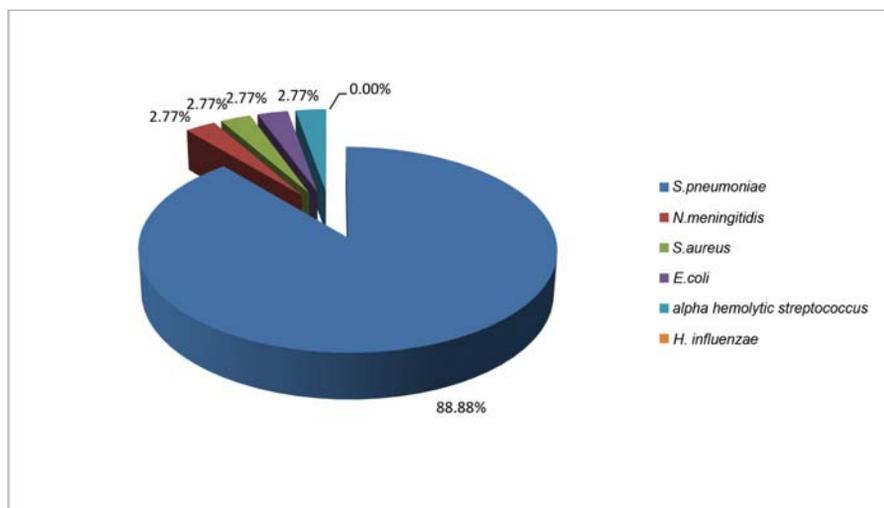
**Outcome**

Eight children (7.9%) died within 48 hours, six were in the age group < 1 year, and one each in the two other groups. Two cases (hydrocephalus, ventriculitis) each in the age group < 1 year and > 5 years (subdural effusion, hemiplegia) developed intracranial complications (both had hearing abnormalities). The rest had a normal recovery.

**Discussion**

ABM is a medical emergency, which warrants early diagnosis and aggressive therapy. The choice of empirical antimicrobial therapy for bacterial meningitis is based on the most common pathogen prevalent in a particular geographic area and age group and its antibiotic sensitivity pattern. We aimed to study the clinical features after dividing the children into three groups and comparing the presenting features and also comparing different microbiological tests used in the diagnosis of meningitis.

The incidence of ABM reported in our study was 1.09%. This is similar to studies by Kabra et al and other Indian studies<sup>3,4,13,15</sup> that reported a similar incidence ranging from 0.5 to 2.6%.



**Fig. 1** Organisms isolated from culture positive cases.

**Table 6** Comparison of various tests taking culture as the gold standard

	Cytospin Cyto-Tek gram staining	CSF PCR	LAT	Gram staining (conventional)
Sensitivity	65.6%	86.1%	72%	11.1%
Specificity	44.6%	87.7%	80%	95.4%
Positive predictive value	35.7%	79.5%	66.7%	57.1%
Negative predictive value	64.5%	91.9%	83.9%	66.1%
Accuracy	48.5%	87.1%	77.2%	65.4%
Kappa	0.002	0.725	0.513	0.079

Abbreviations: CSF, cerebrospinal fluid; LAT, latex agglutination test; PCR, polymerase chain reaction.

Most children who suffered from meningitis were < 5 years, that is, 75% of the total cases when compared with older children (5–12 years). Similar results were reported by Pulickal et al<sup>15</sup> who reported that 42.5% of their ABM were infants. Sex distribution of cases did not show any significant difference. However, Pulickal et al<sup>15</sup> reported 70% meningitis cases in males which could be due to referral bias.

Short history of fever (< 7 days) was the universal presenting symptom among all the age groups. But presence of fever is not sufficient to diagnose bacterial meningitis, since it is a very nonspecific symptom. Refusal to feed, seizure, and vomiting formed the major constellation of symptoms seen in the < 1-year age group. Whereas, headache was most commonly seen in > 5-year age group (76.9%). Altered sensorium was also common in the 1 to 5years (57.6%) and > 5 years (69.2%) age group. This is in contrast to the study by Chinchankar, who reported altered sensorium in majority of their cases (98%).<sup>3</sup> This could possibly be due to majority of children being infants or due to early presentation of cases in our study. Seizures were more commonly seen in children less than 5 years of age (76.2% overall), which is similar to the study by Chinchankar et al. It is noteworthy that children in this age group can present with seizures due to other illnesses like febrile seizures, cerebral malaria, hypocalcemia, etc. Focal neurological deficit did not have any statistical association with any age group and signified some intracranial complication.

Signs of vitamin deficiency and hypertension due to raised intracranial tension were seen in minority of cases. Motor and sensory system abnormalities were not seen in any case.

Cranial nerve palsies were seen in 23.1% and fundus abnormalities in 11.5% cases, all of whom were above 5 years age. This is similar to the study by Farag et al<sup>16</sup> who reported in 16.8% cases. Neck signs were mostly seen in the age group 1 to 5 years (45.5%) and > 5 years (80.8%). Chinchankar et al<sup>3</sup> reported meningeal signs were elicited in 26% cases, but they were all below 5 years, which is in contrast to our study.

However, we are not sure if they included infants too. Another study by Pulickal et al<sup>15</sup> reported neck pain to be present in 15% cases. This variability could be due to heterogeneity of our cases and difference in reporting our cases (dividing into three age groups).

### Analysis of Microbiological Tests

About 35.6% of the CSF cultures were positive. Most of the cases were isolated from the age group 1 to 5 years (45.5%). CSF culture positivity rates have been variable, like Pulickal et al<sup>15</sup> reported 47.5% positivity, and Chinchankar et al<sup>3</sup> isolated the causative organism in 50% cases. Some changing trends in etiological agents for community-acquired ABM have been reported worldwide in recent times. The most common organism isolated in our study was *Streptococcus pneumoniae* which is similar to the study by Chinchankar et al<sup>3</sup> and Mani et al.<sup>13</sup> However, in contrast, Pulickal et al<sup>15</sup> reported *Staphylococcus aureus* to be the most common organism. This again could be due to referral bias or under-reporting of *Streptococcus pneumoniae* meningitis cases. Some Indian authors have reported a high-incidence of *H. influenzae* in the pediatric age group,<sup>3</sup> while others have experienced a low-incidence.<sup>4</sup> The plausible explanation for absent Hib meningitis could be due to the following:

True biologically low rate of Hib meningitis in children due to

1. Genetic factors in children resulting in reduced infection rates.
2. Early exposure to *Hemophilus* and other bacterial with cross-reacting antigens, leading to natural immunity.
3. Local variation in bacterial virulence and transmission.

We performed many other tests on CSF like LAT, PCR, and Gram staining with and without cytospin. PCR was found to have a very high sensitivity and specificity similar to studies like that by Mani et al<sup>13</sup> but the main limitation for its use is its limited availability and high-cost.<sup>10</sup> The PCR test has the ability to pick up cases which were not positive by conventional techniques like culture and Gram staining. On the flip side, PCR requires primers for each organism, for example, if an unconventional organism (like *legionella*, *listeria*) is the causative agent and a primer for that organism is not available, the result would be negative.

LAT has an advantage of being a rapid diagnostic test, which is easy to perform, and can test for various microorganisms at a time, but it has poor sensitivity and specificity compared with PCR. In contrast to our results, Chinchankar<sup>3</sup> found 2/3rd of their patients were positive for LAT. That is why its utility is being questioned in various published studies, which could be due to changes in the epidemiology related to the use of vaccines for capsulated organism. LAT is mainly directed against capsular organisms and has limited application.

Gram staining has poor sensitivity but good specificity for bacterial meningitis and is still used for being a rapid, accurate bedside test. The yield of bacteria on Gram staining depends on several factors like the number of organism's present, prior use of antibiotics, technique used for smear preparation, staining technique, and observer's skill and experience.<sup>13</sup>

When Gram staining was done with cytospin (Cyto-Tek cytocentrifuge), the positivity improved to 55.4%, as reported by Mani et al.<sup>13</sup> They recorded a high-yield on Gram staining with cytospin use. A cytospin also provides several other diagnostic benefits like good preservation of morphology of cells and bacteria and increased rate of detection of bacterial pathogens, especially in partially treated pyogenic meningitis, which can mimic tuberculous meningitis, posing a diagnostic dilemma for clinicians. Comparing cytospin Gram staining with PCR, there was 53.5% concordance between the two tests and 44.6% discordance. The cytospin centrifuge provides a simple, rapid, and effective means of concentrating CSF for Gram staining. Shanholtzer et al<sup>14</sup> reported that cytospin centrifuge is effective in detecting microorganisms and in increasing the number of bacteria found per smear, as compared with unconcentrated smears. Therefore, it is a useful supplement to conventional Gram staining. Even 0.5 mL or less of specimen is enough to do the test and aids in early diagnosis of bacterial meningitis.

### Outcome

The mortality rate in our study was approximately 7.9%, which is similar to the study by Pulickal et al<sup>15</sup> who reported approximately 8.75% in their study. Severe neurological sequelae were reported in 2.9% cases, which is in contrast to the above authors who reported it among 32.5% patients, which can be explained due to improved supportive care and better use of empirical antibiotics.

### Conclusion

Younger children presented with complaints of fever, refusal to feed, and altered sensorium, whereas older children had fever, neck rigidity, and cranial nerve palsies. Younger age, low-socioeconomic status, overcrowding, and smoking exposure were the most important risk factors for meningitis in children. The most common etiological agent isolated was *Streptococcus pneumoniae*. CSF culture was positive in only 36.5%. CSF PCR performed best amongst the tests studied for diagnosis of bacterial meningitis. But, cytospin Gram staining was found to have a high-sensitivity and specificity compared with conventional techniques. It is a viable alternative in low-income countries and aids in the early diagnosis of bacterial meningitis.

### What is Already Known on this Topic?

- Bacterial meningitis is present with nonspecific findings like fever, anorexia, headache, poor feeding, and sometimes rashes.
- There are discrepancies with regard to the isolated causative organism.

### What does this Study Add?

- Fever, refusal to feed, headache, altered sensorium, vomiting, and blurring of vision were significantly associated with bacterial meningitis in children (< 5 years age).

- Cranial nerve palsies and neck rigidity were significantly higher in older children.
- Cytospin-prepared Gram staining increased the yield of conventional Gram staining and was a cost-effective alternative for early diagnosis of meningitis.

### Ethical Approval

This study was approved by the Institute Ethics Committee (IEC) at Vardhaman Mahavir Medical College (VMMC) and Safdarjung Hospital. All the authors shared their consent to publish the article in an indexed journal.

### Availability of Data and Materials

The data published in this study is original to the authors, and the raw data are available with R.H., as he is the primary author of this study.

### Authors' Contributions

All the authors have equally contributed to the manuscript.

### Funding

None.

### Conflict of Interest

None declared.

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