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Prospective multi-centre sentinel surveillance for *Haemophilus influenzae* type b & other bacterial meningitis in Indian children

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***Background & objectives*: *Haemophilus influenzae* type b (Hib) is one of the leading bacterial causes of invasive disease in populations without access to Hib conjugate vaccines (Hib-CV). India has recently decided to introduce Hib-CV into the routine immunization programme in selected States. Longitudinal data quantifying the burden of bacterial meningitis and the proportion of disease caused by various bacteria are needed to track the impact of Hib-CV once introduced. A hospital-based sentinel surveillance network was established at four places in the country and this study reports the results of this ongoing surveillance.**

***Methods*: Children aged 1 to 23 months with suspected bacterial meningitis were enrolled in Chennai, Lucknow, New Delhi, and Vellore between July 2008 and June 2010. All cerebrospinal fluid (CSF) samples were tested using cytological, biochemical, and culture methods. Samples with abnormal CSF (≥10 WBC per µl) were tested by latex agglutination test for common paediatric bacterial meningitis pathogens.**

***Results*: A total of 708 patients with abnormal CSF were identified, 89 of whom had a bacterial pathogen confirmed. Hib accounted for the majority of bacteriologically confirmed cases, 62 (70%), while *Streptococcus pneumoniae* and group B *Streptococcus* were identified in 12 (13%) and seven (8%) cases, respectively. The other eight cases were a mix of other bacteria. The proportion of abnormal CSF and probable bacterial meningitis that was caused by Hib was 74 and 58 per cent lower at Christian Medical College (CMC), Vellore, which had a 41 per cent coverage of Hib-CV among all suspected meningitis cases, compared to the combined average proportion at the other three centres where a coverage between 1 and 8 per cent was seen ($P<0.001$ and $P= 0.05$, respectively).**

***Interpretation & conclusions*: Hib was found to be the predominant cause of bacterial meningitis in young children in diverse geographic locations in India. Possible indications of herd immunity was seen at CMC compared to sites with low immunization coverage with Hib-CV. As Hib is the most common pathogen in bacterial meningitis, Hib-CV would have a large impact on bacterial meningitis in Indian children.**

Key words *Haemophilus influenzae* type B - meningitis - surveillance

Haemophilus influenzae type b (Hib) was one of the leading causes of invasive bacterial disease and pneumonia in children in 2000, killing over 370,000 children globally, with the highest burden of disease in low and middle income countries[1]. In 2000, only four low income countries had introduced Hib conjugate vaccines (Hib-CV) into their universal childhood immunizations[2]. However, recently there has been an increased use of Hib-CV. By the end of 2009, 83 per cent of low income countries had introduced Hib-CV universally, and several others have announced plans to introduce the vaccine[3]. In all countries that have introduced Hib-CV, immunization has resulted in remarkable reductions in invasive Hib disease and purulent meningitis[4-6], and proven effective at preventing 5 per cent of clinical and 21 per cent of X-ray confirmed pneumonia[1].

India is one of the few developing countries and the last remaining country in the South Asian sub-continent to have not introduced Hib-CV into its routine immunization programme. Although Hib-CV is used widely in the private market in India and is universally recommended by the Indian Academy of Pediatrics[7], it is not available to the poorest children who are most in need of the vaccine[8]. The burden of Hib disease in India, estimated to be 72,000 deaths and 2.4 million cases of severe disease in 2000[1], has likely changed little in the last decade. Recent studies continue to identify pneumonia and meningitis as leading causes of childhood mortality in India, responsible for 22 per cent of under 5 deaths[9], and confirm Hib as the leading cause of purulent meningitis[10-13].

India has recently decided to introduce Hib-CV into the universal immunization programme (UIP) in selected States. Continuous surveillance for Hib and other common causes of bacterial meningitis is important to define the major causes of meningitis in children, their relative burden, and assess the need for prevention and treatment strategies. Uninterrupted surveillance before, during, and after introduction is important to track the impact of vaccination on Hib disease. To help reach these goals, a multi-site geographically diverse hospital based sentinel surveillance network was established to provide longitudinal data on bacterial meningitis. Here we report the results of bacterial meningitis in young children focusing on the aetiology, relative importance of Hib as a pathogen, clinical aspects and mortality from this ongoing surveillance.

Material & Methods

This study took place at four hospitals between July 2008 and June 2010 - Christian Medical College (CMC), a 2517 bed private hospital with 171 paediatric beds located in Vellore, Tamil Nadu; Chhatrapati Shahuji Maharaj Medical University (CSMMU), a 2424 bed public hospital with 100 paediatric beds located in Lucknow, Uttar Pradesh; Institute of Child Health and Hospital for Children (ICH&HC), a 537 bed public paediatric hospital located in Chennai, Tamil Nadu, and Kalawati Saran Children's Hospital (KSCH), a 370 bed public paediatric hospital located in New Delhi. Children admitted to the study hospitals with clinically suspected meningitis between ages >30 days to <24 months were enrolled. At CSMMU, ICH&HC, and KSCH, all children fitting the age criteria were screened for suspected meningitis as they were admitted through daily surveillance in the hospital wards. At CMC, all children who had CSF submitted to the microbiology laboratory with any WBC present in the CSF were screened for enrollment.

The criteria for clinically suspected meningitis were based on WHO criteria which defines clinically suspected meningitis as any child with acute fever and one of the following signs: neck stiffness, altered consciousness and other meningeal signs[14]. Acute fever was defined as fever with an onset within the last 5 days confirmed at admission (>38 °C at CMC, ICH, KSCH and >38.5°C at CSMMU) and / or from history taken from the child's guardian. Other signs for inclusion included any of the following: seizure, altered consciousness, bulging fontanelle, neck stiffness. In addition, children suspected to have meningitis based on the attending physician's clinical judgment, regardless of the above mentioned symptoms, were also enrolled. At CMC, children were excluded if they were hospitalized in the past 10 days due to a non-related illness.

Once enrolled, demographic data, health history, and clinical characteristics were obtained. Demographic information was obtained by questioning the patient's caregiver. Pre-admission clinical history was obtained from the patients' case sheets, but in the event of missing or unclear information, it was obtained from the caregiver. Physical findings and other clinical information were obtained from the patient's case sheet or, if information was missing, from the attending physicians. Antibiotics used prior to hospitalization and recent lumbar puncture (LP) were determined based on visual verification of antibiotics, hospital records/

receipts, or parent recall. Immunization records were taken from immunization cards when available or parent recall. Medical decision making and care for the patients was provided by the regular clinical staff following the hospitals' standards of care. LPs were performed at the treating physician's discretion and with guardian's consent. LP kits and attendants were made available around the clock to assist with logistical aspects of LP such as immediate transportation of samples and delivery of laboratory results to physicians. Final clinical diagnosis and outcome were taken from hospital records.

CSF samples were examined for complete white blood cell count (WBC), protein and glucose concentrations, Gram stain and bacterial culture (all sites except CSMMU) using chocolate agar plates incubated in a CO_2 enriched atmosphere. In addition, cases with abnormal CSF findings (CSF with ≥10 WBC per µl) were tested using a latex agglutination test (LAT) kit specific for Hib, *Streptococcus pneumoniae*, *Escherichia coli* K1, group B *Streptococcus* and *Neisseria meningitidis* groups A, B, C, Y and W135 (either Directigen™ Meningitis Combo Test; Becton, Dickinson and Company, USA or Wellcogen® Bacterial Antigen Test Kit, Remel Products, UK). In the case of limited CSF volume, samples were prioritized for WBC, LAT, and biochemistry. Care was taken to assure that laboratory methods were of high quality and performed as soon as possible after the LP. Internal and external quality control procedures were in place, and training of research staff was undertaken as part of the ongoing surveillance programme.

Clinically suspected cases of bacterial meningitis were categorized as follows: abnormal CSF (CSF with ≥10 WBC per µl), probable meningitis [Following WHO criteria[14]; CSF findings of either ≥100 WBC per µl, or 10-99 WBC per µl with increased protein concentration (≥100 mg/dl) or decreased glucose concentration (<40 mg/dl), or visually cloudy CSF], and confirmed bacterial meningitis (CSF with demonstrated bacterial pathogen positive via culture and / or LAT).

Technical assistance and on site evaluation was provided by Johns Hopkins School of Public Health (JHSPH), USA and the All India Institute of Medical Sciences (AIIMS), New Delhi to ensure the standardization and quality of clinical surveillance, laboratory methods, and data collection between centers. Databases were managed using Access 2007® (Microsoft®, USA). Data were analyzed using Stata IC 10© (StataCorp) and Excel 2007® (Microsoft®, USA). Differences between proportions of positive cases among sites were determined using Fisher's exact two sided test.

Ethical clearance was obtained from the Institutional Review Board at each site. Additional clearance was obtained from the Indian Council of Medical Research and JHSPH for CSMMU and KSCH, and from the International Clinical Epidemiology Network for CMC and ICH&HC. Informed consent was taken from caregivers prior to obtaining demographic information, pre-admission clinical history and for sample storage.

Results

A total of 2,912 patients with suspected meningitis were enrolled over the course of 21 months at CMC (n=382, July 2008 to March 2010), 13 months at CSMMU (n=445, March 2009 to March 2010), 18 months at ICH&HC (n=1614, January 2009 to June 2010), and 6 months at KSCH (n=471, March to September 2009).

The demographics of the children admitted to the hospitals with suspected meningitis varied greatly between sites (Table I). At CSMMU and KSCH, 37 and 50 per cent of children were severely malnourished, defined using WHO criteria[15], while only 34 and 35 per cent of the children's mothers had more than primary education. In contrast, both CMC and ICH&HC had higher rates of education, with >75 per cent of mothers having higher than primary education, and less than 15 per cent children had severe malnutrition. The proportion of children who had received one or more diphtheria, tetanus, pertussis (DTP) immunization was high at CMC and ICH&HC, with both sites approaching 90 per cent, where as only 54 and 56 per cent at CSMMU and KSCH had received any DTP. When questioned about vaccines other than those covered by National Immunization Schedule, CMC reported a relatively higher immunization with any dose of Hib-CV; 41 per cent when compared to lower rates of 0.1, 8 and 1 per cent, respectively by CSMMU, ICH & HC and KSCH. Of children immunized with Hib-CV, 95 per cent received 2 or more doses. Only one child had received a pneumococcal vaccine. The proportion of clinically suspected meningitis cases who received antibiotics prior to LP varied from 34 to 89 per cent across sites (average 65.2%), while overall 35 per cent received antibiotics prior to hospital admission (Table I).

A total of 2,584 (89%) children underwent LP and subsequent laboratory testing. LP rates were generally high at all sites; at CSMMU, ICH&HC, and KSCH,

Table I. Demographics of patients enrolled on clinical suspicion of meningitis

Characteristic	CMC		CSMMU		ICH&HC		KSCH	
	N	(%)[a]	N	(%)[a]	N	(%)[a]	N	(%)[a]
Total enrolled	382		445		1614		471	
Female gender	143	(37)	139	(31)	687	(43)	171	(36)
Age >12 months	109	(29)	162	(36)	627	(39)	67	(14)
Severe malnutrition[b]	32	(9)	166	(37)	228	(14)	235	(50)
Maternal education, >1 primary school	340	(89)	141	(34)	1168	(75)	127	(35)
Vaccination								
DTP, one or more immunization	333	(89)	239	(54)	1436	(90)	229	(56)
Hib, one or more immunization	152	(41)	2	(<1)	124	(8)	2	(1)
Clinical information								
Antibiotics received prior to LP	131	(34)	245	(55)	1110	(72)	413	(89)
LP with CSF submitted to laboratory	382	(100)	443	(99)	1317	(82)	442	(94)

[a]Proportions are based on available data in each category, not all enrolls. The number of missing values in each category follows; gender: 1 (<1%), malnutrition: 31 (1%), maternal education: 189 (6%), DTP immunization: 80 (3%), Hib immunization: 111 (4%), antibiotics: 78 (3%); [b]Severe malnutrition was defined as a weight to age Z-score >3 standard deviations below WHO average[14]
LP, lumbar puncture; DTP, diphtheria, tetatus, pertussis; CMC, Christian Medical College; CSMMU, Chhatrapati Shahuji Maharaj Medical University; ICH&HC, Institute of Child Health & Hospital for Children; KSCH, Kalawati Saran Children's Hospital

99, 82 and 94 per cent of suspected meningitis patients underwent LP, respectively. The most common reasons for not performing a LP were the physician's decision that an LP was not necessary (66%), parental refusal (11%), and contraindication (10%). All children enrolled at CMC had an LP done since a CSF sample at the lab was the starting point for enrollment. The WHO[14] sets criteria for quality indicators of bacterial meningitis surveillance, which include the proportion of probable bacterial cases who were tested with either culture or LAT (target: >90%, this study: 86%), the proportion of patients with abnormal CSF in which bacterial pathogen was identified (target: >15%, this study: 13%), the proportion of patients with WBC of >100,(target: >40%, this study 25%), and the proportion of CSF isolates are Hib positive (target: >20%, this study 62/89 70%).

Of the children who underwent an LP, 708 cases (27.4 %) with abnormal CSF were found, 441 (62%) of whom were defined as probable bacterial meningitis. LAT was performed on 592 (84%) of cases with abnormal CSF findings. Bacterial pathogens were confirmed in 89 patients. The majority of bacterial pathogens 79 (89%), were isolated from patients with CSF findings indicative of probable bacterial meningitis, with most others found in patients with abnormal CSF. Two pathogens were identified in CSF with <10 WBC per µl, both of which had raised protein and low glucose levels. The case fatality rate increased from 9 per cent (n=64) in patients with abnormal CSF to 16 per cent (n=14) among cases of confirmed bacterial meningitis. The case fatality rate for Hib meningitis was 11 per cent. There was no clear seasonal distribution of probable or confirmed bacterial meningitis.

Of the 89 confirmed bacterial cases, 62 were found to be Hib (70%), 12 *S. pneumoniae* (13%), seven GBS (8%), and eight (9%) other bacteria: three *Neisseria meningitidis* (two from groups A, C, Y or W135, one isolate that was either *Neisseria meningitidis* group B or *Escherichia coli* K1; the LAT could not differentiate between these two bacteria, and culture and Gram stains were negative), two *Enterobacter* sp., and one each *Citrobacter freundii*, *Pseudomonas* sp., and Group D *Salmonella* (Table II). Bacterial meningitis was most common in young infants, with 87 per cent of cases occurring in infants 12 months of age or younger (Fig. 1). The distribution of Hib cases showed a clear peak between 4 to 9 months. Hib was identified in 8.7 per cent (n=62) of those with abnormal CSF, while any organism identification in this category was 12.6 per cent (n=89). In the probable meningitis group Hib was identified in 13.2 per cent while overall organism identification was 19.3 per cent (n=85).

Table II. Confirmed bacterial meningitis cases by isolation method

Pathogen	Isolates	LAT positive No. (%)		Gram stain positive No. (%)		Culture positive No. (%)	
H. influenzae type B	62	62	(100)	7	(11)	1	(2)
S. pneumoniae	12	12	(100)	2	(17)	2	(17)
Group B Streptococcal	7	7	(100)	0	(0)	0	(0)
N. meningitis[a]	3	3	(100)	2	(67)	0	(0)
Other bacteria[b]	5	0	(0)	0	(0)	5	(100)
Total	89	84	(94)	11	(12)	8	(9)

[a]Two isolates are from groups A, C, Y or W135, one isolate that was from either *N. meningitidis* group B or *Escherichia coli* K1 (LAT could not differentiate between these two bacteria; [b]Isolates were as follows: two *Enterobacter* sp., and one each *Citrobacter freundii*, *Pseudomonas* sp., and Group D *Salmonella*

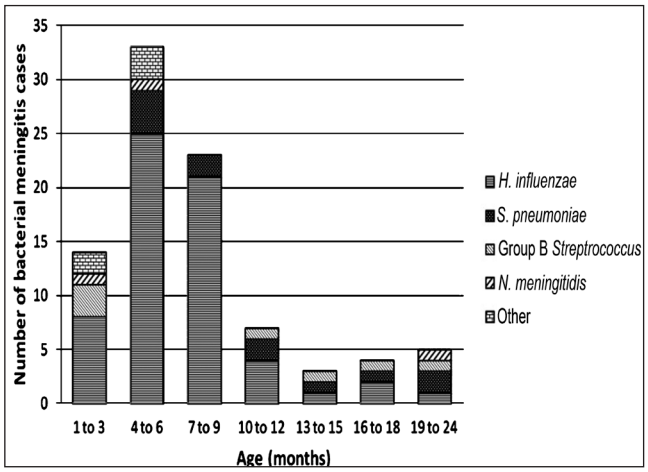

Fig. 1. Age distribution of confirmed bacterial meningitis cases.

The clinical symptoms found in confirmed Hib meningitis cases were generally non-specific: fever, seizure, and altered consciousness were most common (Table III). Stiff neck and bulging fontanelle, were found in 10 (16%) and 9 (15%) of cases, respectively. Nine (15%) of Hib cases were severely malnourished, 51 (82%) Hib cases showed >100 WBC per µl of CSF and 58 (94%) fit under the WHO definition of probable bacterial meningitis. However, 4 (6%) had only abnormal CSF findings. All Hib cases were positive by LAT. Seven Hib cases (11%) had positive Gram stains, all of whom were also LAT positive. Only one Hib case was culture positive for Hib in addition to being Gram stain and LAT positive.

The proportion of meningitis cases caused by Hib was lower at CMC as compared to CSMMU, ICH&HC, and KSCH (Fig. 2). All individual comparisons of CSMMU, ICH&HC or KSCH to CMC were statistically significant ($P<0.05$) except for the comparison of probable bacterial meningitis between CMC and CSMMU. When the combined data from CSMMU, ICH&HC and KSCH were compared against CMC, proportions of abnormal CSF and probable bacterial meningitis caused by Hib were respectively 12 and 15 per cent, all of which were significantly higher than the proportions seen at CMC, respectively 3 and 6 per cent ($P<0.001$, $P<0.015$). No difference was seen in the proportion of meningitis caused by pneumococcus between sites. However, the number of Hib cases isolated per pneumococcal case was lower at CMC compared to the other sites. CMC identified 1.8 cases of Hib for each pneumococcal case identified, while 7, 6.3, and 7.5 Hib cases were identified for each

Table III. Clinical and laboratory findings in confirmed Hib meningitis cases

Clinical inclusion criteria	N	(%)
Temperature	61	(98)
Seizure	40	(65)
Altered consciousness	40	(65)
Bulging fontanelle	9	(15)
Stiff neck	10	(16)
≥3 inclusion symptoms	32	(53)
CSF findings		
WBC count, 10-99 per µl	11	(18)
WBC count, >100 per µl	51	(82)
Protein >100 mg/dl	39	(63)
Glucose <40 mg/dl	30	(48)
Probable bacterial meningitis	58	(94)

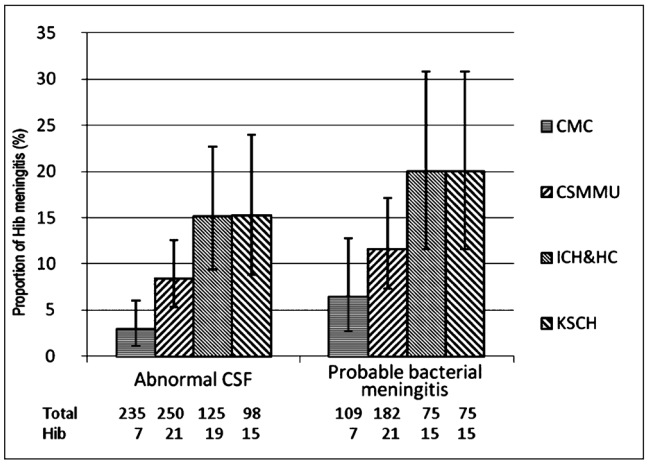

Fig. 2. Proportion of meningitis due to Hib. The proportion of abnormal CSF or probable bacterial meningitis cases with confirmed Hib comparing CSMMU, ICH&HC, or KSCH to CMC were significantly different (P<0.05), except for comparison of probable bacterial meningitis between CMC and CSMMU.

pneumococcal case at CSMMU, ICH&HC and KSCH, respectively. When combined data from CSMMU, ICH, and KSCH were compared with CMC, CMC demonstrated a 74 per cent lower proportion of Hib isolates per case with abnormal CSF, 58 per cent lower proportion per probable meningitis case, and a 75 per cent lower proportion of confirmed Hib cases per pneumococcal isolate.

Discussion

Multiple sentinel surveillance centres were established for monitoring bacterial meningitis in young children in India. A total of 2912 patients in the age group of 1 to 23 months with suspected meningitis were enrolled at four surveillance centres. Findings from this study support earlier studies establishing Hib as the leading cause of bacterial meningitis in Indian infants, and was associated with mortality in confirmed cases[10-13,16,17]. Only a few bacterial pathogens were isolated using culture methods, likely due to high rates of antibiotic use prior to hospitalization and LP, making sensitive methods such as LAT critical for the identification of Hib disease. Additionally, use of Hib-CV outside the routine vaccines under National Immunization Programme may have made an impact on Hib meningitis at CMC with modest coverage, suggesting herd immunity.

Other studies in India examining bacterial meningitis in the last decade using sensitive methods such as LAT or polymerase chain reaction (PCR) have detected Hib in 18 to 35 per cent of likely childhood bacterial meningitis cases[10-13]. The exact definitions of likely bacterial meningitis varied between studies based on clinical and laboratory cut-offs but were similar to the definition of probable bacterial meningitis presented here, and match the proportionate range seen at the sites with low Hib-CV coverage: CSMMU, ICH&HC, and KSCH (12 to 20%). Across these sites an average of 15 per cent of probable bacterial meningitis, as defined by the WHO, were due to Hib. This is similar to results of a surveillance study in Sri Lanka which used similar methods, where 18 per cent of probable bacterial meningitis in children 2 months to 2 years old was caused by Hib[18]. The limited variation between these two countries and between Indian studies when using similar benchmarks and detection methods, suggests that these findings are generally indicative of hospitalization due to bacterial meningitis in the region.

Eleven per cent of children with Hib meningitis altogether died despite being admitted to hospitals. This may be an underestimate of case fatality, since the outcome for 21 per cent of children with confirmed Hib meningitis could not be determined because the children were lost to follow up. In addition, 15 patients who were diagnosed with acute central nervous system infections died before they could be stabilized for LP. Despite the possibility of underestimation, the overall fatality rate was similar to other Indian studies, which have shown a combined case fatality rate of 15 per cent among hospitalized Hib cases[11,16,17]. The case fatality rate in Hib cases was lower than that seen for other confirmed bacterial meningitis cases, similar to earlier studies in India[19,20]. However, because Hib causes the majority of bacterial meningitis cases, it remains the dominant cause of all-cause bacterial meningitis mortality despite its lower fatality rate. It should be noted that the studies described here represent hospital based studies and likely do not represent the mortality of bacterial meningitis cases who do not receive hospital care, which are thought to have a much higher mortality rate[1].

Possible herd immunity effect of Hib-CV was demonstrated at CMC, where a lower relative proportion of disease was seen when compared to Hib-CV vaccination rate. A 41 per cent Hib-CV coverage rate was noted among all enrolled patients, but the proportion of Hib isolates per abnormal CSF and probable bacterial meningitis was 74 and 58 per cent lower, respectively, when compared to the combined data from CSSMU, ICH&HC and KSCH. Additionally, the number of Hib

cases isolated per pneumococcal case, which has been used as a parameter to judge the impact of Hib vaccine in low income settings[6,21,22], was 75 per cent lower at CMC compared to the other sites. The proportion of patients with abnormal CSF and non-Hib confirmed pathogens was 4 per cent at CMC, and ranged from 2 to 8 per cent at the other sites, suggesting that non-Hib pathogens were present at similar proportions regardless of the lower proportion of Hib cases. This is consistent with the herd effect from Hib vaccine seen in other countries[23-25]. A study at Vellore examining ecological trends in bacterial meningitis rates over the period when Hib vaccination rates increased found a 65% decline in the mean number of Hib cases per year and a 78 per cent reduction in the number of Hib cases per pneumococcal case with vaccination coverage of 35 per cent; this suggests a herd immunity effect of 30 to 43 per cent[26]. The differences between the sites may be due to other factors aside from vaccination. Most notably, patients from CSMMU and KSCH showed higher rates of severe malnutrition and lower maternal education than was seen at CMC. Maternal education and malnutrition at CMC and ICH&HC were more comparable, suggesting social factors alone may not play much of a role in any aetiological differences between these sites.

One patient with confirmed Hib disease at CMC had received three doses of Hib-CV, indicating breakthrough disease. None of the other children with Hib meningitis at CMC received any doses of Hib-CV. Evaluation of the child's immune system was not done. Hib-CV is considered to provide approximately 94 per cent direct protection against invasive Hib disease[1], therefore, some vaccine failures are to be expected especially in settings with low vaccination coverage where nasopharyngeal carriage of Hib is common.

This study highlights the need for highly sensitive diagnostics for detecting bacterial illness in India. Only one Hib case would have been detected in this study if culture was the only method used to confirm bacterial aetiology. Prior to this study, no specific methods for detecting Hib were used at KSCH and no cases of Hib were identified. ICH&HC demonstrated Hib burden in a study in the early 1990s using LAT where 25 per cent of patients with a high suspicion of bacterial meningitis were found to be Hib positive[18]. Studies from CMC where LAT is routinely used for detecting bacterial pathogens, have consistently found Hib as an important cause of meningitis[26,27].

Based on WHO indicators for bacterial meningitis surveillance, a lower than expected proportion of patients who had abnormal CSF had pathogens isolated. The widespread treatment with antibiotics prior to LP is likely a factor in this. The sensitivity of LAT is known to be affected by antibiotic use, although to a lesser extent than culture[10,17,19,28]. PCR offers a more sensitive method to detect Hib in CSF samples of patients who have been previously treated. A study in Chandigarh, where 79 per cent of probable bacterial meningitis cases received antibiotics, found that using LAT alone missed 38 per cent of Hib meningitis cases when compared with PCR[10]. A large study in Vietnam, South Korea, and China determined that LAT only found 45 per cent of Hib cases when compared to PCR[28]. Additionally, 14 per cent of patients with abnormal CSF were not tested by LAT or culture, mainly because of limited CSF volume taken from patients. PCR, which needs lower volume, could potentially improve this. Studies have demonstrated varying sensitivity of LAT tests for different pathogens, ranging from high sensitivity for *N. meningitis* (93%) compared to *H. influenzae* (83%) and *S. pneumoniae* (77%)[29] and lower sensitivity of *N. meningitis* (39%) compared to *S. pneumoniae* (60%) and *H. influenzae* (93%)[30].

Some of the bacteria-negative cases may be viral infections of the central nervous system that are often indistinguishable based on cell and biochemical markers alone[31]. In India, most studies on meningitis have not used sensitive methods to detect bacterial pathogens[32,33]. Further research on this subject is warranted. Specific follow up to assess sequelae was not done in this study, but other Indian studies have found sequelae in 31 and 36 per cent of Hib meningitis survivors[11,34].

Several methodological differences existed between sites which might have affected the comparability of results. At CMC, children were screened for enrollment if they had CSF submitted to the laboratory, rather than at admission to the hospital. As it is, there is no way to assess the number of children who would fit the clinical definition of suspected meningitis, but did not have an LP performed. This is likely to underestimate the number of suspected cases, but does not affect the overall aetiological findings, since the criteria for the LAT test was based on laboratory findings. CMC excluded patients who had been hospitalized within the last 10 days with a non-related illness, other sites did not. This is likely to have affected isolation of pathogens associated with nosocomial meningitis,

such as *S. aureus*. However, since the majority of pathogens found at all sites were community acquired pathogens, it is unlikely that criteria that affected nosocomial infections significantly biased the overall aetiological findings of this study. CSMMU used a higher temperature threshold for enrolment compared to the other sites, which is likely to have excluded patients who would have been eligible at the other institutions. However, because history of fever and physical suspicion of meningitis were inclusion criteria, regardless of temperature at hospitalization, the bias is likely minimal: only 24 (5%) patients at CSMMU were enrolled based on temperatures without a history of fever or physician suspicion. Additionally, CSMMU did not have bacterial culture performed due to lack of infrastructure. Overall, only five of the 89 confirmed bacterial meningitis cases were identified using culture alone, making it unlikely that the lack of culture at CSMMU biased the results significantly.

The proportion of clinically suspected bacterial meningitis cases that had abnormal CSF findings varied widely between sites. This variation may be due to several factors, including the characteristic of the patients, severity of the disease level of clinical suspicion of the doctors, and the disease profile of the population, as well as the differences in enrolment criteria. This suggests that the populations may not have comparable level of clinically suspected meningitis. However, abnormal CSF laboratory findings were used as the criteria for further testing and comparison between sites, which should have selected for cases with the highest probability of central nervous system involvement and made the data, at this level, more comparable across sites.

In summary, the findings of the present study suggest that Hib accounts for a substantial proportion of bacterial meningitis in Indian children. Since 70 per cent of confirmed bacterial meningitis cases were found to be caused by Hib, the burden of Hib meningitis in children is likely to be substantial. It is, therefore, important to continue such surveillance to quantify the impact of Hib-CV when it is introduced into the universal immunization programme in India.

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