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RESEARCH ARTICLE

Meningococcal Meningitis: A Multicentric Hospital-based Study in Kathmandu, Nepal

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Abstract:

Background:

The global epidemiology of meningococcal disease varies markedly by region and over time. In Nepal, information on serogroup of meningococci is not available since the 1983 serogroup A epidemic in Kathmandu.

Objective:

To provide some fundamental data on the circulating serogroups of meningococci for potential meningococcal immunization programs in Nepal.

Methods:

This cross-sectional prospective study was conducted from January 2017 to December 2018 among 387 clinically suspected meningitis cases. Cerebrospinal fluid samples were collected by lumbar puncture technique at five referral hospitals of Kathmandu and processed by conventional cultural techniques. *Neisseria meningitidis* was identified by colony morphology, Gram staining and oxidase test. Serogrouping of meningococci was performed by slide agglutination test. Antibiotic susceptibility testing was done by the modified Kirby Bauer disc diffusion method. The data was entered into IBM SPSS Statistics 21 software and a *p*-value of <0.05 was considered significant.

Results:

Thirty-two samples were positive by culture for a bacterial pathogen with 2.3% of meningococci. All except one meningococcal meningitis cases were aged below 15 years. All *N.meningitidis* isolates belonged to serogroup A and were susceptible to ceftriaxone, chloramphenicol, meropenem and minocycline; however, 22% isolates showed resistance to cotrimoxazole and 11% intermediate resistance to ciprofloxacin.

Conclusion:

The circulating serogroup of *N. meningitidis* in Kathmandu has not changed over the past 35 years. The prevalence of meningococcal meningitis in Kathmandu is low but might be underestimated due to the sole use of culture-based diagnostic methods. Detection of meningococci by alternative methods may be useful in the precise estimation of actual disease burden.

Keywords: Meningococci, Prevalence, Antibiotics, CSF, Nepal, Serogroup.

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1. INTRODUCTION

Neisseria meningitidis is one of the leading causes of bacterial meningitis worldwide [1]. The global epidemiology of meningococcal meningitis varies markedly by region and over time [2]. The highest incidence occurs in the "meningitis belt" of sub-Saharan Africa [3]. Epidemiological data on the burden of meningococcal meningitis in South-East Asia are scarce [4]. In Nepal, a large serogroup A meningococcal outbreak occurred in the Kathmandu valley in 1982 [5]. Since then, only a few studies have been published on meningococcal meningitis in

Nepal [6, 7].

Of the twelve serogroups of *N. meningitidis*, 6 (A, B, C, W, X and Y) can cause epidemics [3]. The global distribution of serogroups of meningococci is variable. Serogroups B and C are most common in America, Europe and Australia, whereas serogroup A has caused the majority of the disease in Asia, as well as in Africa, before the introduction of a monovalent conjugate vaccine in large mass campaigns starting in 2010. Sometimes, some serogroups can emerge in certain regions or countries like serogroup C in China and serogroup Y in North America [3]. Two cases of serogroup B have been reported in India, a neighboring country of Nepal [8, 9].

Information on circulating serogroups of meningococci at the present time and in the local context is essential for the authorities to make evidence-based decisions whether to use vaccines or other modalities of control measures in the national program. In Nepal, meningococcal vaccination has not yet been introduced into the national immunization program. Therefore, this study was conducted to provide some fundamental data on the circulating serogroups of meningococci for potential meningococcal immunization program in Nepal.

2. MATERIALS AND METHODS

2.1. Study Design

This cross-sectional prospective study was conducted from January 2017 to December 2018 among clinically suspected meningitis cases attending five major hospitals located at Kathmandu, Nepal - Bhaktapur Hospital, Bir hospital, Kanti Children's Hospital (KCH), Sukraraj Tropical and Infectious Diseases Hospital (STIDH) and Tribhuvan University Teaching Hospital (TUTH).

2.2. Sample Size

Considering the estimated prevalence of bacterial meningitis (p) to be 7.2% with 95% confidence interval (z), 3% maximum tolerable error and 10% drop out, the minimum sample size calculated was 314 [10]. Altogether, 387 samples were included in this study.

2.3. Sample Collection and Processing

Cerebrospinal Fluid (CSF) sample was collected by the attending physician/medical officer by lumbar puncture from each clinically suspected meningitis case at the respective study site. A sterile wide-bore needle was inserted between the lumbar vertebrae L4 and L5, and the CSF sample was allowed to drip into the sterile container. A loopful of specimen was inoculated immediately within 30 minutes into blood agar and chocolate agar (Hi-Media Laboratories, Pvt. Limited, India) plates and incubated in candle jar (5-10% CO_2) at 37ºC for 24 hours.

2.4. Identification of Bacteria

Identification of bacterial isolates was done at National Public Health Laboratory, Teku, Kathmandu, by standard microbiological techniques including observation of colony characteristics, Gram staining, catalase, oxidase and other required biochemical tests. Additionally, bile solubility test and optochin sensitivity test were performed for identification of *S. pneumoniae* and X and V factor requirement test for *H. influenzae*.

Serogrouping of *N. meningitidis* isolate was done by slide agglutination test using group-specific antisera (Remel Europe Ltd, UK). Briefly, bacterial suspension was prepared by emulsifying 2-3 colonies of *N. meningitidis* in a drop of sterile normal saline on a disposable card. A drop of polyvalent antisera A-D and X-Z, W135 was added, appropriately mixed and rotated at 120 revolutions per minute (rpm). The appearance of any agglutination within 2 minutes was observed. If positive, it was tested for serogroups A, B, C, W and Y using group-specific monovalent antisera. *N. meningitidis* Z1503, Z5163, Z6432 and Z6434 were used as control strains [11].

Antibiotic susceptibility testing was done by the modified Kirby Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Antibiotic discs (Hi-Media Laboratories, Pvt. Limited, India) used were ceftriaxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), meropemem (10 µg), minocycline (30 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg). *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247, *N.meningitidis* Z1503 and *Escherichia coli* ATCC 25922 were used as control organisms for antibiotic susceptibility testing.

2.5. Data Analysis

The data were entered into Microsoft Office Excel 2007 and IBM SPSS Statistics 21 software. Data on variables like age group and gender were calculated as percentages and compared using the chi-square test. A p-value of <0.05 was considered to be statistically significant.

3. RESULTS

Out of 387 CSF samples, 32 (8.27%) were positive by culture for a bacterial pathogen. The proportion of meningococci among clinically suspected meningitis cases was 2.3% (9/387) among clinically suspected meningitis cases (Table **1**). All *N. meningitidis* isolates belonged to serogroup A.

Table 1. Bacteria isolated from CSF culture (n=32).

Type of Bacteria	Number	Percentage
Streptococcus pneumoniae	12	37.5
Neisseria meningitidis	9	28.1
Haemophilus influenzae	8	25.0
Escherichia coli	3	9.4

The clinically suspected meningitis cases were between 2 days and 89 years of age, with a median age of 12 months. The highest number of culture-positive bacterial meningitis cases was from the neonates. The meningococcal meningitis cases were between 5 days to 89 years old with a median age of 30 months. All except one meningococcal meningitis cases were below 15 years of age (Table **2**).

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Table 2. Age distribution of clinically suspected, bacterial culture positive and meningococcal meningitis cases.

Age Group	Clinically Suspected Meningitis Cases n (%)	Culture Positive Meningitis Cases n (%)	Meningococcal Meningitis Cases n (%)
Neonate < 1 month	128 (33.1)	13 (10.2)	1 (0.8)
Infant 1 month-1 year	74 (19.1)	4 (5.4)	1 (1.3)
Child 1-10 years	40 (10.3)	6 (15.0)	3 (7.5)
Adolescent 10-19 years	33 (8.5)	6 (18.2)	3 (9.1)
Adults 19-45 years	80 (20.7)	1 (1.2)	0 (0.00)
Adults above 45 years	32 (8.3)	2 (6.2)	1 (3.1)

There was a slight predominance of clinically suspected male (56.8%) meningitis cases with male:female ratio of 1.32:1. Of the 9 meningococcal meningitis cases, 77.8% (7/9) were male (Table **3**).

Table 3. Gender wise distribution of clinically suspected and culture positive bacterial meningitis cases.

Gender	Clinically Suspected Meningitis Cases n (%)	Culture Positive Meningitis Cases n (%)	Meningococcal Peningitis Cases n (%)	*p*-value (Calculated Using Chi-square Test)
Female	167 (43.1)	14 (43.7)	2 (22.2)	0.93
Male	220 (56.8)	18 (56.2)	7 (77.8)	

The meningococcal isolates were susceptible to the commonly used antibiotics. All the meningococcal isolates were susceptible to ceftriaxone, chloramphenicol, meropenem and minocycline; however, 22% isolates showed resistance to cotrimoxazole and 11% intermediate resistant to ciprofloxacin (Table **4**).

Table 4. Antibiotic susceptibility pattern of *N. meningitidis* isolates (n=9).

Antibiotics	Susceptible %	Intermediate %	Resistant %
Ceftriaxone	100	0	0
Chloramphenicol	100	0	0
Ciprofloxacin	89	11	0
Cotrimoxazole	78	0	22
Meropenem	100	0	0
Minocycline	100	0	0

4. DISCUSSION

The laboratory diagnosis of bacterial meningitis imposes a challenge. The actual estimate of bacterial meningitis in a resource-limited country like Nepal is difficult to establish. The proportion of culture positivity among 387 clinically suspected meningitis cases in this study (8.3%) was similar to other studies conducted in Nepal [7, 10]. The prevalence of bacterial meningitis varies worldwide, with reports from Asia, Europe and Africa [13 - 17]. A relatively lower prevalence (3-4.5%) of bacterial meningitis was reported by some researchers of our region [17 - 20]. The true burden of bacterial meningitis might have been underestimated due to the sole use of culture-based diagnostic methods. Though culture is considered as the gold standard for confirmation of cases, the positive rate is relatively low because of the fastidious and delicate nature of the major etiological agents and suboptimal storage and transportation conditions of specimens [21]. Bacteria couldn't be isolated from 355 CSF samples in this study. This low culture yield of CSF might be partly due to the treatment of cases with antibiotics prior to the collection of samples, as 58% of cases were referred from other health care settings to our study sites, which are tertiary care centres. The incorporation of newer diagnostic methods such as latex agglutination and polymerase chain reaction are likely to improve the laboratory confirmation of clinical diagnosis. A hospital-based study in Kathmandu, Nepal, similar to our study reported 14% of confirmed bacterial meningitis cases by using molecular methods [22]. Therefore, it is important to introduce molecular methods for surveillance of vaccine-preventable diseases, whenever possible.

With the introduction of and widespread use of vaccines, the etiology of bacterial meningitis has dramatically changed in the past few decades [23]. Pneumococci, meningococci and *H. influenzae* were the most common bacterial pathogens isolated in this study. These findings are similar to the other reports on bacterial meningitis from Kathmandu, Nepal [18, 20, 22, 24, 25]. *E.coli* were isolated from neonates in our study. Few other studies in Nepal have documented the isolation of other gram-positive (*Staphylococcus aureus*, coagulase-negative *S.aureus*, alpha and beta haemolytic streptococci, viridians streptococci and enterococci) and gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Aeromonas, Acineto-bacter*) from meningitis cases [7, 10, 20, 22]. Isolation of only four genera could be due to the exclusion of nosocomial, post-surgical and traumatic meningitis in this study.

Meningococcal meningitis epidemics have regularly recurred across the sub-Saharan African countries for over a century [26, 27]. To date, three studies explained the meningococcal epidemic (1982-1984) that occurred in the Kathmandu valley of Nepal. The first recorded meningitis epidemic caused by *N. meningitidis* serogroup A took place in April-May,1982 in Kathmandu valley of Nepal, resulting in 875 cases and 95 deaths [28]. The epidemic continued until Feb 1984 and then declined after mass vaccination [5, 28]. In 1989, an intercontinental spread of meningitis epidemic occurred in Nepal along with Saudi Arabia and Chad and it was thought to be introduced through pilgrims on their return from the hajj [29]. Since then, some hospital-based studies and case reports have reported meningococcal meningitis in Nepal [7, 10, 18 - 20, 22, 30, 31]. Reports of the low prevalence of meningococcal meningitis in India are comparable to our findings indicating geographical similarity [32, 33]. However, none of the hospital-based studies in Nepal have reported the information on circulating serogroups. Our study confirms that the circulating serogroup of *N. meningitidis* in Kathmandu, Nepal is serogroup A which has not changed over the past 35 years. Since meningococcal vaccination has not yet been

included in the national immunization program and considering the epidemic potential of this organism, continuous surveillance should be done.

Similar to other studies, all except one meningococcal meningitis cases in this study were below 15 years [10, 18]. In contrast to the findings of previous studies, our study showed that bacterial meningitis in infants accounted for 53% of all cases [20, 34]. The incidence of meningitis in infants might be due to the vulnerability of their choroid plexus to penetration by bacteria during the septicemic process and to low immunological status [35]. One meningococcal meningitis case in this study was 89 years of age. There is an increased multifactorial risk of bacterial meningitis for older adults, which includes both a greater susceptibility for underlying acute and chronic diseases and immunosenescence, a decline in immune function related to aging [36].

This study has demonstrated that meningitis is more common in males than in females. The male preponderance of 1.32: 1 in this study agrees with the findings in a tertiary hospital of Kathmandu [10]. This male predominance might be due to the sex-related physiology and gender-specific behavior. There is a relative absence of gene locus for the elaboration of immunoglobulin, which is located on the X chromosomes [37].

The emergence and rapid spread of antibiotic-resistant isolates are of great concern globally. Fortunately, all the meningococcal isolates in this study were susceptible to ceftriaxone, chloramphenicol, meropenem and minocycline. This is in agreement with other studies conducted in Nepal [7, 18]. This offers the clinicians of our region a range of choice for empiric treatment in emergency situations. In contrast, there are previous reports of rare chloramphenicol-resistant *N. meningitidis* isolates in Nepal as well as in Australia, France, and Vietnam [10, 38, 39]. Similar to our study, cotrimoxazole resistance was reported in a tertiary care hospital of Nepal [10]. One meningococcal isolate in our study was intermediate resistant to ciprofloxacin. Diminished fluoroquinolone (*e.g.*, ciprofloxacin) susceptibility has been described only in few instances due to mutations in the gene (*gyrA*) encoding the gyrase A fluoroquinolone target [40, 41].

To the best of our knowledge, this is the first multicentric study on bacterial meningitis including the five major hospitals of our region. The strength of our study is that all age groups have been included at multicentric sites making the study population representative. The major limitation of our study is that laboratory diagnosis is based only on conventional culture-based methods, which might have contributed to the under-estimation of actual disease burden.

CONCLUSION

The circulating *N. meningitidis* isolates in Kathmandu, Nepal is serogroup A which has not changed over the past 35 years. All isolates are susceptible to the commonly used antibiotics. The prevalence of meningococcal meningitis in Kathmandu, Nepal is low, but might have been underestimated due to the sole use of culture-based diagnostic methods. Therefore, detection of meningococci in CSF samples by alternative sensitive methods like PCR may be useful in the precise estimation of the actual disease burden.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethical approval of this study was obtained from the Nepal Health Research Council (Reg. No. 465/2016), Nepal. Parents or guardians were assured about the non-disclosure of information collected from them and were also informed about the use of data for analysis and using the results for improving patient care activities as well as publication without disclosing the name or identity of cases.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

At the time of enrollment, consent was taken from the caregivers or guardians on behalf of the patients.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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