Original article

Streptococcus pneumoniae among children with clinical meningitis in Nepal

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Abstract

Introduction: Invasive bacterial disease is a significant cause of morbidity and mortality worldwide and it is a major cause of childhood deaths in Nepal. *Streptococcus pneumoniae* is responsible for invasive and non-invasive pneumococcal diseases worldwide, such as pneumonia, bacteremia and meningitis. The aim of the study was to isolate, identify and determine antimicrobial susceptibility pattern of *Streptococcus pneumoniae* along with use of rapid immunochromatographic test "Binax NOW", to detect antigen for diagnosis of bacterial meningitis.

Methods: The study was carried out from October 2013 to September 2015 in Children's Hospital, Teaching Hospital-Department of Child Health and Public Health Research Laboratory, Institute of Medicine, Kathmandu, Nepal. Cerebrospinal fluid sample from 339 suspected cases of meningitis from children below 15 years of age were examined for identification by Gram staining, Culture and by Binax Now test. The identification of bacteria was done following standard method recommended by American Society for Microbiology. Antibiotic sensitivity testing was done by modified Kirby-Bauer disk diffusion method.

Results: Of total 339 suspected cases, 24(7.08%) bacterial meningitis was detected by Gram staining and culture methods whereas BinaxNow method detected 28(8.26%). On the basis of age, the highest numbers of the positive cases were found in the age group between 0-23 months (9.30%) followed by age group 49-60 months (8.16%)

Conclusions: In conclusion, a significant rate of bacterial meningitis was found in this study prompting concern for national wide surveillance.

Keywords: Children, Meningitis, *S. pneumoniae*, Nepal

Introduction

Streptococcus pneumoniae is a major cause of bacterial meningitis in the developing world accounting for 800,000 deaths annually among children 5 years of age. [1-4] Despite the availability of newer antibiotics, the mortality rate due to acute bacterial meningitis remains significantly high in developing countries, ranging from 16-32%. [5-8] Risk of complications and sequelae do vary by causative agent, being higher for meningitis due to *S. pneumoniae* than for other causative agents. [9-12]

There is a wide variation in *S. pneumoniae* capsule polysaccharides: currently 94 serotypes have been identified. Different *S. pneumoniae* serotypes have different propensities to cause disease. [13] The distribution

of its serotypes varies by country; age or ethnic group is frequently associated with poor neurologic outcomes as a consequence of cortical and subcortical injury. [14-20] The exact etiological diagnosis is often not possible, because of poor culture facilities in developing countries due to variety of reasons. [21-23] Antigen detection in CSF specimens provides a useful adjunct to culture-based diagnosis. [24,25] Antigen detection test can demonstrate the presence of non-viable bacteria. The latex agglutination test and countercurrent immunoelectrophoresis have both been applied to *S. pneumoniae* identification, but both are insufficient as routine diagnostic methods in general practice because of their lack of sensitivity and specificity. [26-29] At present, polymerase chain reaction (PCR) is considered the most sensitive and specific test, but expensive and requires a complicated process. [30]

The NOW *S. pneumoniae* Antigen Test (Binax) is an in vitro rapid immunochromatographic test (ICT) for detection of pneumococcal antigen in the urine of adult patients with pneumonia and in the CSF of patients of all ages with meningitis. The tool has demonstrated high sensitivity and specificity in testing CSF samples from patients with culture-confirmed meningitis. [31, 32]

Methods

The study was carried out from October 2013 to September 2015 in Children's Hospital, Teaching Hospital-Department of Child Health and Public Health Research Laboratory, Institute of Medicine, Kathmandu, Nepal. In this period CSF sample was collected by lumbar puncture from 339 suspected cases of meningitis in children under the age of 15 years and identification by Gram staining and culture was done following standard methods recommended by American Society for Microbiology. [33]

Volume and gross appearance was noted from the sample and sample greater than 1 ml was centrifuged at the rate of 2,500 revolutions per minute (rpm) for 10 minutes to concentrate if any organisms were present. The sediment was used for culture as well as for Gram staining. [31] The supernatant was kept for detection of antigen by rapid immunochormatographic Binax NOW test. Sample less than 1 ml was inoculated directly to the culture media like MacConkey agar (MA), blood agar (BA) and chocolate agar (CA) plates and incubated overnight at 37 °C aerobically and the CA plates were incubated up to 48 hours at 37 °C in 5 % CO_2 atmosphere. The bacterial growth obtained was examined for colony as well as Gram staining and identification was done following standard microbiological methods recommended by American Society for Microbiology. [33]

The antibiotic sensitivity test of the pathogens isolated from the clinical specimen against different antibiotics was done using Mueller Hinton agar (MHA) by the standard disk diffusion technique of modified Kirby-Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI). [34] Antibiotic disks used were: Penicillin (10 units), amoxicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), cefotaxime (30 µg). *Staphylococcus aureus* ATCC 25923 and *E. coli* 25922 were used as control organisms for antibiotic susceptibility testing. [34]

Pneumococcal antigen test was done by the Binax NOW test (Binax, Inc., Portland, ME) is a rapid in vitro immunochromatographic assay which that detects pneumococcal antigen in CSF. The test kit contains a nitrocellulose membrane onto which rabbit antipneumococcal antibody is attached. The test sample is mixed with reaction buffer and then placed on the test membrane. If the test sample contains pneumococcal antigen, a pink line will appear within 15 min. The antigen against which the Binax NOW test reacts is a cell wall polysaccharide on *S. pneumoniae*, which is present in all clinically relevant strains. [31, 32]

Ethical aspects and Data analysis

Institutional Review Board (IRB) of Institute of Medicine approved this study; and samples were collected after written consent from parents/guardians of participating children.

All the results were entered in the worksheet of statistical package for social science (SPSS) software-16.0 version and the results were determined.

Results

During the study period, 339 CSF samples were collected and processed for the detection of pneumococcus by Gram staining, culture and antigen detection method. Gram stain and culture revealed 24 (7.07%) cases but Binax NOW test for detection of antigen revealed 28 (8.25%) positive cases. [p value=0.6654]

Table 1 Comparison of detection methods

Methods	Number of specimens	Number of positive cases (%)
Gram staining	339	24 (7.08%)
Culture	339	24 (7.08%)
Binax Now	339	28 (8.26%)

Age and Gender-wise distribution of patients

On the basis of age, the highest numbers of positive cases were found among age group between 0-23 months (9.30%) followed by age group 49-60 months (8.16%), as shown in below table.

Table 2 Age wise distributions of patients

Age in months	Total cases	Positive cases (%)
0-23	215	20 (9.30%)
24-48	55	3 (5.45%)
49-60	49	4 (8.16%)
>60	20	1(5.00%)

Comparison of detection methods

All culture positive sample was also positive by Binax NOW test and 4 culture negative samples were positive by Binax NOW test.

Table 3 Evaluation of Binax NOW *S. pneumoniae* antigen detection test

S.N	Test result	*Streptococcus pneumoniae*		Total
		Culture positive	Culture negative	
1.	Binax NOW positive	24	4	28
2.	Binax NOW negative	0	311	311
	Total	24	315	339

Antibiotic susceptibility pattern of isolates

Only 1(4.16%) of the isolates was intermediate sensitive and no isolates were resistant to penicillin and cefotaxime

Overall, 5(20.83%) isolates were resistant and 4(16.66%) were intermediate sensitive to cotrimoxazole.

Only 1(4.16%) of the isolates was resistant to chloramphenicol and erythromycin.

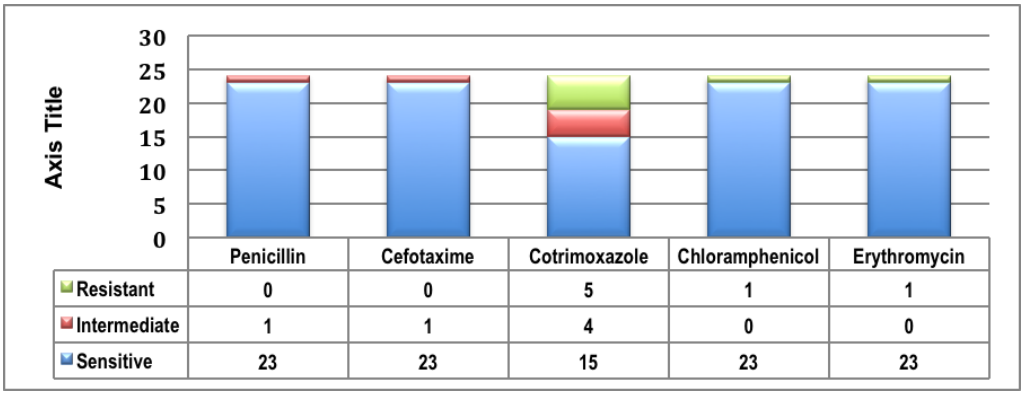

Figure 1. Antibiotic susceptibility pattern of isolates

Discussion

In the developing world, invasive pneumococcal disease (including meningitis) is a leading cause of morbidity and mortality, with an estimated 0.7 to 1.0 million deaths annually among children less than 5 years of age. [35] Risk of complications and sequelae do vary by causative agent, being higher for meningitis due to *S. pneumoniae* than for other causative agents. [36-38] Acute bacterial meningitis is a medical emergency, which warrants early diagnosis and aggressive therapy. Most often therapy for bacterial meningitis has to be initiated before the etiology is known. [39]

In this study, 24 cases were found to be culture and gram staining positive whereas higher number; 28 positive cases was found by Binax NOW antigen detection test. There is considerable variation in culture positivity in the study from the developing countries and culture is positive in not more than 15-35% patients. [40-42] Gram stain is regarded as useful techniques in resource limited settings although the sensitivity varies depending on the organism causing infection. The Binax NOW test identified the majority of culture-positive samples, independent of sample type. Furthermore, it was able to identify pneumococci in samples from treated patients with negative cultures. The only other method available in these cases is the PCR assay. Although sensitive, the PCR technique is time-consuming, especially compared with the Binax NOW test. It also has the disadvantage of being more expensive, requiring well trained staff. [43]

On the basis of the age the highest numbers of the positive cases were found in the age group between 0-23 months (9.30%) followed by age group 49- 60 months (8.16%).

The most remarkable finding of this study is the high cotrimoxazole resistance and the result was in accordance with the findings of Bangladesh. [44] Currently, the antibiotic resistance patterns of *S. pneumoniae* isolates vary widely from one country to another within Europe. Rates of resistance to penicillin have been reported to be increasing in such countries but resistance has remained at very low levels in other central European countries. [45,46] Whereas the study showed some variation from other countries, which showed none of the strains, were resistant to penicillin and cefotaxime, which was consistent with study done in Hungary. [46]

The increased rate of cotrimoxazole resistance can be possibly correlated with the wide use this antibiotic in the communities because of its dose convenience, cost effectiveness, and easy availability over the counter. Although multiple factors influence the emergence and spread of antimicrobial resistance, antimicrobial consumption is one of the most important. However, majority of *S. pneumoniae* in South Asia are now cotrimoxazole resistant raising the question of whether WHO should shift from cotrimoxazole to more expensive drugs for treatment.[47] This remarkable difference may have occurred because of the misuse of antibiotics, the lack of antibiotic policy in these countries, or as a result of the different antibiotic distribution policies.[44]

Conclusion

A significant rate of bacterial meningitis was found in this study prompting concern for national wide surveillance and a more detailed study over a wider area and for a longer period of time should be conducted. The data obtain from the study is essential for continued enhanced surveillance especially for the implementation of polyvalent conjugate vaccine.

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Conflict of interest: None declared

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