**INTRODUCTION**

*Streptococcus pneumoniae* is the most common community-acquired pathogen causing respiratory tract infections, such as otitis media, sinusitis and pneumonia, and it invades the bloodstream causing meningitis (Hoa et al., 2010). Severe invasive disease may be life threatening and accounts for 1 to 2 million deaths annually worldwide at both extremes of age (Mulholland, 1999).

*S. pneumoniae* was considered as sensitive to most antibiotics, especially penicillins (Hoa et al., 2010). In 1967 the first clinically significant isolate of penicillin-resistant pneumococci (PRP) was reported from Australia followed by reports from Papua New Guinea, South Africa and in the 1970’s and 1980’s...
from the Western hemisphere and Spain (Hansman and Bullen, 1967; Klugman, 1990; Lister, 1995; Schreiber and Jacobs, 1995). Despite the emergence of PRP in resource surplus parts of the globe, for a long time there were no reports of penicillin resistance among clinical isolates of *S. pneumoniae* from Nepal. However, in a recent study conducted by the South Asian Pneumococcal Alliance (SAPNA), a network formed to monitor pneumococci in Nepal and other South Asian countries, 3.8% of invasive pneumococcal isolates from a children’s hospital in Nepal were found to be resistant to penicillin (Shah et al, 2009). In another study conducted at BP Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal 4% of 71 pneumococcal isolates were resistant to penicillin (Khanal et al, 2002).

With the emergence of PRP, there have been worldwide reports of treatment failure and other classes of antibiotics have been increasingly used for treatment of pneumococcal infections (Hoa et al, 2010). PRP strains resistant to other routinely used antibiotics have started to appear resulting in the emergence of multidrug-resistant *S. pneumoniae* (Lalitha et al, 2002). The widespread and inappropriate use of antibiotics for various infections was probably the main factor responsible for the emergence of drug resistance among *S. pneumoniae* (Hoa et al, 2010). The emergence of high-level resistance to penicillin, particularly in combination with resistance to other antibiotics, is a serious threat to current treatment strategies. Although there are a few studies reporting the resistance patterns of *S. pneumoniae* from Eastern Nepal, none of these studies have followed the trends in antibiotic resistance over a long time period (Khanal et al, 2002; Shah et al, 2009).

Documentation of antibiotic resistance in *S. pneumoniae* infection from Western Nepal is limited to date.

A knowledge of local antibiotic susceptibilities of *S. pneumoniae* is essential for the development of effective treatment protocols and monitoring drug resistance. We conducted this study to determine the prevalence of antibiotic resistance among clinical isolates of *S. pneumoniae* in a population in whom the pneumococcal vaccine is not yet available. We monitored the changing trends in antibiotic resistance among *S. pneumoniae* isolated from patients in Western Nepal.

**MATERIALS AND METHODS**

**Study setting**

This study was conducted from January 2000 to August 2007 at Manipal Teaching Hospital (MTH), Manipal College of Medical Sciences (MCOMS), Pokhara, western Nepal. The hospital is a major healthcare provider for the region. The institution serves 10 of the 15 districts of western Nepal. The population of these 10 districts was approximately 2 million in a 2001 census. The hospital has an average daily patient load of 500 outpatients and 150 inpatients with seasonal variation.

**Subjects and sample size**

The clinical isolates of *S. pneumoniae* were isolated from respiratory specimens (sputum and bronchoalveolar lavage), blood and ocular specimens collected from patients suffering from lower respiratory tract infections, blood stream infections and ocular infections, respectively, and were included in the study. All sputum samples were graded as satisfactory based on Bartlett scoring before inclusion in the study (Winn et al, 2006).
Processing of specimens and identification

A Gram’s stain was done on each specimen and they were simultaneously inoculated on sheep blood agar and chocolate agar. The isolates were identified as *S. pneumoniae* based on characteristic Gram’s stain morphology, alpha hemolysis on blood agar, greenish discoloration (bleaching) on chocolate agar, bile solubility and sensitivity to optochin (Mackie and McCartney, 1996).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar with 5% sheep blood, according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007). Antibiotics tested were: oxacillin (1 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (1.25 µg/23.75 µg), chloramphenicol (30 µg) and tetracycline (30 µg). Due to a change in our hospital’s antibiotic policy, from 2004 onwards chloramphenicol and tetracycline were not used for treatment of infections caused by *S. pneumoniae*, therefore these antibiotics were not tested after 2004. Isolates with oxacillin zone sizes ≥ 20 mm were considered as penicillin susceptible pneumococci. Isolates showing an oxacillin zone ≤ 19 mm were reported as resistant after determining the minimum inhibitory concentration (MIC) for penicillin using the agar dilution method. Isolates resistant to penicillin and at least two other antibiotic classes were defined as multi-drug resistant (MDR) *S. pneumoniae* (Balakrishnan, 2006). The reference strain *S. pneumoniae* ATCC 49619 was used for quality control of antibiotic susceptibility testing.

Statistical analysis

Statistical analysis was done using SPSS version 16.0 statistics software (SPSS, Chicago, IL). Percentages were calculated for categorical variables. A chi-square test or a Fisher’s exact test were used to study the difference in antibiotic resistance among penicillin-resistant and penicillin-susceptible isolates. A *p*-value < 0.05 was considered statistically significant.

RESULTS

During the study period, there were 312 *S. pneumoniae* isolates. The demographics of the 312 patients with pneumococcal infection are summarized in Table 1. The majority of patients with pneumococcal infection were male (56.7%). The ages of the patients ranged from 1 to 99 years with a mean (± SD) of 56.61 (± 21.0) years. Sputum was the most common specimen from which *S. pneumoniae* was isolated.

Antibiotic susceptibility testing revealed 173 isolates (55%) were sensitive to all the antibiotics tested. Of the remaining isolates, 108 (35%) were resistant to one
### Table 2
Comparison of resistance of penicillin-resistant and penicillin-susceptible pneumococcal isolates to various antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Penicillin-resistant Pneumococci</th>
<th>Penicillin-susceptible Pneumococci</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates tested</td>
<td>No. of resistant isolates (%)</td>
<td>No. of isolates tested</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15</td>
<td>4 (26.7)</td>
<td>297</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>15</td>
<td>5 (33.3)</td>
<td>297</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>11</td>
<td>1 (9.1)</td>
<td>241</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>11</td>
<td>0 (0)</td>
<td>241</td>
</tr>
</tbody>
</table>

### Table 3
Co-resistance among *Streptococcus pneumoniae* isolates.

<table>
<thead>
<tr>
<th>Co-resistance</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-trimoxazole, tetracycline</td>
<td>11 (3.5)</td>
</tr>
<tr>
<td>Co-trimoxazole, erythromycin</td>
<td>9 (2.9)</td>
</tr>
<tr>
<td>Co-trimoxazole, penicillin</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>Co-trimoxazole, chloramphenicol</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Erythromycin, tetracycline</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Erythromycin, penicillin</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Co-trimoxazole, erythromycin, tetracycline</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Penicillin, erythromycin, Co-trimoxazole</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Penicillin, erythromycin, tetracycline</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>

antibiotic, 27 (9%) were resistant to two antibiotics and 4 (1%) were resistant to three antibiotics tested.

Of the 312 isolates, 297 (95%) were susceptible to penicillin and 15 (5%) were penicillin-resistant. Table 2 shows the resistance patterns of the penicillin-susceptible and penicillin-resistant isolates to other antibiotics. Of the 312 isolates, 107 (34.3%) and 23 (7.4%) were resistant to trimethoprim-sulfamethoxazole and erythromycin, respectively. Of the 252 isolates screened, 28 (11.1%) and 1 (0.4%) were resistant to tetracycline and chloramphenicol, respectively. Figs 1 and 2 show the resistance to erythromycin, penicillin, trimethoprim-sulfamethoxazole, tetracycline and chloramphenicol. There was a significant increase in penicillin resistance from 0% in 2000 to 21.4% in 2007 ($p = 0.0175$). Table 3 shows co-resistance to two or more antibiotics among the isolates. Three isolates were resistant to penicillin and two other classes of antibiotics and were considered as MDR *S. pneumoniae*.
Pneumococcal infections, such as pneumonia, bacteremia, meningitis, and otitis media, are major causes of morbidity and mortality in persons of all age groups. The prevalence of pneumococcal disease is extremely high in developing countries and among indigenous and disadvantaged minorities in developed countries (Berman, 1991; Cortese et al, 1992).

Surveillance of trends in pneumococcal susceptibility in different geographic areas is necessary to aid clinicians in choosing the best drug for empiric therapy (Centers for Disease Control and Prevention, 1996). Susceptibility patterns can help evaluate efforts to decrease resistance rates through judicious antibiotic use. In this study, conducted at a teaching hospital in western Nepal, 55% of the isolates were susceptible to all antibiotics tested.

In a recent study from the United Arab Emirates (UAE), 57% of the isolates were penicillin susceptible, while the remaining 43% were resistant (Senok et al, 2007). In our study 95% of the 312 isolates were found susceptible to penicillin. Similarly, in a study conducted at Kanti Children’s Hospital, Kathmandu, only 3.8% of the isolates from 50 children with invasive pneumococcal infection were resistant to penicillin (Shah et al, 2009). This better susceptibility of the isolates to penicillin could reflect a decreased utilization of the drug in Nepal. Penicillin resistance among isolates of S. pneumoniae increased to a maximum of 21.4% during 2007. A study from Spain showed use of both macrolides and β-lactams accounted for penicillin resistance (Garcia-Rey et al, 2002). Stephenson (1996) observed after an aggressive campaign on the need for shrewd use of antibiotics, the rate of penicillin resistant pneumococci declined significantly (Stephenson, 1996). Therefore, judicious use of macrolides and β-lactams should be emphasized to prevent development of penicillin resistance in our population.
In our study, 7.4% of isolates had resistance to erythromycin. In Norway, a country known for its low prevalence of antimicrobial-resistant bacteria, 6.0% of isolates were resistant to macrolides (Littauer et al, 2005). In the UAE, 30% of isolates were resistant to erythromycin (Senok et al, 2007). Increased macrolide resistance is disturbing since erythromycin, azithromycin, and clarithromycin are commonly prescribed antibiotics for outpatient treatment of community acquired pneumonia and low-level macrolide resistance has been associated with clinical failure (Fogarty et al, 2000).

The progression of penicillin and macrolide resistance is of concern. Factors contributing to increased penicillin and erythromycin resistance among *S. pneumoniae* are clonal spread, cyclic turnover of circulating serotypes, natural fluctuations and antibiotic consumption (Stingemore et al, 1989; Munoz et al, 1991; Marco et al, 2000)

In a study by Senok et al (2002) 77% of isolates were resistant to trimethoprim-sulfamethoxazole and 16.9% were resistant to tetracycline. In our study 34.3% of isolates were resistant to trimethoprim-sulfamethoxazole and 11.1% resistant to tetracycline. However, there was a significant reduction in resistance of pneumococcal isolates to these antibiotics in recent years in our study. The reason for this favorable change in the susceptibility of isolates is not clear. At a children’s hospital in Nepal 34.6% of children were found to be nasopharyngeal carriers of *S. pneumoniae* (Sherchand et al, 2010). In that study cefotaxime, chloramphenicol and erythromycin were found to be the most effective antibiotics against *S. pneumoniae*, while Co-trimoxazole was the least effective (60%).

We studied the occurrence of co-resistance in our isolates. We found penicillin-resistant *S. pneumoniae* was significantly co-resistant to erythromycin. Penicillin and erythromycin resistance in *S. pneumoniae* are often associated (Goldstein, 1999). In our study *S. pneumoniae* had co-resistance to two or more antibiotics. Co-resistance to tetracycline or erythromycin was commonly observed among trimethoprim-sulfamethoxazole resistant isolates. Acquisition of the conjugative transposable element *Tn1545* is known to confer resistance to non-beta-lactam antibiotics, such as erythromycin and tetracycline (Courvalin and Carlier, 1986; Jabes et al, 1989; Seral et al, 2001). This may be the resistance mechanism of our multi-drug resistant strains.

In conclusion, resistance to erythromycin, tetracycline, chloramphenicol and trimethoprim-sulfamethoxazole was either low or showed a downward trend. However, penicillin resistance showed a steady rise. Therefore, measures to ensure judicious use of macrolides and β-lactams should be advocated to control the development of penicillin-resistant *S. pneumoniae*.

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