STREPTOCOCCUS PNEUMONIAE INFECTIONS IN WESTERN NEPAL

Joshy Maducolil Easow[1], Noyal Mariya Joseph[1], Pathiyil Ravi Shankar[2], Asish Purushothaman Rajamony[3], Banodita Acharya Dhungel[4] and PG Shivananda[5]

[1]Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Pillaiyarkuppam, Pondicherry, India; [2]Department of Clinical Pharmacology, [4]Department of Microbiology and Immunology, KIST Medical College, Lalitpur, Nepal; [3]Department of Microbiology and Immunology, UEIMS School of Medicine and Dentistry, Ras Al Khaimah, UAE; [5]Chair of Microbiology, Manipal University, Manipal, India

Abstract. We conducted a study to determine the prevalence of antibiotic resistance among clinical isolates of *S. pneumoniae*. This study was conducted from January 2000 to August 2007 at a tertiary care teaching hospital in Nepal. The isolates were identified based on standard bacteriological techniques. Antibiotic susceptibility testing used the Kirby-Bauer disc diffusion method; penicillin resistance was confirmed by agar dilution method. During the study period, there were 312 *S. pneumoniae* isolates. Penicillin, trimethoprim-sulfamethoxazole, erythromycin, tetracycline and chloramphenicol resistance were observed in 5, 34.3, 7.4, 11.1 and 0.4% of isolates, respectively. Resistance to all tested antibiotics declined with time except for penicillin, in which resistance increased. Penicillin-resistant *S. pneumoniae* were significantly co-resistant to erythromycin. Co-resistance to tetracycline and erythromycin were observed in trimethoprim-sulfamethoxazole resistant isolates. Penicillin resistance is increasing; therefore, measures to ensure judicious use of β-lactams and macrolides (inducers of penicillin resistance) should be advocated to control the development of penicillin-resistant *S. pneumoniae*.

Keywords: *Streptococcus pneumoniae,* antibiotic resistance, Nepal

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

Correspondence: Dr Joshy Maducolil Easow, Department of Microbiology, Mahatma Gandhi Medical College and Research Institute, Pillaiyarkuppam, Pondicherry - 607 402, India.
Tel: +91 9486008500
E-mail: dr.jmeasow@gmail.com

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 mornitored 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.

Table 1
Demographic data of 312 cases with pneumococcal infections.

Demographic data	Number (%)
Age	
Children (1-12)	9 (2.9)
Adolescents (13-18)	14 (4.5)
Adults (19 and above)	289 (92.6)
Mean ± standard deviation	56.61 ± 21.0
Sex	
Male	177 (56.7)
Female	135 (43.3)
Specimens	
Sputum	294 (94.2)
Bronchoalveolar lavage	8 (2.6)
Blood	7 (2.2)
Ocular specimens	3 (1.0)

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.

Antibiotic	Penicillin-resistant Pneumococci		Penicillin-susceptible Pneumococci		*p*-value
	No. of isolates tested	No. of resistant isolates (%)	No. of isolates tested	No. of resistant isolates (%)	
Erythromycin	15	4 (26.7)	297	19 (6.4)	0.0179
Trimethoprim-sulfamethoxazole	15	5 (33.3)	297	102 (34.3)	0.8428
Tetracycline	11	1 (9.1)	241	27 (11.2)	1.0000
Chloramphenicol	11	0 (0)	241	1 (0.4)	1.0000

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

Co-resistance	Frequency (%)
Co-trimoxazole, tetracycline	11 (3.5)
Co-trimoxazole, erythromycin	9 (2.9)
Co-trimoxazole, penicillin	3 (1.0)
Co-trimoxazole, chloramphenicol	1 (0.3)
Erythromycin, tetracycline	2 (0.6)
Erythromycin, penicillin	1 (0.3)
Co-trimoxazole, erythromycin, tetracycline	1 (0.3)
Penicillin, erythromycin, Co-trimoxazole	2 (0.6)
Penicillin, erythromycin, tetracycline	1 (0.3)

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*.

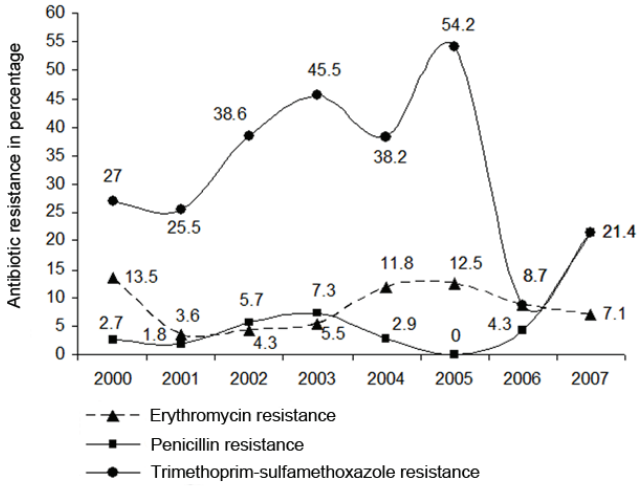

Fig 1–Erythromycin, penicillin and trimethoprim-sulfamethoxazole resistance during 2000-2007.

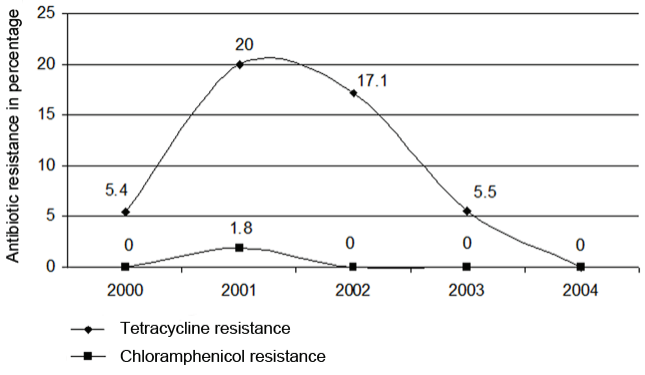

Fig 2–Tetracycline and chloramphenicol resistance during 2000-2004.

DISCUSSION

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 *Tn*1545 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*.

REFERENCES

Balakrishnan I. *Streptococcus pneumoniae*. In: Gillespie SH, Hawkey PM, eds. Principles and practice of clinical bacteriology. 2nd ed. West Sussex: John Wiley & Sons, 2006: 41-57.

Berman S. Epidemiology of acute respiratory infections in children of developing countries. *Rev Infect Dis* 1991; 13(suppl): 454-62.

Centers for Disease Control and Prevention. Defining the public health impact of drug-resistant *Streptococcus pneumoniae*: report of a working group. *Morb Mortal Wkly Rep MMWR* 1996; 45: 1-21.

Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. CLSI document M100 - S 16. Wayne, PA: CLSI, 2007.

Cortese MM, Wolff M, Meido-Hill J, Reid R, Ketcham J, Santosham M. High incidence rates of invasive pneumococcal disease in the White Mountain Apache population. *Arch Intern Med* 1992; 152: 2277-82.

Courvalin P, Carlier C. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. *Mol Gen Genet* 1986; 205: 291-7.

Fogarty C, Goldschmidt R, Bush K. Bacteremic pneumonia due to multidrug-resistant pneumococci in 3 patients treated unsuccessfully with azithromycin and successfully with levofloxacin. *Clin Infect Dis* 2000; 31: 613-5.

Garcia-Rey C, Aguilar L, Baquero F, Casal J, Dal-Re R. Importance of local variations in antibiotic consumption and geographical differences of erythromycin and penicillin resistance in *Streptococcus pneumoniae*. *J Clin Microbiol* 2002; 40: 159-64.

Goldstein FW. Penicillin-resistant *Streptococcus pneumoniae*: selection by both beta-lactam and non-beta-lactam antibiotics. *J Antimicrob Chemother* 1999; 44: 141-4.

Hansman D, Bullen MM. A resistant pneumococcus. *Lancet* 1967; 2: 264-5.

Hoa NQ, Trung NV, Larsson M, *et al*. Decreased *Streptococcus pneumoniae* susceptibility to oral antibiotics among children in rural Vietnam: a community study. *BMC Infect Dis* 2010; 10: 85.

Jabes D, Nachman S, Tomasz A. Penicillin-binding protein families: evidence for the clonal nature of penicillin resistance in clinical isolates of pneumococci. *J Infect Dis* 1989; 159: 16-25.

Khanal B, Sharma SK, Deb M, Bhattacharya SK. Pneumococcal infections: Report of a hospital based study. *J Nepal Med Assoc* 2002; 41: 397-400.

Klugman KP. Pneumococcal resistance to antibiotics. *Clin Microbiol Rev* 1990; 3: 171-96.

Lalitha MK, Pai R, Manoharan A, Appelbaum PC. Multidrug-resistant *Streptococcus pneumoniae* from India. *Lancet* 2002; 359: 445.

Lister PD. Multiply-resistant pneumococcus: therapeutic problems in the management of serious infections. *Eur J Clin Microbiol Infect Dis* 1995; 14 (suppl 1): 18-25.

Littauer P, Sangvik M, Caugant DA, Hoiby EA, Simonsen GS, Sundsfjord A. Molecular epidemiology of macrolide-resistant isolates of *Streptococcus pneumoniae* collected from blood and respiratory specimens in Norway. *J Clin Microbiol* 2005; 43: 2125-32.

Mackie TJ, McCartney JE. Practical medical microbiology. 14th ed. New York: Churchill Livingstone, 1996.

Marco F, Bouza E, Garcia-de-Lomas J, Aguilar L. *Streptococcus pneumoniae* in community-acquired respiratory tract infections in Spain: the impact of serotype and geographical, seasonal and clinical factors on its susceptibility to the most commonly prescribed antibiotics. The Spanish Surveillance Group for Respiratory Pathogens. *J Antimicrob Chemother* 2000; 46: 557-64.

Mulholland K. Strategies for the control of pneumococcal diseases. *Vaccine* 1999; 17 (suppl 1): S79-84.

Munoz R, Coffey TJ, Daniels M, *et al*. Intercontinental spread of a multiresistant clone of serotype 23F *Streptococcus pneumoniae*. *J Infect Dis* 1991; 164: 302-6.

Schreiber JR, Jacobs MR. Antibiotic-resistant pneumococci. *Pediatr Clin North Am* 1995; 42: 519-37.

Senok A, Al-Zarouni M, Al-Najjar J, Nublusi A, Panigrahi D. Antimicrobial resistance among *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates in the United Arab Emirates: 2004-2006. *J Infect Dev Ctries* 2007; 1: 296-302.

Seral C, Castillo FJ, Rubio-Calvo MC, Fenoll A, Garcia C, Gomez-Lus R. Distribution of resistance genes *tet*(M), *aph3'*-III, catpC194

and the integrase gene of *Tn*1545 in clinical *Streptococcus pneumoniae* harbouring *erm*(B) and *mef*(A) genes in Spain. *J Antimicrob Chemother* 2001; 47: 863-6.

Shah AS, Knoll MD, Sharma PR, *et al*. Invasive pneumococcal disease in Kanti Children's Hospital, Nepal, as observed by the South Asian Pneumococcal Alliance network. *Clin Infect Dis* 2009; 48 (suppl): S123-8.

Sherchand JB, Joshi AR, Gauchan P, Amatya J. Distribution of serotypes and antimicrobial resistance of *Streptococcus pneumoniae* in a children's hospital in Nepal. *J Nep Pediatr Soc* 2010; 28: 45-8.

Stephenson J. Icelandic researchers are showing the way to bring down rates of antibiotic-resistant bacteria. *JAMA* 1996; 275: 175.

Stingemore N, Francis GR, Toohey M, McGechie DB. The emergence of erythromycin resistance in *Streptococcus pyogenes* in Fremantle, Western Australia. *Med J Aust* 1990; 150: 621-31.

Winn WC, Allen SD, Janda WM, *et al*. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Baltimore: Lippincott Williams & Wilkins, 2006: 15-7.