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Full Length Research Paper

Ten-years surveillance of antimicrobial resistance pattern of *Streptococcus pneumoniae* in Nepal

Geeta Shakya and Bal Ram Adhikari*

Department of Health Service, National Public Health Laboratory, Teku, Kathmandu, Nepal.

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Resistance to first-line antimicrobial agents in *Streptococcus pneumoniae* is increasing worldwide. The study was carried out in 11 major hospital laboratories representing all geographical region of the country. The aim of this study is to carry out cross-sectional study of Antimicrobial resistance pattern of the *S. pneumoniae* isolates from 1999 to 2008. Identification of the *S. pneumoniae* isolates was done following standard microbiological techniques in all participating laboratories and antimicrobial susceptibility testing was done in Reference laboratory only. A total of 934 *S. pneumoniae* from different specimens were isolated during 10 years of period from all age group patients (age ranging from 2 to 72 years). A total of 57.4% of isolates were cotriomoxazole resistant with highest (78%) resistance in 2002 followed by 74% in 2008. An increasing trend of resistance to penicillin with highest (10%) in 2007 followed by Ampicillin, Erythromycin, ciprofloxacin and Ceftriaxone was observed. Multi-drug resistant pattern was observed from 2007 only.

Key words: *Streptococcus pneumoniae*, Nepal, surveillance, antimicrobial resistance.

INTRODUCTION

Streptococcus pneumoniae is one of the most common causes of bacterial meningitis in adults along with *Neisseria meningitidis*. It is also one of the most common causes of respiratory tract infection, ear infection and otitis media (Dagon, 2000). Pneumococcal pneumonia is more common in the very young and the very old people. The organism is part of the normal upper respiratory tract flora, but, as with many natural flora, it can become pathogenic under the suppressed immune condition. The genome of *S. pneumoniae* is a closed, circular DNA structure that contains between 2.0 and 2.1 million base pairs depending on the strain. It has a core set of 1553 genes plus 154 genes in its virulome which contribute to virulence and 176 genes that maintain a noninvasive phenotype. Genetic information can vary up to 10% between strains (Van der Poll and Opal, 2009).

Resistance to first-line antimicrobial agents in *S. pneumoniae* is increasing worldwide. Until a few years ago, penicillin was the treatment of choice for infections caused by this microorganism. However, since the middle of the 1980s, pneumococci with structural changes in the penicillin binding protein (PBP) have been detected in many parts of the world which has resulted in a decrease in susceptibility not only to penicillin but also to other ß-lactam antibiotics (Baquero et al., 1999; Thornsberry et al., 1999; Doern et al., 1998; Hsueh et al., 2000).

This study was undertaken to evaluate AMR trends of *S. pneumoniae* isolates in Nepal with the aim to describe the susceptibility pattern of *S. pneumoniae* in a nationwide antimicrobial surveillance study.

MATERIALS AND METHODS

The study was conducted in 11 laboratories attached to major hospitals distributed across Nepal from 1990 to 2008. Five laboratories based in Kathmandu valley: Bir Hospital Laboratory, Patan Hospital Laboratory, Kanti Children's Hospital (KCH) Laboratory, and Tribhuvan University Teaching Hospital (TUTH) Laboratory including National Public Health Laboratory (National Co-ordinating laboratory for this study); one in eastern region of Nepal- B. P. Koirala Institute of Health Sciences (BPKIHS) Laboratory, Dharan; four in Western Region of Nepal- Western Regional Hospital Laboratory, Pokhara, Manipal Teaching Hospital Laboratory, Pokhara, United Mission Hospital Laboratory, Palpa, Lumbini Zonal Hospital laboratory, Butwal and Dhulikhel Hospital

*Corresponding author. E-mail: bradhikari81@gmail.com.

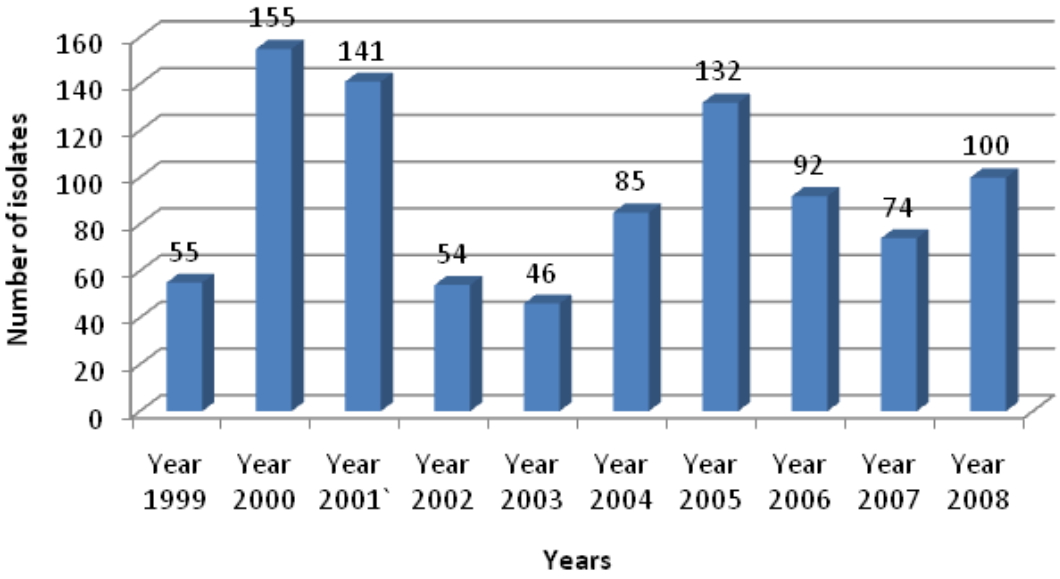

Figure 1. *Streptococcus pnumoniae* isolates reported during 1999 to 2008.

Laboratory, Kavre. Each participating laboratory had collected and sent the isolates to National Public Health Laboratory (NPHL), the National Collaborating (focal point) Laboratory. Specimen collection and identification of the isolates was done following standard microbiological techniques in all participating laboratories. Sample was streaked onto sheep blood agar (5% sheep blood in blood agar base), chocolate agar and Mac-Conkey agar (all media by MAST, UK) by the quadrant isolation technique (Spaulding and Truant, 1974). The plates were incubated in an 8% CO_2 atmosphere at 37°C for 24 h. Isolates were identified by Gram's staining, colony characteristics, optochin sensitivity and bile solubility test (Austrian, 1976). After identification of isolates, they are subcultured onto chocolate agar slants in screw capped tube, incubated overnight and then transported to National Public Health Laboratory (NPHL) in a cold box. All isolates of *S. pneumoniae* were re-confirmed and antimicrobial susceptibility testing was done at National Public Health Laboratory (NPHL). Isolates were preserved at -80°C.

Antimicrobial Susceptibility Testing (AST) was done by Kirby Bauer Disc Diffusion method. *S. pneumoniae* ATCC 49619 was used as control strain for AST. The antibiotics used in this study were: Penicillin (Oxoid, UK), Ampicillin (Oxoid, UK), Erythromycin (Oxoid, UK), Chloramphenicol (Oxoid, UK), Cotrimoxazole (Oxoid, UK) used from 1999 to 2008. Ceftriaxone (Oxoid, UK), Ciprofloxacin (Oxoid, UK) and Ofloxacin (Oxoid, UK) were included from 2004. Similarly, Cefotaxime (Oxoid, UK), Gentamicin (Oxoid, UK), Amikacin (Oxoid, UK), Azithromycin (Oxoid, UK) and Cefalexin (Oxoid, UK) were included only in 2008. For the uniformity among the participating laboratories, external quality control program was conducted quarterly by National Public Health Laboratory.

RESULTS

The results of susceptibility study are summarized from Figures 3 to 6. During 1999 to 2008, a total of 934 isolates of *S. pneumoniae* were identified (Figure 1). Nearly half of the isolates (42%) were from Sputum sample followed by blood (33%) (Figure 2). For Cotrimoxazole, Penicillin, Ampicillin, Erythromycin and Chloramphenicol, the overall resistance percentage was 56.6, 4.7, 5, 5.3 and 2.4% respectively with variation in some year. Cotrimoxazole showed the highest resistance pattern among the antibiotics used in this study, 65% in 1999 to 74% in 2008. Macrolides resistance was established from the beginning of the study as 11% isolates were resistance to Erythromycin in 1999 (Figure 3) while 25% isolates were resistance to Azithromycin (included only in 2008) (Figure 6). Increasing trend of resistance to Beta lactam antibiotics (Penicillin and Ampicillin) was observed from 1999 to 2008 (Figure 3). Increasing resistance towards Ceftriaxone (2004 to 2008), 3rd generatioin cephalosporin was reported during the study period from 0% in 2004 to 8% in 2007(Figure 5). Second generation fluoroquinolones: Ciprofloxacin and Ofloxacin was added only from 2004 which showed increasing resistance pattern from 2004 to 2008 (Figure 5). Resistance towards Ciprofloxacin was 0% in 2004 and 8% in 2008, whereas Ofloxacin showed 0% resistance in 2004 and 5% in 2007.

Cefotaxime, Gentamicin, Amikacin and Cefalexin were included in the study from 2008 only; susceptibility pattern obtained from 2008 surveillance showed that the resistance pattern of Cefotaxime, Gentamicin, Amikacin and Cefalexin was 0, 44, 4 and 88% respectively (Figure 6). Co-resistant pattern of Penicillin with other antibiotics was presented in Table 1.

DISCUSSION

Antimicrobial resistance problem is more frequent in the developing countries like Nepal where indiscriminate, inadequate and inappropriate use of antimicrobials and self-medication are quite common. Although, antimicrobials were effective in the initial phase of their

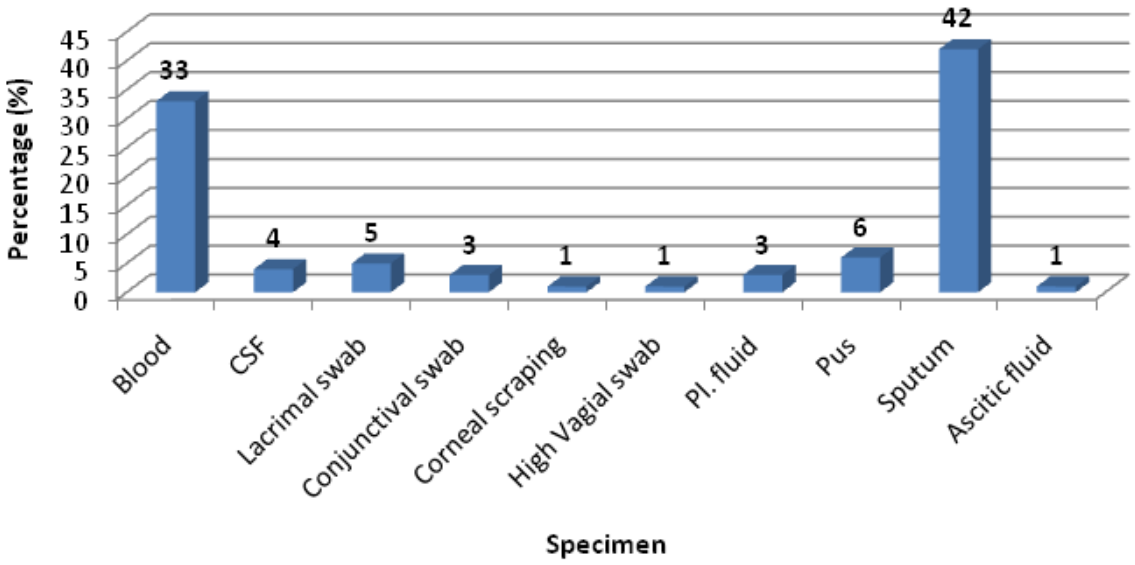

Figure 2. *Streptococcus pneumonia* isolates from different specimens (n = 924).

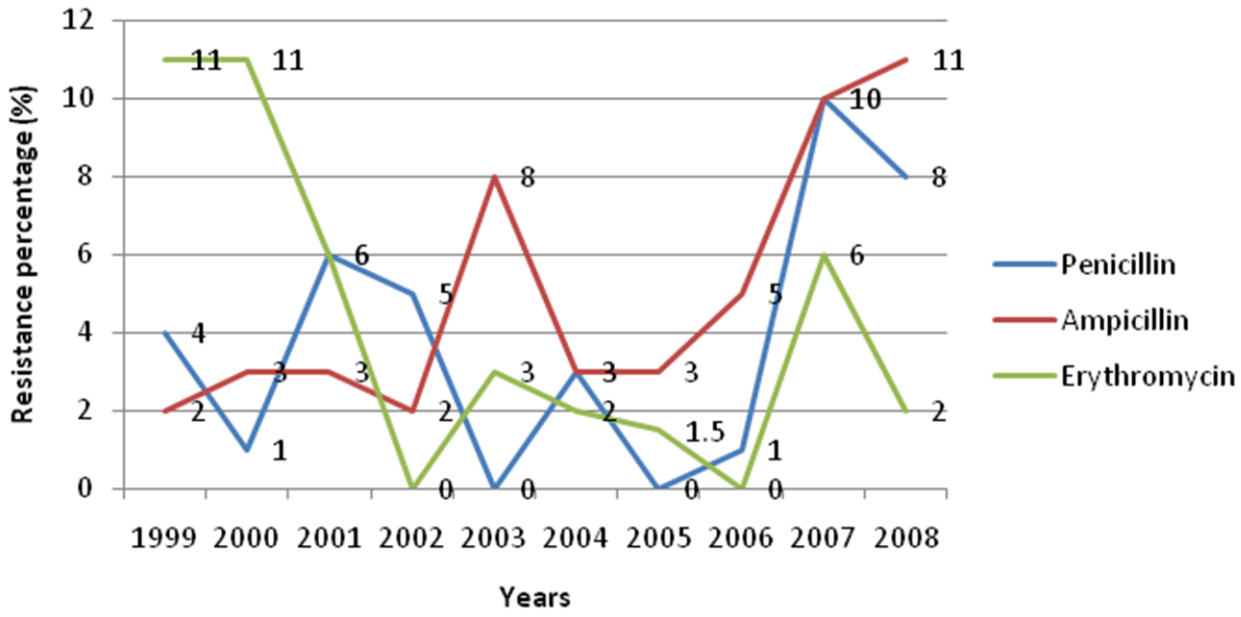

Figure 3. Antimicrobial resistance pattern of Penicillin, Ampicillin and Erythromycin from 1999 to 2008.

development, the fast emergence of drug resistant *S. pneumonia* strains have created a problem in the control and treatment of various infections (Breiman, 1994). In this study, the level of Cotrimoxazole resistance among *S. pneumonia* isolates was higher than the other antibiotics followed by ampicillin, penicillin and Erythromycin. The finding of this study is supported by other studies where prevalence of co-trimoxazole was 43.2% (Sener et al., 2007). Cotrimoxazole interfere with the biosynthesis of folic acid; Trimethoprim inhibits bacterial dihydrofolate reductase (DHFR) and Sulfamethoxazole inhibits dihydropteroate synthase (DHPS) (Widdowson and Klugman, 1999; Adrian and Klugman, 1997; Maskell et al., 1997). 50 of 158 co-otrimoxazole-resistant isolates were analysed for mutations in the DHFR gene as well as for duplications within *sul*A which codes for DHPS. The DHFR genes were sequenced and all 50 isolates displayed the amino acid change Ile-100→Leu which causes Trimethoprim resistant (Widdowson and Klugman, 1999; Adrian and

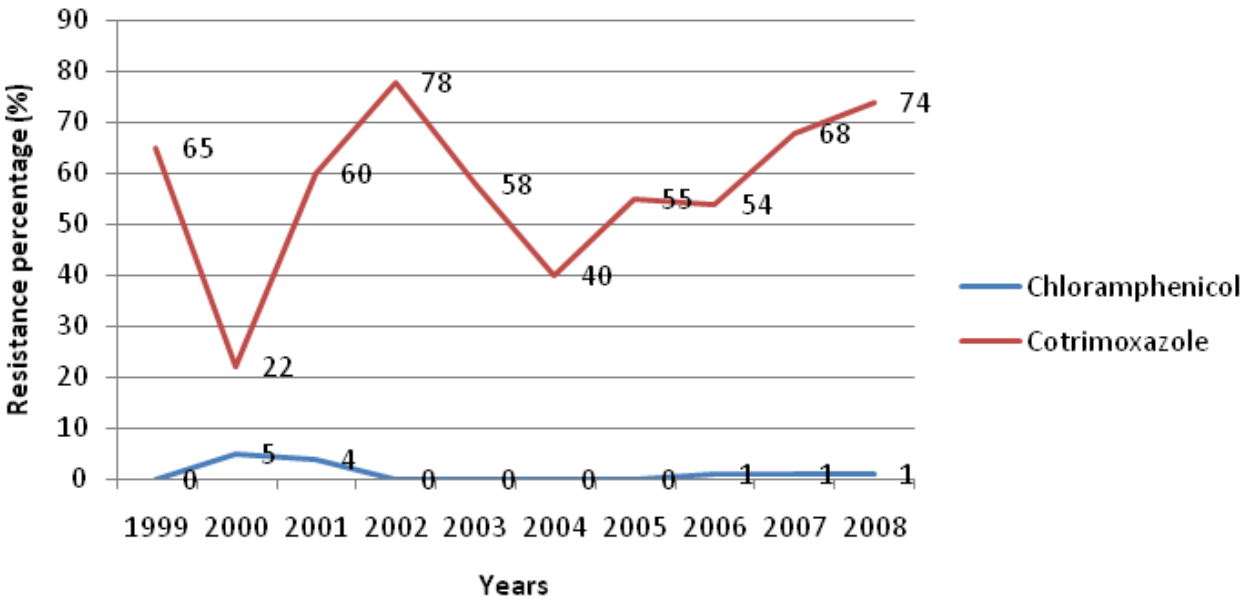

Figure 4. Antimicrobial resistance pattern of Chloramphenicol and Cotrimoxazole from 1999-2008.

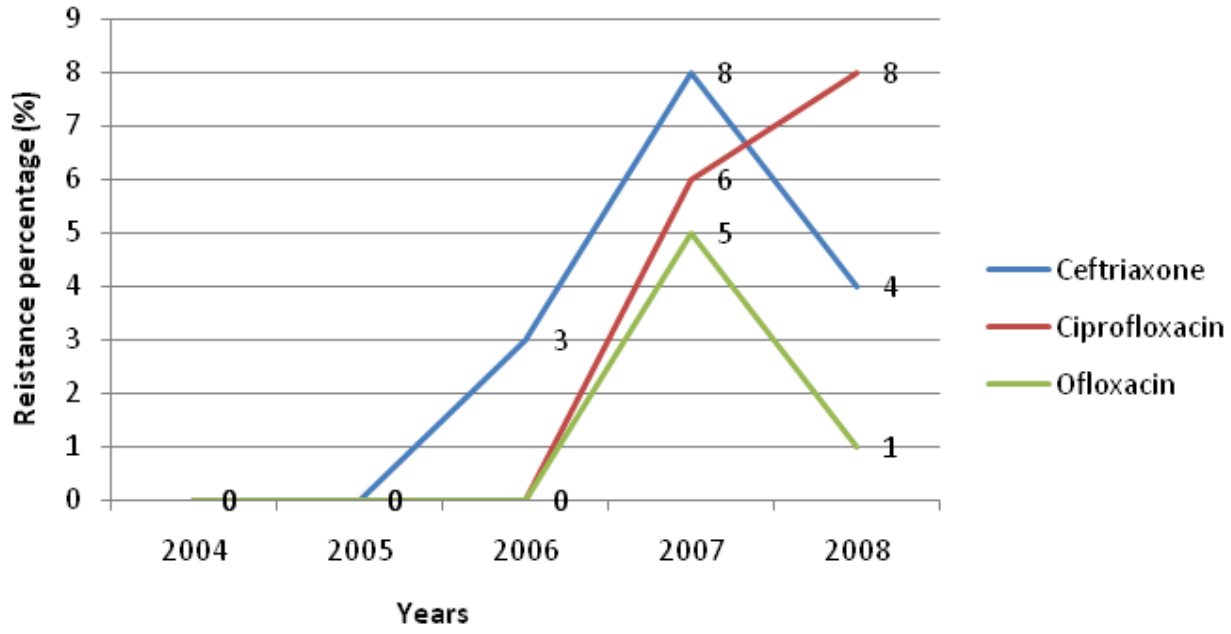

Figure 5. Antimicrobial resistance pattern of ceftriaxone, ciprofloxacin and ofloxacin from 2004-2008.

Klugman, 1997; Maskell et al., 2001). Sulphonamide resistance in a laboratory mutant revealed the duplication of amino acids 66 and 67 (termed *sul*-d) in *sul*A, the chromosomal DHPS gene. Transformation experiments showed that the duplications are sufficient to confer high-level sulphonamide resistance (Widdowson and Klugman, 1999; Maskell et al., 1997). An increasing trend of resistance to ß-lactams was observed in this study: Penicillin and Ampicillin showed the overall resistance of 4.7 and 5% respectively with highest 10% in 2007 and 11% in 2008. Other ß-lactam antibiotics of Cephalosporin group: Ceftriaxone and Cefotaxime were included from 2004 and 2008 respectively.

Ceftriaxone resistant was noted from 2006 (3%). Resistance pattern was increased from 3% in 2006 to 8% in 2007 followed by 4% in 2008 (Figure 5). In contrast to Ceftriaxone, all the isolates were susceptible to Cefotaxime which was included only in 2008. These

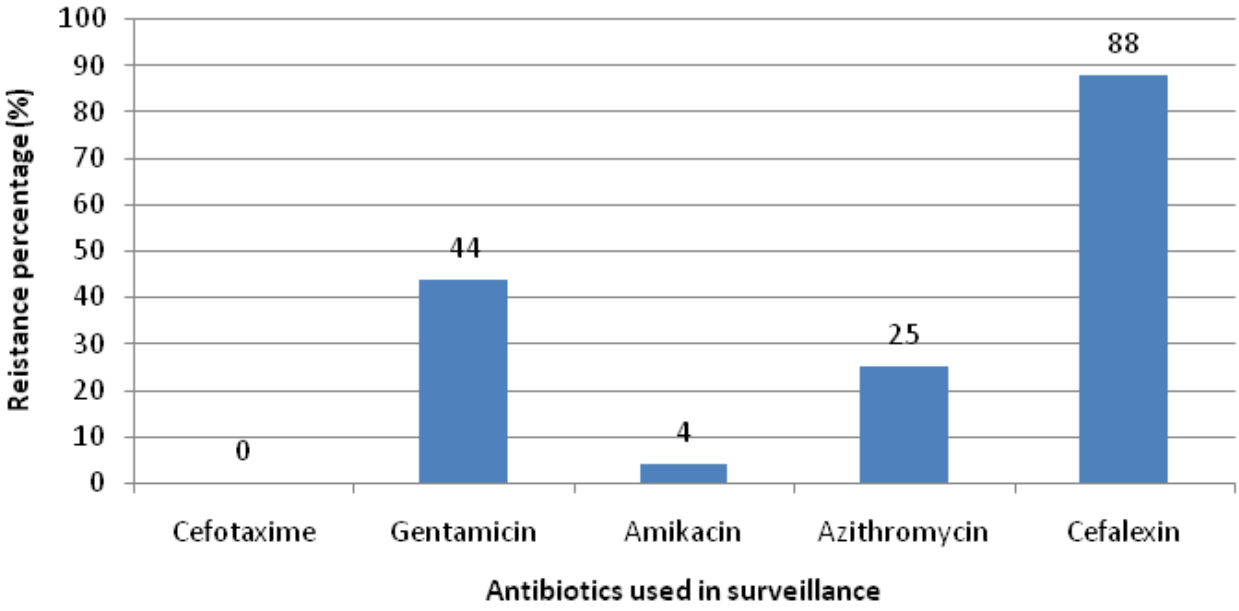

Figure 6. Antimicrobial resistance pattern of antibiotics used only in 2008.

Table 1. Pattern of co-resistance to tested antibiotics from 2004-2008.

Antibiotics	No of resistant isolates (%)				
	2004 (n = 85)	2005 (n = 132)	2006 (n = 92)	2007 (n = 74)	2008 (n = 100)
Pen, Cotri	3 (3.5)	0	0	7 (9.46)	6 (6)
Pen, Cip	0	0	0	2 (2.7)	3 (3)
Pen, Cip, Ery	0	0	0	2 (2.7)	0
Pen, Cip, Azith	-	-	-	-	4 (4)

results were consistent with findings in other studies: the intermediate- and fully-resistant rates for penicillin increased from 12.5 and 0% in the period of 1989 to 1991 to 27.3 and 22.7% in 1994, respectively (Chang et al., 1997). Cefotaxime and ceftriaxone are the most active Cephalosporins against pneumococci. Despite marked escalation in PNSP, rates of resistance to cefotaxime (MICs ≥ 2 µg/ml) globally remained low (Doern et al., 2005; Karlowsky et al., 2003; Song et al., 2004; Hoban et al., 2001; Doit et al., 1999; Jones et al., 2007). The major mechanism of resistance to ß-lactams involve in the introduction of mutations in genes encoding penicillin-binding proteins (Albrich et al., 2004). A prevalence of resistance to macrolides: Erythromycin was observed as 11% in 1999 to 6% in 2007, similarly 25% isolates were resistance towards Azithromycin in 2008. The most frequent molecular mechanism for macrolide resistance in pneumococci is ribosomal methylation mediated by the presence of the *erm* gene (Baquero et al., 1999). During 10 years of Antimicrobial surveillance study, chloramphenicol showed least resistance percentage in comparison with other antibiotics with some exception (5% resistance in 2000 and 4% in 2001). Chloramphenicol is active against the three main bacterial causes of meningitis: *Neisseria meningitidis*, *S. pneumoniae* and *Haemophilus influenzae*. In the West, chloramphenicol remains the drug of choice in the treatment of meningitis in patients with severe penicillin or cephalosporin allergy. In low income countries, the WHO recommends that chloramphenicol be used first-line to treat meningitis. The steadily increasing resistance of second generation fluoroquinolones: Ciprofloxacin and Ofloxacin was noted from 2007 while all the isolates were susceptible in the year 2004 to 2006. Increased pattern of Ciprofloxacin resistance (5% in 2007 to 8% in 2008) indicate towards possibility of treatment failure in the coming future. The first treatment failure due to fluoroquinolone-resistant *S. pneumoniae* was reported in Turkey in 2003 (Ak et al., 2003). Among invasive *S. pneumoniae* isolated during 2000 to 2001, only 3.5% of isolates were ofloxacin resistant, all of the intermediate-resistant type but high resistivity to ciprofloxacin has been recorded (Oncu et al., 2004).

Co-resistant pattern of Penicillin with other antibiotics is presented in Table 1; except with cotrimoxazole, co-resistant pattern was observed only from 2007 where 2

isolates were Multi-Drug resistant with Penicillin, Ciprofloxacin and Erythromycin in the year 2007 followed by 4 isolates resistant with Penicillin, Ciprofloxacin and Azithromycin in 2008. Increasing resistance trend of penicillin and other antimicrobials including macrolides and Quinolones is an emerging problem in Nepal and should be addressed accordingly. The overuse of antibiotics such as Quinolones with children suffering from otitis media has given rise to a breed of super bacteria that are resistant to antibiotics entirely (Froom et al., 1997). All medical personnel should be aware about the resistivity developed by bacteria to different antibiotics and this type of study should be broad.

Conclusion

High Cotrimoxazole resistance in this study suggests the low usefulness of its use in the pneumococcal infections. Increasing resistance pattern of Penicillin, Ciprofloxacin and Ceftriaxone towards *S. pneumonia* isolates is an emerging problem in Nepal. Chloramphenicol is consistently highly susceptible during the entire period of study. Though Cefotaxime included only in 2008, it is most effective cephalosporin than Ceftriaxone. Findings of the study could be decisive in establishing treatment guidelines of pneumococcal infection in Nepal.

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