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ORIGINAL ARTICLE

Antimicrobial susceptibility trends among *Streptococcus pneumoniae* over an 11-year period in an Iranian referral children Hospital

Mohammad Taghi Haghi Ashtiani[1], Majid Sadeghian[2], Bahram Nikmanesh[3], Babak Pourakbari[2], Shima Mahmoudi[2], Setareh Mamishi[2,4*]

[1]Pediatrics Center of Excellence, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran. [2]Pediatric Infectious Disease Research Center, Tehran University of Medical Sciences, Tehran, Iran. [3]Departement of parasitology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran. [4]Department of Infectious Disease, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and Objectives: The appearance of antibiotic resistance in *Streptococcus pneumoniae* has raised a global concern over the past three decades. This study was conducted to determine the antimicrobial susceptibility of *S. pneumoniae* isolated from patients in Children´s Medical Center (CMC) Hospital during 2001 to 2011.

Materials and Methods: During the 11 years period, a total of 194 *S. pneumoniae* isolates were collected in CMC Hospital. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method and time-series analysis was used to evaluate the antimicrobial susceptibility changes over the time.

Results and Conclusion: Antimicrobial susceptibility of *S. pneumoniae* to different antibiotics decreased from 2001 to 2011 as: penicillin from 78% to 32%, erythromycin from 75% to 35%, chloramphenicol from 94% to 55%, ampicillin from 70% to 62%, ceftriaxone from 100% to 87%, sulfametoxazole from 57% to 40%. We did not find any significant difference between the susceptibility of isolates from sterile and non-sterile sources. It would be an important key to consider antimicrobial stewardship as an essential factor to prevent the development of antimicrobial resistance.

Keywords: *S. pneumonia*, antibiotic resistance, Iran

INTRODUCTION

Streptococcus pneumoniae is a major etiologic agent of infections from mild mucosal infections such as otitis media and sinusitis to more serious infections like pneumonia and meningitis (1). This organism is the cause of 700,000 to 1,000,000 annual deaths in children aged 1 to 59 months, which is about 11% of all deaths of children in this age group (2). The appearance of antibiotic resistance in *S. pneumoniae* has raised a global concern over the past three decades. Antimicrobial resistance among this microorganism is a serious problem especially for frequently utilized beta-lactams, macrolides and fluoroquinolones (3,4). Emergence of multi-resistance isolates and the potential spread of these organisms have crucial implications for the treatment and clinical outcome of the patients (5-8). With recently different bacterial susceptibility patterns introduced from various geographical areas, it is essential to find the antimicrobial patterns in order to control the spread of resistant bacteria (9). This is more significant in developing countries where a

*Corresponding author: Setareh Mamishi, MD
Address: Children's Medical Center Hospital, 62 Qarib St, Keshavarz Blvd, Tehran, Iran.
Tel /fax: +98-21-66428996
E-mail: smamishi@sina.tums.ac.ir

severe misuse of antibiotics at all levels, as a result of the over-the-counter services without prescription in many cases, has been observed (10,11). There are some reports of concerning the antimicrobial susceptibility patterns of *S. pneumoniae strains* in Iran (6,10,12). However, no valid data to show the changes of the antimicrobial susceptibility during different years is available. In addition, there is no valid data with a large sample size from tertiary center hospital which have the cases from different areas of Iran.

This study was conducted to determine the antimicrobial susceptibility of *S. pneumoniae* isolated from patients in Children´s Medical Center Hospital (CMC) during 2001 to 2011.

MATERIALS AND METHODS

We retrospectively reviewed laboratory records of all patients with S. *pneumoniae* positive culture during 2001-2011 in CMC Hospital. This hospital is one of the referral tertiary centers of Tehran University of Medical Sciences and patients are admitted from all regions of Iran representing a wide spectrum of socioeconomic levels. Data of microorganisms and antibacterial susceptibility were obtained from the records of clinical microbiology laboratory were filled out on a prepared datasheet.

Isolation and identification of *S. pneumoniae*. Blood was inoculated into a single 20-mL bottle of brain heart infusion broth with sodium polyanethol sulphonate and was incubated at 37˚C in air for 7 days, with routine subcultures onto sheep blood agar in 5% CO_2 at 24 hour, 48 hour, and 7 days. Turbid bottles were examined by Gram statining and subcultured onto appropriate media. Cerebrospinal fluid was analyzed by Gram stains if the white blood cell count was >10/mm^3. All samples were cultured on sheep blood agar for 48 hour under aerobic and microaerophilic conditions. Organisms were identified in the microbiology laboratory of CMC Hospital using standard identification methods including alpha-haemolysis, bile solubility and optochin sensitivity (13-15).

Antimicrobial agents and susceptibility testing. Antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion method, which is the predominant assay used in Iran. Bacterial sensitivity was tested for the following antimicrobials: vancomycin, ceftriaxone, cefotaxime, cefazolin, ampicillin, chloramphenicole, oxaciilin, erythromycin, gentamicin and trimethoprim-sulfamethoxazole. The guidelines of clinical and laboratory standard institute (CLSI) was followed to determine the disk zone diameters as susceptible or resistant (16). Intermediate susceptibility to antimicrobial agents was categorized as resistant in the data analysis and presentations. Quality control organisms were used in the CMC microbiology laboratory to ensure accurate performance of the laboratory tests.

Data analysis was performed using SPSS, version 17 (Inc, Chicago IL, USA). Time series analyses were used to predict the changes of the variables during the time.

RESULTS

During the time period of 2001–2011 (11 years), a total of 194 *S. pneumoniae* isolates were collected in CMC Hospital. Nearly two-thirds of the total isolates (n=126 [65%]) were obtained from bloodstream infections, and the remaining isolates were cultured from sputum (n=19 [10%]), eye (n=16 [8%]), CSF (n=13 [7%]), ascites (n=6 [3%]), ear (n=6 [3%]) and others (n=8 [4%]).

The overall susceptibility of *S. pneumoniae* to different antibiotics was as: 100% to vancomycin, 88% to ceftriaxone, 87% to cefotaxime, 78% to cefazolin, 76% to ampicillin, 70% to chloramphenicol, 54% to penicillin, 49% to erythromycin, 37% to gentamicin and 28% to trimethoprim-sulfamethoxazole.

Variable susceptibility profiles for different antibiotics were observed over the 11 years, the prevalence of susceptibility to penicillin decreased from 78% (23 of 30) in 2001 to 32% (13 of 45) in 2011. In the same time period, susceptibility to erythromycin declined from 75% (12 of 16) in 2001 to 35% (15 of 43) in 2011 and susceptibility to chloramphenicol started to decrease from 94% (15 of 16) to 55% (23 of 42). In addition, during these couple of years, susceptibility to ampicillin declined from 70% (14 of 20) to 62% (26 of 42). Beside this, susceptibility to ceftriaxone started to fall from 100% (8 of 8) to 87% (35 of 40) and susceptibility to sulfametoxazole came down from 57% (10 of 18) to 40% (17 of 42).

Time series analysis of the 5 antibiotics of penicillin, erythromycin, chloramphenicol, ceftriaxone, and

trimethoprim-sulfamethoxazole showed an overall decreasing trend for *S. pneumoniae* susceptibility during 2001 to 2011 and even forecasting prediction for 2016 (Fig. 1-5). Individual susceptibility to these 5 antibiotics has declined during 2001-2011 by 2.4, 2.1, 1.7, 1.1, and 1.4 times. On the other hand, cefotaxime resistance increased from initially low levels in 2002 to reach a plateau from 2005 onward; however, after decreasing slightly in 2006 this rate was stable until 2008 and the rate increased again in 2011. Susceptibility of *S. pneumoniae* to gentamicin was 14% (3 of 21) in 2001, 33% (7 of 21) in 2003, 15% (2 of 13) in 2005, 28% (8 of 29) in 2010, and 49% (21 of 43) in 2011.

Susceptibility of *S. pneumoniae* to ampicillin was different during various years; it was about 70% in 2001 but it did not stay stable very long time and the percentages declined to around 55% in 2002 and 2003. Further on, the percentages increased to 85% in 2005 and stayed steady until 2010, finally the percentage dropped to 62% (26 of 42) in 2011. Concurrent resistance to ampicillin and erythromycin was not detected in 2001 but reached to 18.4% (7 of 38) in 2011. At the same period of time, ampicillin-chloramphenicol resistance was starting to rise from zero to 10.5% (4 of 38) as well as ampicillin-ceftriaxone resistance which was increased from zero to 8% (3 of 37). Moreover erythromycin-ceftriaxone resistance was starting to increase from zero to 5.2% (2 of 38) and ceftriaxone-chloramphenicol resistance went up from zero to 7.6% (3 of 39). Moreover, erythromycin-chloramphenicol resistance was raised dramatically from zero to 45% (18 of 40).

DISCUSSION

This study reveals the development of antibiotic resistance of *S. pneumoniae* in CMC Hospital over an 11-year period (2001–2011). Overall, the susceptibility rate to 5 main antibiotics of penicillin, erythromycin, chloramphenicol, ceftriaxone and trimethoprim-sulfamethoxazole has shown striking changes through the 11 years from high levels in 2001 to low levels in 2011.

International studies by Asian Network For surveillance of Resistant Pathogens (ANSORP) documented extremely high prevalence of penicillin and erythromycin resistance among clinical cases of *S. pneumoniae* in Korea, Japan, Thailand, Taiwan and Sri Lanka (5). The increase in prevalence of antimicrobial resistant in *S. pneumoniae* is related to increase of invasive *S. pneumoniae* infections in children and more clinical failures of antimicrobial treatments (5,17). A study by Mera *et al,* reported a decline in *S. pneumoniae* susceptibility during 1992-2001 in the United States (18). Another study by Whitney *et al.* reported a similar reduction during 1995-1998 in United States (19). This study found that if the upsurge of antibiotics usage continues, we might lose the efficacy of important antibiotics such as penicillin, erythromycin and trimethoprim-sulfamethoxazole by 2016.

In our study the susceptibility to penicillin was 32% in 2011 that was similar to other reports from north, central America and Asia (20-22) while there are some reports that shows higher susceptibility for this antibiotic (12, 23). According to ANSORP reports, although penicillin non-susceptibility (40–60%) is common in Asia, reduced susceptibility could not be related to a worse clinical outcome (5,24). Sangthawan *et al*. reported that prior antibiotic use was significantly associated with penicillin resistance in *S. pneumoniae* isolates in Thailand but this outcome was not related to in vitro penicillin susceptibility pattern (24).

Despite various reports on penicillin resistance in pneumococcus, it is difficult to demonstrate a clear relationship between penicillin resistance and clinical failure. It can be explained because the susceptibility breakpoints were initially established on the basis of achievable levels of this antibiotic in the cerebrospinal fluid while most pneumococcal infections occur in the lung or the bloodstream (25).

Similar to other studies, we found the overall susceptibility to vancomycin as 100%. That means the most resistant strains of *S. pneumoniae* still remain sensitive to this antibiotic (12, 19, 26). Our data showed that cefotaxime and ceftriaxone (Fig. 4) which recommended for empiric pneumonia therapy still have good effect due to low prevalence of resistance. The overall susceptibility to cephalosporins was 88% to ceftriaxone and 87% to cefotaxime but the important point is that the susceptibility to cephalosporins was about 100% in 2001 and decreased to 88% in 2011 and the forecasting analysis shows that it would be lower in the future.

Harrison *et al.* found the susceptibility of 86% to 95% for ceftriaxone in the United States (27). A study by Kohanteb *et al*. reported a higher susceptibility rate for cephalosporins (cefotaxime 96% and ceftriaxone

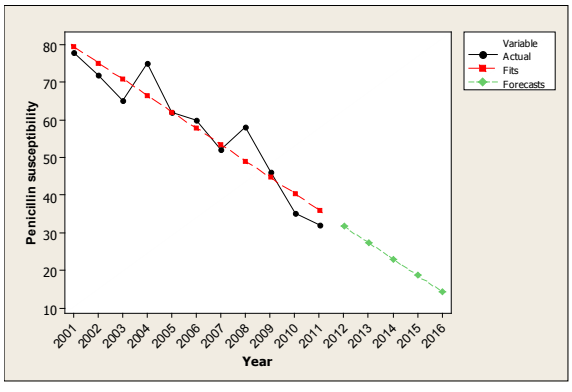

Figure 1.Time series for penicillin susceptibility percentile during 2001-2011 and forecasting diagram during 2012-2016.

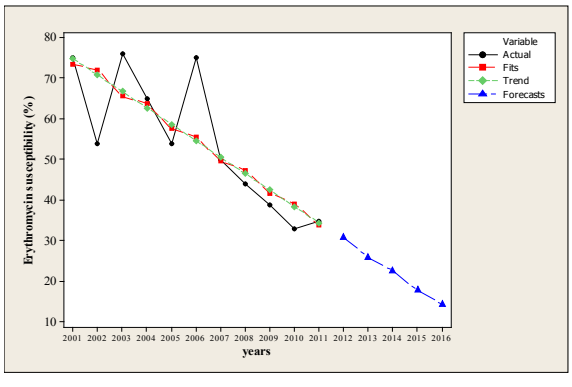

Fig. 2. Time series for Erythromycin susceptibility percentile during 2001-2011 and forecasting diagram during 2012-2016.

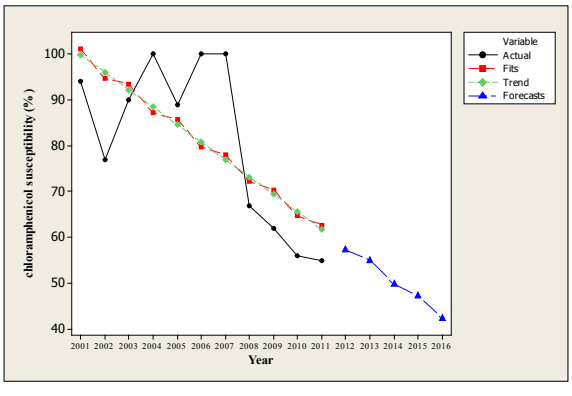

Fig. 3. Time series for Chloramphenicol susceptibility percentile during 2001-2011 and forecasting diagram during 2012-2016.

94%) in Iran (12). Moreover, ANSORP reported 92% susceptibility to ceftriaxone in Asia (5).

Macrolides are considered as the alternative to β-lactams in the treatment of *S. pneumoniae*. Although ANSORP reported very low erythromycin susceptibility rate in Asian countries including 8%

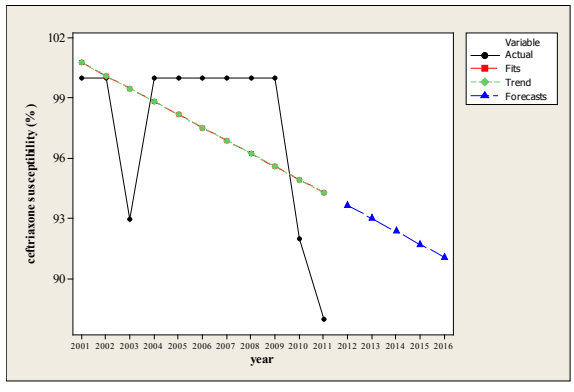

Fig. 4. Time series for Ceftriaxone susceptibility percentile during 2001-2011 and forecasting diagram during 2012-2016.

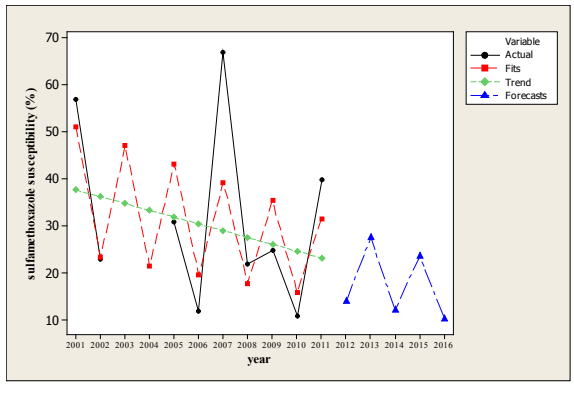

Fig. 5. Time series for trimethoprim - sulfamethoxazolesusceptibility percentile during 2001-2011 and forecasting diagram during 2012-2016.

in Vietnam, 14% in Taiwan, 19% in Korea and 26% in China (5), it should be highlighted that macrolide resistance among pneumococci appears to be increasing in prevalence. In this study the overall susceptibility to erythromycin was 49% which was dramatically decreased from 75% in 2001 to 35% in 2011. Further on, it is predicted to be under the 20% in 2016 (Fig. 2).

This study identifies unstable patterns of resistance to available antimicrobial drugs during 11 years. Continued epidemiological surveillance appears to be prudent practice to guide effective chemotherapy. Moreover, it would be an important key to consider antimicrobial stewardship as an essential factor to prevent the development of antimicrobial resistance.

REFERENCES

1. Hussain M, Melegaro A, Pebody RG, George R, Edmunds WJ, Talukdar R, et al. A longitudinal household study of *Streptococcus pneumoniae* nasopharyngeal carriage in a UK setting. *Epidemiol*

Infect 2005 Oct;133(5):891-8.
2. Green MC, Mason EO, Kaplan SL, Lamberth LB, Stovall SH, Givner LB, et al. Increase in prevalence of *Streptococcus pneumoniae* serotype 6C at eight children's hospitals in the United States from 1993 to 2009. *J Clin Microbiol* 2011 Jun;49(6):2097-101.
3. Jones RN, Jacobs MR, Sader HS. Evolving trends in *Streptococcus pneumoniae* resistance: implications for therapy of community-acquired bacterial pneumonia. *Int J Antimicrob Agents* 2010; 36: 197-204.
4. Jones RN, Sader HS, Moet GJ, Farrell DJ. Declining antimicrobial susceptibility of *Streptococcus pneumoniae* in the United States: report from the SENTRY Antimicrobial Surveillance Program (1998-2009). *Diagn Microbiol Infect Dis* 2010; 68: 334-6.
5. Song JH, Jung SI, Ko KS, Kim NY, Son JS, Chang HH, et al. High prevalence of antimicrobial resistance among clinical *Streptococcus pneumoniae* isolates in Asia (an ANSORP study). *Antimicrob Agents Chemother* 2004 Jun;48(6):2101-7.
6. Pourakbari B, Sadr A, Ashtiani MT, Mamishi S, Dehghani M, Mahmoudi S, et al. Five-year evaluation of the antimicrobial susceptibility patterns of bacteria causing bloodstream infections in Iran. *J Infect Dev Ctries* 2012 Feb;6(2):120-5.
7. Patel SN, McGeer A, Melano R, Tyrrell GJ, Green K, Pillai DR, et al. Susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. *Antimicrob Agents Chemother* 2011 Aug;55(8):3703-8.
8. Mera RM, Miller LA, Daniels JJ, Weil JG, White AR. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States over a 10-year period: Alexander Project. *Diagn Microbiol Infect Dis* 2005 Mar;51(3):195-200.
9. Pottumarthy S, Fritsche TR, Sader HS, Stilwell MG, Jones RN. Susceptibility patterns of *Streptococcus pneumoniae* isolates in North America (2002-2003): contemporary in vitro activities of amoxicillin/clavulanate and 15 other antimicrobial agents. *Int J Antimicrob Agents* 2005; 25: 282-9.
10. Mamishi S, Pourakbari B, Ashtiani MH, Hashemi FB. Frequency of isolation and antimicrobial susceptibility of bacteria isolated from bloodstream infections at Children's Medical Center, Tehran, Iran, 1996-2000. *Int J Antimicrob Agents* 2005; 26: 373-9.
11. Brown SD, Rybak MJ. Antimicrobial susceptibility of *Streptococcus pneumoniae, Streptococcus pyogenes* and *Haemophilus influenzae* collected from patients across the USA, in 2001-2002, as part of the PROTEKT US study. *J Antimicrob Chemother* 2004 Aug;54 Suppl 1:i7-15.
12. Kohanteb J, Sadeghi E. Penicillin - resistant *Streptococcus pneumoniae* in Iran. *Med Princ Pract* 2007; 16: 29-33.
13. Luh KT, Hsueh PR, Teng LJ, Pan HJ, Chen YC, Lu JJ, et al. Quinupristin-dalfopristin resistance among gram-positive bacteria in Taiwan. *Antimicrob Agents Chemother* 2000;44:3374-80.
14. Feizabadi MM, Aliahmadi A, Mobasheri F, Asgharzadeh A, Asadi S, Etemadi G. Phenotypic characteristics and population genetics of *Enterococcus faecalis* cultured from patients in Tehran during 2000-2001. *Can J Microbiol* 2003; 49: 645-9.
15. Huang SS, Hinrichsen VL, Stevenson AE, Rifas-Shiman SL, Kleinman K, Pelton SI, et al. Continued impact of pneumococcal conjugate vaccine on carriage in young children. *Pediatrics* 2009;124:e1-11.
16. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - eighth edition, M07-A8. Wayne, PA: 2009.
17. Mamishi M, Moradkhani S, Mahmoudi S, Hosseinpour Sadeghi R, Pourakbari B. Penicillin-Resistant trend of *Streptococcus pneumoniae* in Asia: A systematic review. *Iran J Microbiol* 2014; 6: 198-210.
18. Mera RM, Miller LA, Daniels JJ, Weil JG, White AR. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States over a 10-year period: Alexander Project. *Diagn Microbiol Infect Dis* 2005; 51: 195-200.
19. Whitney CG, Farley MM, Hadler J, Harrison LH, Lexau C, Reingold A, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000; 28;343:1917-1924.
20. Poulakou G, Katsarolis I, Matthaiopoulou I, Tsiodras S, Kanavaki S, Hatzaki D, et al. Nationwide surveillance of *Streptococcus pneumoniae* in Greece: patterns of resistance and serotype epidemiology. *Int J Antimicrob Agents* 2007;30:87-92.
21. Cardozo DM, Nascimento-Carvalho CM, Souza FR, Silva NM. Nasopharyngeal colonization and penicillin resistance among pneumococcal strains: a worldwide 2004 update. *Braz J Infect Dis* 2006; 10: 293-304.
22. Hoa NQ, Trung NV, Larsson M, Eriksson B, Phuc HD, Chuc NT, et al. Decreased *Streptococcus pneumoniae* susceptibility to oral antibiotics among children in rural Vietnam: a community study. *BMC Infect Dis* 2010;10:85.
23. Reinert RR, Reinert S, van der LM, Cil MY, Al-Lahham A, Appelbaum P. Antimicrobial susceptibility of *Streptococcus pneumoniae* in eight European countries from 2001 to 2003. *Antimicrob Agents Chemother* 2005; 49: 2903-13.
24. Sangthawan P, Chantaratchada S, Chanthadisai N, Wattanathum A. Prevalence and clinical significance of community-acquired penicillin - resistant pneumococcal pneumonia in Thailand. *Respirology* 2003; 8: 208-12.
25. Moore M, McNulty C. European Antibiotic Awareness Day 2012: TARGET antibiotics through guidance, education, and tools. *Br J Gen Pract* 2012; 62: 621-2.
26. Saha SK, Naheed A, El Arifeen S, Islam M, Al-Emran H, Amin R, et al. Surveillance for invasive *Streptococcus pneumoniae* disease among hospitalized children in Bangladesh: antimicrobial susceptibility and serotype distribution. *Clin Infect Dis* 2009 Mar 1;48 Suppl 2:S75-81.
27. Harrison CJ, Woods C, Stout G, Martin B, Selvarangan R. Susceptibilities of Haemophilus influenzae, Streptococcus pneumoniae, including serotype 19A, and Moraxella catarrhalis paediatric isolates from 2005 to 2007 to commonly used antibiotics. *J Antimicrob Chemother* 2009; 63: 511-9.Ebes,