RESEARCH ARTICLE

Increase in Penicillin and multidrug resistance in *Streptococcus pneumoniae* (1993-2016): Report from a tertiary care hospital laboratory, Pakistan

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Abstract

Objective: To determine the trend of resistance to antimicrobials in *Streptococcus pneumoniae* infections, and the impact of new Clinical and Laboratory Standards Institute guidelines on 1211 among meningeal isolates.

Method: The descriptive observational retrospective study was conducted at the Aga Khan University Hospital laboratory in Karachi, and comprised *Streptococcus pneumoniae* isolation and antimicrobial susceptibility data over a period of 24 years, from 1993 to 2016, which was compared in terms of pre-2008 and post-2008 data, which was analysed using SPSS 19.

Results: Of the 7415 non-duplicate isolates identified, 4700(63.4%) were from male patients and 2,715(36.6%) were from female patients. The overall mean age of the patients was 38±27 years. Penicillin resistance in non-meningeal isolates during the two periods was not significantly different ($p>0.05$), but a significant rise in penicillin resistance in meningeal isolates was observed in the second period ($p<0.05$). High resistance rates were observed for co-trimoxazole, tetracycline and erythromycin, and an increased trend of multi-drug resistant strains was also noted from 1999 {n=35/317(11%)} to 2016 {n=110/314 (36%)}.

Conclusion: The emergence of multi-drug resistant strains was evident. The spike in penicillin-resistant *Streptococcus pneumoniae* in meningeal isolates may have been due to the revised guidelines by the Clinical and Laboratory Standards Institute.

Keywords: *Streptococcus pneumoniae*, Resistance, MDR, Penicillin, Revised CLSI breakpoints. (JPMA 71: 2767; 2021)

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Introduction

Streptococcus (S.) pneumoniae results in many serious infections including pneumonia, peritonitis, meningitis, bacteraemia and septicaemia. Morbidity and mortality is high among paediatric, elderly and immunosuppressed populations.[1] The World Health Organisation (WHO) estimates that pneumococcal diseases result in 1.6 million deaths annually, more significantly in children aged <5 years residing in underdeveloped countries.[2]

During the initial years of penicillin discovery and its use, *S. pneumoniae* remained susceptible to beta lactam antibiotics. In 1967, the first penicillin-resistant *S. pneumoniae* (PRSP) clinical isolate was reported in the United Kingdom.[3] This was followed by reports from all over the world, including South Africa, Europe and the United States.[4,5] Simultaneously, there was the emergence of multidrug-resistant (MDR) *S pneumoniae*, meaning resistance to three or more different classes of antibiotics, worldwide.[5-7] Usually higher prevalence of resistant *pneumococcal* isolates in a community is influenced by injudicious use of antibiotics.[8-10]

Until 2008, the Clinical and Laboratory Standards Institute (CLSI) guidelines for susceptibility testing of penicillin from all sources, including meningeal *pneumococcal* isolates, were the same. *S. pneumoniae* was considered susceptible when the minimum inhibitory concentration (MIC) was <0.6ug/ml, intermediate resistant when MIC ranged 0.12-1ug/ml, and resistant when MIC was >2ug/ml.[11] Given the limited penetration and bioavailability of penicillin through meninges, CLSI in 2008 revised MICs of *pneumococci* isolated from the cerebrospinal fluid (CSF). As per new recommendations, meningeal isolates with MIC <0.06 ug/ml are considered susceptible and >0.12 ug/ml are considered resistant. For non-meningeal isolates, breakpoints for intravenous (IV) penicillin susceptibility is defined as <2ug/ml, intermediate as <4ug/ml and resistant >8ug/ml. However, for oral penicillin, the breakpoints for sensitivity are defined as <0.06ug/ml, 0.12-1ug/ml and >2ug/ml respectively[12] for sensitive, intermediate resistant and resistant isolates.

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For effective treatment of serious *pneumococcal* infections, it is imperative to know the trend of penicillin and other drug resistance among the local isolates. The current study was planned to analyse the changing trends of penicillin resistance in meningeal and non-meningeal *S. pneumoniae* isolates and to assess resistance to erythromycin, tetracycline, co-trimoxazole, ofloxacin, ceftriaxone and vancomycin. It also planned to determine the impact of new CLSI susceptibility breakpoints on the clinical isolates.

Materials and Methods

The descriptive, observational, retrospective study was conducted at the Aga Khan University Hospital (AKUH) laboratory, Karachi, and comprised S. pneumoniae isolation and antimicrobial susceptibility data over a period of 24 years, from 1993 to 2016, which was compared in terms of pre-2008 and post-2008 periods. The laboratory receives clinical samples from inpatients and outpatients mostly from within Karachi, but also processes samples from different collection points all over the country.

Data from clinical isolates of *S. pneumoniae* and their antimicrobial susceptibility was retrieved from the laboratory information system after obtaining exemption from the institutional ethics review committee. Patient demographics, date of specimen collection and source of specimen were recorded. Isolates were categorised as invasive when isolated from CSF, blood, synovial fluid, pus from sterile body sites, deep tissue and wounds. Isolates from sputum, tracheal aspirates, bronchial lavage, eye and ear were considered non-invasive. The data was then categorised into four age groups; ≤5 years, >5-≤15 years, >15-≤59 years and ≥60 years.

For all antimicrobials, data was analysed to see the trend of antimicrobial resistance (AMR). For benzyl penicillin, data was divided into two groups: pre-2008 and post-2008, corresponding to the CLSI guideline change.[12]

S. pneumoniae identification was based on colonial morphology, gram stain, catalase test, optochin susceptibility and bile solubility. Resistance against various antimicrobial agents, including erythromycin, ofloxacin, tetracycline, co-trimoxazole and vancomycin, was determined using disc diffusion (Kirby-Bauer) method. Zone diameters were interpreted according to CLSI standards.[12] Penicillin susceptibility was reported using 1ug oxacillin disc. Penicillin MICs against pneumococcal isolates were checked when zone diameter of oxacillin was ≤20mm. MICs were checked using agar dilution method and E-test (BioMerieux, France). From 2008 onwards, penicillin MICs were interpreted using the new CLSI breakpoints. Penicillin MICs were performed on all meningeal and blood isolates. Ceftriaxone MICs were only performed on penicillin-resistant isolates.

Data was analysed using SPSS 19. For descriptive analysis, mean and standard deviation of continuous variables, such as age and MICs, were determined. For categorical variables, like gender and antibiotic resistance, frequencies and percentages were calculated. P<0.05 was considered significant.

Results

Of the 7415 non-duplicate isolates identified, 4700(63.4%) were from male patients and 2,715(36.6%) were from female patients. The overall mean age of the patients was 38±27 years. Overall, there were 2158(29%) invasive and

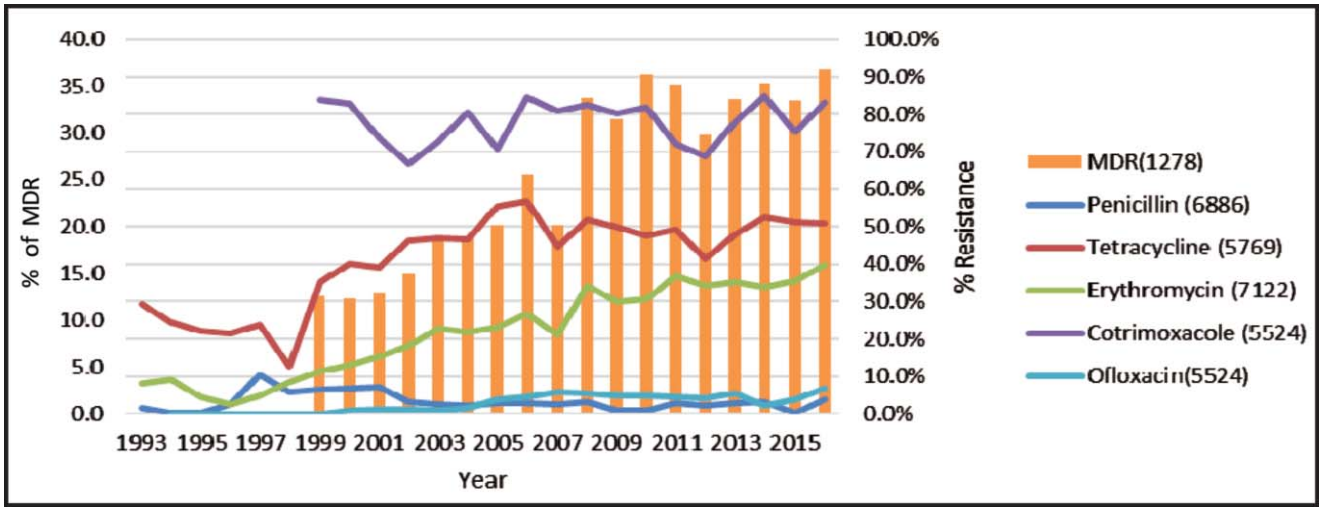

Figure-1: Antimicrobial resistance among *Streptococcus pneumoniae* (1993-2016) and multi-drug resistance (MDR) from 1999-2016.

Table: Penicillin susceptibility in *Streptococcus pneumoniae* in cerebrospinal fluid (CSF) and non-CSF isolates prior to and after 2008 guidelines by the Clinical and Laboratory Standards Institute (CLSI).

Isolates	CSF		Non CSF	
	Pre 2008	Post 2008	Pre 2008	Post 2008
Susceptible (n)	183	37	3811	2165
% Susceptible	87.6	63.8	87.3	96.0
Intermediate (n)	20	0	383	42
% Intermediate	9.6	0.0	8.8	1.9
Resistant (n)	6	21	169	49
% Resistance	2.9	36.2	3.9	2.2

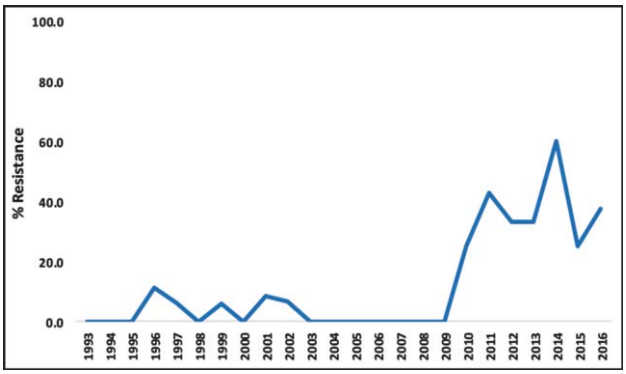

Figure-2: Trend of penicillin resistance among meningeal *Streptococcus pneumoniae* (1993-2016).

5257(71%) non-invasive isolates. The invasive isolates were from blood 1430(19.28%), CSF 294(3.96%), pus 312(4.20%), pleural fluid 86(1.15%) and ascitic fluid 36(0.48%). The non-invasive isolates were from sputum 2885(39%), tracheal aspirate 444(6%), bronchial lavage 289(3.89%), ear 740(10%), eye 537(7.24%), nasal swab 85(1.14%) and other sources 277(3.73%), like bone marrow, pericardial, synovial and umbilical fluid, tissue, high vaginal swab, placenta and maxillary sinuses etc.

Resistance to penicillin, erythromycin, tetracycline, co-trimoxazole and ofloxacin among the isolates was noted (Figure-1). The trend of penicillin resistance in meningeal isolates is given in Figure-2.

No resistance to ceftriaxone and vancomycin was observed.

Penicillin resistance emerged in 2004 and remained around 1-2% in the latter part of the study period (Table-1).

Higher numbers of meningeal *S. pneumoniae* were found resistant to penicillin in the second phase of the study, while a slight non-significant (p=0.789) decline in penicillin resistance in non-meningeal isolates was noted in the post-2008 phase of the study.

Penicillin resistance was highest 1216(4.1%) among *S. pneumoniae* isolated from children ≤5 years of age. It significantly (p<005) declined with increasing age. Isolation of MDR *S. pneumoniae* could not be determined due to inconsistent data till 1999 when it was first noted at around {n=35/317 (11%)}, which, by 2016, rose up to {n=110/314 (36%)}. Isolation of MDR *S. pneumoniae* remained, and it was the highest 1102(29%) in children aged ≤5 years. It declined to 1858(23.7%) in those aged 5-15 years and then increased to 414(25.3%) and 1575(26%) respectively in patients aged 15-59 and >60 years.

Discussion

Among the *S. pneumoniae* isolates collected between 1993 and 2016 in the current study, the resistance to penicillin and ofloxacin was overall found to be low. High resistance to co-trimoxazole, tetracycline and erythromycin was observed, particularly in the latter half of the study, which in turn led to a high MDR rate. No resistance to ceftriaxone and vancomycin was detected.

The current finding of low resistance to penicillin in non-meningeal isolates, which is similar to data published by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) study and Taiwan, reported 0.7% and 0.5% resistance in non-meningeal isolates respectively.[13,14] This is in contrast to data published from the United States, various parts of Europe, Saudi Arabia and Lebanon.[5,15-19]

Due to the revised CLSI breakpoints in 2008, penicillin resistance in meningeal isolates increased from 2.9% to 36.2% in 2015. These results are consistent with the finding of studies conducted in other parts of the world, including Saudi Arabia where a rise in penicillin resistance of 68% in meningeal isolates was reported.[20] Reports from Belgium and Germany also highlight similar findings.[16,17] Treatment of pneumococcal meningitis becomes challenging in settings with high penicillin resistance as vancomycin along with ceftriaxone become the recommended empirical treatment.[21]

Previously, resistance to third-generation cephalosporin has been reported from other countries,[22-24] but local pneumococcal isolates in the current cohort remained susceptible to ceftriaxone. These findings are also verified by a recent study that assessed ceftriaxone breakpoint in invasive pneumococcal isolates (2011-14) in Pakistan and reported very low MICs.[25] These findings support empirical use of ceftriaxone and vancomycin in pneumococcal meningitis with de-escalation to ceftriaxone after availability of MIC results.[21]

Consistent with previously published studies from the South Asian Association for Regional Cooperation (SARC)

countries,[26] frequency of penicillin resistance was highest in isolates from children <5 years of age. Excess use of antimicrobials due to frequent respiratory tract infections amongst children could be a possible reason for this finding. Furthermore, our findings advocate cautious use of antimicrobials in children. Many reports attribute the emergence of penicillin-resistant *S. pneumoniae* serotypes to the initiation of vaccination.[13,27-29] Some Asian countries have reported high prevalence of erythromycin-resistant and MDR *S. pneumoniae* serotype 19A after the introduction of pneumococcal conjugate vaccine (PCV7).[13] Although baseline data on prevalent S. pneumoniae serotypes in children from Pakistan[30] is available, the impact of immunisation on the emergence of resistance could not be assessed due to the recent addition of pneumococcal vaccine to the childhood immunisation programme.

Similar to reports published previously, resistance to co-trimoxazole remained very high in the current study.[18,19,31-33] Resistance to tetracycline and erythromycin also increased to around 50% and 30% respectively over the years. These results are consistent with global and regional data that report increased resistance to these agents.[13,19,33,34] Of note is the fact that this finding limits empirical use of co-trimoxazole, erythromycin and tetracycline in respiratory tract infections despite their low cost and widespread availability.

MDR *S. pneumoniae* rates were reliably analysed from year 1999 onwards and were observed to have increased at an alarming pace to around 35% in 2015. It is important to note that the rise in isolation of MDR *S. pneumoniae* and erythromycin resistance was concurrent in the current study for the last 18 years. Regional data on MDR *S. pneumoniae* reported a very high prevalence from India and the Asia Pacific region.[13,33] This is very worrisome and highlights the dire need for implementing an antimicrobial stewardship programme among community physicians, including paediatricians and family physicians.

Resistance to fluoroquinolone remained low (6%) throughout the study period, which is consistent with reports published from other Asian countries.[13] This is encouraging as family physicians working in primary healthcare settings as well as clinicians based in tertiary care hospitals can use respiratory fluoroquinolone empirically for lower respiratory tract infections.

Since the current study was a retrospective analysis of a prospectively collected database, it could not eliminate all the biases linked to such an analysis. Despite the limitation, however, the resistance trends of commonly used antimicrobials for *S. pneumoniae* isolates will be crucial in drafting treatment guidelines for pneumococcal infections. The findings can be used in the development of local and national guidelines for the management of pneumococcal infections.

Conclusion

The emergence of MDR strains was evident. The spike in penicillin-resistant *S. pneumoniae* in meningeal isolates may have been due to the revised CLSI guidelines. An increasing trend in isolation of erythromycin-, tetracycline-resistant and MDR *S. pneumoniae* is a cause of concern. Low resistance to penicillin and fluoroquinolones in non-meningeal isolates permits use of these drugs in the community setting. High penicillin resistance in meningeal isolates justifies the empiric use of ceftriaxone and vancomycin in pneumococcal meningitis with de-escalation to ceftriaxone after availability of susceptibility results.

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References

1. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1997; 46; 1-24.
2. Gibson PC, Cohen C, Klugman KP, Gouveia L, Anne von Gottberg, Group for Enteric. Pneumococcal diseases. Accessed from World Health Organization. [Online] [Cited 2018 June 23]. Available from: URL:https://www.who.int/ith/diseases/pneumococcal/en/.
3. Hansman D, Bullen MM. A resistant pneumococcus. Lancet. 1967; 2:264-5.
4. Jacobs MR, Koornhof HJ, Robins-Browne RM, Stevenson CM, Vermaak ZA, Frieman I, et al. Emergence of multiple resistant Pneumococci. N Engl J Med. 1978; 299:735-40.
5. Goldstein FW, Acar JF. Antimicrobial resistance among lower respiratory tract isolates of Streptococcus pneumoniae: Results of a 1992-1993 Western Europe and USA collaborative study. The Alexander project collaborative group. J Antimicrob Chemother. 1996; 38:S71-84.
6. Crowther GP, Cohen C, Kluqman KP, Gouveial L, von Gottberg A. Risk factors for multidrug-resistant invasive pneumococcal disease in South Africa, a setting with high HIV prevalence, in the prevaccine era from 2003 to 2008. Antimicrob Agents Chemother. 2012; 56:5088-95.
7. Fenoll A, Granizo JJ, Aguilar L, Giménez MJ, Aragoneses-Fenoll L, Hanquet G et al. Temporal trends of invasive Streptococcus pneumoniae serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007. J Clin Microbiol. 2009; 47:1012-20.
8. Serisier DJ. Risk of population antimicrobial resistance associated with chronic macrolide use for inflammatory airway disease.

Lancet Respir Med. 2013; 1:263-72.
9. Wun YT, Lam TP, Lam KF, Ho PL, Yung WH. The public's perspective on antibiotic resistance and abuse among Chinese in HongKong. Pharmacoepidimiol Drug Saf. 2013; 22:241-9.
10. Riedel S, Beekmann SE, Heilmann KP, Richter SS, Garcia-de-Lomas J, Ferech M et al. Antimicrobial use in Europe and antimicrobial resistance in Streptococcus pneumoniae. Eur J Clin Microbiol Infect Dis. 2007; 26:485-90.
11. Yasuda M, Ishikawa K, Uehara S. Performance standards for antimicrobial susceptibility testing. Seventeenth informational supplement. Document M100-S18. PA: Wayne, 2007.
12. Hamasuna R, Yasuda M, Ishikawa K, Uehara S, Takahashi S, Hayami H, et al. Performance standards for antimicrobial susceptibility testing. Eighteenth informational supplement. Document M100-S18. PA: Wayne, 2008.
13. Kim SH, Song JH, Chung DR, Thamlikitkul V, Yang Y, Wang H, et al. Changing trends in antimicrobial resistance and serotypes of Streptococcus pneumoniae isolates in Asian Countries: an Asian network for surveillance of resistant pathogens (ANSORP) Study. Antimicrob Agents Chemother.2012; 56: 1418-26.
14. Li CF, Liu MF, Shi ZY, Hsueh PR, CH Liao. Changing trends in antimicrobial susceptibility of Streptococcus pneumoniae isolates in Taiwan, 2006-2007. J Microbiol Immunol Infect. 2012; 45:305-10.
15. 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; 343:1917-24.
16. Goosens MC, Catry B, Verhaegen J. Antimicrobial resistance to benzylpenicillin in invasive pneumococcal disease in Belgium, 2003–2010: the effect of altering clinical breakpoints. Epidemiol Infect. 2013; 141:490-5.
17. Imöhl M, Reinert RR, van der Linden M. New penicillin susceptibility breakpoints for Streptococcus pneumoniae and their effects on susceptibility categorisation in Germany (1992-2008). Int J Antimicrob Agents. 2009; 34:271-3.
18. Al-Sheikh YA, Gowda KL, Mohammed Ali MM, John J, Khaled HMD, Chikkabidare SP. Distribution of serotypes and antibiotic susceptibility patterns among invasive pneumococcal diseases in Saudi Arabia. Ann Lab Med.2014; 34:210-5.
19. Hanna-Wakim R, Chehab H, Mahfouz I. Epidemiologic characteristics, serotypes, and antimicrobial susceptibilities of invasive Streptococcus pneumoniae isolates in a nationwide surveillance study in Lebanon. Vaccine. 2012; 30:G11-7.
20. Al-Waili BR, Al-Thawadi S, Hajjar SA. Impact of the revised penicillin susceptibility breakpoints for Streptococcus pneumoniae on antimicrobial resistance rates of meningeal and non-meningeal pneumococcal strains. Ann Saudi Med.2013; 33:111-5.
21. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, et al. Practice guidelines for the management of bacterial meningitis. Clin Infect Dis. 2004; 39:1267-84.
22. Centres for Disease Control and Prevention. Streptococcus pneumoniae. Active Bacterial Core Surveillance Report, Emerging Infections Program Network. 2012. [Online] [Cited 2018 June 11]. Available from: URL: https://www.cdc.gov/abcs/reports-findings/survreports/spneu12.pdf.
23. Chiu CH, Su LH, Huang YC, Lai JC, Chen HL, Wu TL, et al. Increasing ceftriaxone resistance and multiple alterations of penicillin-binding proteins among penicillin-resistant Streptococcus Pneumoniae Isolates in Taiwan. Antimicrob Agents Chemother. 2007; 51:3404-6.
24. Klugman KP, Friedland IR, Bradley JS. Bactericidal activity against cephalosporin-resistant Streptococcus pneumoniae in cerebrospinal fluid of children with acute bacterial meningitis. Antimicrobial Agents Chemother.1995; 39:1988-92.
25. Kumar H, Irfan S, Farooqui J, Fasih N, Zafar A. In-vitro evaluation of penicillin and ceftriaxone resistance amongst Streptococcus pneumoniae isolates causing meningitis, a cross sectional study at the Aga Khan University Hospital clinical laboratory, Karachi, Pakistan. Infect Dis J Pak.2015; 24:842-5.
26. Jaiswal N, Singh M, Das RR, Jindal I, Agarwal A, Thumburu KK. Distribution of serotypes, vaccine coverage, and antimicrobial susceptibility pattern of Streptococcus pneumoniae in children living in SAARC countries: A systematic review. PLoS One. 2014; 9:e108617.
27. Pérez-Trallero E, Marimón JM, Alonso M. Decline and rise of the antimicrobial susceptibility of Streptococcus pneumoniae isolated from middle ear fluid in children: Influence of changes in circulating serotypes. Antimicrob Agents Chemother. 2012; 56:3989-91.
28. Siira LJ, Jalava P, Tissari M, Vaara M, Kaijalainen T, Virolainen A. Clonality behind the increase of multidrug-resistance among non-invasive pneumococci in Southern Finland. Eur J Clin Microbiol Infect Dis. 2012; 31:867-71.
29. Song JH, Dagan R, Klugman KP, Fritzell B. The relationship between pneumococcal serotypes and antibiotic resistance. Vaccine. 2012; 30:2728-37.
30. Shakoor S, Kabir F, Khowaja AR, Qureshi SM, Jehan F, Qamar F, et al. Pneumococcal serotypes and serogroups causing Invasive disease in Pakistan, 2005–2013. PloS One.2014; 9:e98796.
31. Mills RO, Twum-Danso K, Owusu-Agyei S, Donkor ES. Epidemiology of pneumococcal carriage in children under five years of age in Accra, Ghana. Infect Dis. 2015; 47:326-31.
32. Thummeepak R, Leerach N, Kunthalert D, Tangchaisuriya U, Thanwisai A, Sitthisak A. High prevalence of multi-drug resistant Streptococcus pneumoniae among healthy children in Thailand. J Infect Public Health. 2015; 8:274-81.
33. Shariff M, Choudhary J, Zahoor S, Deb M. Characterization of Streptococcus pneumoniae isolates from India with special reference to their sequence types. J Infect Dev Ctries.2013; 7:101-9.
34. Jenkins SG, Farrell DJ. Increase in pneumococcus macrolide resistance, United States. Emerg Infect Dis. 2009; 15:1260-64.