# Oro-Pharyngeal Carriage And Antimicrobial Susceptibility Of Streptococcus Pneumoniae From Healthy Children

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**Citation**

Z Rashid, I Isahak, C Jeann, M Suki, N Mohamad, D Hussain, T Sim, H Jaafar, Y Yusof, M Rahman. *Oro-Pharyngeal Carriage And Antimicrobial Susceptibility Of Streptococcus Pneumoniae From Healthy Children*. The Internet Journal of Microbiology. 2013 Volume 11 Number 1.

**Abstract**

Objectives:
Streptococcus pneumoniae causes acute otitis media, pneumonia, meningitis and bacteraemia. This study aimed to determine the prevalence of Streptococcus pneumoniae oropharyngeal carriage in healthy children and the antimicrobial susceptibility in a daycare nursery and a government-managed orphanage in Kuala Lumpur during 2010.Methods: Throat swabs were obtained from 36 children of daycare nursery (open community) and from 84 orphans from orphanage (closed community) those did not receive any pneumococcal vaccine. Children were between births to 6 years of age. Antibiotic susceptibility of isolated strains was determined using disk diffusion method and Etest® (minimum inhibitory concentration). Results: Overall prevalence of Streptococcus pneumoniae of the children was 1.7% (2 out of 120). Prevalence of the bacteria in open community was 5.6% (2 out of 36) and no positive cases were recorded in orphanage (closed community, (p=0.161). Prevalence was 15.4% (2 out of 13) in children aged below 2 years in the open community. There was no association was found to exist between Streptococcus pneumoniae carriage with age (p=0.432) and gender (p=0.418). Serotyping showed serotype 11F for one isolate, while the other was non-typable. Both isolates were susceptible to penicillin, azithromycin, ceftriaxone and vancomycin. The serotype 11F isolate was susceptible while the non-typable isolate was resistant to erythromycin.Conclusions: The results demonstrated low prevalence of Streptococcus pneumoniae in healthy children. These findings may complement other studies to explore further risk factors for colonisation, antimicrobial susceptibility and serotype distribution of Streptococcus pneumoniae to help for the planning of immunization strategies.

## INTRODUCTION

Streptococcus pneumoniae (S. pneumoniae) causes acute otitis media, pneumonia, meningitis and bacteraemia. It is one of the major causes of respiratory tract disease in children, with mortality worldwide estimated at 700,000 to 1 million annually in children aged less than 5 years.[1] The incidence of invasive pneumococcal bacteraemia in children less than 5 years in Malaysia was estimated at least 750 cases annually with 2% mortality.[2] Hospital-based studies on pneumococcal infection in Malaysia have shown pneumonia to be the most common clinical presentation, with the highest morbidity and mortality in children below two years of age.[3,4,5]

Colonisation of the upper respiratory tract is the prerequisite for the development of infection and invasive pneumococcal disease. Epidemiology studies in western countries had shown a significant correlation between invasive pneumococcal disease in children with identification of asymptomatic carrier of S. pneumoniae in the pharynx.[6,7] During pneumococcal outbreaks in day care centres, the corresponding pathogen strain was found up to 86% of the healthy children in the same centre.[6] The link between S. pneumoniae colonisation and pneumococcal disease were reviewed by Bogaert et al,[8] with the peak incidence of colonisation found during the first three years of life.

Recent years resistance of S. pneumoniae strains in this country has been increased due to frequent use of antibiotics especially penicillin[3,4,9,10,11,] and macrolides.[12,13] High prevalence of antibiotic-resistant strains was found among healthy young children.[14,15]

The aim of this study was to determine the prevalence and

antimicrobial susceptibility of S. pneumoniae isolates from the oropharynx of healthy children of day-care nursery and orphanage.

## MATERIALS AND METHODS

This cross-sectional study was conducted in the daycare nursery of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and Tengku Budriah Orphanage, Jalan Yaakob Latif, Kuala Lumpur in April 2010. This research was approved by the Research and Ethical Committee of UKMMC. Informed consent was taken from parents or guardians of each child to take part in this study. The Director of the Department of Social Welfare and staff of the orphanage were briefed on the purpose of the study and consent was sought. The subjects were selected using convenience sampling. Inclusion criteria of this study were children aged between birth to 6 years old and their parents or guardians were able to speak and understand Bahasa Malaysia or English. Children with primary or acquired immunodeficiency or on steroids or immunosuppressive drugs were excluded from this study.

Children in 'open community' were those who attended the UKMMC daycare nursery during office hours. They were exposed to family members at home, other children and staff in the nursery. The children from the UKMMC daycare nursery age ranged between birth to 4 years old, and they were cared for by their parents after working hours. Children in 'closed community' were children who lived together in the same place and had less exposure or contact hours with the outside community. Tengku Budriah Orphanage is a government-managed orphanage under the Department of Social Welfare and the Ministry of Women, Family and Community Development, Malaysia. It adopts and provides shelter to children with problematic socio-economic background. These children stay full time in the centre, while attending the kindergarten set up within the orphanage.

Five final year medical students were involved in conducting this study, and were trained by Otorhinolaryngology surgeons in this centre on the correct throat swab procedure. Throat swabs were taken from all healthy children aged between birth to 6 years old after obtaining informed consents from the parents or the authorities of Department of Social Welfare, Malaysia. Sterile swabs were used to collect the samples from their oropharynx, and with the assistance of microbiologists the specimens were inoculated immediately onto 5% sheep blood agar plates and transported in a cooler box to the laboratory.

In the laboratory, the plates were streaked and incubated in 5% $CO_2$ incubator at 37°C for 20-24 hours. The isolation and identification of S. pneumoniae from the specimens were performed in the laboratory by standard bacteriologic culture methods and sensitivity to optochin disc. Antimicrobial susceptibility was determined using disk diffusion method and minimum inhibitory concentrations (MIC), according to the Clinical Laboratory Standards Institute (CLSI) recommendations and breakpoints for interpretations.[16] Mueller-Hinton 5% sheep blood agar was used for susceptibility testing and the plates were incubated in 5% $CO_2$ incubator at 37°C for 20-24 hours. The isolates were screened for susceptibility to penicillin using 1µg oxacillin disks. Isolates with zones of inhibition of ≥20mm were considered susceptible to penicillin. Antibiotic disks tested also include erythromycin 15µg, azithromycin 15µg, chloramphenicol 30µg, trimethoprim-sulfamethoxazole 1.25/23.75µg, clindamycin 2µg and vancomycin 30µg (Oxoid and BD BBL™ Sensi-Disc™). MIC for penicillin, ceftriaxone and vancomycin were determined using Etest® method (AB bioMérieux, Solna, Sweden). The isolates were then stored at -70°C for further analyses. The isolates were subcultured and sent to the Institute of Medical Research, Kuala Lumpur for serotyping.

Data was analyzed using SPSS version 13.0. Percentages of oropharyngeal carriage of S. pneumoniae were calculated and comparison was made between variables (age, gender and community) using Chi-square test with p value <0.05 as the significant level.

## RESULTS

A total of 120 children were included, where 84 were from the orphanage and 36 were from the daycare nursery. The mean age of these children was 30.8 months, where the oldest was 74 months old and the youngest was 2 weeks old. The demographics of the children are shown in Table 1. There were 64 males and 56 females (53.3% and 46.7% respectively). The children were of different ethnicity, namely Malay (78.8%), Indian (11.0%), Chinese (3.4%), and others (6.8%).

S. pneumoniae was isolated from 2 out of 120 children. The first child was 17 months old while the second child was 24 months old. The overall carriage rate of S. pneumoniae among healthy children in this population was 1.7%. Prevalence of S. pneumoniae oropharyngeal carriage by

different variables has been shown( Table 2). Carriage of S. pneumoniae was present in 2 out of 57 (3.5%) children ≤2 years old and none present in children >2 years old. Carriage was found in 2 of the 56 girls (3.6%) and none in boys. In comparing between open and closed community, carriage was present in 2 out of the 36 children (5.6%) in open community and none in closed community. There was a higher prevalence of S. pneumoniae oropharyngeal carriage in children ≤2 years old residing in open community (15.4%) where 2 out of 13 children had positive culture. However, by using Chi-square analysis with Yates correction, there was no association between positive culture for Streptococcus pneumoniae with all the variables, namely gender, age and community (p values >0.05).

Serotyping showed serotype 11F for one isolate, while the other isolate was non-typable. Both isolates were susceptible to penicillin, and the MIC was 0.006 µg/mL (serotype 11F isolate) and 0.125 µg/mL (non-typable isolate). The serotype 11F isolate was susceptible while the non-typable isolate was resistant to erythromycin. The full antibiotic susceptibility results by disk diffusion method are shown in Table 3, and MIC results are shown in Table 4.

### Figure 1

Table 1:Demographics of the 120 children sampled for oropharyngeal carriage of

| Characteristics | Number of children (%) |
|---|---|
| **Gender** | |
| Male | 64 (53.3) |
| Female | 56 (46.7) |
| **Race** | |
| Malay | 93 (78.8) |
| Chinese | 4 (3.4) |
| Indian | 13 (11.0) |
| Others | 8 (6.8) |
| **Age** | |
| ≤2 years old | 65 (53.3) |
| >2 years old | 55 (46.7) |
| **Community** | |
| Nursery (open community) | 36 (30.0) |
| Orphanage (closed community | 84 (70.0) |

### Figure 2

Table 2: Prevalence of oropharyngeal carriage by different variables

| Variables | Number of children | *Streptococcus pneumoniae* carriage (%) | p value |
|---|---|---|---|
| **Gender** | | | 0.418 |
| Male | 64 | 0 | |
| Female | 56 | 2 (3.6) | |
| **Age** | | | 0.432 |
| ≤2 years old | 57 | 2 (3.5) | |
| >2 years old | 63 | 0 | |
| **Community** | | | 0.161 |
| Nursery (open community) | **36** | 2 (5.6) | |
| ≤2 years old | 13 | 2 (15.4) | |
| >2 years old | 23 | 0 | |
| Orphanage (closed community) | **84** | 0 | |
| ≤2 years old | 44 | 0 | |
| >2 years old | 40 | 0 | |

### Figure 3

Table 3: Antimicrobial susceptibilities of by disk diffusion method

| Antibiotics | Isolate 1 (serotype 11F) | Isolate 2 (non-typable) |
|---|---|---|
| Penicillin | S | S |
| Erythromycin | S | R |
| Azithromycin | S | S |
| Chloramphenicol | S | S |
| Trimethoprim-sulfamethoxazole | R | R |
| Clindamycin | S | S |
| Vancomycin | S | S |

Legends: S = Susceptible, R = Resistant

### Figure 4

Table 4: Minimum inhibitory concentrations (MIC) of antibiotics for isolates

| Antibiotics | MIC (µg/mL) | |
|---|---|---|
| | Isolate 1 (serotype 11F) | Isolate 2 (non-typable) |
| Penicillin | 0.006 | 0.125 |
| Ceftriaxone | 0.016 | 0.50 |
| Vancomycin | 0.38 | 0.50 |

## DISCUSSION

This was a community-based study in Malaysia. The results showed that the prevalence of S. pneumoniae oropharyngeal carriage was 2 out of 13 (15.4%) children in the open community aged below 2 years, and 2 out of 57 (3.5%) in all children below 2 years. A previous study involving pre-school children in Kota Bharu found 36 out of 355 nasopharyngeal samples (10%) isolated S. pneumoniae and all isolates were susceptible to penicillin except one.[17] Our prevalence rate was considered low when compared to other studies and those carried out in other countries that were conducted among the same age group and the same clinical specimens. Similar studies in Turkey with a population sizes of 1022 and 683 children showed that the prevalence rates were 23.4% and 4.2% respectively.[18,19]

The colonisation rates of S. pneumoniae reported from all over the world varies widely. Certain risk factors contribute to the higher frequency of colonisation, including overcrowding, environmental features and socioeconomic factors,[8] and higher risk in children attending daycare centres[20], suggesting horizontal spread.

The method used to obtain our oropharyngeal specimens for the isolation of S. pneumoniae was via throat swab. Precautions were taken in order to increase our isolation rate by taking the samples before the children's meal time. This was because food remnants present at the throat may affect the yield. However, the sensitivity of throat swabs could be lower than nasopharyngeal swab in detecting S. pneumoniae. A study had shown that detection of colonisation of S. pneumoniae by nasopharyngeal swab and wash were much higher, up to 3 times than that of oropharyngeal swab.[21] In a study comparing four different sampling methods for pharyngeal carriage detection, oropharyngeal swabs had the lowest sensitivity of 20% compared to nasopharyngeal swabs (30%), nasal swabs (32%) and nasopharyngeal aspirates (33%).[22] Another study also concluded that S. pneumoniae was mainly carried on the nasopharynx of healthy children compared to oropharynx.[23]

Although the results showed that there was no significant correlation between the age group of children and the prevalence of S. pneumoniae oropharyngeal carriage, numerous studies conducted in the past had reported that age is the most important factor in the rate of colonisation in healthy children. Most studies concluded that children under age of 2 are at a greater risk of being colonised by S. pneumoniae. [15,21,24,25]

The findings showed that there was a higher prevalence of carrier rate in the daycare centre or nursery compared to Tengku Budriah orphanage could mean that the children with more exposure to the community could be more susceptible to colonisation. S. pneumoniae is a community-circulating bacterium that spreads via droplet transmission. The children in the nursery were exposed to family members at home and also individuals in the nursery, both adults and children. Similar studies concluded that exposure within daycare centres and families were main risk factors for colonisation. [26,27]Whereas children up to age of 6 years from the orphanage formed part of an enclosed community, as they had yet to start attending school. However, high colonisation rate of up to 82% has been reported in infants living in an orphanage.[28]

Limitations in our study include the small sample size, a total of 120 children up to age of 6 years. The study was conducted in a short span of time with a single sampling session. A likely explanation for the low prevalence in our population compared to other studies could also be attributed to the sampling method used, with the lower sensitivity of oropharyngeal compared to nasopharyngeal specimens.

Both our isolates were sensitive to penicillin. The serotype 11F isolate was susceptible while the non-typable isolate was resistant to erythromycin, while both were susceptible to azithromycin. The rate of penicillin-nonsusceptible S. pneumoniae in this country was reported as 0.8% in 1988[9] and 10.9% in 1996-1997.[10] Desa et al reported nonsusceptibility to penicillin of 31% (resistant 20%, intermediate 11%).[11] A retrospective review of invasive pneumococcal disease in a Malaysian university hospital from 1999-2004 reported high nonsusceptibility to penicillin (resistant 16%, intermediate 45%).[5] The Asian Network for Surveillance of Resistant Pathogens (ANSORP) data on patterns of resistance in Asian countries showed that S. pneumoniae serotypes in Malaysia have increasing resistance to penicillin ranging from 9-39%, and resistance to erythromycin ranging from 3% (1996-1997) to 37% (1998-2001)[12,13]

Approved vaccines for pneumococcal infections contained 23 serotypes (polysaccharide vaccine, PPV23), 7 and 10 serotypes (pneumococcal conjugate vaccines, PCV7 and PCV10). A recently approved 13-valent pneumococcal vaccine (PCV13) now succeeds PCV7 which has been used

in routine childhood immunization schedule in several countries, such as the United States since 2000.[29] PCV13 contains six other serotypes (1, 3, 5, 6A, 7F and 19A) in addition to the seven serotypes in PCV7 (4, 6B, 9V, 14, 18C, 19F and 23F).

Based on previous studies, serotype 11F has never been reported in Malaysia.[3,4,9,11] Surveillance S. pneumoniae serotypes is necessary to monitor the circulating serotypes in the community and serotypes that cause invasive pneumococcal disease. This is in view of the serotype coverage of currently available pneumococcal vaccines, and issues of the changing epidemiology of circulating serotypes or serotype replacement in the era of conjugate vaccines,[14,30,31,32] both in vaccinated and unvaccinated populations.

The results demonstrated a low prevalence of S. pneumoniae in healthy children. These findings may complement other studies that may further explore risk factors for colonisation, antimicrobial susceptibility and serotype distribution of S. pneumoniae in larger populations of children and adults. The epidemiology of the isolates involved in colonisation and invasive pneumococcal disease, their resistance patterns and serotypes, as well as the burden of disease, are essential information for future preventive strategies and planning of immunization programs.

## ACKNOWLEDGEMENTS

This study was conducted with the grant from UKMMC Fundamental Research Fund, project code FF-158-2010. Authors are thankful to Director of the Department of Social Welfare, Principal of Government-managed orphanage and Principal of UKMMC Nursery. Authors are also thankful to Otorhinolaryngology surgeons, Mr Jeevanan Jahendran and Dr Mohd Iszuari Md Ismail who had trained the five medical students the technique of obtaining oropharyngeal specimens via throat swab. Authors are grateful to Dr Rohaidah Hashim, Bacteriology Unit, Institute of Medical Research, Kuala Lumpur for assistance in serotyping the isolates. Authors acknowledge the help of laboratory technologist Sukeri Kasni, postgraduate students Dr Adilahtul Bushro Zaini and Dr Hasni Mahayidin, and Infection Control Nurse Nor Rina Mahawar, during the study.

### References

1. World Health Organization. Pneumococcal conjugate vaccine for childhood immunization. WHO position paper. Wkly Epidemiol Rec 2007; 82:93-104.
2. Ministry of Health, Malaysia. Health Technology Assessment Report on Childhood Immunisation. Health Technology Assessment Unit, Medical Development Division. MOH/P/PAK/47.02 (TR) 2001. Available at http://www.moh.gov.my/health_assesments/13 (accessed 24 January 2011).
3. Jamal F, Pit S, Isahak I, Abdullah N, Zainal Z, Abdullah R, Henrichsen J. Pneumococcal Infection in Hospitalized Patients: A Four-Year Study in Malaysia. Southeast Asian J Trop Med Public Health 1987;18(1): 79-84.
4. Rohani MY, Raudzah A, Ng AJ, Ng PP, Zaidatul AA, Asmah I, Murtaza M, Parasakthy N, Mohd Yasmin MY, Cheong YM. Epidemiology of Streptococcus pneumoniae infection in Malaysia. Epidemiol Infect 1999; 122: 77-82.
5. Lim LH, Lee WS, Parasakthi N. Childhood invasive pneumococcal disease: a hospital-based study from Malaysia. J Paediatr Child Health 2007; 43: 366–9.
6. Berkovitch M, Bulkowstein M, Zhovtis D, Greenberg R, Nitzan Y, Barzilay B, Boldur I. Colonization rate of bacteria in the throat of healthy infants. Int J Pediatr Otorhinolaryngol 2002; 63: 19-24.
7. Lucarevschi BR, Baldacci ER, Bricks LF, Bertoli CJ, Teixeira LM, Mendes CMF, Oplustil C. Oropharyngeal carriage of Streptococcus pneumoniae by children attending day care centers in Taubate, SP: Correlation between serotypes and the conjugated heptavalent pneumococcal vaccine. J Pediatr (Rio J) 2003; 79(3): 215-220.
8. Bogaert D, De Groot R, Hermans PWM. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis 2004; 4 (3): 144-54.
9. Cheong YM, Jegathesan M, Henrichsen J, Wong YH, Ng AJ, Louis A. Antibiotic susceptibility and serotype distribution of Streptococcus pneumoniae in Malaysian children. J Trop Pediatr 1988; 34:182–5.
10. Rohani MY, Parasakthi N, Raudzah A, Yasim MY. In-vitro susceptibilities of Streptococcus pneumoniae strains isolated in Malaysia to six antibiotics. J Antimicrob Chemother 1999; 44 (6): 852-3.
11. Desa MNM, Lin TK, Yasin RM, Parasakthi N. Penicillin susceptibility and molecular characteristics of clinical isolates of Streptococcus pneumoniae at the University of Malaya Medical Center, Kuala Lumpur, Malaysia. Int J Infect Dis 2003; 7 (3): 190-7.
12. Song J-H, Jung S-I, Ko KS, et al. High prevalence of antimicrobial resistance among clinical Streptococcus pneumoniae isolates in Asia (an ANSORP study). Antimicrob Agents Chemother 2004; 48(6):2101–7.
13. Song J-H, Chang H-H, Suh JY, Ko KS, Jung S-I, Oh WS, et al. Macrolide resistance and genotypic characterization of Streptococcus pneumoniae in Asian countries: a study of the Asian Network for Surveillance of Resistant Pathogens (ANSORP). J Antimicrob Chemother 2004; 53(3):457–63.
14. Marchisio P, Esposito S, Schito GC, Marchese A, Cavagna R, Principi N, Hercules Project Collaborative Group. Nasopharyngeal carriage of Streptococcus pneumoniae in healthy children: Implications for the use of heptavalent pneumococcal conjugate vaccine. Emerg Infect Dis 2002; 8(5): 479-484.
15. Chiu SS, Ho PL, Chow FK, Yuen KY, Lau YL. Nasopharyngeal Carriage of Antimicrobial- Resistant Streptococcus pneumoniae among Young Children Attending 79 Kindergarten and Day Care Centers in Hong Kong. Antimicrob Agents Chemother 2001; 2765-2770.
16. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twentienth Informational Supplement. CLSI Document M100-S20. Wayne PA: Clinical Laboratory Standards Institute; 2010.

17. Malik AS, Ismail A, Pennie RA, Naidu JV. Susceptibility pattern of Streptococcus pneumoniae among pre-school children in Kota Bharu, Malaysia. J Trop Pediatr 1998; 44 (1): 10-4.
18. Gazi H, Kurutepe S, la Surueuoglu S, Teker A, Ozbakkaloglu B. Antimicrobial susceptibility of bacterial pathogens in the oropharynx of healthy school children in Turkey. Indian J Med Res 2003; 120: 489-494.
19. Oguzkaya Artan M, Baykan Z, Artan C. 2008. Antimicrobial susceptibility of Streptococcus pneumoniae in the oropharynx of healthy preschool children and identification of risk factors. Jpn J Infect Dis 61: 302-303.
20. Bogaert D, Engelen MN, Timmers-Reker AJ, et al. Pneumococcal carriage in children in the Netherlands: a molecular epidemiological study. J Clin Microbiol 2001; 39: 3316-20.
21. Lieberman D, Shleyfer E, Castel H, et al. Nasopharyngeal Versus Oropharyngeal Sampling for Isolation of potential Respiratory Pathogens in Adults. J Clin Microbiol 2005; 525-528.
22. Rapola S, Salo E, Kiiski P, et al. Comparison of four different sampling methods for detecting pharyngeal carriage of Streptococcus pneumoniae and Haemophilus influenzae in children. J Clin Microbiol 1997; 35: 1077-79.
23. Greenberg D, Broides A, Blancovich I, Peled N, Givon-Lavi N, Dagan R. Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of Streptococcus pneumoniae and Haemophilus influenzae from healthy and sick individuals varies with age. J Clin Microbiol 2004; 42(10): 4604-4609.
24. Monteros LEE, Rojas VJ, Ituarte FA, Cruz MC, Lopez AR, Suarez RR, Morales PK, Conyer RT, Barreto DG. Streptococcus pneumoniae isolates in healthy children attending day-care centers in 12 states in Mexico. Salud Publica de Mexico 2007; 49(4): 249-255.
25. Bogaert D, Sluijter M, Toom NL, et al. Dynamics of pneumococcal colonization in healthy Dutch children. Microbiology 2005; 152:377-85.
26. Leino T, Auranen K, Jokinen J, Terronen P, Takala AK. Pneumococcal carriage in children during their first two years: important role of family exposure. Pediatr Infect Dis 2001; 20(11): 1022-7.
27. Hoti F, Erasto P, Leino T, Auranen K. Outbreaks of Streptococcus pneumoniae carriage in day care cohorts in Finland-implications for elimination of transmission. BMC Infect Dis 2009; 9:102: 1-13.
28. Raymond J, Le Thomas I, Moulin F, Commeau A, Gendrel D, Berche P. Sequential colonisation by Streptococcus pneumoniae of healthy children living in an orphanage. J Infect Dis 2000; 181: 1983-88.
29. CDC. Licensure of a 13-valent pneumococcal conjugate vaccine(PCV13) and recommendations for use among children- Advisory Committee on Immunization Practices (ACIP), 2010. MMWR 2010; 59: 258-61.
30. Dagan R. Serotype replacement in perspective. Vaccine 2009; 27 Suppl 3: C22-4.
31. Rodrigues F, Nunes S, Sá-Leão R, Gonçalves G, Lemos L, de Lencastre H. Streptococcus pneumoniae nasopharyngeal carriage in children attending day-care centers in the central region of Portugal, in the era of 7-valent pneumococcal conjugate vaccine. Microb Drug Resist 2009; 15 (4): 269-77.
32. Vasoo S, Singh K, Chow C, Lin RTP, Hsu LY, Tambyah PA. Pneumococcal carriage and resistance in children attending day care centers in Singapore in an early era of PCV-7 uptake. J Infect 2010; 60 (6): 507-9.

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