The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Original article

Characteristics of hospitalized children infected with macrolide-resistant *Mycoplasma pneumoniae*

Haruki Komatsu[a,b,*], *Tomoyuki Tsunoda*[b], *Ayano Inui*[b], *Tsuyoshi Sogo*[b], *Tomoo Fujisawa*[b]

[a] *Department of Pediatrics, Toho University Sakura Medical Center, Japan*
[b] *Division of Hepatology and Gastroenterology, Department of Pediatrics, Eastern Yokohama Hospital, Japan*

ARTICLE INFO

Article history:
Received 13 August 2013
Accepted 26 September 2013
Available online 3 January 2014

Keywords:
Antibiotics
Minocycline
Mutation
Young children

ABSTRACT

Background: The aim of this study was to clarify retrospectively the characteristics of children hospitalized for respiratory tract infection caused by macrolide-resistant *Mycoplasma pneumoniae* (*M. pneumoniae*).
Methods: Children who were hospitalized for respiratory tract infection due to *M. pneumoniae* were enrolled in this study. The diagnosis of *M. pneumoniae* infection was made on the grounds of polymerase chain reaction results.
Results: Thirty-three children were hospitalized due to lower respiratory tract infection with *M. pneumoniae*. Of the 33 children, 31 (median age five years) were identified as being infected with macrolide-resistant *M. pneumoniae* (A2063G:30, A2064G:1) by sequence analysis. Of the 31 children infected with macrolide-resistant *M. pneumoniae*, 21 (68%) had received 14- or 15-membered macrolide antibiotics and four (13%) had received minocycline before hospitalization. During hospitalization, minocycline was administered to 16 (52%) of the 31 children infected with macrolide-resistant *M. pneumoniae*. Of the 20 children infected with macrolide-resistant *M. pneumoniae* under eight years of age, six (30%) were treated with minocycline during hospitalization. The difference in total febrile days between children receiving minocycline treatment before hospitalization and children not receiving minocycline treatment was three days.
Conclusions: The majority of hospitalized children with respiratory tract infection due to macrolide-resistant *M. pneumoniae* infection was of preschool age and had received 14- or 15-membered macrolide antibiotics before hospitalization. Because macrolide-resistant *M. pneumoniae* is widespread in Japan, the administration of minocycline as a second-line antibiotic in children under eight years of age cannot be withheld when clinical symptoms do not improve with macrolide antibiotics.

* *Corresponding author at*: Department of Pediatrics, Toho University Sakura Medical Center, 564-1 Shimoshizu Sakura, Chiba 285-8741, Japan.
E-mail address: haruki-komatsu@chive.ocn.ne.jp (H. Komatsu).
1413-8670/$ – see front matter © 2013 Elsevier Editora Ltda. All rights reserved.
http://dx.doi.org/10.1016/j.bjid.2013.09.004

Introduction

Mycoplasma pneumoniae (*M. pneumoniae*) is a major cause of respiratory infection in school-age children and young adults. Because *M. pneumoniae* is sensitive to macrolides (14-membered ring: erythromycin, clarithromycin; 15-membered ring: azithromycin), *M. pneumoniae* illness is usually mild, and hospitalization is infrequently required. Since 2000, however, the emerging of macrolide-resistant *M. pneumoniae* has been reported in Japan, China, United States, and European countries, and recently, adults and children have been suffering from macrolide-resistant *M. pneumoniae* infections.[1–12] Macrolide resistance rates in children with respiratory tract infection due to *M. pneumoniae* were reported to be 30.6% in Japan and 9.8% in France.[4,6–8,10] The emergence of macrolide-resistant *M. pneumoniae* changes the clinical courses of children with respiratory tract infection.

Tetracyclines and fluoroquinolones can be used for the treatment of *M. pneumoniae* infections;[13] however, these drugs are not recommended in children due to concerns about possible adverse effects. One of the adverse effects of tetracyclines is incorporation into tissues that are calcifying at the time of administration. Incorporation of tetracyclines into teeth, cartilage, and bone results in permanent discoloration varying from yellow to brown.[14–18] Because the calcification of permanent teeth is not completed until 7–8 years of age, tetracyclines are not indicated for the treatment of common infection in children under eight years of age.[19,20] In addition, fluoroquinolones have the potential to induce joint/cartilage toxicity in the pediatric population.[21–23] Therefore, macrolides have been considered as first-line drugs for the treatment of *M. pneumoniae* infections in children.

The aim of this study was to clarify retrospectively the characteristics of children hospitalized for respiratory tract infection caused by macrolide-resistant *M. pneumoniae* in a regional hospital in Japan, where macrolide-resistant *M. pneumonia* infections are endemic. In addition, we analyzed the treatment options for macrolide-resistant *M. pneumoniae* infections in young children under eight years of age.

Patients and methods

Patients

Children who were hospitalized in Eastern Yokohama Hospital between September 2010 and February 2012 for respiratory tract infection due to *M. pneumoniae* were enrolled in this study. All of them were diagnosed with pneumoniae or bronchitis on the basis of chest X-ray findings. The diagnosis of *M. pneumoniae* infection was made on the grounds of PCR results of nasopharyngeal or throat material collected with a swab. A febrile day was defined as a day in which the child's body temperature exceeded 38.0 °C at least once. Clinical information was collected from medical records at Eastern Yokohama Hospital. Informed written consent for study participation was obtained from all of the children's parents or guardians. The study protocol was approved by the ethics committee of Eastern Yokohama Hospital (2012041) and conformed to the ethical guidelines of the Declaration of Helsinki.

PCR assay

Nasopharyngeal or throat swabs collected from patients were vigorously mixed with 500 μL of PBS. Of the 500 μL, 200 μL was used for the extraction of *M. pneumoniae* DNA, which was accomplished using QIAamp DNA Blood Mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The extracted DNA was dissolved in 100 μL of elution buffer and stored at −30 °C. PCR assays were performed as described by Abele-Horn et al.[24] In brief, PCR was performed in a 50 μL reaction mixture containing 2.5 U of Taq DNA polymerase (TaKaRa EX Taq, Takara Bio, Shiga, Japan) with 0.2 μM primers and 10 μL extracted DNA. The amplification was performed for 40 cycles (denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s) with a sense primer (MP-1: 5′-GAA GCT TAT GGT ACA GGT TGG-3′) and an antisense primer (MP-2: 5′-ATT ACC ATC CTT GTT GTA AGG-3′). PCR assay was performed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). The amplified PCR product was a 144-bp DNA fragment of the *M. pneumoniae* ATPase operon gene. A product of the predicted size (272 bp) was observed after electrophoresis on a 2% agarose gel, ethidium bromide staining, and visualization under ultraviolet light.

Sequence analysis of the 23S rRNA gene

To identify the mutations of domain V of 23S rRNA, we performed nested PCR as previously described by Matsuoka et al.[5] The first-round PCR product (927 bp) was obtained using an outer sense primer (MN23SDVF; 5′-GCAGTGAAGAACGAGGGG-3′ [1758–1775]) and an outer antisense primer (MN23SDVR; 5′-GTCCTCGCTTCGGTCCTCTCG-3′ [2664–2684]). To detect the point mutation at 2063 and 2064 in domain V, we used an inner sense primer (MN23SF1937; 5′-ACTATAACGGTCCTAAGGTA-3′ [1918–1937]) and an inner antisense primer (MN23SR2128; 5′-ACCTATTCTCTACATGATAA-3′ [2108–2177]) for a second-round amplification, and a 210-bp PCR product was obtained. For the detection of point mutation at 2617 in domain, the inner sense primer (MN23SF2577; 5′-TACGTGAGTTGGGTTCAAA [2577–2595]) and antisense primer (MN23SR2664; 5′-GTCCTCGCTTCGGTCCTCTCG-3′ [2664–2684]) were used for a second round amplification, and a 108-bp PCR product was obtained. Nucleotide positions were designated on the basis of nucleotide sequences from *M. pneumonia* M129 (GenBank/EMBL accession number X68422). A product of the predicted size was observed after electrophoresis on a 2% agarose gel, ethidium bromide staining, and visualization under ultraviolet light. The DNA band was excised from the gel, and the DNA was purified using a QIAquick gel extraction and DNA purification kit (Qiagen, Hilden, Germany). All sequencing reactions were performed using the ABI Prism Big Dye Terminator Cycle Sequencing kit (Applied Biosystems).

Table 1 – Patient characteristics of hospitalized children infected with macrolide-resistant *Mycoplasma pneumonia*.

Male/female		14/17
Age (yr.)	Median (range) Mean	5 (1–15) 6.2
White blood cell counts (/μL)	Median (range) Mean	6520 (3410–15,570) 6913
Serum C-reactive protein levels (mg/dL)	Median (range) Mean	2.5 (0.3–12.6) 3.4
Number of days with fever before hospitalization	Median (range) Mean	6 (3–11) 6.1
No. of patients prescribed 14- or 15-membered macrolide antibiotics before hospitalization		21 (68%)
Days of administration of 14-membered macrolide antibiotics before hospitalization ($n = 11$)	Median (range) Mean	3 (2–5) 3.3
No. of patients prescribed minocycline before hospitalization		3 (10%)
Days of administration of minocycline before hospitalization ($n = 3$)	Median (range) Mean	3 (2–6) 3.7
Mutations in 23S rRNA gene	A2063G A2064G C2617G	30 1 0

Results

Patients infected with macrolide-resistant M. pneumoniae

Between September 2010 and February 2012, 33 children (male/female = 16/17, age range 1–15 years, median age five years) hospitalized for respiratory tract infection were diagnosed with *M. pneumoniae* infection by PCR assay. The nucleotide sequence analysis of the 23S rRNA gene of *M. pneumoniae* showed that 31 children (94%) were infected with macrolide-resistant *M. pneumoniae* and the remaining two children (6%) were infected with wild-type *M. pneumoniae*. The mutations of A2063G and A2064G were detected in 30 children and in one child, respectively. The mutation of C2617G was not detected in any of the children.

Patients' characteristics of the 31 children with macrolide-resistant *M. pneumoniae* at the first day of hospitalization are shown in Table 1. Febrile days before hospitalization ranged from three to 11, with a median of six. Of the 31 children infected with macrolide-resistant *M. pneumoniae*, 30 (97%) were treated with antibiotics as shown in Table 2. Of the 31 children infected with macrolide-resistant *M. pneumoniae*, 21 (68%) had a history of receiving erythromycin, clarithromycin, or azithromycin. The duration of administration of 14-membered macrolides ranged from two to five days, with a median of three days. Four of the children had a history of receiving minocycline before hospitalization. Of the four children administered with minocycline, three were under eight years of age (Table 2).

Treatment of macrolide-resistant M. pneumoniae infection during hospitalization

The antibiotics administered during hospitalization to the children in this study are shown in Table 3. As initial

Table 2 – Antimicrobial agents against macrolide-resistant *Mycoplasma pneumoniae* used before hospitalization.

	Under 8 years of age ($n = 20$)	8 years old or older ($n = 11$)
β-Lactam → clarithromycin	3	2
β-Lactam → azithromycin	1	2
Clarithromycin → azithromycin	2	1
Clarithromycin → minocycline	2	0
Tosufloxacin	2	0
β-Lactam	2	2
Azithromycin	2	1
Clarithromycin	1	1
Clarithromycin → azithromycin → tosufloxacin	1	0
β-Lactam → erythromycin	1	0
β-Lactam → josamycin	1	0
β-Lactam → minocycline	1	0
Norfloxacin → rokitamycine	1	0
Minocycline	0	1
No antibiotics	0	1

Table 3 – Antimicrobial agents against macrolide-resistant *Mycoplasma pneumoniae* duration hospitalization.

	Under 8 years of age ($n = 20$)	8 years old or older ($n = 11$)
Minocycline	5	10
Tosufloxacin	4	0
Azithromycin	2	1
Clarithromycin	2	0
Clindamycin hydrochloride	2	0
Azithromycin → tosufloxacin	2	0
Azithromycin → minocycline	1	0
Clindamycin hydrochloride + clarithromycin → tosufloxacin	1	0
No antibiotics	1	0

antibiotics during hospitalization, minocycline was administered to 15 children, azithromycin to six children, tosufloxacin (quinolone) to four children, clarithromycin to two children, clindamycin to two children, and clarithromycin plus clindamycin to one child. One child did not receive any antimicrobial agents during hospitalization. Of the 11 children who were aged eight or more, 10 (91%) were treated with minocycline as the initial antibiotic during hospitalization. In four of the 20 children under eight years of age, the initial antibiotics were switched to other antimicrobial agents during hospitalization. As second antibiotics during hospitalization, minocycline was administered to one child and tosufloxacin to three children. The initial antibiotics were not changed in any children who were over 8 years of age.

Evaluation of the effectiveness of minocycline

To evaluate the effectiveness of minocycline, we compared the number of febrile days among the children administered minocycline before hospitalization, the children administered minocycline during hospitalization (excepting the children to whom minocycline was administered before hospitalization), and the children who did not receive minocycline (Table 4). There was no difference in the number of days with fever before hospitalization (median from 6 to 6.5 days) among the three groups. The number of days with fever during hospitalization ranged from 0 to 1 (median 0.5 day) among children who received minocycline before hospitalization. In the children who received minocycline during hospitalization, the number of days with fever during hospitalization ranged from one to five (median two days). On the other hand, the number of days with fever during hospitalization ranged from zero to seven (median four days) in children who did not receive minocycline. Similarly, the median of total number of days with fever in children who received minocycline was 3-day lower than that in children who had not received minocycline. These findings suggest that minocycline contributed to a 3-day-earlier resolution of fever in children infected with macrolide-resistant *M. pneumoniae*.

Clinical manifestations in children with macrolide-resistant M. pneumoniae infection

All 31 children infected with macrolide-resistant *M. pneumoniae* had cough and dyspnea. The pulsed oxygen saturation ranged from 86% to 98% (median 93%). Of the 31 children, 21 (65%) required oxygen supplementation due to hypoxia during hospitalization. None of them required mechanical ventilation. All of them were cured without consequences.

Discussion

Of the 33 hospitalized children with respiratory tract infection caused by *M. pneumonia*, 31 (94%) were infected with

Table 4 – Days with fever of patients infected with macrolide-resistant *Mycoplasma pneumonia*.

		Minocycline administered before hospitalization ($n = 4$)	Minocycline administered during hospitalization ($n = 12$)	Minocycline not administered ($n = 15$)
Age (yr.)	Median (range)	4.5 (4–11)	8.5 (3–15)	4 (1–11)
	Mean	6.0	8.6	4.3
No. of patients prescribed 14- or 15-membered macrolide antibiotics before hospitalization		2 (50%)	9 (75%)	9 (60%)
Days with fever before hospitalization	Median (range)	6.5 (5–8)	6 (5–9)	6 (3–11)
	Mean	6.5	6.1	6.1
Days with fever during hospitalization	Median (range)	0.5 (0–1)	2 (1–5)	4 (0–7)
	Mean	0.5	2.6	4.3
Total days with fever	Median (range)	7 (6–8)	8.5 (6–11)	10 (6–16)
	Mean	7	8.5	10.3

macrolide-resistant *M. pneumonia*. Usually, the peak incidence of *M. pneumoniae* occurs in school-aged children and young adults. In this study, however, the median age of the 31 children infected with macrolide-resistant *M. pneumoniae* was five years of age. Of these 31 children, 21 (68%) had already been treated with 14- or 15-membered macrolide antibiotics before admission. In addition, fever had persisted for six days (median) before admission in the 31 children infected with macrolide-resistant *M. pneumoniae*. These findings suggest that the majority of hospitalized children suffering from *M. pneumoniae* infection in Japan are infected with macrolide-resistant strains and pre-school children are likely to show persistent clinical symptoms such as high fever and cough even if macrolide antibiotics are administered in case of macrolide-resistant *M. pneumoniae*.

The total number of days with fever in children infected with macrolide-resistant *M. pneumoniae* in this study was comparable with that reported in a previous study, which showed a median of 10 days.[25] The authors of the previous study reported a median of 3.5 days with fever during 14-membered ring macrolides administration in patients with macrolide-resistant *M. pneumoniae* infection, indicating that four days of administration of macrolides is sufficient to control macrolide-resistant *M. pneumoniae* infection. However, the present study showed that children infected with macrolide-resistant *M. pneumoniae* had already received 14-membered macrolide antibiotics for three days (median) before hospitalization and high fever persisted during hospitalization even with continuing use of macrolides. These findings suggest that it takes four days or more for the treatment with macrolide antibiotics to control macrolide-resistant *M. pneumoniae* infection.

There are currently two choices for pediatricians to treat hospitalized children infected with macrolide-resistant *M. pneumoniae*. One is to continue to use macrolide antibiotics and waiting for the resolution of clinical symptoms, because fatal outcome of *M. pneumoniae* infection is rare. The other choice is to discontinue macrolide antibiotics and try to use tetracyclines or fluoroquinolones. In this study,[23] 31 (74%) children infected with macrolide-resistant *M. pneumoniae* received minocycline or tosufloxacin during hospitalization. These findings indicate that the majority of pediatricians prefer to discontinue macrolide antibiotics during hospitalization. Minocycline has been reported to be effective in macrolide-resistant *M. pneumoniae* infection.[4,9,11,26] The present study showed that the difference in the number of days with fever during hospitalization between children receiving pre-hospitalization minocycline treatment and children not receiving minocycline treatment was of three days. Although this was a retrospective study and not a controlled study, these findings indicate that the early treatment with minocycline was effective against macrolide-resistant *M. pneumoniae* infection in children. In the case of the young child with respiratory failure described above, the administration of minocycline was required to improve clinical symptoms.

Of the 16 children treated with minocycline before or during hospitalization, two (four years old, 11 years old) received minocycline as first-line antimicrobial agent, while the remaining children received minocycline as second-line microbial agent through their clinical courses. Of the 11 children who were aged eight years or older, 10 (91%) were treated with minocycline during hospitalization. Although minocycline is contraindicated or not indicated in children under eight years of age, six (38%) of the 16 children treated with minocycline were under eight years in this study. The prevalence of tetracycline and minocycline tooth staining was reported to be approximately 3–6%.[19] Moreover, there are some reports of minocycline-derivative tooth staining in adults.[14,15,27,28] However, the association between dose and tooth staining is controversial.[19] In contrast, a previous study reported that there was no significant difference in dental staining and defects between children under eight years of age treated with oral minocycline for brucellosis and matched controls.[29] Normally, minocycline should not be administered as a first-line antimicrobial agent against *M. pneumoniae* infection in young children. When minocycline is administered in children under eight years of age after considering the benefits and risks, it is essential to inform parents that minocycline has the potential to stain tooth.

Seven children treated with non-minocycline received tosufloxacin, which is a quinolone antibiotic. In 2010, tosufloxacin was approved for children by the Japanese Health and Labor Ministry. The antimicrobial mechanism of quinolone is different from that of macrolides,[1,6,30–32] and tosufloxacin is considered by some clinicians to be effective against macrolide-resistant *M. pneumoniae* infection. However, further studies are required to determine the effectiveness of tosufloxacin in macrolide-resistant *M. pneumoniae* infection.

In conclusion, preschool children with lower respiratory tract diseases due to macrolide-resistant *M. pneumoniae* infection tend to be hospitalized. The majority of the children with macrolide-resistant *M. pneumoniae* infection in our study had received 14- or 15-membered macrolide antibiotics before hospitalization. The treatment with minocycline contributed to the early resolution of clinical symptoms. Although minocycline should not be used as first-line antimicrobial agent in children under eight years of age, minocycline could be allowed to be administered if the respiratory condition worsens during the administration of macrolide antibiotics.

Ethical approval

The study protocol was approved by the ethics committee of Eastern Yokohama Hospital (2012041).

Authors' contributions

HK contributed to the design of this study and drafted this manuscript. TT, AI, TS and TF participated in data collection and critical revision of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Isozumi R, Yoshimine H, Morozumi M, Ubukata K, Ariyoshi K. Adult community-acquired pneumonia caused by macrolide resistant *Mycoplasma pneumoniae*. Respirology. 2009;14:1206–8.
2. Li X, Atkinson TP, Hagood J, Makris C, Duffy LB, Waites KB. Emerging macrolide resistance in *Mycoplasma pneumoniae* in children: detection and characterization of resistant isolates. Pediatr Infect Dis J. 2009;28:693–6.
3. Wolff BJ, Thacker WL, Schwartz SB, Winchell JM. Detection of macrolide resistance in *Mycoplasma pneumoniae* by real-time PCR and high-resolution melt analysis. Antimicrob Agents Chemother. 2008;52:3542–9.
4. Morozumi M, Iwata S, Hasegawa K, et al. Increased macrolide resistance of *Mycoplasma pneumoniae* in pediatric patients with community-acquired pneumonia. Antimicrob Agents Chemother. 2008;52:348–50.
5. Matsuoka M, Narita M, Okazaki N, et al. Characterization and molecular analysis of macrolide-resistant *Mycoplasma pneumoniae* clinical isolates obtained in Japan. Antimicrob Agents Chemother. 2004;48:4624–30.
6. Cao B, Zhao CJ, Yin YD, et al. High prevalence of macrolide resistance in *Mycoplasma pneumoniae* isolates from adult and adolescent patients with respiratory tract infection in China. Clin Infect Dis. 2010;51:189–94.
7. Dumke R, von Baum H, Luck PC, Jacobs E. Occurrence of macrolide-resistant *Mycoplasma pneumoniae* strains in Germany. Clin Microbiol Infect. 2010;16:613–6.
8. Chironna M, Sallustio A, Esposito S, et al. Emergence of macrolide-resistant strains during an outbreak of *Mycoplasma pneumoniae* infections in children. J Antimicrob Chemother. 2011;66:734–7.
9. Morozumi M, Hasegawa K, Kobayashi R, et al. Emergence of macrolide-resistant *Mycoplasma pneumoniae* with a 23S rRNA gene mutation. Antimicrob Agents Chemother. 2005;49:2302–6.
10. Peuchant O, Menard A, Renaudin H, et al. Increased macrolide resistance of *Mycoplasma pneumoniae* in France directly detected in clinical specimens by real-time PCR and melting curve analysis. J Antimicrob Chemother. 2009;64:52–8.
11. Matsubara K, Morozumi M, Okada T, et al. A comparative clinical study of macrolide-sensitive and macrolide-resistant *Mycoplasma pneumoniae* infections in pediatric patients. J Infect Chemother. 2009;15:380–3.
12. Miyashita N, Maruyama T, Kobayashi T, et al. Community-acquired macrolide-resistant *Mycoplasma pneumoniae* in patients more than 18 years of age. J Infect Chemother. 2013;17:114–8.
13. Okada T, Morozumi M, Tajima T, et al. Rapid effectiveness of minocycline or doxycycline against macrolide-resistant *Mycoplasma pneumoniae* infection in a 2011 outbreak among Japanese children. Clin Infect Dis. 2012;55:1642–9.
14. Berger RS, Mandel EB, Hayes TJ, Grimwood RR. Minocycline staining of the oral cavity. J Am Acad Dermatol. 1989;21:1300–1.
15. Chiappinelli JA, Walton RE. Tooth discoloration resulting from long-term tetracycline therapy: a case report. Quintessence Int. 1992;23:539–41.
16. Fleming P, Witkop Jr CJ, Kuhlmann WH. Staining and hypoplasia of enamel caused by tetracycline: case report. Pediatr Dent. 1987;9:245–6.
17. Grossman ER. Tetracycline and staining of the teeth. JAMA. 1986;255:2442–3.
18. Primosch RE. Tetracycline discoloration, enamel defects, and dental caries in patients with cystic fibrosis. Oral Surg Oral Med Oral Pathol. 1980;50:301–8.
19. Sanchez AR, Rogers 3rd RS, Sheridan PJ. Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. Int J Dermatol. 2004;43:709–15.
20. Ellison MJ. Vancomycin, metronidazole, and tetracyclines. Clin Podiatr Med Surg. 1992;9:425–42.
21. Grady R. Safety profile of quinolone antibiotics in the pediatric population. Pediatr Infect Dis J. 2003;22:1128–32.
22. Grady RW. Systemic quinolone antibiotics in children: a review of the use and safety. Expert Opin Drug Saf. 2005;4:623–30.
23. Sendzik J, Lode H, Stahlmann R. Quinolone-induced arthropathy: an update focusing on new mechanistic and clinical data. Int J Antimicrob Agents. 2009;33:194–200.
24. Abele-Horn M, Busch U, Nitschko H, et al. Molecular approaches to diagnosis of pulmonary diseases due to *Mycoplasma pneumoniae*. J Clin Microbiol. 1998;36:548–51.
25. Suzuki N, Yuyama M, Maeda S, Ogawa H, Mashiko K, Kiyoura Y. Genotypic identification of presumptive *Streptococcus pneumoniae* by PCR using four genes highly specific for *S. pneumoniae*. J Med Microbiol. 2006;55:709–14.
26. Kawai Y, Miyashita N, Yamaguchi T, et al. Clinical efficacy of macrolide antibiotics against genetically determined macrolide-resistant *Mycoplasma pneumoniae* pneumoniae in paediatric patients. Respirology. 2012;17:354–62.
27. McKenna BE, Lamey PJ, Kennedy JG, Bateson J. Minocycline-induced staining of the adult permanent dentition: a review of the literature and report of a case. Dent Update. 1999;26:160–2.
28. Westbury LW, Najera A. Minocycline-induced intraoral pharmacogenic pigmentation: case reports and review of the literature. J Periodontol. 1997;68:84–91.
29. Cascio A, Di Liberto C, D'Angelo M, et al. No findings of dental defects in children treated with minocycline. Antimicrob Agents Chemother. 2004;48:2739–41.
30. Hasegawa M, Sato Y, Kanayama A, et al. Antibacterial activity of tosufloxacin against major organisms detected from patients with the results obtained from organisms isolated about 10 years ago. J Infect Chemother. 2006;12:152–6.
31. Ikejima H, Yamamoto H, Ishida K, Kaku M, Shimada J. Evaluation of in-vitro activity of new quinolones, macrolides, and minocycline against *Mycoplasma pneumoniae*. J Infect Chemother. 2000;6:148–50.
32. Morozumi M, Takahashi T, Ubukata K. Macrolide-resistant *Mycoplasma pneumoniae*: characteristics of isolates and clinical aspects of community-acquired pneumonia. J Infect Chemother. 2010;16:78–86.