Therapeutic efficacy of azithromycin, clarithromycin, minocycline and tosufloxacin against macrolide-resistant and macrolide-sensitive *Mycoplasma pneumoniae* pneumonia in pediatric patients

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¶ Membership of Hokkaido Pediatric Respiratory Infection Study Group is provided in the Acknowledgments.

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Abstract

**Objective**

To clarify therapeutic effects of azithromycin, clarithromycin, minocycline and tosufloxacin against macrolide-resistant *Mycoplasma pneumoniae* (MRMP) pneumonia and against macrolide-sensitive *Mycoplasma pneumoniae* (MSMP) pneumonia in pediatric patients.

**Methods**

A prospective, multicenter observational study was conducted from July 2013 to August 2015. The therapeutic effects of azithromycin, clarithromycin, minocycline and tosufloxacin were evaluated in 59 patients with pneumonia caused by MRMP and in 50 patients with
emerging Infectious Diseases, Labour and Welfare Programs from the Ministry of Health, Labour and Welfare of Japan. Pfizer Inc. provided grants for this study (A Prospective Observational Study of Antibiotic Treatment against Macrolide-Resistant Mycoplasma Pneumoniae Infections in Pediatric Patients, WS2419287) but was not involved in the design of the study or in enrollment of patients, data collection, analysis and interpretation, or preparation of the manuscript.

Competing interests: This research was funded in part by a Grant-in-Aid for Scientific Research (C), 2013 (25461577), from the Ministry of Education, Science, Sports and Culture of Japan and by a Health Science Research Grant (H24-Shinkou-Ippan-014) for Research on Emerging and Re-emerging Infectious Diseases, Labour and Welfare Programs from the Ministry of Health, Labour and Welfare of Japan. Pfizer Inc. provided grants for this study (A Prospective Observational Study of Antibiotic Treatment against Macrolide-Resistant Mycoplasma Pneumoniae Infections in Pediatric Patients, WS2419287) but was not involved in the design of the study or in enrollment of patients, data collection, analysis and interpretation, or preparation of the manuscript. We have declared that no competing interests exist along with any other relevant declarations relating to employment, consultancy, patents, products in development, marketed products, etc. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

pneumonia caused by MSMP. *In vitro* activities of antimicrobial agents against isolates of *Mycoplasma pneumoniae* were also measured.

**Results**

Mean durations of fever following commencement of treatment in patients infected with MRMP and MSMP were 5.2 and 1.9 days, respectively (log-rank test, *P* < 0.0001). Among patients infected with MRMP, mean durations of fever were 4.6, 5.5, 1.0 and 7.5 days for patients treated with azithromycin, clarithromycin, minocycline and tosufloxacin, respectively (log-rank test, *P* < 0.0001). Among patients infected with MSMP, mean durations of fever were 2.5, 1.7, 0.9 and 4.3 days for patients treated with azithromycin, clarithromycin, minocycline and tosufloxacin, respectively (log-rank test, *P* = 0.0162). The MIC90s of azithromycin and clarithromycin among the 27 isolates of MRMP were 64 and 256 μg/ml, respectively, and those among the 23 isolates of MSMP were <0.00125 and 0.001 μg/ml, respectively. The MIC90s of minocycline and tosufloxacin among the 27 isolates of MRMP were 1.0 and 0.25 μg/ml, respectively, and those among the 23 isolates of MSMP were 1.0 and 0.5 μg/ml, respectively.

**Conclusion**

Both minocycline and tosufloxacin showed good *in vitro* activities against MRMP. Minocycline, but not tosufloxacin, shortened the duration of fever in pediatric patients infected with MRMP compared to the duration of fever in patients treated with macrolides.

**Introduction**

*Mycoplasma pneumoniae* is one of the common causative pathogens of community-acquired respiratory tract infections mainly in children and young adults [1]. Macrolides are generally considered to be the drugs of choice for treatment of children with *M. pneumoniae* infection [2]. Since about 2000, macrolide-resistant *M. pneumoniae* (MRMP) has been emerging in Asia, Europe, Canada and the USA [3–6]. The rates of MRMP infection range from 3% to 26% in Europe [7, 8], 63% to 97% in China [9–12] and 25% to 93% in Japan [13–18]. Macrolides are less effective against MRMP infection than against macrolide-sensitive *M. pneumoniae* (MSMP) [14, 19, 20]. Recently, the incidence of extra-pulmonary complications in patients with MRMP infection was reported to be significantly higher than that in patients with MSMP infection [21].

Minocycline and fluoroquinolones were shown to be more effective than macrolides in adult patients infected with MRMP [22]. Minocycline and tosufloxacin have also been used for treatment of pediatric patients infected with MRMP [14, 23–26]. Tetracyclines including minocycline are incorporated into teeth, cartilage and bone, resulting in discoloration of both primary and permanent dentitions [27]. Therefore, tetracyclines are contraindicated in children aged less than 8 years [27]. Fluoroquinolones including tosufloxacin have a potential risk of inducing cartilage and joint toxicity in children [28]. Although the Japanese guidelines for management of respiratory infectious diseases in children recommend the use of minocycline or tosufloxacin instead of macrolides when MRMP pneumonia is suspected and when there is a lack of defervescence within 48 h after the initiation of macrolide therapy [29], the clinical effects of tosufloxacin in pediatric patients infected with MRMP have been controversial. One
of the reasons for this recommendation was the low MIC titers of minocycline and tosufloxacin against MRMP [14].

The purpose of this study was to clarify the therapeutic effects of macrolides (azithromycin and clarithromycin), minocycline and tosufloxacin against MRMP and MSMP infection in pediatric patients as well as the in vitro activities of these antibiotics against MRSP and MSMP.

Materials and methods

Ethics statement

All of the necessary ethics approval for this study was obtained from the Institutional Review Board of Hokkaido University Hospital for Clinical Research (012–0174). Written or verbal informed consent was provided by each patient. According to the ethical guidelines for clinical studies in Japan, written informed consent is not necessarily required for research not involving intervention but using human biological specimens. When written informed consent is not obtained, however, the physician must obtain oral informed consent and maintain records of methods for providing information and the content of the information. The acquisition of informed consent was confirmed upon arrival of the clinical specimens. The Institutional Review Board of Hokkaido University Hospital for Clinical Research follows this policy.

Study design

A prospective, multicenter observational study was conducted from July 2013 to August 2015 at 6 pediatric clinics and in the department of pediatrics in 9 hospitals in Asahikawa, Iwamizawa, Ebetsu, Obihiro, Kushiro, Sapporo, Chitose and Muroran cities, Hokkaido, Japan. Patients aged under 18 years of age who were afflicted with pneumonia due to *M. pneumoniae* were enrolled in this study. Diagnosis of pneumonia due to *M. pneumoniae* was made when all of the following criteria were met: (1) body temperature above 38 degrees Celsius, (2) signs and symptoms of the respiratory system (cough, dyspnea or abnormal breath sounds), (3) abnormal findings on chest X-ray (lobar or segmental consolidation, tiny centrilobular nodules and bronchovascular thickening) and (4) detection of *M. pneumoniae* DNA by real-time PCR (described below) or at least a four-fold increase in IgG antibody against *M. pneumoniae* from acute phase serum to convalescent phase serum.

The choice of antibiotics was made according to standard-of-care based on decisions by the treating physicians. The attending pediatrician subsequently chose one of the following antibiotics: azithromycin at 10 mg/kg/day for 3 days, clarithromycin at 10–15 mg/kg/day for 3–7 days, minocycline at 2–4 mg/kg/day for 2–4 days and tosufloxacin at 12mg/kg/day for 3–7 days. Minocycline was not chosen for patients aged less than 8 years because of side effects such as tooth discoloration. Laboratory data including results of a serological test for *M. pneumoniae*, radiographic findings, selection and dosage of antibiotics, and outpatient/inpatient status were recorded by the pediatricians. The age and sex of each patient and the time of onset (the first time that the patient had a fever of more than 37.5˚C) were recorded by the parents of children. The parents were also instructed to take their children’s axillary body temperatures several times and to record the peak daily body temperatures.

Real-time PCR assay

Nasopharyngeal swab samples were collected from patients and suspended in three ml of BD universal viral transport medium (Becton Dickinson, Sparks, MD, USA). DNA was extracted with a QIAamp DNA mini kit (Qiagen, Venlo, The Netherlands) from one ml of BD universal viral transport medium and was finally resuspended in 50 μl of a buffer. DNA of *M. pneumoniae*
was identified by real-time PCR with Mp181-F and Mp181-R primer pairs and an Mp181-P probe using one µl of DNA as described elsewhere [30].

Detection of macrolide-resistant point mutations in domain V of 23S rRNA gene
Mutations associated with resistance to macrolides at sites 2063, 2064, and 2617 in the M. pneumoniae 23S rRNA gene domain V region were detected by a sequencing method described elsewhere [31]. M. pneumoniae showing a point mutation in domain V of the 23S rRNA gene was defined as MRMP.

Isolation of M. pneumoniae by culture
Modified Hayflick medium was used for the isolation of M. pneumoniae from patients [32].

Antibiotic susceptibility
MICs of antibiotics were determined by a broth microdilution method based on the method of the National Committee for Clinical Laboratory Standards [31].

Sample size and power calculation
For the primary analysis, 32 patients in each antibiotics group were required for a power of 80% at a two-sided alpha of 0.05 to detect a treatment difference of 1 day with a 1.4 standard deviation regarding fever duration. Therefore, the targeted required number of patients with MRMP infection was 128. The number of patients with M. pneumoniae infection was estimated to be 50% of those enrolled. The final number of patients with M. pneumoniae infection was expected to be 256. Since this study has an observational nature, we recruited as many patients as possible from the collaborative clinics and hospitals during the period from July 2013 to August 2015.

Statistical analysis
Demographic data are expressed as means +- SD or proportions. Continuous variables were compared using Student’s t-test. Frequency analysis was performed by the chi-square test. The distributions of fever duration were depicted by the Kaplan–Meier method, and the log-rank test was used for comparisons of fever duration. To adjust for confounding, we set the duration of fever as a dependent variable and set the following factors as clinically relevant independent variables in multivariate Cox’s regression analysis: age, sex, hospital admission during the course, days from onset of fever to administration of antibiotics, antibiotic initially chosen, change in antibiotics during the course and macrolide resistance of M. pneumoniae. To be understandable intuitively, we inversed the value of the hazard ratio in this text; that is, a value less than 1.0 means that the duration of fever is shorter than that of the reference. A two-sided \( P \) value of \(<0.05\) was considered statistically significant. All statistical analyses were performed using JMP software version 12.0.1 (SAS Institute, Cary, NC, USA).

Results
Patient characteristics and macrolide resistance of M. pneumoniae
During the two-year enrollment period, recruitment was slower than expected, and a total of 109 patients who were diagnosed with pneumonia due to M. pneumoniae were enrolled in this study (S1 Table). In 42 of the 92 M. pneumoniae-positive samples, the presence of A2063G
A single-base mutation that is known to confer macrolide resistance to *M. pneumoniae*, was detected, but other mutations (A2063C, A2063T, A2064G and C2617G) were not detected. These mutations were not detected in the remaining 50 *M. pneumoniae*-positive samples. Although nasopharyngeal swab samples were not available from seventeen patients who had been shown to have pneumonia due to *M. pneumoniae* by serological tests in Kushiro City from July 2013 to January 2014, these patients were regarded as being infected with MRMP (see discussion). The 42 patients from whom MRMP was detected and the 17 patients in Kushiro City in whom *M. pneumoniae* infection was serologically demonstrated were incorporated into the MRMP group. Fifty patients from whom *M. pneumoniae* was detected but single-base mutations conferring macrolide resistance to *M. pneumoniae* were not detected were incorporated into the MSMP group.

No significant differences were found between the MRMP and MSMP patients in baseline status items: age, sex, time from onset of fever to administration of antibiotics and the antibiotic initially chosen (Table 1).

### Antibiotic susceptibility

*In vitro* anti-mycoplasma activities of eight agents against 50 isolates of *M. pneumoniae* with or without A2063G mutation in the 23S rRNA gene were measured (Table 2). The MIC90s of erythromycin, clarithromycin, azithromycin and clindamycin among the 27 isolates of MRMP were 0.002–0.0078 μg/ml, 0.0005–0.003 μg/ml, <0.000125–0.00025 μg/ml and 0.13–0.5 μg/ml, respectively. The MIC90s of levofloxacin, ciprofloxacin, tosufloxacin, minocycline and clindamycin among the 22 isolates of MSMP were 0.25–0.5 μg/ml, 0.5–2 μg/ml, 0.13–0.25 μg/ml, 0.13–1 μg/ml and 0.13–1 μg/ml, respectively.

### Table 1. Background characteristics of patients diagnosed with pneumonia due to *Mycoplasma pneumoniae*.

|                | MRMP patients | MSMP patients | P value |
|----------------|---------------|---------------|---------|
| No. of patients| 59            | 50            |         |
| Mean age (yr)  | 9.0 ± 3.2 (3–17) | 9.2 ± 3.3 (2–15) | 0.7745  |
| No. of males/females | 33/26 | 29/21 | 0.8280  |
| Mean duration (days) of fever before administration of antibiotics ± SD (range) | 3.1± 1.8 (0–6) | 3.6 ± 2.1 (0–8) | 0.2056  |
| Antibiotics initially chosen | AZM 18 (30.5%) | 11 (22.0%) | 0.2217  |
| CAM 29 (49.2%) | 26 (52.0%) |
| MINO 4 (6.8%) | 9 (18.0%) |
| TFLX 8 (13.5%) | 4 (4.0%) |

AZM, azithromycin; CAM, clarithromycin; MINO, minocycline; TFLX, tosufloxacin; MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-sensitive *Mycoplasma pneumoniae*.

https://doi.org/10.1371/journal.pone.0173635.t001

### Table 2. *In vitro* anti-mycoplasma activities against clinical isolates of *M. pneumoniae* with or without A2063G mutation in the 23S rRNA gene.

| Antimicrobial agent | MIC (μg/ml) for MRMP (n = 27) | MIC (μg/ml) for MSMP (n = 23) |
|---------------------|-------------------------------|-------------------------------|
|                     | Range | 50% | 90% | Range | 50% | 90% | Range | 50% | 90% | Range | 50% | 90% | Range | 50% | 90% |
| Erythromycin        | 128 - >256 | 256 | >256 | 0.002–0.0078 | 0.0039 | 0.0039 |
| Clarithromycin      | 64 - >256 | 256 | >256 | 0.0005–0.0039 | 0.001 | 0.001 |
| Azithromycin        | 16–128 | 32 | 64 | <0.000125–0.00025 | <0.000125 | <0.000125 |
| Clindamycin         | 16–256 | 64 | 128 | 0.13–0.5 | 0.25 | 0.5 |
| Levofloxacin        | 0.25–0.5 | 0.5 | 0.5 | 0.25–0.5 | 0.5 | 0.5 |
| Ciprofloxacin       | 0.5–1 | 1 | 1 | 0.5–2 | 1 | 1 |
| Tosufloxacin        | 0.13–0.25 | 0.25 | 0.25 | 0.13–0.5 | 0.25 | 0.5 |
| Minocycline         | 0.13–1 | 0.5 | 1 | 0.13–2 | 0.5 | 1 |

MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-sensitive *Mycoplasma pneumoniae*.

https://doi.org/10.1371/journal.pone.0173635.t002
were >256, 256, 64 and 128 μg/ml, respectively, and those among the 23 isolates of MSMP were 0.0039, 0.001, <0.000125 and 0.5 μg/ml, respectively. The MIC90s of levofloxacin, ciprofloxacin, tosufloxacin and minocycline among the 27 isolates of MRMP were 0.5, 1.0, 0.25 and 1.0 μg/ml, respectively, and those among the 23 isolates of MSMP were 0.5, 1.0, 0.5 and 1.0 μg/ml, respectively. The results for resistant gene mutation of the 50 cultural isolates were consistent with those for the original isolates.

Duration of fever following commencement of treatment

The durations of fever following commencement of treatment with azithromycin, clarithromycin, minocycline and tosufloxacin were evaluated by Kaplan-Meier estimates (Fig 1). The mean durations were 3.8, 3.7, 0.9 and 16.4 days for the azithromycin, clarithromycin, minocycline and tosufloxacin groups, respectively (log-rank test, \( P < 0.0001 \)) (Fig 1A). Among the patients with MRMP, the mean durations were 4.6, 5.5, 1.0 and 7.5 days for the azithromycin, clarithromycin, minocycline and tosufloxacin groups, respectively (log-rank test, \( P < 0.0001 \)) (Fig 1B). Among the patients with MSMP, the mean durations were 2.5, 1.7, 0.9 and 4.3 days for the azithromycin, clarithromycin, minocycline and tosufloxacin groups, respectively (log-rank test, \( P = 0.0162 \)) (Fig 1C).

The durations of fever following commencement of treatment in patients infected with MRMP and patients infected with MSMP were evaluated by Kaplan-Meier estimates (Fig 2). The mean durations were 5.2 and 1.9 days for the MRMP and MSMP groups, respectively (log-rank test, \( P < 0.0001 \)) (Fig 2A). The durations of fever were significantly different between MRMP patients and MSMP patients treated with azithromycin (4.6 and 2.5 days, respectively, \( P = 0.0175 \)) (Fig 2B) and between MRMP patients and MSMP patients treated with clarithromycin (5.4 and 1.7 days, respectively, \( P < 0.0001 \)) (Fig 2C). No statistically significant difference in the duration of fever was found between MRMP patients and MSMP patients treated with minocycline (1.0 and 0.9 days, respectively, \( P = 0.7496 \)) (Fig 2D) or between MRMP patients and MSMP patients treated with tosufloxacin (7.5 and 4.3 days, respectively, \( P = 0.3166 \)) (Fig 2E).

Fever subsided within two days following commencement of treatment in 9 (15%) of the 59 patients infected with MRMP and in 39 (78%) of the 50 patients infected with MSMP (Fig 3).

The Cox proportional hazards model showed that the duration of fever following commencement of treatment correlated with hospital admission during the course (hazard
ratio = 0.48, 95% confidence interval of 0.29 to 0.78, \( P = 0.0031 \)) and with macrolide resistance of \textit{M. pneumoniae} (hazard ratio = 0.41, 95% confidence interval of 0.26 to 0.64, \( P < 0.0001 \)) (Table 3). There was a statistically significant association between duration of fever following commencement of treatment and the antibiotic initially chosen. Patients who were treated with minocycline had a shorter duration of fever than did patients treated with azithromycin (hazard ratio = 0.35, 95% confidence interval of 0.15 to 0.85, \( P = 0.0215 \)), clarithromycin (hazard ratio = 0.26, 95% confidence interval of 0.11 to 0.61, \( P = 0.0024 \)) or tosufloxacin (hazard ratio = 0.16, 95% confidence interval of 0.06 to 0.43 \( P = 0.0004 \)). Patients treated with azithromycin had a shorter duration of fever than did patients treated with tosufloxacin (hazard ratio = 0.45, 95% confidence interval of 0.20 to 0.91, \( P = 0.0256 \)). There was no statistically significant association between duration of fever following commencement of treatment and age (\( P = 0.9896 \)), sex (\( P = 0.2810 \)), days from fever onset to commencement of treatment (\( P = 0.0621 \)) or change in antibiotics during the course (\( P = 0.2273 \)).

The durations of fever following commencement of treatment in the 59 patients infected with MRMP were sub-analyzed using the Cox proportional hazards model (Table 3). The duration of fever following commencement of treatment correlated with hospital admission during the course (hazard ratio = 0.50, 95% confidence interval of 0.25 to 0.98, \( P = 0.0404 \)) and with the antibiotic initially chosen. Patients treated with minocycline had a shorter duration of fever than did patients treated with azithromycin (hazard ratio = 0.03, 95% confidence interval of 0.00 to 0.20, \( P = 0.0003 \)), clarithromycin (hazard ratio = 0.02, 95% confidence interval of
0.00 to 0.13, \( P < 0.0001 \)) or tosufloxacin (hazard ratio = 0.01, 95% confidence interval of 0.00 to 0.11, \( P < 0.0001 \)). The durations of fever following commencement of treatment in the 50 patients infected with MSMP were also sub-analyzed using the Cox proportional hazards model (Table 3). The duration of fever following commencement of treatment correlated with hospital admission during the course (hazard ratio = 0.39, 95% confidence interval of 0.17 to 0.87, \( P = 0.0240 \)) and with days from fever onset to administration (hazard ratio = 0.79, 95% confidence interval of 0.65 to 0.97, \( P = 0.0256 \)). There was no statistically significant association between duration of fever following commencement of treatment and age (\( P = 0.9496 \)), sex (\( P = 0.1075 \)), or the antibiotic initially chosen.

**Discussion**

In the present study, the therapeutic effects of azithromycin, clarithromycin, minocycline and tosufloxacin against MRMP and MSMP infection in pediatric patients were investigated. In patients treated with macrolides (azithromycin and clarithromycin), the duration of fever following commencement of treatment for MRMP was longer than that following commencement of treatment for MSMP (Figs 2 and 3). These results were consistent with the results of the antibiotic susceptibility tests (Table 2). Similar findings have been reported [14, 19, 20, 24].

In patients infected with MRMP, the duration of fever following commencement of treatment with minocycline was significantly shorter than the duration of fever following commencement of treatment with macrolides (azithromycin and clarithromycin) and tosufloxacin (Fig 1, Table 3). Minocycline is well known to be effective for treatment of MRMP infection in
pediatric patients aged more than 8 years [14, 23, 26]. The clinical effects of tosufloxacin in patients infected with MRMP have been controversial. Kawai et al. reported that the 48-h defervescence rate after initiation of treatment with tosufloxacin (43 of 62 patients, 69%) was significantly higher than that after initiation of treatment with azithromycin (11 of 27 patients, 41%) [14]. Okada et al. reported that 24-h defervescence rates after initiation of treatment with tosufloxacin and macrolides were 31% (4 of 13 patients) and 31% (4 of 13 patients), respectively [26]. In our study, the duration of fever following commencement of treatment with tosufloxacin in patients infected with MRMP was not significantly different from the duration of fever following commencement of treatment with macrolides (azithromycin and clarithromycin) (Fig 1, Table 3). Additionally, the duration of fever following commencement of treatment with tosufloxacin in patients infected with MRMP was almost same as that for patients infected with MSMP (Fig 2). In this study, we could not demonstrate a therapeutic advantage of tosufloxacin for treatment of MRMP.

Both minocycline (MIC90, 1.0 μg/ml) and tosufloxacin (MIC90, 0.25 μg/ml) had higher antibacterial efficacies than those of azithromycin (MIC90, 64 μg/ml) and clarithromycin (MIC90, 256 μg/ml) against isolates of MRMP (Table 2). Therefore, antibacterial efficacy alone could not explain the differences in clinical effects of minocycline and tosufloxacin in patients infected with MRMP. Minocycline has a relatively high blood concentration (2.3 μg/ml after oral administration of 4 mg/kg) and a long half-time (10 hours) [33]. Minocycline penetrates efficiently into lung tissues and bronchial mucus; mean tissue or mucus concentration to plasma concentration ratios were 3.78 +/- 1.10 for lung parenchyma, 4.04 +/- 1.31 for bronchial walls and 1.99 +/- 1.80 for intraluminal mucus collected from bronchi.

### Table 3. Results of analysis using the Cox proportional hazards model to determine factors influencing duration of fever following commencement of treatment.

| Independent factors | Unit/Control | Patients infected with either with MRMP or MSMP (n = 109) | Patients infected with MRMP (n = 59) | Patients infected with MSMP (n = 50) |
|---------------------|-------------|--------------------------------------------------------|-------------------------------------|-------------------------------------|
|                     |             | Hazard ratio (95% Confidence interval) P                | Hazard ratio (95% Confidence interval) P | Hazard ratio (95% Confidence interval) P |
| Age                 | Per one year | 1.00 (0.93–1.07) 0.9896                               | 1.00 (0.91–1.10) 0.9467             | 1.00 (0.90–1.11) 0.9496             |
| Sex                 | Female      | 1.25 (0.83–1.89) 0.2810                               | 1.14 (0.62–2.08) 0.6772             | 1.69 (0.88–3.25) 0.1075             |
| Admitted patients or outpatients | Admitted patients | 0.48 (0.29–0.78) 0.0031 | 0.50 (0.25–0.98) 0.0404 | 0.39 (0.17–0.87) 0.0240 |
| Days from fever onset to administration | Per one day | 0.89 (0.79–1.01) 0.0621 | 0.92 (0.76–1.10) 0.3452 | 0.79 (0.65–0.97) 0.0256 |
| Antibiotic initially chosen | AZM CAM | 0.73 (0.45–1.22) 0.2279 | 0.66 (0.35–1.28) 0.2228 | 0.75 (0.34–1.72) 0.4785 |
|                      | MINO AZM    | 0.35 (0.15–0.85) 0.0215 | 0.03 (0.00–0.20) 0.0003 | 0.70 (0.21–2.22) 0.5529 |
|                      | MINO CAM    | 0.26 (0.11–0.61) 0.0024 | 0.02 (0.00–0.13) <0.0001 | 0.52 (0.18–1.54) 0.2378 |
|                      | AZM TFLX    | 0.45 (0.20–0.91) 0.0256 | 0.48 (0.17–1.27) 0.1410 | 0.33 (0.08–1.06) 0.0639 |
|                      | CAM TFLX    | 0.61 (0.29–1.19) 0.1554 | 0.72 (0.26–1.82) 0.5060 | 0.44 (0.12–1.23) 0.1269 |
|                      | MINO TFLX   | 0.16 (0.06–0.43) 0.0004 | 0.01 (0.00–0.11) <0.0001 | 0.23 (0.04–1.01) 0.0511 |
| Change in antibiotics during the course | Without change | 1.41 (0.82–2.50) 0.2273 | 1.12 (0.58–2.22) 0.7350 | 1.72 (0.63–5.56) 0.3269 |
| Macrolide resistance of M. pneumoniae | Macrolide resistant | 0.41 (0.26–0.64) <0.0001 | - | - | - | - |

AZM, azithromycin; CAM, clarithromycin; MINO, minocycline; TFLX, tosufloxacin; MRMP, macrolide-resistant *Mycoplasma pneumoniae*; MSMP, macrolide-sensitive *Mycoplasma pneumoniae*.

https://doi.org/10.1371/journal.pone.0173635.t003
after oral administration of 100 mg for 3 days [34]. In contrast, the maximum blood concentra-
tion of tosufloxacin is relatively low (1.0 μg/ml after administration of 6 mg/kg) and its
half-time is short (3.8 hours) [35]. Tosufloxacin does not penetrate efficiently into lung sput-
tum and bronchial mucus; maximum sputum concentration to serum concentration ratios
were 0.34/0.94 in one patient and 0.31/0.51 in another patient after oral administration of
150 mg [36]. These differences can partially explain the differences in clinical effects of
these two antibiotics in MRMP infections.

Seventeen patients in Kushiro City for whom *M. pneumoniae* infection was serologically
demonstrated but nasopharyngeal swab samples were not available were considered to be
patients with MRMP for the following reasons. First, the rate of MRMP infection in Kushiro
City was 100% in this period (July 2013 to January 2014) [16]. Second, there was no statistically
significant difference in fever duration between MRMP patients and the 17 patients who were
shown to have pneumonia due to *M. pneumoniae* by serological tests.

In patients infected with MSMP, the durations of fever following commencement of treat-
ments with four antibiotics were different (*P* = 0.0162) (Fig 1). The durations of fever following
commencement of treatments with azithromycin, clarithromycin and minocycline were
shorter than the duration of fever following commencement of treatment with tosufloxacin,
though the difference was not statistically significant (Table 3).

Our study has several limitations. First, this study was a nonrandomized trial to compare
the efficacies of several antibiotics against MRMP strains. Selection of antibiotics was made
by the attending physicians. The lack of evaluation of pneumonia severity could be a con-
founding factor for comparison of fever duration and choice of the antibiotic for treating
MRMP or MSMP. We started surveillance of MRMP among pediatric patients in Hokkaido
in 2012 [16], and physicians therefore knew the prevalence of macrolide resistance in their
region. That might have influenced their choice of antibiotic for treating *M. pneumoniae*
pneumonia. Physicians in Kushiro City were inclined to select either minocycline or tosu-
flexacin because the macrolide resistance rate in Kushiro City was 100% in that period (July
2013 to January 2014). Six of thirteen patients prescribed minocycline and six of twelve
patients prescribed tosufloxacin resided in Kushiro City. Second, we assessed the clinical
outcome only by using fever, not by using respiratory symptoms. Third, we could not totally
exclude colonization of *M. pneumoniae* instead of infection in the *M. pneumoniae* DNA-
positive patients because of the lack of an IgG antibody against *M. pneumoniae* between
acute and convalescent phase serum. Finally, we could not achieve the target sample size in
this study. If we had calculated the sample size to detect the difference between 3.8 days vs.
0.9 days with 1.4 standard deviation, the required sample size becomes 7 patients with *M.
* pneumoniae* for each antibiotic (28 in total) with 0.5 of 2-sided alpha and 90% power. Thus,
this post-hoc power calculation shows that relatively modest differences among groups
could be detected with 59 MRMP patients in the present study despite the failure to reach
the planned sample size.

In conclusion, minocycline was clinically more effective against MRMP infection in pediat-
ric patients than were the other three antibiotics (azithromycin, clarithromycin and tosufloxa-
cin). Therefore, minocycline could be one of the choices for treatment of MRMP infection in
children over 8 years of age. Treatment of MRMP infection in children less than 8 years of age
should be investigated.

**Supporting information**

S1 Table. Data set for this manuscript.
(XLS)
Acknowledgments

We thank Stewart Chisholm for proofreading the manuscript. This research was funded in part by a Grant-in-Aid for Scientific Research (C), 2013 (25461577), from the Ministry of Education, Science, Sports and Culture of Japan and by a Health Science Research Grant (H24-Shinkou-Ippan-014) for Research on Emerging and Re-emerging Infectious Diseases, Labour and Welfare Programs from the Ministry of Health, Labour and Welfare of Japan. Pfizer Inc. provided grants for this study but was not involved in the design of the study or in enrollment of patients, data collection, analysis and interpretation, or preparation of the manuscript. We have declared that no competing interests exist along with any other relevant declarations relating to employment, consultancy, patents, products in development, marketed products, etc. This does not alter our adherence to PLOS ONE policies on sharing data and materials. The members of the Hokkaido Pediatric Respiratory Infection Study Group are as follows: Nobuhisa Ishiguro, Naoko Koseki, Miki Kaiho, Hideaki Kikuta, Takehiro Togashi, Keisuke Morita, Naoko Nagano, Masanori Nakanishi, Kyosuke Hazama, Toru Watanabe, Satoshi Sasaki, Tadashi Ariiga, Akiko Okamura, Shigeru Yamazaki, Satoru Shida, Naofumi Kajii, Tetsuo Nagashima, Mikiyo Yoshioka, Yutaka Takahashi, Mutsuko Konno, Akihito Ishizaka, Takeyasu Takebayashi, Mutsuo Shibata, Hideto Furuyama, Hiroyuki Sawada, Yoshihiro Matsuzono, Mari Murashita, Tatsuru Yamanaka, Hiroyuki Naito, Yasushi Akutsu, Hayato Aoyagi, Katsuyuki Tobise, Chie Tobise, Koichi Yasojima, Akira Tsukihomi, Kiyotaka Kosugiyama, Yoshikazu Kinugawa, Yasuo Tahara, Kazue Yasuda, Susumu Iizuka, Hidefumi Tonoki, Yasuhisa Odagawa, Yoshiaki Oka, Mitsuo Narita, Suguru Ogawa, Yoshinori Kuniya, Yoko Tsuda, Noriyuki Uetsuji, Yoshihiro Itakura, Akira Tsuchiyama and Katsumi Azuma.

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