Original Article

Nationwide survey of Streptococcus pneumoniae drug resistance in the pediatric field in Japan

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Abstract

Background: Streptococcus pneumoniae is a major causative pathogen of pneumonia in children. The Drug-Resistant Pathogen Surveillance Group in Pediatric Infectious Disease conducted a nationwide surveillance of S. pneumoniae in 2000–2001, 2004, 2007, 2010 and 2012, and investigated changes in drug resistance of S. pneumoniae.

Methods: All strains of S. pneumoniae were isolated from clinical specimens collected from pediatric patients. The minimum inhibitory concentration was measured and the strains were classified according to the Clinical Laboratory Standards Institute criteria. The isolation rates of penicillin-intermediate resistant S. pneumoniae (PISP) and penicillin-resistant S. pneumoniae (PRSP) were compared based on seven patient factors. Logistic regression analysis was also performed.

Results: The sum of the isolation rates for PISP and PRSP for each period was 64.6%, 67.0%, 56.2%, 76.9% and 49.5%, respectively. Among the patient factors, age category 1 (<3 years, ≥3 years), age category 2 (infant, toddler and preschooler, schoolchild), siblings (absence, presence), and pre-treatment with antimicrobial agents (absence, presence) were associated with significant differences in the isolation rate of PISP + PRSP. An interaction was observed between pre-treatment with antimicrobial agents and schoolchild, and the isolation rate of PISP + PRSP was higher in patients with both pre-treatment with antimicrobial agents and schoolchild.

Conclusion: Although some changes were observed in the rate of resistance of S. pneumoniae, an increasing trend was not observed. Both pre-treatment with antimicrobial agents and age were associated with resistance, and an interaction was observed between pre-treatment with antimicrobial agents and schoolchild.

Key words interaction, pediatric infectious disease, Streptococcus pneumoniae, surveillance, susceptibility.
Here we report the results of a retrospective analysis of changes in the drug susceptibility of *S. pneumoniae* and the relationship between the development of resistance by *S. pneumoniae* and patient characteristics, based on the results of the surveillance previously conducted in periods 1–5.

**Methods**

**Bacterial strains**

All strains of *S. pneumoniae* were isolated from clinical specimens collected from pediatric patients at 19–28 institutions that participated in the surveillance conducted in periods 1–5. Among those institutions, 10 institutions participated continuously. Nasopharyngeal, pharyngeal, and sputum specimens were mainly collected. After the strains isolated from the patients at each institution were cryopreserved with the Microbank system, they were transferred to the Research Center for Anti-infectious Drugs, Kitasato University.

**Antimicrobial susceptibility testing**

The minimum inhibitory concentration (MIC) was measured using the broth microdilution method according to Clinical Laboratory Standards Institute (CLSI) methods at the Research Center for Anti-infectious Drugs, Kitasato University, using frozen plates containing six predetermined antimicrobial agents (Eiken Chemical, Tokyo, Japan). Information on the drug susceptibility in periods 1–5 has already been reported. During these periods, the following 26 drugs were tested: penicillin G (PCG), ampicillin, amoxicillin, piperacillin, cefaclor (CCL), cefditoren, cefcapene (CFPN), cefpodoxime, cefdinir, cefotiam, ceftriaxone, cefotaxime (CTX), ceferam, faropenem (FRPM), panipenem, meropenem, doripenem, tebipenem (TBPM), azithromycin (AZM), clarithromycin (CAM), rokitamycin (RKM), vancomycin, teicoplanin, norfloxacine, levofloxacine and tosufloxacin (TFLX).

**Susceptibility testing**

The strains were classified according to the CLSI 2007 criteria (non-meningitis, oral penicillin). PCG sensitivity of \(\leq 0.06 \mu\text{g/mL}\) was classified as PSSP, sensitivity of \(0.12 \text{–} 1.0 \mu\text{g/mL}\) was classified as PISP, and sensitivity of \(\geq 2.0 \mu\text{g/mL}\) was classified as PRSP. Although PCG sensitivity \(\leq 2.0 \mu\text{g/mL}\) was classified as PSSP, sensitivity \(4 \mu\text{g/mL}\) as PISP, and sensitivity \(\geq 8.0 \mu\text{g/mL}\) as PRSP according to the CLSI 2013 guidelines, we used the CLSI 2007 guidelines in order to compare all data in periods 1–5 in the present report.

**Patient characteristics and analysis**

The isolation rates of PISP and PRSP were compared based on seven patient factors: sex, age category 1 (<3 years, \(\geq 3\) years), age category 2 (infant, toddler and preschooler, schoolchild), siblings (presence, absence), group day care (attendance, non-attendance), group day care (siblings) (attendance, non-attendance), and pre-treatment with antimicrobial agents within 1 month (presence, absence). Two-sided chi-squared test was used to identify whether significant difference existed. The level of significance was set at 5%.

Logistic regression analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Variables were pre-treatment with antimicrobial agents, sex, age category 2, siblings, group day care, and interaction between pre-treatment with antimicrobial agents and each other factor.

**Results**

**S. pneumoniae isolation rate**

Table 1 lists the number of strains of *S. pneumoniae* isolated from various specimens. The majority of specimens were collected from the nasopharynx, and the total number of strains of PSSP, PISP and PRSP isolated from the nasopharynx was 506, 565 and 268, respectively.

| Strain | Specimen | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Total |
|--------|----------|---------|---------|---------|---------|---------|-------|
| PSSP   | Nasopharynx | 72      | 87      | 107     | 89      | 151     | 506   |
|        | Pharynx   | 26      | 11      | 4       | 3       | 5       | 49    |
|        | Sputum    | 8       | 6       | 8       | 7       | 19      | 48    |
|        | Others    | 12      | 5       | 4       | 7       | 12      | 40    |
|        | Unknown   | 10      | 1       | 1       | 0       | 0       | 12    |
| PISP   | Nasopharynx | 66      | 82      | 117     | 187     | 113     | 565   |
|        | Pharynx   | 31      | 21      | 11      | 11      | 8       | 82    |
|        | Sputum    | 18      | 10      | 13      | 19      | 23      | 83    |
|        | Others    | 8       | 7       | 7       | 12      | 1       | 35    |
|        | Unknown   | 3       | 3       | 0       | 0       | 0       | 6     |
| PRSP   | Nasopharynx | 65      | 71      | 9       | 100     | 23      | 268   |
|        | Pharynx   | 19      | 7       | 2       | 2       | 3       | 33    |
|        | Sputum    | 12      | 20      | 0       | 15      | 10      | 57    |
|        | Others    | 7       | 0       | 0       | 7       | 2       | 16    |
|        | Unknown   | 5       | 2       | 0       | 0       | 0       | 7     |

PISP, penicillin-intermediate resistant *Streptococcus pneumoniae*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*.
Change in drug susceptibility

Table 2 lists the distribution of *S. pneumoniae* according to degree of resistance. The number of strains of PSSP in periods 1–5 was 128 (35.4%), 110 (33.0%), 124 (43.8%), 106 (23.1%), and 187 (50.5%), respectively. The number of strains of PISP + PRSP was 234 (64.6%), 223 (67.0%), 159 (56.2%), 353 (76.9%), and 183 (49.5%), respectively. The number of PRSP strains was 108 (29.8%), 100 (30.0%), 11 (3.9%), 124 (27.0%), and 38 (10.3%), respectively. The number of strains of PISP + PRSP was higher in patients pre-treated with antimicrobial agents, was higher in toddler and preschooler than in infant, was lower in schoolchild than in infant, and was lower in patients with siblings. Among the interactions, the isolation rate of PISP + PRSP was higher in patients pre-treated with antimicrobial agents and schoolchild, and was lower in patients pre-treated with antimicrobial agents and patients with siblings.

Susceptibility to antimicrobial agents

Tables 3 and 4 list the MIC$_{50}$ as well as MIC$_{90}$ (the MIC at which 50% and 90% strains are inhibited, respectively) for antimicrobial agents against *S. pneumoniae*. From periods 1 to 5, the drugs for which MIC$_{50}$ increased more than fourfold were CFPN, CTX, AZM, CAM, and RKM against PSSP strains; FRPM, AZM, CAM, and RKM against PRSP strains; and AZM, CAM, and RKM against PRSP strains. The drug for which MIC$_{50}$ increased more than fourfold was CAM against PRSP strains. The other drugs have in general retained susceptibility. The MIC$_{50}$ and MIC$_{90}$ of both TBPM and TFLX, which were newly launched after period 3, were $\leq 0.125$ μg/mL and 0.25 μg/mL, respectively. The MIC$_{50}$ and MIC$_{90}$ of CCL against PSSP and PISP strains decreased to below one-fourth of the original values.

Patient factors

Table 5 lists the number of patients for each patient characteristic in periods 1–5. Among the patient factors, age category 1 (<3 years), age category 2 (infant, toddler and preschooler, schoolchild), siblings (absence, presence), and pre-treatment with antimicrobial agents (absence, presence) were associated with significant differences in the isolation rate of PISP + PRSP. The isolation rate of PISP + PRSP was higher in patients in age category 1 (<3 years) in period 1 ($P = 0.0002$) and period 3 ($P = 0.0039$); in patients in age category 2 (infant, toddler and preschooler) in period 1 ($P < 0.0001$) and period 4 ($P = 0.0105$); in patients with siblings (absence) in period 3 ($P = 0.0048$); and in patients with pre-treatment with antimicrobial agents (presence) in period 1 ($P = 0.0014$), period 2 ($P = 0.0365$), and period 5 ($P = 0.0370$). No significant difference was found in period 4.

Table 6 lists the isolation rates of PISP + PRSP for the whole period overall by patient characteristics. The isolation rate of PISP + PRSP was higher in patients in age category 1 (<3 years; $P < 0.0001$); in patients in age category 2 (infant, toddler and preschooler; $P < 0.0001$); in patients without siblings ($P = 0.0032$); and in patients pre-treated with antimicrobial agents ($P < 0.0001$).

Logistic regression analysis

Table 7 shows the results of logistic regression including interaction between pre-treatment with antimicrobial agents and each other factor. The isolation rate of PISP + PRSP was higher in patients pre-treated with antimicrobial agents, was higher in toddler and preschooler than in infant, was lower in schoolchild than in infant, and was lower in patients with siblings. Among the interactions, the isolation rate of PISP + PRSP was higher in patients pre-treated with antimicrobial agents and schoolchild, and was lower in patients pre-treated with antimicrobial agents and patients with siblings.

Discussion

Among the pathogens evaluated by the Surveillance Group from 2000 to 2012, we paid particular attention to *S. pneumoniae*. We retrospectively analyzed the changes in drug susceptibility of *S. pneumoniae* as well as the relationship between the development of resistance by *S. pneumoniae* and patient characteristics.

From periods 1 to 5, the change in MIC$_{50}$ and MIC$_{90}$ of β-lactams against PRSP strains was less than fourfold. The drug for which MIC$_{50}$ increased more than fourfold was CAM against PSSP strains, while the drugs for which MIC$_{50}$ increased more than fourfold were CAM, AZM and RKM against PSSP, PISP, and PRSP strains. From period 1 to 2, the MIC$_{50}$ of CAM and AZM increased from 0.25–1 to 128 μg/mL against PSSP strains, from 0.5–1 to >128 μg/mL against PISP strains, and from 0.5–4 to 4–32 μg/mL against PRSP strains, respectively. After period 3, MIC$_{50}$ and MIC$_{90}$ of both these drugs were >64 μg/mL. In Japan, the survey conducted from 2005 to 2006 in the otolaryngological field showed a high prevalence of CAM- and erythromycin-resistant strains, and the proportion of strains carrying both *mefA* and *ermB*, which are macrolide-resistant genes, increased in 2007. Therefore, it was considered that *S. pneumoniae* had become resistant to the macrolides before period 3 based on the results of surveillance.

The isolation rates of PISP + PRSP ranged from 49.5% to 76.9% for the whole period overall. The isolation rates of PRSP ranged from 3.9% to 30.0%, and lower rates were observed in periods 1 to 5.
| Drugs  | MIC\(_{50}\) (μg/mL) | PSSP | PISP | PRSP |
|--------|----------------------|------|------|------|
|        | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 |
| PCG    | 0.03     | 0.06    | ≤0.06   | ≤0.06   | ≤0.06   | 0.5      | 0.5      | 0.5      | 0.5      | 0.5      | 2       | 2       | 2       | 2       | 2       |
| ABPC   | 0.03     | 0.03    | ≤0.06   | ≤0.06   | ≤0.06   | 0.5      | 0.5      | 1        | 0.5      | 1        | 2       | 2       | 2       | 2       | 2       |
| AMPC   | –        | –       | ≤0.06   | ≤0.06   | ≤0.06   | –        | –        | 0.5      | 0.25     | 0.5      | –       | –       | 1       | 1       | 1       |
| PIPC   | –        | –       | ≤0.06   | ≤0.06   | ≤0.06   | –        | –        | 0.5      | 1        | 1        | –       | –       | 2       | 2       | 2       |
| CCL    | 4        | 0.5     | 0.5     | 1       | 0.5     | 64       | 8        | 16       | 16       | 8        | >128   | 64      | 128     | 64      | 64      |
| CDTR   | 0.06     | 0.125   | ≤0.06   | 0.125   | 0.125   | 0.25     | 0.5      | 0.25     | 0.25     | 0.25     | 0.5    | 0.5      | 0.5      | 0.5      | 0.5     |
| CFPN   | 0.06     | 0.125   | 0.125   | 0.25    | 0.25    | 0.5      | 0.5      | 0.5      | 0.5      | 0.5      | 0.5    | 0.5      | 0.5      | 0.5      | 0.5     |
| CPDX   | 0.125    | 0.25    | 0.25    | 0.25    | 0.25    | 1        | 2        | 2        | 2        | 1        | 1      | 1        | 1        | 1        | 1       |
| CFDN   | 0.25     | 0.25    | 0.25    | 0.25    | 0.25    | 2        | 2        | 2        | 2        | 1        | 1      | 1        | 1        | 1        | 1       |
| CTM    | 0.125    | 0.125   | 0.125   | 0.25    | 0.25    | 0.5      | 0.5      | 0.5      | 0.5      | 0.5      | 1      | 1        | 1        | 1        | 1       |
| CTRX   | 0.125    | 0.125   | 0.125   | 0.25    | 0.25    | 0.5      | 0.5      | 0.5      | 0.5      | 0.5      | 1      | 1        | 1        | 1        | 1       |
| CTX    | 0.06     | 0.25    | 0.125   | 0.25    | 0.125   | 0.25     | 0.5      | 0.5      | 0.5      | 0.5      | 0.5    | 1        | 1        | 1        | 1       |
| CFTM   | –        | 0.125   | 0.125   | 0.25    | 0.125   | –        | 1        | 0.5      | 0.5      | 0.5      | 0.5    | 1        | 1        | 1        | 1       |
| DRPM   | –        | –       | –       | ≤0.06   | ≤0.06   | ≤0.06    | ≤0.06    | ≤0.06    | ≤0.06    | ≤0.06    | ≤0.06  | ≤0.06    | ≤0.06    | ≤0.06    | ≤0.06    |
| FRPM   | ≤0.02    | ≤0.02   | ≤0.06   | ≤0.06   | ≤0.06   | 0.06     | 0.125    | 0.25     | 0.125    | 0.25     | 0.25    | 0.5      | 0.5      | 0.5      | 0.5     |
| PAPM   | ≤0.02    | ≤0.02   | ≤0.06   | ≤0.06   | ≤0.06   | 0.06     | 0.125    | ≤0.06    | ≤0.06    | ≤0.06    | 0.125  | 0.25     | ≤0.06    | ≤0.06    | 0.125    |
| MEPM   | ≤0.02    | ≤0.02   | ≤0.06   | ≤0.06   | ≤0.06   | 0.125    | 0.125    | 0.125    | 0.125    | 0.125    | 0.25    | 0.5      | 0.5      | 0.5      | 0.5     |
| DRP    | –        | –       | –       | ≤0.06   | ≤0.06   | –        | –        | –        | ≤0.06    | ≤0.125   | –      | –        | 0.25     | 0.25     | 0.25    |
| TBPM   | –        | –       | –       | ≤0.06   | ≤0.06   | –        | –        | –        | ≤0.06    | ≤0.06    | –      | –        | ≤0.06    | ≤0.06    | ≤0.06   |
| AZM    | 1        | 128     | >64     | >64     | >64     | 1        | >128     | >64      | >64      | >64      | 4      | 32       | >64      | >64      | >64     |
| CAM    | 0.25     | 128     | >64     | >64     | >64     | 0.5      | >128     | >64      | >64      | >64      | 0.5    | 4        | >64      | >64      | >64     |
| RKM    | 0.125    | 1       | 2       | 8       | 4       | 0.25     | 8        | 1        | 2        | 1        | 0.5    | 1        | >32      | 2        | 2       |
| VCM    | 0.125    | 0.5     | 0.25    | 0.25    | 0.25    | 0.25     | 0.25     | 0.25     | 0.25     | 0.25     | 0.25   | 0.5      | 0.25     | 0.25     | 0.25    |
| TEIC   | –        | 0.06    | –       | –       | –       | –        | 0.06     | –        | –        | –        | –      | 0.06     | –        | –        | –       |
| NFLX   | 8        | 8       | –       | –       | –       | 8        | 4        | –        | –        | –        | 8      | 8        | –        | –        | –       |
| LVFX   | –        | 1       | 1       | 1       | 1       | –        | –        | 1        | 1        | 1        | –      | –        | 1        | 1        | 1       |
| TFLX   | –        | –       | –       | 0.25    | 0.25    | –        | 0.25     | 0.25     | 0.25     | 0.25     | –      | –        | 0.25     | 0.25     | 0.25    |

ABPC, ampicillin; AMPC, amoxicillin; AZM, azithromycin; CAM, clarithromycin; CCL, cefaclor; CDTR, cefditoren; CFDN, cefdinir; CFPN, cefcapene; CFTM, cefteram; CPDX, cefpodoxime; CTM, cefotiam; CTRX, ceftriaxone; CTX, cefotaxime; DRPM, doripenem; FRPM, faropenem; LVFX, levofloxacin; MEPM, meropenem; MIC\(_{50}\), minimum inhibitory concentration at which 50% strains are inhibited; NFLX, norfloxacin; PAPM, panipenem; PCG, penicillin G; PIPC, piperacillin; PISP, penicillin-intermediate resistant *Streptococcus pneumoniae*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*; RKM, rokitamycin; TBPM, tebipenem; TEIC, teicoplanin; TFLX, tosufloxacin; VCM, vancomycin.
Table 4  MIC<sub>90</sub> of antimicrobial agents for *Streptococcus pneumoniae* (μg/mL)

| Drugs | PSSP | PISP | PRSP |
|-------|------|------|------|
|       | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 |
| PCG   | 0.06 | 0.06 | ≤0.06 | ≤0.06 | ≤0.06 | 1 | 1 | 1 | 1 | 1 | 4 | 4 | 2 | 2 | 2 |
| ABPC  | 0.06 | 0.06 | ≤0.06 | 0.125 | 0.125 | 2 | 2 | 2 | 2 | 2 | 4 | 4 | 4 | 4 | 8 |
| AMPC  | – | – | – | ≤0.06 | ≤0.06 | – | – | 1 | 1 | 1 | – | – | 4 | 2 | 2 |
| PIPC  | – | – | – | ≤0.06 | 0.125 | – | – | 2 | 2 | 2 | – | – | 2 | 4 |
| CCL   | 8 | 1 | 4 | 2 | 1 | >128 | 64 | 64 | 32 | 32 | >128 | 128 | 128 | 64 |
| CDTR  | 0.25 | 0.125 | 0.25 | 0.5 | 0.25 | 1 | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 2 | 0.5 | 1 | 1 |
| CFN   | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 |
| CFDX  | 1 | 0.5 | 1 | 2 | 1 | 4 | 2 | 2 | 2 | 2 | 4 | 8 | 4 | 4 | 8 |
| CFDN  | 0.5 | 0.25 | 1 | 1 | 0.5 | 8 | 4 | 4 | 4 | 4 | 8 | 16 | 8 | 8 |
| CTM   | 0.5 | 0.25 | 1 | 0.5 | 0.25 | 8 | 4 | 4 | 4 | 4 | 8 | 8 | 8 | 8 |
| CTRX  | 0.5 | 0.25 | 0.5 | 0.5 | 0.5 | 1 | 1 | 1 | 1 | 1 | 2 | 4 | 1 | 2 | 2 |
| CTX   | 0.25 | 0.25 | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 |
| CFTM  | – | 0.5 | 0.5 | 0.5 | 0.5 | – | – | 1 | 1 | 1 | – | 2 | 1 | 2 | 4 |
| FRPM  | ≤0.02 | ≤0.02 | ≤0.06 | ≤0.06 | ≤0.06 | 0.5 | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | 1 | 0.5 | 0.5 | 0.5 |
| PAPM  | ≤0.02 | ≤0.02 | ≤0.06 | ≤0.06 | ≤0.06 | 0.25 | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 | 0.25 | 0.25 | 0.25 | 0.25 |
| MEPM  | ≤0.02 | 0.5 | ≤0.06 | ≤0.06 | ≤0.06 | 0.25 | 0.5 | 0.25 | 0.25 | 0.5 | 0.5 | 2 | 0.5 | 0.5 | 1 |
| DRPM  | – | – | – | ≤0.06 | ≤0.06 | – | – | – | – | – | – | – | – | 0.5 |
| TBPM  | – | – | – | ≤0.06 | ≤0.06 | – | – | – | – | – | ≤0.06 | ≤0.06 | – | – |
| AZM   | >128 | >128 | >64 | >64 | >64 | >128 | >128 | >64 | >64 | >64 | >128 | >128 | >64 | >64 | >64 |
| CAM   | 16 | >128 | >64 | >64 | >64 | >128 | >128 | >64 | >64 | >64 | >128 | >128 | >64 | >64 | >64 |
| RKM   | 16 | >128 | >32 | >32 | >32 | >128 | >128 | >32 | >32 | >32 | >128 | >128 | >32 | >32 | >32 |
| VCM   | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 |
| TEIC  | – | 0.125 | – | – | – | 0.125 | – | – | – | – | 0.25 | – | – | – |
| NFLX  | 16 | 16 | – | – | – | 16 | 8 | – | – | – | 16 | 16 | – | – |
| LVFX  | – | 2 | 2 | 2 | 2 | – | – | 1 | 2 | 2 | – | – | 2 | 2 | 2 |
| TFLX  | – | – | – | 0.25 | 0.25 | – | – | – | 0.25 | 0.25 | – | – | – | 0.25 | 0.25 |

ABPC, ampicillin; AMPC, amoxicillin; AZM, azithromycin; CAM, clarithromycin; CCL, cefaclor; CDTR, cefditoren; CFDN, cefdinir; CFN, cefcapene; CFTM, cefteram; CPDX, cefpodoxime; CTM, cefotiam; CTRX, ceftriaxone; CTX, cefotaxime; FRPM, faropenem; DRPM, doripenem; LVFX, levofloxacin; MEPM, meropenem; MIC<sub>90</sub>, minimum inhibitory concentration at which 90% strains are inhibited.; NFLX, norfloxacin; PAPM, panipenem; PCG, penicillin G; PIPC, piperacillin; PISP, penicillin-intermediate resistant *Streptococcus pneumoniae*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*; RKM, rokitamycin; TBPM, tebipenem; TEIC, teicoplanin; TFLX, tosufloxacin; VCM, vancomycin.
Table 5  Patient characteristics vs isolation rates

| Patient characteristics | Period 1 | Period 2 | Period 3 | Period 4 | Period 5 |
|-------------------------|----------|----------|----------|----------|----------|
|                         | PSSP     | PISP + PRSP | P-value | PSSP     | PISP + PRSP | P-value | PSSP     | PISP + PRSP | P-value | PSSP     | PISP + PRSP | P-value |
| Sex                     | Male     | 70 (35.9) | 125 (64.1) | 0.8267   | 60 (32.4) | 125 (67.6) | 0.4760   | 66 (44.6) | 82 (55.4) | 0.7590   | 65 (23.9) | 207 (76.1) | 0.6441   | 110 (51.6) | 103 (48.4) | 0.6646   |
|                         | Female   | 56 (34.8) | 105 (65.2) | 0.0002   | 50 (36.2) | 88 (63.8)  | 0.53   | 53 (42.7) | 71 (57.3) | 0.0039   | 41 (22.0) | 145 (78.0) | 0.4714   | 76 (49.4) | 78 (50.6)  |          |
| Age category 1          | <3 years | 64 (28.1) | 164 (71.9) | 0.0001   | 67 (32.2) | 141 (67.8) | 0.7955   | 69 (37.7) | 114 (62.3) | 0.0039   | 74 (22.2) | 259 (77.8) | 0.4714   | 121 (48.2) | 130 (51.8) | 0.1923   |
|                         | ≥3 years | 62 (47.3) | 69 (52.7)  | 0.393    | 39 (33.6) | 77 (66.4)  | 0.556    | 55 (55.6) | 44 (44.4) | 0.0554   | 32 (25.4) | 94 (74.6)  | 0.105    | 66 (55.5) | 53 (44.5)  |          |
| Age category 2          | Infant   | 21 (28.4) | 53 (71.6)  | 0.0001   | 23 (35.9) | 41 (64.1)  | 0.2361   | 25 (43.9) | 32 (56.1) | 0.0554   | 21 (21.4) | 77 (78.6)  | 0.0105   | 41 (47.7) | 45 (52.3)  | 0.7664   |
|                         | Toddler and preschooler Schoolchild | 80 (32.1) | 169 (67.9) | 0.841    | 64 (30.7) | 156 (69.3) | 0.516    | 84 (41.1) | 119 (58.9) | 0.715    | 71 (21.5) | 259 (78.5) | 0.129    | 129 (51.0) | 124 (49.0) |          |
|                         | Siblings Absence | 38 (29.7) | 90 (70.3)  | 0.0882   | 41 (31.5) | 89 (68.5)  | 0.5969   | 45 (34.9) | 84 (65.1) | 0.0048   | 35 (18.9) | 150 (81.1) | 0.0812   | 65 (45.5) | 78 (54.5)  | 0.4252   |
|                         | Presence | 86 (38.7) | 136 (61.3) | 0.2458   | 67 (34.4) | 128 (65.6) | 0.795    | 79 (51.6) | 74 (48.4) | 0.715    | 71 (25.9) | 203 (74.1) | 0.835    | 83 (50.0) | 83 (50.0)  |          |
| Group day care          | Non-attendance | 58 (32.6) | 120 (67.4) | 0.2458   | 56 (35.2) | 103 (64.8) | 0.6641   | 51 (45.9) | 60 (54.1) | 0.9820   | 44 (24.4) | 136 (75.6) | 0.5377   | 74 (55.2) | 60 (44.8)  | 0.0616   |
|                         | Attendance | 67 (38.5) | 107 (61.5) | 0.53   | 53 (32.9) | 108 (67.1) | 0.458    | 71 (45.8) | 84 (54.2) | 0.55    | 55 (21.9) | 196 (78.1) | 0.766    | 76 (44.4) | 95 (55.6)  |          |
| Group day care (siblings) | Non-attendance | 25 (36.8) | 43 (63.2)  | 0.5978   | 14 (30.4) | 32 (69.6)  | 0.4666   | 12 (48.0) | 13 (52.0) | 0.5663   | 10 (22.2) | 35 (77.8)  | 0.4933   | 14 (42.4) | 19 (57.6)  | 0.1980   |
|                         | Attendance | 60 (40.5) | 88 (59.5)  | 0.53   | 53 (36.3) | 93 (63.7)  | 0.353    | 63 (54.3) | 53 (45.7) | 0.56    | 56 (27.2) | 150 (72.8) | 0.655    | 65 (55.1) | 53 (44.9)  |          |
| Pre-treatment of antimicrobial agents | Absence | 71 (44.1) | 90 (55.9)  | 0.0014   | 61 (39.6) | 93 (60.4)  | 0.0365   | 61 (48.8) | 64 (51.2) | 0.1283   | 51 (25.0) | 153 (75.0) | 0.3861   | 101 (55.2) | 82 (44.8)  | 0.0370   |
|                         | Presence | 52 (27.7) | 136 (72.3) | 0.4826   | 48 (28.6) | 120 (71.4) | 0.0365   | 62 (39.7) | 94 (60.3) | 0.55    | 55 (21.6) | 200 (78.4) | 0.52    | 52 (43.0) | 69 (57.0)  |          |

PISP, penicillin-intermediate resistant *Streptococcus pneumoniae*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*. 

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The isolation rate of PRSP decreased in period 5, and two possible causes can be suggested. First, this be due to the presence of the pediatric heptavalent pneumococcal conjugate vaccination (PCV7), which became a government-supported vaccine in February 2011 following its introduction in February 2010. More than 90 serotypes of S. pneumoniae have been identified so far based on the immunogenicity of the capsule that encloses the cell,22 and the PCV7 vaccine was developed to induce antibodies against seven serotypes (4, 6B, 9 V, 14, 18C, 19 F, and 23 F),23 which are commonly detected in S. pneumoniae, which is responsible for causing IPD. In the West, PCV7 had already been introduced in the 2000s.24 In the USA, 1 year after the introduction of PCV7 (year 2000), the incidence of IPD in patients <5 years of age decreased by 59% compared with 1998–1999,23 and the incidence of IPD caused by serotypes covered by PCV7 in patients <5 years decreased by 98% in 2005.25 In addition, the isolation rate of PRSP was 21.5% in 1999–2000, and decreased to 14.6% in 2004–2005.26 Similarly, according to surveillance conducted in Spain in patients <14 years, the isolation rate of PISP + PRSP was >45% before the introduction of PCV7, and decreased to 27.4% in 2008 after the introduction of PCV7. Furthermore, the isolation rate of PRSP was in the range of 10% before the introduction of

Table 6  Overall isolation rates of PISP and PRSP vs patient characteristics

| Background                     | n   | PSSP, n (%) | PISP + PRSP, n (%) | P-value† |
|-------------------------------|-----|-------------|--------------------|---------|
| Sex                           |     |             |                    |         |
| Male                          | 1013| 371 (36.6)  | 642 (63.4)         | 0.8450  |
| Female                        | 763 | 276 (36.2)  | 487 (63.8)         |         |
| Unknown                       | 31  | 8 (25.8)    | 23 (74.2)          |         |
| Age category 1                |     |             |                    |         |
| <3 years                      | 1203| 395 (32.8)  | 808 (67.2)         | <0.0001 |
| ≥3 years                      | 591 | 254 (43.0)  | 337 (57.0)         |         |
| Unknown                       | 13  | 6 (46.2)    | 7 (53.8)           |         |
| Age category 2                |     |             |                    | <0.0001 |
| Infant                        | 379 | 131 (34.3)  | 248 (65.4)         |         |
| Toddler and preschooler       | 1258| 431 (34.3)  | 827 (65.7)         |         |
| Schoolchild                   | 157 | 87 (55.4)   | 70 (44.6)          |         |
| Unknown                       | 13  | 6 (46.2)    | 7 (53.8)           |         |
| Siblings                      |     |             |                    | 0.0032  |
| Absence                       | 715 | 224 (31.3)  | 491 (68.7)         |         |
| Presence                      | 1010| 386 (38.2)  | 624 (61.8)         |         |
| Unknown                       | 82  | 45 (54.9)   | 37 (45.1)          |         |
| Group day care (patient)      |     |             |                    | 0.4371  |
| Non-attendance                | 762 | 283 (37.1)  | 479 (62.9)         |         |
| Attendance                    | 912 | 322 (35.3)  | 590 (64.7)         |         |
| Unknown                       | 133 | 50 (37.6)   | 83 (62.4)          | 0.1176  |
| Group day care (siblings)     |     |             |                    |         |
| Non-attendance                | 217 | 75 (34.6)   | 142 (65.4)         |         |
| Attendance                    | 734 | 297 (40.5)  | 437 (59.5)         |         |
| Unknown                       | 856 | 283 (33.1)  | 573 (66.9)         |         |
| Pre-treatment with antimicrobial agents | | | | |
| Absence                       | 827 | 345 (41.7)  | 482 (58.3)         | <0.0001 |
| Presence                      | 888 | 269 (30.3)  | 619 (69.7)         |         |
| Unknown                       | 92  | 41 (44.6)   | 51 (55.4)          |         |

†Chi-squared test (with the exception of Unknown). PISP, penicillin-intermediate resistant Streptococcus pneumoniae; PRSP, penicillin-resistant Streptococcus pneumoniae; PSSP, penicillin-susceptible Streptococcus pneumoniae.

Table 7  Interaction between pre-treatment with antimicrobial agents and other factors (logistic regression analysis) (n = 1589)

| Variables                                      | Level  | Estimates | SE  | Wald χ² | P-value† |
|------------------------------------------------|--------|-----------|-----|---------|---------|
| Intercept                                      |        | 0.4155    | 0.0772 | 28.9697 | <0.0001 |
| Pre-treatment of antimicrobial agents (Reference = Absence) | Presence | 0.3778 | 0.0772 | 23.9519 | <0.0001 |
| Sex (Reference = Male)                        | Female | 0.0033    | 0.0542 | 0.0037  | 0.9515  |
| Age category 2 (Reference = Infant)           | Toddler and preschooler | 0.2482 | 0.0837 | 8.7997  | 0.0030  |
|                                               | Schoolchild | −0.6084 | 0.1291 | 22.2067 | <0.0001 |
| Siblings (Reference = Absence)                | Presence | −0.1627 | 0.0558 | 8.5118  | 0.0035  |
| Group day care (patient) (Reference = Non-attendance) | Attendance | 0.0692 | 0.0565 | 1.4999  | 0.2207  |
| Sex × Pre-treatment of antimicrobial agents   | Female × Presence | −0.0170 | 0.0542 | 0.0983  | 0.7539  |
| Age category 2 × Pre-treatment of antimicrobial agents | Toddler and preschooler × Presence | −0.1032 | 0.0837 | 1.5228  | 0.2172  |
| Siblings × Pre-treatment of antimicrobial agents | Presence × Presence | −0.1116 | 0.0558 | 4.0088  | 0.0453  |
| Group day care (patient) × Pre-treatment of antimicrobial agents | Attendance × Presence | −0.0528 | 0.0565 | 0.8733  | 0.3500  |

†Patients with at least one unknown factor were excluded from analysis. ×, interaction.
providing strong antibacterial activity. 28,29 Recent surveys of antibiotic (PBP), PBP1A, PBP2X, and PBP2B of *Streptococcus pneumoniae* that has a broad spectrum of activity against bacteria including *S. pneumoniae* and which prevent cross-resistance.

TBPM, launched in August 2009, is a β-lactam antibiotic that has a broad spectrum of activity against bacteria including *S. pneumoniae*, and has higher affinity for penicillin-binding protein (PBP), PBP1A, PBP2X, and PBP2B of *S. pneumoniae*, providing strong antibacterial activity. 28,29 Recent surveys have clearly shown the absence of drug resistance to TBPM in *S. pneumoniae*.14,15,30,31

TFLX, launched in 1990, is a fluoroquinolone that has been used for a wide variety of bacterial infections mainly in the field of adult respiratory tract infection, and has a high degree of clinical efficacy.32–34 TFLX shows strong antibacterial activity, inhibiting bacterial DNA gyrase and topoisomerase IV,35–37 and preventing cross-resistance of *S. pneumoniae*.38 TFLX also has low joint toxicity in juvenile animals, and was launched for pediatric patients in 2010 as a fine granular formulation.

These two newly launched antimicrobial agents, which have strong bactericidal activities and are likely to prevent cross-resistance in *S. pneumoniae* to β-lactams and macrolides, are considered to be one of the reasons for the decrease in the isolation rate of PRSP in period 5.

It has already been reported that the patient factors associated with resistance were age category 1 (<3 years), age category 2 (infant, toddler and preschooler), presence of siblings, attendance of patients at group day care, attendance of siblings at group day care, and pre-treatment with antimicrobial agents.13–15,38 During the surveillance, evaluation of the relationship between PISP + PRSP isolation rate and each patient factor indicated a higher isolation rate of PISP + PRSP in patients from age category 1 (<3 years), in patients from age category 2 (infant, toddler and preschooler), in patient without siblings, and in patients pre-treated with antimicrobial agents. Among these resistance factors, three of the four factors were consistent with those already reported, and the exception was absence of siblings.

The PISP + PRSP isolation rate in patients with siblings was lower in period 3 (P = 0.0048) and for the overall period (P=0.0032). The analysis of this result was affected by the confounding factor of age, because the surveillance had been conducted with pediatric patients for whom the sibling’s existence would depend on the age of the patient. We therefore analyzed the relationship between siblings (absence, presence) and age category 2 (infant, toddler and preschooler, schoolchild). Table 8 lists the isolation rate of PISP + PRSP by patient, taking into account the variables of siblings and age category 2. This analysis showed that the isolation rate of PISP + PRSP was lower in schoolchild than in infant or toddler and preschooler, despite the presence or absence of siblings in period 3 and in the overall period. An obvious difference was not observed according to the presence or absence of siblings. In previous studies, presence of siblings has been reported to be associated with resistance, but in the present surveillance the presence of siblings was not associated with resistance.

These results are based on data obtained in the retrospective analysis of the surveillance previously conducted in periods 1–5, and do not indicate an increasing tendency for the occurrence of PISP or PRSP. There has been an increasing number of *S. pneumoniae* serotypes not covered by PCV7 since its introduction;16,39 the pediatric 13-valent pneumococcal conjugate vaccination (PCV13) was launched in 2013, and new drug-resistant bacteria might be predicted to emerge due to the launch of new antimicrobial agents such as TBPM and TFLX. For these reasons, we believe it will be necessary to conduct a prospective analysis of the relationship between drug-resistant *S. pneumoniae* isolation rate and drug susceptibility, serotype,

Table 8 Isolation rates of PISP and PRSP in period 3 and overall vs siblings and age category 2

| Period 3         | Siblings | Presence | PISP | PRSP | PISP + PRSP | Subtotal | PISP | PRSP | PISP + PRSP | Subtotal | Overall period | Siblings | Presence | PISP | PRSP | PISP + PRSP | Subtotal | PISP | PRSP | PISP + PRSP | Subtotal |
|------------------|----------|----------|------|------|-------------|----------|------|------|-------------|----------|----------------|----------|----------|------|------|-------------|----------|------|------|-------------|----------|
| Infant, n (%)    | Toddler and preschooler, n (%) | Schoolchild, n (%) | Total, n (%) |
| **PISP**         |          |          |      |      |             |          |      |      |             |          |                |          |          |      |      |             |          |      |      |             |          |
| Period 3         | Siblings | Presence | PISP | PRSP | PISP + PRSP | Subtotal | PISP | PRSP | PISP + PRSP | Subtotal | Overall period | Siblings | Presence | PISP | PRSP | PISP + PRSP | Subtotal | PISP | PRSP | PISP + PRSP | Subtotal |
| 16 (47.1)        | 52 (50.5) | 51 (49.5) | 103  | 4 (26.7) | 79 (52.0) | 34       | 9 (39.1) | 32 (32.0) | 4 (56.7) | 2 (48.0) | 152 |
| 18 (52.9)        | 51 (49.5) | 103     | 4 (26.7) | 73 (48.0) | 34 |
| Subtotal         |          |          |      |      |             |          |      |      |             |          |                |          |          |      |      |             |          |      |      |             |          |
| 23 (103)         | 2 (33.3) | 6 (65.1) | 129  |
| Overall period   | Siblings | Presence | PISP | PRSP | PISP + PRSP | Subtotal | PISP | PRSP | PISP + PRSP | Subtotal | Overall period | Siblings | Presence | PISP | PRSP | PISP + PRSP | Subtotal | PISP | PRSP | PISP + PRSP | Subtotal |
| 78 (34.1)        | 246 (36.6) | 60 (57.7) | 384 (38.2) | 78 (34.1) | 246 (36.6) | 60 (57.7) | 384 (38.2) | 78 (34.1) | 246 (36.6) | 60 (57.7) | 384 (38.2) | 78 (34.1) | 246 (36.6) | 60 (57.7) | 384 (38.2) |
| 151 (65.9)       | 427 (63.4) | 44 (42.3) | 622 (61.8) | 151 (65.9) | 427 (63.4) | 44 (42.3) | 622 (61.8) | 151 (65.9) | 427 (63.4) | 44 (42.3) | 622 (61.8) | 151 (65.9) | 427 (63.4) | 44 (42.3) | 622 (61.8) |
| Subtotal         |          |          |      |      |             |          |      |      |             |          |                |          |          |      |      |             |          |      |      |             |          |
| 229              | 673       | 104     | 1006 |
| Absence          |          |          |      |      |             |          |      |      |             |          |                |          |          |      |      |             |          |      |      |             |          |
| 45 (32.8)        | 154 (29.1) | 22 (52.4) | 221 (31.2) | 45 (32.8) | 154 (29.1) | 22 (52.4) | 221 (31.2) | 45 (32.8) | 154 (29.1) | 22 (52.4) | 221 (31.2) | 45 (32.8) | 154 (29.1) | 22 (52.4) | 221 (31.2) |
| 92 (67.2)        | 376 (70.9) | 20 (47.6) | 488 (68.9) | 92 (67.2) | 376 (70.9) | 20 (47.6) | 488 (68.9) | 92 (67.2) | 376 (70.9) | 20 (47.6) | 488 (68.9) | 92 (67.2) | 376 (70.9) | 20 (47.6) | 488 (68.9) |
| Subtotal         |          |          |      |      |             |          |      |      |             |          |                |          |          |      |      |             |          |      |      |             |          |
| 137              | 530       | 42      | 709  |

PISP, penicillin-intermediate resistant *Streptococcus pneumoniae*; PRSP, penicillin-resistant *Streptococcus pneumoniae*; PSSP, penicillin-susceptible *Streptococcus pneumoniae*. © 2015 The Authors. Pediatrics International published by John Wiley & Sons Australia, Ltd on behalf of Japan Pediatric Society.
and history of pediatric pneumococcal conjugate vaccination, based on the results of surveillance.

In conclusion, on retrospective evaluation of the surveillance conducted from 2000 to 2012, some changes were observed in the rate of resistance of *S. pneumoniae* in pediatric patients, but an increasing trend was not observed. In addition, both pre-treatment with antimicrobial agents and age were associated with resistance, and an interaction between pre-treatment with antimicrobial agents and schoolchild was observed. Therefore, when treating infections it is important to considering the characteristics of the pediatric patients being treated, and to continue the surveillance in order to clarify the development of resistance in *S. pneumoniae*.

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