Non-antibiotic treatment for pediatric outpatients with common cold inhibits the emergence of drug resistant pneumococci

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NON-ANTIBIOTIC TREATMENT FOR PEDIATRIC OUTPATIENTS WITH COMMON COLD INHIBITS THE EMERGENCE OF DRUG RESISTANT PNEUMOCOCCI

AYUMI MATSUMOTO[1)], AYA TAKEYAMA[2)], KOICHI HASHIMOTO[1)], MASAKI ITO[1)], MASAHIKO KATAYOSE[2)], KAZUO KATO[3)], YUKIHIKO KAWASAKI[1)] and MITSUAKI HOSOYA[1)]

[1)]*Department of Pediatrics, Fukushima Medical University, Fukushima, Japan,* [2)]*Department of Pediatrics, Soma General Hospital, Fukushima, Japan,* [3)]*Department of Pediatrics, Fujita General Hospital, Fukushima, Japan*

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Abstract : The occurrence of drug resistant *Streptococcus pneumoniae (S. pneumoniae)* is very high in Japan. Unnecessary use of antibiotics had been thought to cause this problem but previous studies had not clearly showed that the decreasing rate of antibiotic use had been related to the reduction of the prevalence of resistant strains. In this study, we tried to prove that non-antibiotic treatment for common cold would reduce the antibiotic resistant *S. pneumoniae* in nasopharynx in children. Forty-five children with the common cold were randomly selected from pediatric patients who had taken antibiotics within the past three months. We collected nasopharyngeal swabs from all of the participants and once again after a period of 2 to 3 months without using any antibiotics. Twenty-four of these patients had the *S. pneumoniae* strains isolated. Then these strains were undergone a susceptibility test and drug-resistant gene detection. The susceptibility test reveled that patients with penicillin-resistant strains decreased from 17 to 7 ($p<0.01$). The test also reveled that the decreased number of patients had strains that were resistant to cefditren. The gene detection revealed that none of the patients acquired a higher resistance to penicillin. Our study suggests that the treatment without antibiotics reduces the drug-resistant *S. pneumoniae*. Controlled antibiotic use in children might prevent children from carrying the antibiotic resistant *S. pneumoniae*.

Key words : Steptococcus pneumoniae, antibiotics, common cold, child

INTRODUCTION

The occurrence of multi-drug resistant *Streptococcus pneumoniae (S. pneumoniae)* is very high in Japan. We previously reported about characteristics of nasopharyngeal isolates

松本歩美，武山　彩，橋本浩一，伊藤正樹，片寄雅彦，加藤一夫，川崎幸彦，細矢光亮
Corresponding author : Ayumi Matsumoto　E-mail : b-walk@fmu.ac.jp
http://fmu.ac.jp/home/lib/F-igaku/　http://www.sasappa.co.jp/online/

from children. The study revealed that 88.5% of *S. pneumoniae* had more than one *pbp* gene alteration that indicated strains would be resistant to beta-lactams and 84.5% of *S. pneumoniae* had *mefA* gene or/and *ermB* gene alteration that indicated the strains would be resistant to macrolides[1]. We also found that more than half of the patients who came to our hospital with common cold were given antibiotics without signs of bacterial infection and that previous antibiotic use had a close relationship to the alteration of *pbp* genes in *S. pneumoniae*. Then, we considered if we would medicate the children with less antibiotics, the resistant rate would decrease.

Worldwide, risk factors for the carriage of resistant *S. pneumoniae* have been well investigated in the last couple of decades[2-16] and those studies reported that the most influencing factor was the previous use of antibiotics. Unnecessary use of antibiotics had been thought to cause this emergence of antibiotic resistant *S. pneumoniae*. On the basis of this theory, previous studies were performed to show the dynamics between antibiotic use and the rate of resistant *S. pneumoniae*[17, 18]. But it seemed to be difficult to demonstrate that the less use of antibiotic clearly reduce the prevalence of resistant strains. We considered one of the reasons why they could not demonstrate the reduction of resistant strains was that the researchers investigated a large number of the mass but not the change in individuals. Therefore, we tried to investigate the nasopharyngeal *S. pneumoniae* in specific patients. We did not prescript the antibiotics to them within a certain period and tested their isolates. The purpose of this study is to analyze the change of drug-resistance in those isolates before and after the antibiotic free term.

METHOD

Study design

This study was performed as a prospective trial in Soma general hospital, in the northern part of Japan, from November 2003 to July 2004. In this area, there is only one pediatric clinic and seven clinics that treat children. Patients with common cold on "walk-in" were excluded. We also excluded the patients that showed signs of bacterial infection, such as the high concentration of C reactive protein (CRP) more than 5 mg/dl and an increasing numbers of white blood cell (WBC) more than 20,000/μL. We chose patients who had taken antibiotics within the past three months and 134 patients were considered to be suitable for this study. Out of those 134 patients, 45 patients were chosen at random to enroll into this study. Informed consent was administered to all the participants and their families. Nasopharyngeal swabs were also collected at this time. We did not use any type of antibiotic treatment and the participants agreed that they would not receive any antibiotics treatment from other clinics or hospitals during the term of this trial if it would not be necessary. Follow-up examinations were performed after 2 or 3 months to all patients.

Bacterial isolates

The nasopharyngeal swabs were plated on 5% sheep blood agar, and then incubated for 24 hours. Out of colonies showed alpha-hemolysis, a major colony was picked up and incu-

Table 1. Characteristics of patients

Characteristics	*n* [%] (*N*=24)
Male	19 [79.2]
Age (months old)	
Maximum	60
Minimum	2
Mean	15.3
Day care attendance	17 [70.8]
Having sibling	16 [66.7]
Type of antibiotic	
Beta-lactams	19 [79.2]
Macrolides	5 [20.8]
Term of antibiotic medication (days)	
Maximum	7
Minimum	3
Mean	4.9

bated for another 24 hours, and identified as *S. pneumoniae* by Optochin test (Optochin Showa Disc ; Nissui Phamaceutical, Tokyo) and a latex coagulating reaction test (Slidex pneumo-Kit ; BioMerieux, France) was also done to these samples. Antimicrobial susceptibility to four antibiotics, such as penicillin, cefditren, erythromycun, clarithromycin were also tested with a broth micro dilution test (MICroFAST Panel Type3J-T MicroScan ; Dade Behring, USA), in accordance with the previous standards of National Committee for Clinical Laboratory Standards (currently called Clinical Laboratory and Standards Institute). Then each of the individual strains was determined by minimum inhibitory concentration (MIC) against antibiotics. These strains were also tested to detect antibiotic-resistant genes. Altered *pbp* genes, namely, *pbp1a, pbp2x,* and *pbp2b*, and macrolide-resistant genes, namely, *mefA* and *ermB* were determined by polymerase chain reaction (PCR), using a commercially available primer mixture (Wakunaga Pharmaceutical, Hiroshima, Japan) that included the oligonucleotide primers for detecting the resistant genes. We could confirm the strains as *S. pneumonia* at a molecular level since the primer mixture also included the primer for detecting *lytA,* a specific gene in *S. pneumoniae*.

Patients' background

The patients' background including, age, sex, daycare attendance, sibling and antibiotic used within past 3 months were taken into account by a questionnaire and/or by referring to medical records.

Statistical Analysis

We assessed the differences using the T test, the outcomes of MIC test and the number of strains with drug resistant genes using the χ 2 test. If the value of P was less than 0.05, then the result was considered statistically significant.

RESULTS

S. pneumoniae isolation

Out of the 45 participants, we detected 30 strains at the initial test. Out of the 30 strains, 24 strains were still evident in the second examination.

Characteristics of host patients

All of the patients recovered without any problem. Table 1 shows the characteristics of the patients. There were 79.2%[19] male and the median age was 15.3 months. Day care attendance was 70.8%[17]. Children with sibling(s) were 66.7%[16]. There were 79.2%[19] patients who had taken beta-lactams and 20.8%[5] patients had taken macrolides within the past 3 months before the test. The mean term of antibiotic medication was 5 days.

Outcome of the MIC range

Fig. 1 shows outcome of the MIC distribution against four antibiotics. Fig. 1 (a) shows the number of penicillin-resistant strains (MIC ≧ 0.12 mg/L) that were decreased from 70.8%[17] to 29.2%[7] ($p<0.01$). Also, it clearly shows a peak of MIC shifts from 2 mg/L to 0.06 mg/L. Fig. 1 (b) shows the decrease in the number of strains those showed resistant to cefditren (MIC ≧ 0.5 mg/L) from 66.7%[16] to 25.0%[6] ($p<0.01$) and it shows a peak of MIC shifts

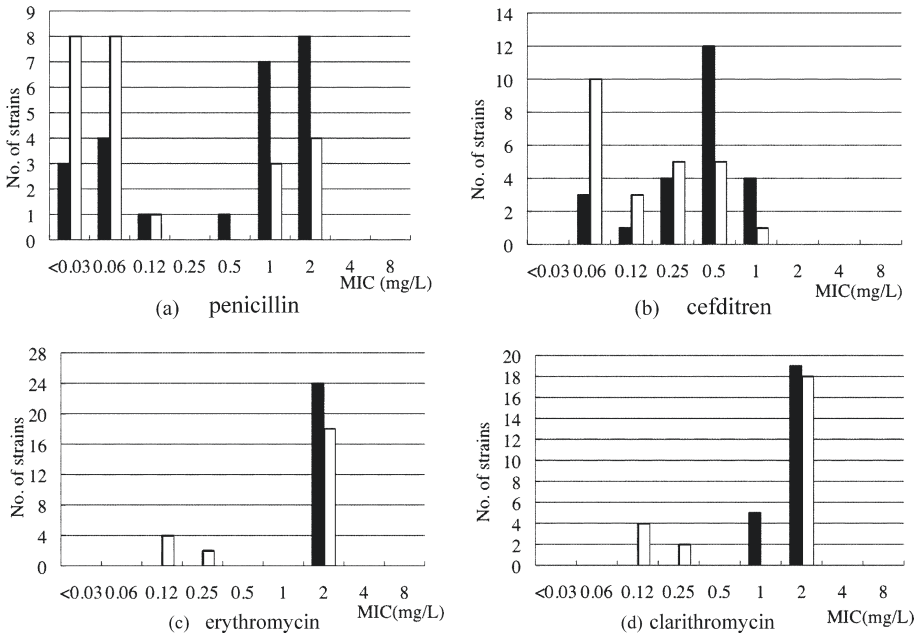

Fig. 1. MIC distribution for antibiotics against beta-lactams. *Streptococcus pneumoniae* isolated at the initial and second test were determined their MIC by penicillin (a), cefditren (b), erythromycin (c), and clarithromycin (d). Black bars and white bars indicate the initial and second test, respectively.

Table 2. Results of altered *pbp* gene detection at the initial and second tests

	initial	second	*P* value
1a+2x+2b	15	7	0.005
1a+2x	2	3	0.194
2x+2b	0	1	0.105
2x	4	4	0.354
no altered gene	3	9	0.013

Table 3. Results of macrolide-resistant gene detection at the initial and second tests

	initial	second	*P* value
mefA+ermB	5	1	0.024
mefA	4	5	0.158
ermB	14	13	0.217
none	1	5	0.024

from 0.5 mg/L to 0.06 mg/L. Fig. 1 (c) and 2 (d) show the decrease in the number of strains those showed resistant to erythromycin (MIC $\geqq$ 1 mg/L) from 24 (100%) to 18 (75.0%) ($p<0.01$), and to clarithromycin (MIC $\geqq$ 1 mg/L) from 24 (100%) to 18 (75.0%) ($p<0.01$). However, peaks of MIC to those macrolides were located at the similar point during testing.

Outcome of gene detection

Table 2 displays the results of *pbp* gene detection during testing. There was a decrease in numbers of strain with 3-points alteration (1a+2x+2b) of *pbp* genes from 62.5%[15] to 29.2%[7] ($p<0.01$). Strains without altered *pbp* gene showed an increase in the number from 12.5%[3] to 37.5%[9] ($p=0.04$). Table 3 displays that the results of macrolide-resistant gene detection in both groups. There was a decrease in numbers of strains with both *mefA* and *ermB* genes from 20.8%[5] to 4.2%[1] ($p=0.02$). Strains without macrolide-resistant gene showed an increase in numbers from 4.2%[1] to 20.8%[5] ($p=0.02$).

Change of the resistant gene of S. pneumoniae in individuals

Fig. 2 shows dynamics of the *pbp* gene alteration of *S. pneumoniae* in 24 patients. The graph clearly exhibits that there is no patient who had the strain acquired altered *pbp* gene(s). In the 11 patients (group A), the strains had a decreased number of *pbp* gene alteration and in the rest of the 13 patients (group B) the strains remained the same gene(s) during the two tests. Table 4 shows the term from the medication to the initial test (the antibiotic-free term) in the both group A and group B. There is no statistical difference between the two groups. Fig. 3 shows change of the macrolide-resistant gene in those patients. In the 2 patients, the strains acquired a macrolide-resistant gene and in the rest of 22 patients, the strains had the less resistant gene or the same gene(s) during the two tests.

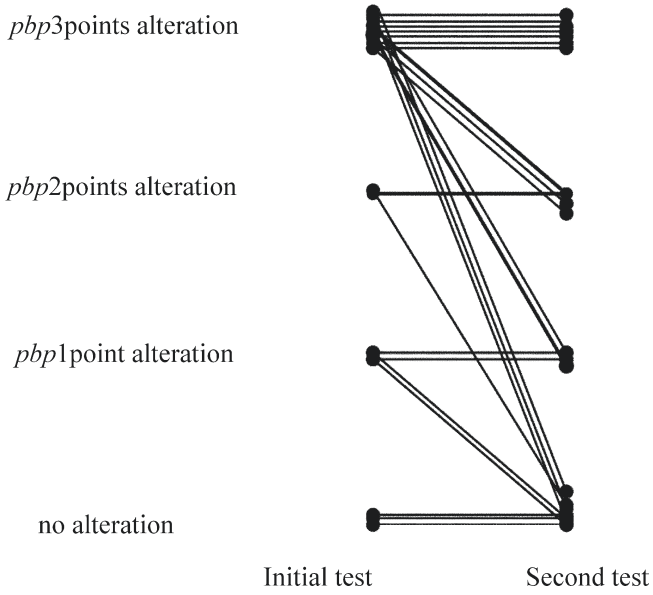

Fig. 2. Change of the pbp gene alteration of *Streptococcus pneumoniae* at the initial and the second test in individuals. The black dots indicate the each patient.

DISCUSSION

Penicillin-resistant *S. pneumonie* was first isolated in the late 1980's and has rapidly spread throughout our country[19]. Currently, it is known that the prevalence of those strains is very high as recent reports showed the rate is higher than 80%[20,21]. Other reports also mentioned that the rate of macrolide-resistant strains has increased and that the rate is higher than 80%[22]. It has been considered that unnecessary use of antibiotics in our country cause these problems[19]. We believe that the most important factor is controlled antibiotic use to prevent the emergence of resistant *S. pneumonaie*. In this study, we have tried to prove that drug-resistant *S. pneumoniae* in nasopharynx of children would improve with no antibiotic use.

According to the results of the MIC test, penicillin-resistant strains and strains with resistance against cephalosporin antibiotics showed a decrease. These are consistent with the results of gene detection. There were 11 patients in whom the numbers of altered *pbp* gene had decreased and of those eleven patients, 6 patients showed an improvement from strains with altered *pbp* genes to strains having no *pbp* gene alteration. The detection also revealed that there was no patient who had the strain acquired altered *pbp* gene(s). The MIC peaks against to erythromycin and clarithromycin did not change afterwards. The macrolide-resistant gene detection showed a decline in the strains with both *mefA* and *ermB* but two strains had acquired a macrolide-resistant gene. These outcomes propose that the antibiotic free term might inhibit the emergence of resistant strains especially against beta-lactams but the further control study is needed to prove the effect of antibiotic free term on resistant

Table 4. The antibiotic free term in the group A and the group B

	group A	group B	*P* value
maximam	174	165	
minimum	81	90	
median	143	116	0.850
mean	131.3	122.4	0.447

* The group A includes the patients who had the strain with a decreased number of altered pbp gene(s)

** The group B includes the patients who had the strain with the same altered pbp gene(s) during the two tests

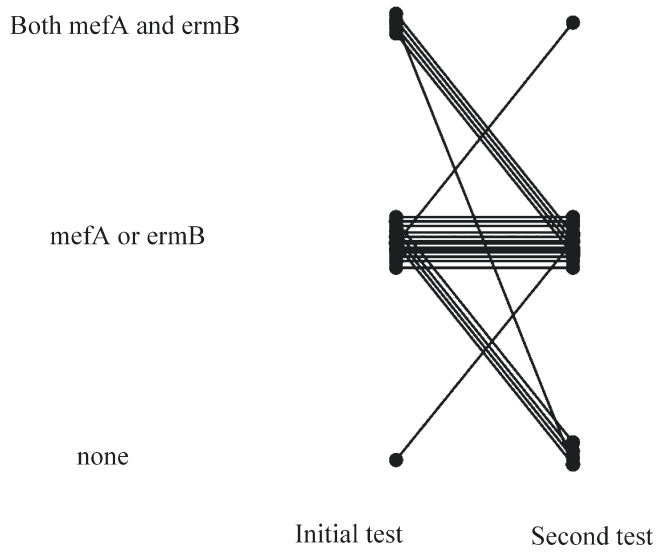

Fig. 3. Change of the macrolide-resistant gene of *Streptococcus pneumoniae* at the initial and the second test in individuals. The black dots indicate the each patient.

genes to be replaced by normal genes.

In this study, we found there were 11 patients in whom the numbers of altered *pbp* gene had decreased but on the other side, the strains remained to have the same gene in more than half of all patients. We compared the antibiotic free term between the patients with less altered *pbp* gene(s) and the patients with same gene(s) but there was no statistical difference. We should test the samples more frequently and follow up longer to define how long will genes take to change. Or some environmental facts as having siblings, attending daycare nursery and age might influence on the patients in those the strains did not show an improvement. We should also continue to analyze more patients to define the reason why some strains did not recover antibiotic susceptibility even after the period of free from antibiotic selecting pressure.

This study has several limitations. Firstly, the rate of resistant strains was quite high at

the initial test. We predicted a high resistant rate when we started this study since the occurrence of resistant *S. pneumoniae* was very high in this area ; more than 80%[1] and all the patients enrolled in this study had taken antibiotics before the study. Secondly, the method of *S. pneumoniae* isolation could cause a possible bias because only a single colony of *S. pneumoniae* was examined from each patient in this study. Since strains from the nasopharynx are considered to have various susceptibilities against antibiotics this single colony might not reflect a suitable resistant rate. It would be logical to assume that susceptible strains became predominant under no antibiotic selecting pressure.

Some studies prescribe a pneumococcal conjugate vaccine as an effective way not to materialize of invasive pneumococcal disease[24,25]. This may reduce incidences of persistent antibiotic-resistant *S. pneumoniae* infection but does not mean antibiotic-resistant *S. pneumoniae* will vanish. Even though human being will continue producing new drugs in the future, the resistances to those drugs will also follow in time. Therefore, we consider that the best way to prevent the emergence of antibiotic-resistant pneumococci is not to use antibiotics for all patients who do not require the medication as a source of treatment. We believe this is a simple way that will contribute to improve the threatening state of *S. pneumoniae* around Japan and the world.

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