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Original Research Article

Relationship between Clinical Characteristics and Multidrug-Resistant Patterns of *Streptococcus pneumoniae* in Japan

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Abstract	Keywords
<p><i>Streptococcus pneumoniae</i> is the leading cause of invasive diseases such as pneumonia, meningitis, and sepsis. Furthermore the emergency of multidrug-resistant <i>Streptococcus pneumoniae</i> has been focused worldwide. The objective of this study was to determine the relationship between clinical characteristics and multidrug-resistant patterns of <i>Streptococcus pneumoniae</i> in Japan. We determined the <i>in vitro</i> susceptibilities of 10 antimicrobial agents for 153 <i>Streptococcus pneumoniae</i> in Japan. From antimicrobial susceptible results, we categorized six multi-drug resistant patterns as follows [pattern A (39:25.5 %); erythromycin, tetracycline, imipenem, pattern B (25:16.3%); erythromycin, tetracycline, trimethoprim-sulfamethoxazole, pattern C (17:11.1%); erythromycin, tetracycline, ofloxacin, pattern D (16:10.5%); erythromycin, tetracycline, chloramphenicol, Pattern E (11:7.2 %); erythromycin, tetracycline, amoxicillin, pattern F (10:6.5 %); erythromycin, tetracycline, cefotaxime]. These results indicated the most multidrug-resistant <i>Streptococcus pneumoniae</i> had macrolide and tetracycline resistant ability. Our results also revealed that the risk factors of multidrug-resistant <i>Streptococcus pneumoniae</i> were (1) nasal discharge, (2) among 1-6 years year-old children, and (3) department of paediatrics. The emergence of multi-drug resistant <i>Streptococcus pneumoniae</i> suggests the need for continuing surveillance of antimicrobial resistance.</p>	<p>Antimicrobial susceptibility Minimal Inhibitory Concentration Multidrug-resistant <i>Streptococcus pneumoniae</i></p>

Introduction

Streptococcus pneumoniae is a major source of morbidity and mortality worldwide. It is estimated that about 1 million children die of pneumococcal disease every year in WHO report (WHO, 2007). Pneumococcal infections are the leading cause of death from a vaccine-preventable illness in children aged under 5 years (CDC, 2006). Invasive diseases caused by pneumococci include meningitis, sepsis, and pneumoniae (WHO, 2005). Risk factors for invasive pneumococcal disease (IPD) include age (with incidence being highest in young children aged under 2 years and the elderly aged over 65 years), ethnicity, geographic location, concomitant chronic illnesses, and attendance in day care centres (Fletcher et al., 2006).

During the past 2 decades, multidrug-resistant *Streptococcus pneumoniae* has spread worldwide (Mera et al., 2005; Kim et al., 2012). In recent times, pneumococcal strains with high level of resistance to penicillin have emerged, and these organisms have shown resistance to other antibiotics such as tetracycline, erythromycin, chloramphenicol, ciprofloxacin and clindamycin. Isolates of *Streptococcus pneumoniae* that are resistant to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, erythromycin alone or in combination were being recovered more and more often worldwide (Marchese et al., 2005; Ashley et al., 2011).

The objective of this study was to determine the relationship between clinical characteristics and multidrug-resistant patterns of *Streptococcus pneumoniae* isolated in Japan.

Materials and methods

Strains and clinical data collection

A total of 153 *Streptococcus pneumoniae* was obtained from various clinical specimens from 2013 to 2014 in Japanese private hospital. We used medical records appended to clinical species for the analysis of clinical feature at hospital. We considered several isolates from the same region of the same patient as one isolate per one patient for the analysis in this study. All streptococcal isolates were identified by standard conventional biochemical methods or the VITEK2

system (bioMérieux, Durham NC, USA). Our experimental design was approved by the ethics committee at private hospital in Japan.

Antimicrobial susceptibility analysis

Streptococcus pneumoniae isolates were examined for 10 antibiotic susceptibilities as follows; PCG, penicillin G; AMPC, amoxicillin; CTX, cefotaxime; IPM, imipenem; OFLX, ofloxacin; EM, erythromycin; VCM, vancomycin; TC, tetracycline; CP, chloramphenicol; ST, trimethoprim-sulfamethoxazole. Minimal inhibitory concentration (MICs) was determined at clinical laboratory in private hospital using broth micro dilution methodology with the VITEK2 system. MICs were calculated as MIC₉₀ (MIC causing inhibition of 90% of isolates). Percentage susceptibilities were calculated based on Clinical Laboratory Standard Institute (CLSI, 2014) break point. We used two separate interpretive breakpoints for meningeal and non-meningeal isolates to define penicillin. Multidrug-resistance was defined as non-susceptibility to more than any three antimicrobial agents (Magiorakos et al., 2012).

Statistical analysis of the data

We conducted the statistical analysis with the chi-squared test or Fisher's exact test when appropriate. Differences were considered significant when p was <0.05 .

Results

We screened 153 *Streptococcus pneumoniae* clinical isolates. From those susceptible results, we categorized six multidrug-resistant patterns (Table 1). The multidrug-resistant pattern of most frequently was erythromycin, tetracycline, and imipenem. Most multidrug-resistant *Streptococcus pneumoniae* had macrolide and tetracycline resistant ability.

We first analysed the relationship between gender ratio and multidrug-resistant patterns (Fig. 1). There was no significant difference between male and female in all multidrug-resistant patterns. We next analysed the relationship between age incidence and multidrug-resistant patterns (Fig. 2). In all multidrug-resistant patterns, *Streptococcus pneumoniae* were significantly isolated at the age incidence among 1 - 6 years age group ($p<0.05$). Especially, in pattern E and F,

Streptococcus pneumoniae were only isolated from under 12 years-old children.

We also analysed the relationship between biological source and multidrug-resistant patterns (Fig. 3). Nasal discharge is the most biological source in all

multidrug-resistant patterns ($p < 0.05$). Especially, multidrug-resistant *Streptococcus pneumoniae* were isolated from nasal discharge in pattern A, B, E, F ($p < 0.05$). In pattern D, *Streptococcus pneumoniae* were isolated from other source such as sputum and blood.

Table 1. The multidrug-resistant patterns of *Streptococcus pneumoniae*

Multidrug-resistant patterns	Drugs/Antibiotics	Number of isolates (%)
Pattern A	Erythromycin, tetracycline, imipenem	39 (25.5%)
Pattern B	Erythromycin, tetracycline, trimethoprim-sulfamethoxazole	25 (16.3%)
Pattern C	Erythromycin, tetracycline, ofloxacin	17 (11.1%)
Pattern D	Erythromycin, tetracycline, chloramphenicol	16 (10.5%)
Pattern E	Erythromycin, tetracycline, amoxicillin	11 (7.2%)
Pattern F	Erythromycin, tetracycline, cefotaxime	10 (6.5%)

Fig. 4 demonstrates that *Streptococcus pneumoniae* were isolated from the department of paediatrics in all multidrug-resistant patterns ($p < 0.05$). Especially, in pattern E and F, *Streptococcus pneumoniae* were only isolated from under the department of paediatrics. In pattern B and D, *Streptococcus pneumoniae* were isolated from other department such as general medicine and neurology.

Furthermore, we analysed the relationship between penicillin G MIC values and multidrug-resistant patterns (Fig. 5). In pattern A and E, penicillin G MIC values in *Streptococcus pneumoniae* were significantly higher than 1 $\mu\text{g/mL}$ ($p < 0.05$). In pattern C and D, penicillin G MIC values in *Streptococcus pneumoniae* was significantly lower than 0.06 $\mu\text{g/mL}$ ($p < 0.05$).

Fig. 1: Gender wise distribution of *Streptococcus pneumoniae* isolates.

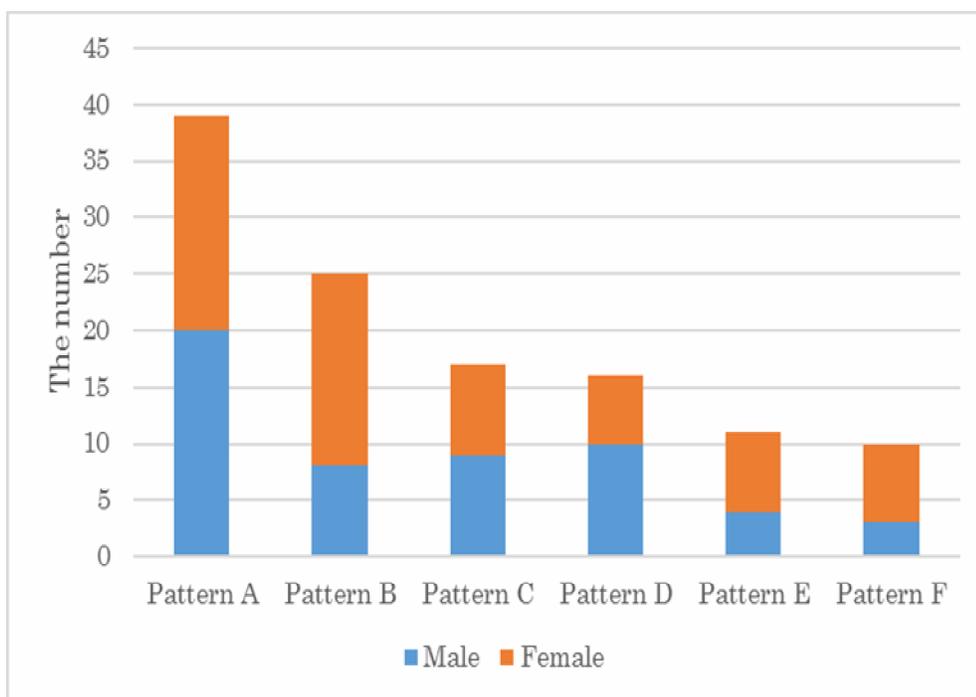


Fig. 2: Age wise distribution of *Streptococcus pneumoniae* isolates.

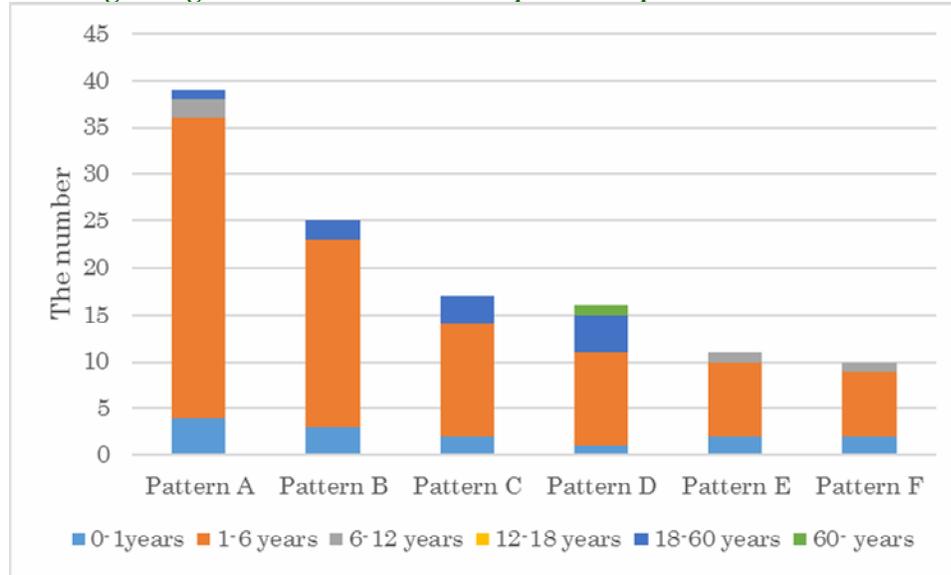
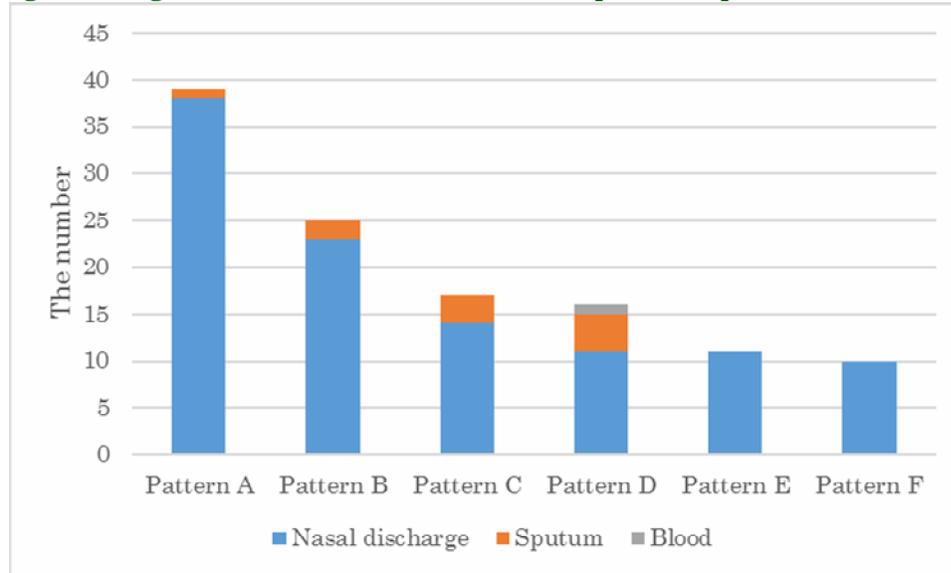


Fig. 3: Biological source wise distribution of *Streptococcus pneumoniae* isolates.



Discussion

Although many researchers reported only antimicrobial susceptible patterns in multidrug-resistant *Streptococcus pneumoniae*, this study is the first report about the relationship between clinical characteristics and multidrug-resistant patterns of *Streptococcus pneumoniae*. In this study, we clarified the relationship between clinical characteristics and multidrug-resistant patterns of *Streptococcus pneumoniae*. Our results demonstrated that quarter of all *Streptococcus pneumoniae clinical* isolates had multidrug-resistant pattern (erythromycin, tetracycline,

and imipenem resistant). Our results also revealed that the risk factors of multidrug-resistant *Streptococcus pneumoniae* were (1) nasal discharge, (2) among 1-6 years year-old children, and (3) department of paediatrics.

The emergency of *Streptococcus pneumoniae* possessing more than three different antibiotic-resistant ability have been focused worldwide. In USA study, about 25% of all isolates were multidrug resistant, defined as resistant two or more of the following agents: cefuroxime, a macrolide, penicillin, tetracycline, and trimethoprim sulfamethoxazole

(Thornsberry et al., 2008). Among multidrug-resistant strains, the most common co-resistance pair was erythromycin and trimethoprim sulfamethoxazole (74% of isolates), although penicillin-erythromycin and penicillin trimethoprim sulfamethoxazole co-resistance patterns were also found in more than 56 %

of multidrug-resistant strains. Resistance to 4 antimicrobial agents tested was observed in 33% of all antibiotic-resistant isolates. Levofloxacin, which was used as a representative of the fluoroquinolone class, was active against at least 98 % of all multidrug-resistant isolates (Thornsberry et al., 2008).

Fig. 4: Clinical department wise distribution of *Streptococcus pneumoniae* isolates.

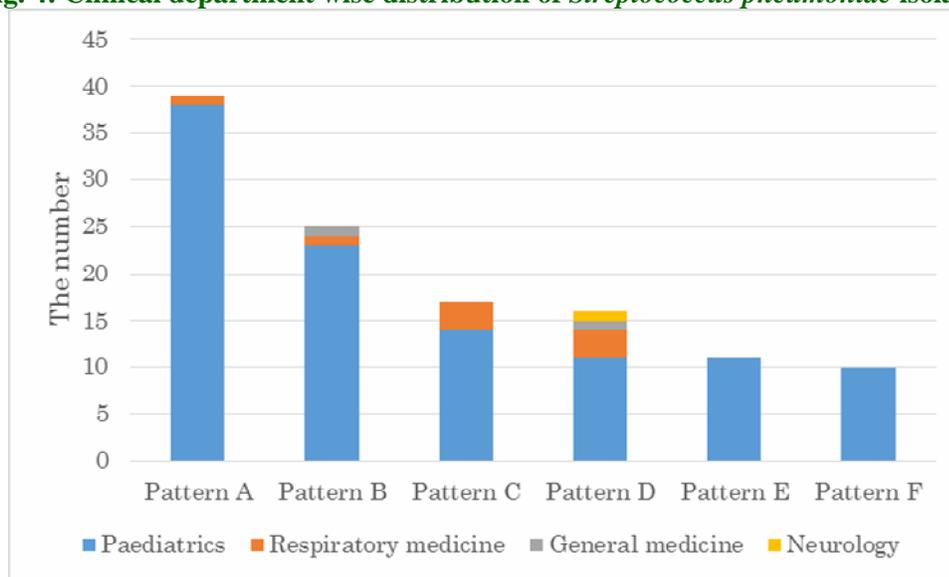
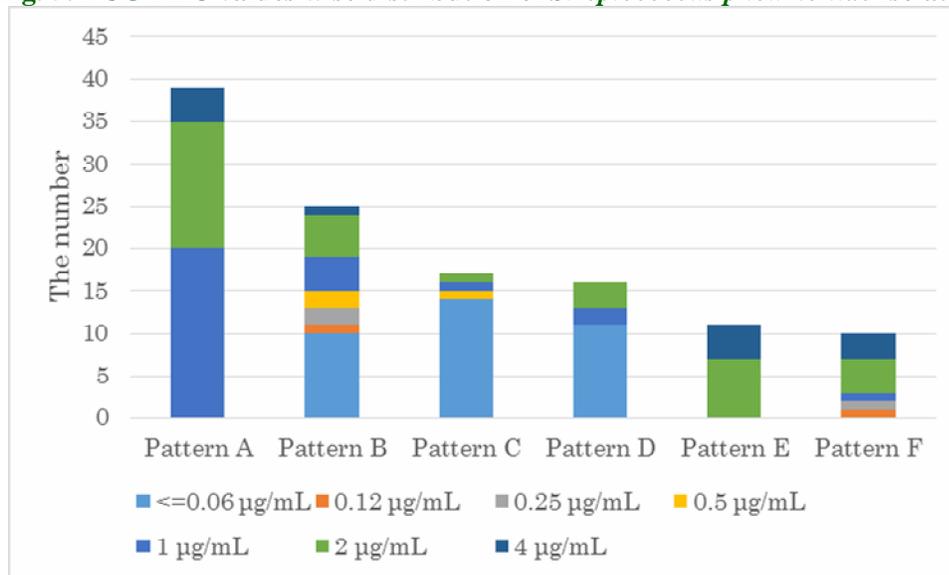


Fig. 5: PCG MIC values wise distribution of *Streptococcus pneumoniae* isolates.



As the ratio of multidrug-resistant pattern B including erythromycin and trimethoprim sulfamethoxazole resistant was 16.3% in our study, there were no significant differences between previous study and our study. In other USA study, the prevalence of multidrug-resistance among *Streptococcus pneumoniae*

isolates was about 30% overall (Farrell et al., 2012). Although the introduction of the paediatric 7-valent pneumococcal conjugate vaccine resulted in a decline of invasive pneumococcal disease, along with associated reductions in penicillin and macrolide non-susceptibility among IPD isolates (Talbot et al., 2004).

However, an increase in antibiotic-resistance was found among the increasing prevalence of non-vaccine serotypes, particularly multidrug-resistant serotype 19A (Pelton et al., 2007). This multidrug-resistant pattern was ceftriaxone, amoxicillin, azithromycin and trimethoprim sulfamethoxazole (Pelton et al., 2007). In Korean study, about 56 % of all the isolates were multidrug-resistant, defined as resistant to three or more of the following agents: penicillin, erythromycin, clindamycin, cefotaxime, tetracycline and levofloxacin. Levofloxacin, as a representative fluoroquinolone, was active against about 88 % of all multidrug-resistant isolates (Lee et al., 2010). Although the ratio of multi drug resistant pattern including ofloxacin-resistant was 11.1% in our study, levofloxacin may be also candidate for multidrug-resistant *Streptococcus pneumoniae*. Although we cannot simply compare our results and previous reports because of different types of antibiotics and different definitions of multidrug-resistance, further investigation will be necessary to elucidate the relationship between clinical characteristics and multidrug-resistant patterns of *Streptococcus pneumoniae* worldwide.

Conclusion

In conclusion, we categorized six patterns of multidrug-resistant *Streptococcus pneumoniae* and we clarified the relationship between clinical characteristics and multidrug-resistant patterns. These results indicated the most multidrug-resistant *Streptococcus pneumoniae* had macrolide and tetracycline resistant ability. Careful monitoring of multidrug-resistant patterns of *Streptococcus pneumoniae* will help guide appropriate therapeutic selection and may provide early detection of changes in resistance to other antimicrobial agents.

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