Characterization of Mucoid and Non-Mucoid Streptococcus pneumoniae Isolated From Outpatients

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Background: Streptococcus pneumoniae causes pneumonia, sepsis, and meningitis. This study aimed to investigate the clinical characteristics of mucoid and non-mucoid isolates of S. pneumoniae, and to explore the relationship between the isolate phenotypes and their antibiotic susceptibility.

Methods: Clinical isolates from 3,453 non-repetitive S. pneumoniae (189 mucoid and 3,264 non-mucoid) infections obtained between January 2008 and December 2012 from outpatients at the Kimitsu-Central Hospital were evaluated.

Results: Compared to the non-mucoid isolates, the mucoid phenotypes were more susceptible to certain antibiotics such as erythromycin, clarithromycin, and tetracycline as opposed to clindamycin, chloramphenicol, and rifampicin. The mucoid phenotype was isolated more frequently from schoolchildren, adults, and elderly adults in a variety of clinical sites, including otorrhea, genitalia, pus, and eye discharge than the non-mucoid phenotype. This suggested that mucoid isolates are more likely to be involved than non-mucoid isolates in various local infections. Systemic infection, which indicates invasiveness, was not associated with the mucoid or non-mucoid phenotype.

Conclusions: The results of this study suggest that mucoid isolates tend to have higher susceptibility than non-mucoid isolates to antibiotics. To the best of our knowledge, mucoid and non-mucoid S. pneumoniae isolates considerably differ in terms of clinical isolation site and age-specific prevalence.

Key Words: Streptococcus pneumoniae, Mucoid colony, Antimicrobial susceptibility

INTRODUCTION

Streptococcus pneumoniae is an important pathogen that causes invasive and non-invasive pneumococcal diseases (e.g., meningitis, sepsis, pneumonia, and otitis media) in individuals of all age groups [1]. In various countries including Japan, pneumococcal conjugate vaccines are routinely used to protect infants, children, and elderly adults from pneumococcal diseases; severe pneumococcal pneumonia and meningitis are still associated with high mortality rates [2]. Furthermore, antimicrobial resistance in S. pneumoniae has been observed globally since 1980s [3]. In particular, the increasing resistance of S. pneumoniae strains to widely used anti-pneumococcal drugs, including β-lactams and macrolides, has become a serious clinical concern in both developing and developed countries, including Japan [4, 5].

Colonies of S. pneumoniae isolates show various morphological features, such as the presence of a central depression or a mucoid appearance [6]. The mucoid phenotype has also been observed in other pathogenic bacteria, including Pseudomonas...
Characterization of phenotypes of *S. pneumoniae* 

**METHODS**

1. Patients and samples

In this study, 3,453 non-repetitive *S. pneumoniae* (189 mucoid and 3,264 non-mucoid) clinical isolates were evaluated; they were obtained between January 2008 and December 2012 from outpatients at the Kimitsu-Central Hospital who were suspected to have infectious diseases. Strains isolated from hospitalized patients within 48 hr were defined as outpatient-derived strains.

To elucidate the age-specific prevalence of different phenotypes, the patients were assigned to one of four different age groups. Patients aged 0-5 yr were assigned to the infant and preschool group; those aged 6-11 yr were assigned to the schoolchildren group; those aged 12-64 yr were assigned to the adult group, and those aged ≥65 yr were assigned to the elderly adult group.

2. Microbiological investigations

All 3,453 strains were identified by using an optochin disk (Eiken Chemical Co. Ltd., Tokyo, Japan) and Slidex pneumo-Kit (Sismex bioMerieux, Tokyo, Japan). Mucoid strains were visually identified on the basis of colony morphology described in the Manual of Clinical Microbiology, 10th edition [9]. Susceptibility to penicillin G (meningitis and nonmeningitis), amoxicillin-clavulanate, cefotaxime (meningitis and nonmeningitis), ceftriaxone (meningitis and nonmeningitis), cefepime (meningitis and nonmeningitis), meropenem, erythromycin, clarithromycin, clindamycin, tetracycline, chloramphenicol, vancomycin, rifampicin, and trimethoprim-sulfamethoxazole was tested by using MicroFAST 5J panels (Siemens Healthcare Diagnostics, Tokyo, Japan). Only susceptibility to ceftriaxone was evaluated in 57 mucoid and 1,226 non-mucoid samples isolated after May 2011. The results were interpreted according to the Clinical Laboratory Standards Institute document M100-S24 [10]. Oral penicillin V is an unapproved antimicrobial agent in Japan; therefore, it was not evaluated in this study. The MicroFAST 5J panels were inoculated and read according to the manufacturer’s recommendations. The inoculated panels were then incubated at 35°C in ambient air for 20-24 hr prior to visual determination of the MIC.

Quality control for MIC determination was performed by using the reference strain *S. pneumoniae* ATCC 49619.

3. Statistical analysis

Data were analyzed by using Microsoft Excel for Mac 2011. Categorical variables were compared by using either chi-squared test or Fisher’s exact test, as appropriate. Two-tailed *P*<0.05 was considered statistically significant. Statistical significance was assessed in comparison between the infant and preschool group and each age group for antibiotic resistance rate with either chi-squared test or Fisher’s exact test. When the infant and preschool group showed significance to all each age group, we defined this was significant.

**RESULTS**

The MIC$_{50}$ of penicillin G (nonmeningitis), cefotaxime (nonmeningitis), ceftriaxone (nonmeningitis), and meropenem in non-mucoid isolates was higher than that in mucoid isolates (Table 1). Similarly, the MIC$_{50}$ of amoxicillin-clavulanate, cefepime (nonmeningitis), and trimethoprim-sulfamethoxazole in non-mucoid isolates was higher than that in mucoid isolates. Since only non-mucoid isolates were detected from patients with meningitis, we compared mucoid isolates and non-mucoid isolates from patients with meningitis with regard to their susceptibility to penicillin G, cefotaxime, ceftriaxone, and cefepime (Table 1). Additionally, non-mucoid isolates from patients with meningitis before 2010 were not tested for ceftriaxone susceptibility (meningitis). The MIC$_{50}$ and MIC$_{90}$ of erythromycin, clarithromycin, tetracycline, vancomycin, levofloxacin, and rifampicin were comparable for mucoid and non-mucoid isolates, although clarithromycin, tetracycline, and vancomycin are not routinely used in the clinical setting. The rate of resistance and MIC of protein synthesis-inhibiting antibiotics, including erythromycin, clarithromycin, clindamycin, tetracycline, and chloramphenicol, were found to be high for both. Moreover, the rate of resistance to clarithromycin, tetracycline, and trimethoprim-sulfamethoxazole was higher in non-mucoid isolates than in mucoid isolates (*P*<0.01); the rate of resistance to clindamycin, chloramphenicol, and rifampicin was higher in mucoid isolates than in non-mucoid isolates (*P*<0.01) (Table 1). However, the rate of resistance to β-lactam antibiotics, including penicillin G, amoxicillin-
clavulanate, cefotaxime, ceftriaxone, cefepime, meropenem, and vancomycin, did not differ significantly between the two phenotypes (Table 1). All mucoid and non-mucoid strains isolated from meningitis samples were susceptible to both penicillin G and cefotaxime, which have two cut-off values in meningitis and non-meningitis samples.

Among the schoolchildren, adult, and elderly adult groups, the frequency of mucoid isolates was significantly higher than that of non-mucoid isolates ($P<0.01$) (Table 2). Non-mucoid isolates were more resistant to erythromycin, clarithromycin, clindamycin, and tetracycline in the infant and preschool group than in the schoolchildren, adult, and elderly adult groups in all samples ($P<0.05$) (Table 3). The results for non-mucoid isolates were similar for all respiratory tract samples (Table 4). The invasiveness and antibiotic resistance rate of non-mucoid isolates did not differ significantly among age groups for all antibiotics (Table 5). The antibiotic resistance rate did not differ significantly among mucoid isolates, too. In contrast, the frequency of mucoid isolates obtained from otorrhea samples, genital swabs, ecthyma

### Table 1. MIC$_{50}$, MIC$_{90}$, and range of various antibiotics for *Streptococcus pneumoniae*

|                    | Mucoid (N = 189) | Non-mucoid (N = 3,264) |
|--------------------|------------------|------------------------|
|                    | MIC$_{50}$ (µg/mL) | MIC$_{90}$ (µg/mL) | MIC range (µg/mL) | Resistant (%) | MIC$_{50}$ (µg/mL) | MIC$_{90}$ (µg/mL) | MIC range (µg/mL) | Resistant (%) |
| Penicillin G nonmeningitis | ≤0.03 ≤0.03 ≤0.03 to 0.06 | 0 | 0.5 ≤0.03 to >2 | 0 | ≤0.03 ≤0.03 ≤0.03 to 0.06 | 0 | ≤0.03 ≤0.03 ≤0.03 to 0.06 | 0 |
| Penicillin G meningitis | ≤0.06 ≤0.06 ≤0.06 to 0.06 | 0 | ≤1 ≤1 ≤1 to >4 | 0.1 | ≤0.06 ≤0.06 ≤0.06 to 0.06 | 0 | ≤0.06 ≤0.06 ≤0.06 to 0.06 | 0 |
| AC | ≤1 ≤1 ≤1 | 0 | ≤1 2 ≤1 to >4 | 0.1 | ≤1 ≤1 ≤1 to >4 | 0 | ≤1 ≤1 ≤1 to >4 | 0.1 |
| Cefotaxime nonmeningitis | 0.12 0.25 ≤0.06 to 0.5 | 0 | 0.25 1 ≤0.06 to >4 | 0.5 | 0.12 0.25 ≤0.06 to 0.5 | 0 | 0.25 1 ≤0.06 to >4 | 0.5 |
| Cefotaxime meningitis | ≤0.06 ≤0.25 ≤0.06 to 0.25 | 0 | ≤0.12 ≤0.12 ≤0.12 to 0.12 | 0.7 | ≤0.06 ≤0.25 ≤0.06 to 0.25 | 0 | ≤0.12 ≤0.12 ≤0.12 to 0.12 | 0.7 |
| Ceftriaxone nonmeningitis | ≤0.12 ≤0.12 ≤0.12 to 0.12 | 0 | ≤0.12 ≤0.12 ≤0.12 to 0.12 | 0.7 | ≤0.12 ≤0.12 ≤0.12 to 0.12 | 0 | ≤0.12 ≤0.12 ≤0.12 to 0.12 | 0.7 |
| Cefepime nonmeningitis | ≤0.5 ≤0.5 ≤0.5 | 0 | ≤0.5 ≤0.5 ≤0.5 to >2 | 0.6 | ≤0.5 ≤0.5 ≤0.5 | 0 | ≤0.5 ≤0.5 ≤0.5 to >2 | 0.6 |
| Cefepime meningitis | ≤0.5 ≤0.5 ≤0.5 | 0 | ≤0.5 ≤0.5 ≤0.5 | 0 | ≤0.5 ≤0.5 ≤0.5 | 0 | ≤0.5 ≤0.5 ≤0.5 | 0 |
| Meropenem | ≤0.12 ≤0.12 ≤0.12 to 0.25 | 0 | ≤0.12 ≤0.12 ≤0.12 to 0.25 | 1.8 | ≤0.12 ≤0.12 ≤0.12 to 0.25 | 0 | ≤0.12 ≤0.12 ≤0.12 to 0.25 | 1.8 |
| Erythromycin | >1 >1 ≤0.12 to >1 | 81.5 | >1 >1 ≤0.12 to >1 | 86.3 | >1 >1 ≤0.12 to >1 | 81.5 | >1 >1 ≤0.12 to >1 | 86.3 |
| Clarithromycin | >1 >1 ≤0.12 to >1 | 60.3 | >1 >1 ≤0.12 to >1 | 73* | >1 >1 ≤0.12 to >1 | 60.3 | >1 >1 ≤0.12 to >1 | 73* |
| Clindamycin | 1 >1 ≤0.12 to >1 | 61.4 | 0.25 >1 ≤0.12 to >1 | 47.2 | 1 >1 ≤0.12 to >1 | 61.4 | 0.25 >1 ≤0.12 to >1 | 47.2 |
| Tetracycline | >4 >4 ≤0.5 to >4 | 59.3 | >4 >4 ≤0.5 to >4 | 82.2* | >4 >4 ≤0.5 to >4 | 59.3 | >4 >4 ≤0.5 to >4 | 82.2* |
| Chloramphenicol | ≤4 >16 ≤4 to >16 | 43 | ≤4 >16 ≤4 to >16 | 13.4 | ≤4 >16 ≤4 to >16 | 43 | ≤4 >16 ≤4 to >16 | 13.4 |
| Vancomycin | 0.5 0.5 ≤0.12 to 0.5 | 0 | 0.5 0.5 ≤0.12 to 0.5 | 0 | 0.5 0.5 ≤0.12 to 0.5 | 0 | 0.5 0.5 ≤0.12 to 0.5 | 0 |
| Levofloxacin | 0.5 1 ≤0.25 to 2 | 0.5 | 0.5 1 ≤0.25 to 2 | 0.7 | 0.5 1 ≤0.25 to 2 | 0.5 | 0.5 1 ≤0.25 to 2 | 0.7 |
| Rifampicin | ≤1 ≤1 ≤1 to >4 | 0.5 | ≤1 ≤1 ≤1 to >4 | 0 | ≤1 ≤1 ≤1 to >4 | 0.5 | ≤1 ≤1 ≤1 to >4 | 0 |
| TMP/SMX | ≤9.5 ≤9.5 ≤9.5 to 7.6 | 1.1 | ≤9.5 ≤9.5 ≤9.5 to 7.6 | 8.6* | ≤9.5 ≤9.5 ≤9.5 to 7.6 | 1.1 | ≤9.5 ≤9.5 ≤9.5 to 7.6 | 8.6* |

$^*$P<0.01.

Abbreviations: MIC, minimal inhibitory concentration; AC, Amoxicillin-clavulanate; TMP/SMX, Trimethoprim-sulfamethoxazole.

### Table 2. Patient age at the time of detection and tissue source for *Streptococcus pneumoniae* isolated from 189 patients infected with mucoid strains and 3,264 patients infected with non-mucoid strains

|                    | Mucoid (N = 189) | Non-mucoid (N = 3,264) |
|--------------------|------------------|------------------------|
| **Age, N (%)**     |                  |                        |
| Infant and preschool group | 63 (33.3)       | 2,577 (79.0)*          |
| Schoolchildren group | 27 (14.3)*       | 162 (4.9)              |
| Adult group         | 39 (20.6)*       | 183 (5.6)              |
| Elderly adult group  | 60 (31.8)*       | 342 (10.5)             |
| **Source of isolates, N (%)** |                  |                        |
| Respiratory tract sample | 168 (88.9)      | 3,161 (96.4)*          |
| Otorrhea sample     | 7 (3.7)*         | 27 (0.8)               |
| Genital swab        | 3 (1.6)*         | 3 (0.3)                |
| Ecthyma and abscess exudate | 3 (1.6)*     | 10 (0.3)               |
| Eye discharge       | 3 (1.6)*         | 15 (0.5)               |
| Invasiveness        | 5 (2.6)          | 47 (1.4)               |

$^*$P<0.01; $^*$P<0.05.
and abscess exudate, and eye discharge was significantly higher than that of non-mucoid isolates (P < 0.05) (Table 2).

**DISCUSSION**

Mucoid colonies of *S. pneumoniae* generate large amounts of capsular polysaccharides [9]. The capsule polysaccharide is the major virulence determinant of *S. pneumoniae* [11], particularly in hosts lacking type-specific antibodies of sufficient quantity or avidity [12]. Moreover, previous studies demonstrated that the mucoid phenotype, which is characterized by the presence of hyaluronic acid capsular polysaccharide in *S. pneumoniae* mucoid phenotype, is a key virulence determinant associated with severe *S. pneumoniae* infections [13, 14]. The mucoid phenotype of *S. pneumoniae* may also show higher pathogenicity than the non-mucoid phenotype; therefore, it is important to characterize *S. pneumoniae* mucoid and non-mucoid isolates. Previous studies demonstrated that *P. aeruginosa* isolates with mucoid phenotype are more susceptible than non-mucoid isolates to multiple antibiotics such as β-lactams and fluoroquinolones [8, 15]. Although the reason for antibiotic resistance was not identified in the present study, Ciofu et al. [8] provided a possible explanation for the antibiotic susceptibility of mucoid-overproducing *P. aeruginosa*; they proposed that non-mucoid resistant isolates co-existing with mucoid strains in biofilms may play a protective role. This explanation could be also applied to *S. pneumoniae*. Although similar findings have been reported in *P. aeruginosa* [8], to the best of our knowledge, this is the first report showing differences in antibiotic susceptibility patterns of mucoid and non-mucoid *S. pneumoniae* isolates.

*S. pneumoniae* infections are more common in infants, children, and elderly adults than in adults. In this study, it was observed that mucoid isolate infections were not age-dependent, whereas non-mucoid isolates were more frequent in infants and preschool children. Pastor et al. [16] reported an age-specific *S. pneumoniae* prevalence of approximately 35-45% in infants and

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**Table 3.** Antibiotic resistance rate (%) of non-mucoid strains isolated from outpatients according to their age group, including those with meningitis and nonmeningitis, in all samples

|                      | Infant and preschool group (N = 2,577) | Schoolchildren group (N = 162) | Adult group (N = 183) | Elderly adult group (N = 342) |
|----------------------|----------------------------------------|-------------------------------|----------------------|-------------------------------|
| Penicillin G nonmeningitis | 0                                      | 0                             | 0                    | 0                             |
| Penicillin G meningitis  | 0                                      | 0                             | 0                    | 0                             |
| AC                   | 0                                      | 0                             | 0                    | 0                             |
| Cefotaxime nonmeningitis | 0.5                                    | 1.2                           | 0.5                  | 0                             |
| Cefotaxime meningitis  | 0                                      | 0                             | 0                    | 0                             |
| Ceftriaxone nonmeningitis | 0.9                                    | 0                             | 0                    | 0                             |
| Ceftriaxone meningitis  | 0.7                                    | 0                             | 1.1                  | 0                             |
| Cefepine nonmeningitis  | 0                                      | 0                             | 0                    | 0                             |
| Cefepine meningitis    | 0                                      | 0                             | 0                    | 0                             |
| Meropenem             | 0.9                                    | 3.0                           | 2.2                  | 0.9                           |
| Erythromycin          | 88.4*                                  | 82.1                          | 72.7                 | 79.8                          |
| Clarithromycin        | 75.7*                                  | 66.7                          | 59.0                 | 63.5                          |
| Clindamycin           | 49.9†                                  | 37.7                          | 35.0                 | 38.3                          |
| Tetracycline          | 84.1†                                  | 75.3                          | 69.9                 | 73.1                          |
| Chloramphenicol       | 12.6                                   | 12.7                          | 8.7                  | 14.3                          |
| Vancomycin            | 0                                      | 0                             | 0                    | 0                             |
| Levofloxacin          | 0.1                                    | 0.6                           | 3.3                  | 3.5                           |
| Rifampicin            | 0                                      | 0                             | 0                    | 0                             |
| TMP/SMX              | 8.5                                    | 4.3                           | 5.5                  | 12.3                          |

*P < 0.05; †P < 0.01.
Abbreviations: AC, Amoxicillin-clavulanate; TMP/SMX, Trimethoprim-sulfamethoxazole.

**Table 4.** Antibiotic resistance rate (%) of non-mucoid strains isolated from outpatients according to their age group, for respiratory tract samples

|                      | Infant and preschool group (N = 2,525) | Schoolchildren group (N = 156) | Adult group (N = 163) | Elderly adult group (N = 317) |
|----------------------|----------------------------------------|-------------------------------|----------------------|-------------------------------|
| Penicillin G         | 0                                      | 0                             | 0                    | 0                             |
| AC                   | 0                                      | 0                             | 0                    | 0                             |
| Cefotaxime           | 0.4                                    | 0.6                           | 0.6                  | 0                             |
| Ceftriaxone          | 0.3                                    | 0                             | 0                    | 0                             |
| Cefepine             | 0.5                                    | 1.2                           | 1.2                  | 0                             |
| Meropenem            | 1.5                                    | 2.6                           | 2.5                  | 1.6                           |
| Erythromycin         | 90.1*                                  | 78.8                          | 71.8                 | 80.1                          |
| Clarithromycin       | 77.1*                                  | 62.2                          | 60.1                 | 61.2                          |
| Clindamycin          | 50.9*                                  | 34.6                          | 35.0                 | 37.9                          |
| Tetracycline         | 86.0*                                  | 73.1                          | 69.3                 | 72.2                          |
| Chloramphenicol      | 14.0                                   | 12.8                          | 8.6                  | 14.2                          |
| Vancomycin           | 0                                      | 0                             | 0                    | 0                             |
| Levofloxacin         | 0.2                                    | 0.6                           | 3.1                  | 4.7                           |
| Rifampicin           | 0                                      | 0                             | 0                    | 0                             |
| TMP/SMX             | 8.9                                    | 5.1                           | 4.9                  | 12.6                          |

*P < 0.01.
Abbreviations: AC, Amoxicillin-clavulanate; TMP/SMX, Trimethoprim-sulfamethoxazole.
Table 5. Antibiotic resistance rate (%) of non-mucoid strains isolated from outpatients according to their age group, including those with meningitis and nonmeningitis, with regard to invasiveness

| Antibiotic          | Infant and preschool group (N = 14) (%) | Schoolchildren group (N = 5) (%) | Adult group (N = 5) (%) | Elderly adult group (N = 23) (%) |
|---------------------|----------------------------------------|---------------------------------|------------------------|---------------------------------|
| Penicillin G        | 0                                      | 0                               | 0                      | 0                               |
| nonmeningitis       |                                        |                                 |                        |                                 |
| Penicillin G meningitis | 0                                      | 0                               | 0                      | 0                               |
| AC                  | 0                                      | 0                               | 0                      | 0                               |
| Cefotaxime nonmeningitis | 0                                      | 0                               | 0                      | 0                               |
| Cefotaxime meningitis | 0                                      | 0                               | 0                      | 0                               |
| Ceftriaxone nonmeningitis | 0                                      | 0                               | 0                      | 0                               |
| Cefepine nonmeningitis | 0                                      | 0                               | 0                      | 0                               |
| Cefepine meningitis | 0                                      | 0                               | 0                      | 0                               |
| Meropenem           | 0                                      | 0                               | 0                      | 0                               |
| Erythromycin        | 64.3                                   | 40.0                            | 60.0                   | 82.6                            |
| Clarithromycin      | 64.3                                   | 40.0                            | 40.0                   | 78.3                            |
| Clindamycin         | 28.6                                   | 0.0                             | 20.0                   | 39.1                            |
| Tetracycline        | 50.0                                   | 40.0                            | 60.0                   | 69.6                            |
| Chloramphenicol     | 7.1                                    | 40.0                            | 40.0                   | 13.0                            |
| Vancomycin          | 0                                      | 0                               | 0                      | 0                               |
| Levofloxacin        | 0                                      | 0                               | 0                      | 0                               |
| Rifampicin          | 0                                      | 0                               | 0                      | 0                               |
| TMP/SMX             | 7.1                                    | 0.0                             | 40.0                   | 13.0                            |

Abbreviations: AC, Amoxicillin-clavulanate; TMP/SMX, Trimethoprim-sulfamethoxazole.

Most non-mucoid isolates were obtained from patients with respiratory infections, which may suggest that mucoid isolates are more variable in terms of clinical infection site than non-mucoid phenotypes isolated from the respiratory system. However, systemic infection, which indicates invasiveness, was not associated with either of the mucoid and non-mucoid phenotypes.

In conclusion, to the best of our knowledge, this study is that mucoid and non-mucoid S. pneumoniae isolates differ significantly in terms of clinical site of isolation and age-specific prevalence. In particular, these results suggest that mucoid isolates tend to have greater antibiotics susceptibility than non-mucoid isolates. However, limited studies on the region-specific epidemiology of mucoid and non-mucoid isolates have been reported. Furthermore, this study was not designed to collect information regarding serotype distributions. Therefore, further studies to confirm the present findings are warranted.

Authors’ Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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