原　著

Characterization of *Streptococcus pneumoniae* Strains Isolated from Systemic Infections in Children

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Summary

Twenty-three cases of systemic pneumococcal infection diagnosed from October 1988 to September 1998 were analyzed retrospectively in order to characterize the epidemiology of systemic pneumococcal infections. The clinical diagnosis of those cases were 8 pneumonia, 8 meningitis, 3 septicemia, 3 septic arthritis, and 1 peritonitis. The patients ranged in age from 6 months to 21 years old (mean ± SD = 3 years, 6 months ± 5 years, 2 months), and 61% of the patients were younger than 24 months. Resistance to penicillin G (PCG) was detected in 57% of all cases. Resistance to cefotaxime (CTX), imipenem (IPM), erythromycin (EM), and clindamycin (CLDM) was 33%, 9%, 70%, and 65%, respectively. Of the 13 isolates resistant to PCG, 2 were resistant to IPM, 11 to EM and 11 to CLDM. Serotyping was performed on 17 isolates. The identified serotypes were 19 (6 isolates), 6 (5 isolates), 23 (3 isolates), 14 (2 isolates), and 5 (1 isolate). Eleven isolates resistant to PCG were limited to serotypes 6, 19, or 23. One patient had a recurrent episode of bacteremic pneumonia 7 months after the first episode. *Streptococcus pneumoniae* isolates from both episodes were compared by serotyping and pulsed-field gel electrophoresis with restriction digestion, and were confirmed as the same strain.

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Introduction

Streptococcus pneumoniae is the most frequent cause of community-acquired bacterial pneumonia, as well as a significant cause of meningitis, bacteremia, and acute otitis media in children. Over the past decade, the incidence of serious infections due to strains of *S. pneumoniae* with decreased susceptibility to penicillin and other beta-lactam antibiotics has been steadily increasing worldwide[1]. In addition to penicillin resistance, resistance to cefotaxime and ceftriaxone and clinical failures of their use in the treatment of pneumococcal meningitis have also been reported[2]. The emergence of these strains has made the selec-

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tion of antibiotics for the treatment of pneumococcal infections more difficult, and vaccination in order to prevent systemic pneumococcal infection is becoming even more important.

With this in mind, we performed a retrospective study of 23 children with systemic pneumococcal infections identified at Chiba Children's Hospital over a 10-year period, from October 1988 to September 1998, to evaluate antimicrobial susceptibility and to identify serotypes of *S. pneumoniae* isolates.

Materials and Methods

All the clinical isolates of *S. pneumoniae* reported here were recovered from October 1988 to September 1998 in the microbiology laboratory of Chiba Children's Hospital, a 200-bed scaled tertiary-care hospital located in the east-central part of Chiba City.

Isolates were identified as *S. pneumoniae* by their susceptibility to optochin and their bile solubility. Minimum inhibitory concentrations (MICs) were determined by broth microdilution assay in cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood, as described by the National Committee for Clinical Laboratory Standards (NCCLS)[3]. For the standard quality control strain, *S. pneumoniae* ATCC49619 was tested. Isolates were defind as antibiotic susceptible, intermediate, or resistant, according to the 1998 guidelines (M100-S8) of NCCLS[3]. Penicillin-resistant pneumococcal isolates included both intermediate and resistant isolates.

Isolates of *S. pneumoniae* were serotyped or serogrouped in Nagasaki University Hospital or Juntendo University Hospital by using the capsular swelling method with antisera obtained from Stantens seruminstitut (Copenhagen, Denmark).

Pulsed-field gel electrophoresis (PFGE) was performed for the isolates from a recurrent case with pneumonia as described previously[4] with the following modifications : isolates were grown for 6 h in 6 ml of Tripticase soy broth. Preparation of agarose-embedded genomic DNA was done by using a GenPath™ group I reagent kit (Bio-Rad, Tokyo). Chromosomal DNA were digested with *Sma*I and *Apa*I (Takara, Tokyo). The patterns obtained with each enzyme were compared. DNA fragments were separated on 1% agarose gels using the GenPath™ system (Bio-Rad, Tokyo), with running conditions of 6 V/cm at 14 ℃ and a switching time of 1 to 14 sec for 18.5 hours. PFGE patterns were digitized and the positions were analyzed using the DENDRON™ version 2.2 (Solltech, IA)

Results

1. Clinical properties

A total of 23 *S. pneumoniae* isolates were recovered from normally sterile sites. The isolates included 10 with blood isolates, 1 with lung tissue isolate, 7 with both cerebrospinal fluid (CSF) and blood isolates, 1 with CSF isolate alone, 3 with synovial fluid, and 1 with continuous ambulatory peritoneal dialysis (CAPD) fluid. The clinical diagnoses were 8 pneumonia, 8 meningitis, 3 septicemia, 3 septic arthritis, and 1 peritonitis.

Twelve patients were male, and eleven were female. Fig. 1 shows the age distribution of all the patients. The patients ranged in age from 6 months to 21 years old (mean ± SD = 3 years, 6 months ± 5 years, 2 months), and 61% of the patients were younger than 24 months. 10 of the 23 children (43%) had underlying conditions that may have contributed to their risk of infection. Three of the 23 patients died (case fatality rate, 13%).

Fig. 1 Number of Cases of Systemic Pneumococcal Infections by Age.

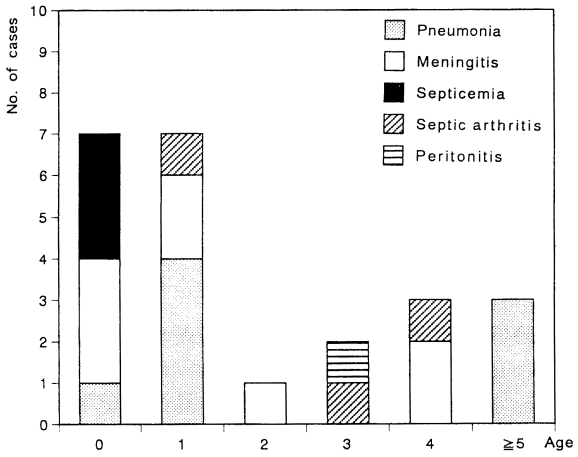

Fig. 2 Number of Isolates of Systemic Pneumococcal Infections by Serotype and Penicillin G Susceptibility.

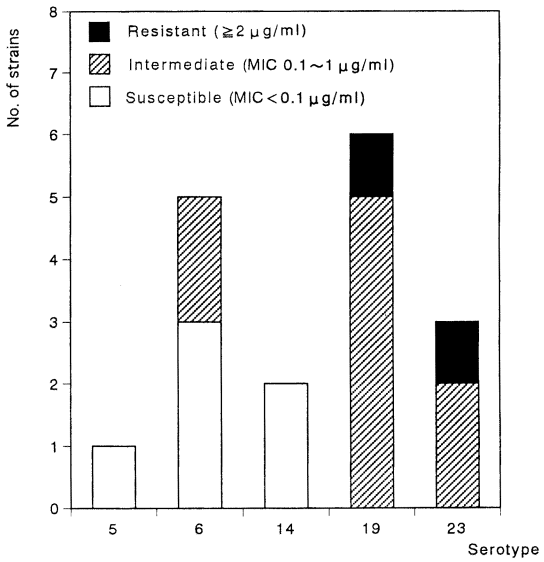

Table Susceptibilities to selected antibiotics of *S. pneumoniae* isolated from systemic infections

Antibiotic	No. of Isolates	MIC Range (μg/ml)	MIC_{50}	MIC_{90}	Breakpoint *	No. of Strains (%)		
						Susceptible	Intermediately resistant	Highly resistant
Penicillin G	23	≦ 0.015 ～ 2	0.5	1	≧ 0.1	10 (44)	11 (48)	2 (9)
Cefotaxime	15	≦ 0.13 ～ 1	0.25	1	≧ 1	10 (67)	5 (33)	0 (0)
Imipenem	23	≦ 0.06 ～ 0.25	≦ 0.06	0.13	≧ 0.25	21 (91)	2 (9)	0 (0)
Erythromycin	23	≦ 0.06 ～≧ 8	2	≧ 8	≧ 0.5	7 (30)	0 (0)	16 (70)
Clindamycin	23	≦ 0.13 ～≧ 16	0.5	≧ 16	≧ 0.5	8 (35)	5 (22)	10 (44)

*NCCLS (M100-S8) standards of intermediate-level resistance

2. Antimicrobial susceptibilities

MICs of five antimicrobial agents tested for the control strain were within the acceptable quality control ranges provided by NCCLS[3].

The table summarizes the susceptibility to five antimicrobial agents examined. The overall rate of resistance to penicillin G was 57%, of which 48% had intermediate resistance and 9% had high-level resistance. There were no significant differences in penicillin resistance level among isolated sites. The proportions of isolates resistant to cefotaxime, imipenem, erythromycin and clindamycin was 33%, 9%, 70%, and 65%, respectively. All isolates resistant to cefotaxime (5 isolates) also demonstrated resistance to penicillin. Seventeen isolates (74% of all the cases) were resistant to 2 or more classes of antimicrobial agents.

3. Serotyping

Serotyping was performed on 17 isolates. The identified serotypes were 19 (6 isolates), 6 (5 isolates), 23 (3 isolates), 14 (2 isolates), and 5 (1 isolate). Fig. 2 shows the relationship between serotypes and peni-

cillin susceptibilities. All eleven isolates resistant to penicillin were limited to serotypes 6, 19, or 23.

4. PFGE analysis of isolates from a case with a recurrent episode

One patient had 2 episodes of bacteremic pneumonia. The time interval between the first and second episodes was about 7 months. Both isolates were the same serotype (6) and showed the same pattern of antimicrobial susceptibility. PFGE analysis with *Sma*I or *Apa*I restriction digestion of those isolates subsequently characterized them as identical isolates.

Discussion

S. pneumoniae that are not only resistant to penicillins and cephems but also multidrug-resistant have increased worldwide in recent years. At our hospital, we have also seen an increase in the frequency of infections due to pneumococci resistant to penicillin, cefotaxime, erythromycin, and clindamycin. We found the overall rates of resistance to penicillin and to cefotaxime to be 57% and 33%, respectively. It is noteworthy that similar rates of resistance to penicillin have been reported in recent nationwide hospital-based surveys of bacterial meningitis in Japan[5]. Therefore, the increasing frequency of penicillin, cefotaxime, and multidrug-resistant *S. pneumoniae* has required changes in the empirical approach to antibiotic selection when treating such infections. In particular, the common antibiotic treatment for bacterial meningitis of ampicillin plus cefotaxime has recently seen a greater failure rate. With this in mind, while waiting for the results of susceptibility testing, we now recommend panipenem/betamipron therapy for meningitis patients admitted to our hospital when Gram-positive cocci are seen on CSF smears and rapid pneumococcal antigen detection is positive[6].

Most pneumococcal infections among children are caused by a limited number of serotypes[1)7)]. Among our patients, most isolates of *S. pneumoniae* belong to serotypes 6, 14, 19, or 23, which are also the most common "childhood types". And, as others have observed, all 11 of our isolates of *S. pneumoniae* that were resistant to penicillin were serotypes 6, 19, or 23. As management of pneumococcal infections has become more difficult, the prevention of infection by vaccination has become more important. In our study, 61% of the systemic pneumococcal infections were found in children younger than 24 months old. Unfortunately, a poor response to the polysaccharide antigen is well recognized in this population[7]. The success of the conjugate vaccines to *Haemophilus influenzae* type b has encouraged work on similar vaccines for *S. pneumoniae*. For that purpose, it is necessary to select candidate vaccine types and then monitor their susceptibility over time with community-based surveillance of pneumococcal serotypes.

In this study, we encountered a patient who had a recurrent episode of bacteremic pneumonia about 7 months after the first episode. Both serotypes and PFGE patterns revealed that the isolates from both episodes were concordant. However, it is possible that the initial therapy may have been adequate to treat pneumonia but inadequate to eradicate the colonization of the nasopharynx and/or sinuses, or perhaps the patient was reinfected with the identical genotyped strain. The findings of this case suggest that cultures of the nasopharynx or sputum are needed as a follow-up for such patients. PFGE is a useful tool not only for epidemiological tracking but also to elucidate the etiology of recurrence.

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小児期肺炎球菌性全身感染症の臨床細菌学的検討

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要　旨

当院開設以来の10年間に経験した肺炎球菌性全身感染症の肺炎8例，化膿性髄膜炎8例，敗血症3例，化膿性関節炎3例，腹膜炎1例の合計23例について臨床細菌学的検討を行った．患者の年齢は6カ月から21歳に分布しており，14名（61%）が2歳以下の乳幼児であった．57%が広義のペニシリン耐性菌であった．cefotaxime（CTX），imipenem（IPM），erythromycin（EM），およびclindamycin（CLDM）に対する耐性率は，33%，9%，70%，および65%の各々であった．血清型の同定を実施した17株の内訳は，19型（6株），6型（5株），23型（3株），14型（2株），および5型（1株）の各々であった．ペニシリン耐性11菌株の血清型は，6，9，23型のいずれかに属していた．最初のエピソードから約7カ月後，再度肺炎（菌血症を伴う）を発症した1例を経験した．この両菌株は，血清型とパルスフィールド電気泳動法を用いたDNAパターンが一致していた．

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