ORIGINAL ARTICLE 10.1111/j.1469-0691.2009.02935.x

High prevalence of multidrug-resistant Pneumococcal molecular epidemiology network clones among *Streptococcus pneumoniae* isolates from adult patients with community-acquired pneumonia in Japan

S. Imai[1], Y. Ito[1], T. Ishida[2], T. Hirai[1], I. Ito[1], K. Maekawa[1], S. Takakura[3], Y. Iinuma[3], S. Ichiyama[3], M. Mishima[1] and the Kansai Community Acquired Pneumococcal Pneumonia Study Group

1) *Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto,* 2) *Kurashiki Central Hospital, Okayama and* 3) *Department of Clinical Laboratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Abstract

A total of 141 *Streptococcus pneumoniae* isolates from patients with community-acquired pneumonia were collected from May 2003 through October 2004. The strains were tested for antimicrobial agent susceptibility, serotype and genotype by multilocus sequence typing (MLST) and the presence of the pilus *rlrA* islet. MLST analysis identified 49 sequence types (STs), of which 19 were novel. eBURST analysis using the MLST database (3773 STs) grouped the isolates into 27 clonal complexes and three singletons. A total of 92 (65.2%) isolates were related to ten of the 43 international Pneumococcal Molecular Epidemiology Network (PMEN) clones; major clones found were multidrug-resistant Netherlands^{3}-31 [clonal complex (CC) 180], Taiwan^{19F}-14 (CC271), Taiwan^{23F}-15 (CC242), and Colombia^{23F}-26 (CC138) (the latter new to Asia). We adopted univariate and multiple logistic regression models to identify factors associated with PMEN CCs. Multivariate analysis showed that multidrug resistance (OR 6.3; 95% CI 2.0–22.9), carriage serogroups (OR 7.2; 95% CI 2.5–23.7), prevalence of *rlrA* (OR 12.6; 95% CI 3.6–59.7) and central nervous system-related disorders (OR 7.7; 95% CI 1.8–48.4) were independently associated with PMEN CCs. Our data indicate that multidrug-resistant PMEN clones are highly prevalent, contributing to the high frequency of resistance to antimicrobial agents in Japan, and suggest that certain predisposing factors in patients contribute to the high frequency of these clones.

Keywords: Carriage, host factor, multidrug resistance, multilocus sequencing typing, *rlrA*, serotype, *Streptococcus pneumoniae*
Original Submission: 2 January 2009; **Revised Submission:** 5 March 2009; **Accepted:** 9 April 2009
Editor: J-L. Mainardi
Article published online: 20 August 2009
Clin Microbiol Infect 2009; **15:** 1039–1045

Corresponding author and reprint requests: Y. Ito, Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, 54, Kawahara, Shogoin, Sakyo-ku, Kyoto, 606-8507, Japan
E-mail: yutaka@kuhp.kyoto-u.ac.jp

Introduction

Several clinical and epidemiological studies of *Streptococcus pneumoniae* have revealed that a small number of dominant resistant clones is responsible for the spread of *S. pneumoniae* resistance to different classes of antimicrobials [1]. Although increased antibiotic use has been the most important selective force driving the appearance and circulation of resistant strains [2], other, as yet unidentified, mechanisms of dissemination and successful establishment of such clones are likely.

Multilocus sequencing typing (MLST) is a nucleotide sequence-based approach for characterizing isolates of bacteria and other organisms via the internet (http://www.mlst.net/), having the advantage over pulsed-field gel electrophoresis of ease of manipulation and unambiguous and convenient comparison [3]. The Pneumococcal Molecular Epidemiology Network (PMEN) includes 26 multidrug-resistant and 17 susceptible *S. pneumoniae* clones found worldwide using MLST (http://www.sph.emory.edu/PMEN/) [4]. In Asian countries, Spain^{23F}-1, Spain^{6B}-2, Taiwan^{19F}-14, Taiwan^{23F}-15 and Netherlands^{3}-31 have been spreading, which could be a major reason for the rapid increases in penicillin- and macrolide-resistant *S. pneumoniae* strains [5–8].

Because the ecological niche of *S. pneumoniae* is the human nasopharynx, horizontal transfer of antimicrobial resistance genes occurs easily via commensal organisms that reside in the nasopharynx [9]. Various pneumococcal virulence factors have a role to play in bacterial colonization and disease [10,11].

Although at least 90 different capsular serotypes have been described, their carriage potentials differ [12]. Bruegg-

eman *et al.* have performed a meta-analysis to calculate the ORs for different serogroups/serotypes causing invasive diseases. Using serotype 14 as the reference for serogroups/serotypes, it was concluded that 1, 5 and 7 cause more invasive disease, whereas 18, 9, 8, 33, 38, 19, 6, 23, 3 and 15 are responsible for carriage, irrespective of any temporal change or major geographical differences [13]. Recent studies have revealed that pneumococcal pili encoded by the *rlrA* islet also enhance adherence to lung epithelial cells [14] and that the presence of the *rlrA* gene was correlated more with MLST genotypes than with serotypes; the *rlrA* gene was also found to be carried in several PMEN clones [15–17].

Furthermore, different host factors, such as male gender, smoking, recent antibiotic use, attendance at day care centres, as well as various underlying diseases, all predispose to pneumococcal colonization [10,18,19].

In the present study, we performed MLST for *S. pneumoniae* isolates collected from patients with community-acquired pneumonia (CAP) in Japan in order to investigate clonal spread of the bacteria, especially the PMEN clones. We then employed univariate and multiple logistic regression models to identify microbiological and clinical risk factors associated with the prevalent PMEN clones compared with the non-PMEN isolates.

Material and Methods

Study design, bacterial strains and clinical data

total of 141 pneumococcal isolates that had been prospectively collected from patients >15 years old diagnosed with CAP between May 2003 and October 2004 in Japan have been previously described [20]. In brief, the sources were sputum (n = 132), blood (n = 6), transtracheal aspiration (n = 2) and bronchoalveolar lavage (n = 1). On the basis of sociodemographic and clinical data, we calculated the Charlson comorbidity index [21] and the Pneumonia Severity Index [22]. The study was approved by the Ethics Committees of each hospital.

MLST

The bacterial strains were analysed by MLST, as described elsewhere [3]. In order to determine nucleotide sequences of new alleles, we used three new primers: xpt2-up, TCGCTCGTAATAGTTTTATC, ddl2-up, AAATGCCTTACGTTGGTTGC, and ddl2-dn, GCGCT TGTCAAAACTTTCCT. The sequence types (STs) were obtained by reference to the MLST database (http://www.mlst.net/). New alleles and new STs were submitted to the curator of the database and were assigned designations. ST180, ST2808 and ST2809 isolates in the present study have already been described in our previous report [6].

eBURST analysis for clonal complexes

To visualize the genetic relationships among the different strains, a dendrogram was generated from the distance matrix between STs by using the unweighted pair group method with arithmetic averages on the PubMLST website (http://pubmlst.org/). eBURST was carried out to estimate the relationship of isolates with software (http://spneumoniae.mlst.net/eburst/). Clonal complexes (CCs) consisted of eBURST sets in which all STs share six of seven identical alleles with at least one other ST within the group [single-locus variants (SLV)]. We ran eBURST with default settings associating each ST with a CC on the entire MLST database (3773STs) and STs newly assigned within our dataset. In this study, we named CCs according to an ST number of the eBURST predicted founder, defined as being the ST with the greatest number of SLVs, a smaller number of STs in a group consisting of two different STs or a singleton itself.

Antimicrobial susceptibility and serotyping

We have already reported the antimicrobial agent susceptibilities and serotypes of 141 isolates [20,23]. In brief, non-susceptibility to penicillin, erythromycin, ceftriaxone, clindamycin, minocycline and trimethoprim-sulfamethoxazole was recorded in 64 (45.4%), 118 (83.7%), five (3.5%), 77 (54.6%), 116 (82.3%) and 41 isolates (29.1%), respectively. Multidrug resistance includes three or more antimicrobial non-susceptibilities. According to a meta-analysis [13], we divided the serotypes into three different groups with different invasive disease potential in our collection. These were high (invasive), serotype 7; medium, 4, 9N, 9V and 14; and low (carriage), 3, 6A, 6B, 15, 19A, 19F, 23A and 23F.

Detection of the *rlrA* islet

The genomic location of the *rlrA* islet was determined by simultaneously assessing five PCR amplifications, as previously reported [15,16]. If the *rlrA* islet or parts of it were absent, the PCR product using these primers would be similar in length to DNA as mainly type A (1310 bp), type B (1912 bp) or type C (2616 bp).

Statistical analysis

Statistical analysis was performed using JMP version 6.03 (SAS Campus Drive, Cary, NC, USA). Related factors and outcomes for PMEN clonal genotypes were identified by comparison with non-PMEN genotypes using univariate analysis.

Associations between categorical variables were tested by the Pearson χ^2 or Fisher's exact test, when appropriate. Means of continuous variables were compared by Student's t-test or Mann–Whitney U-test, as appropriate. ORs and the

respective 95% CIs were computed as an estimate of the relative risk. Variables were selected for further analysis if their probability values were <0.05 by univariate analysis. In order to avoid problems of multi-colinearity, correlations among predictor variables were analysed first and then multiple logistic regression analysis was performed. All tests were two-tailed and probability values of ≤0.05 were considered statistically significant.

Results

MLST and the BURST analysis

Among 141 isolates, 49 STs were found in the MLST analysis and a dendrogram was constructed (Fig. 1). We identified nine new allele sequences (*aroE*112, *gdh*171, *gki*175, *gki*192, *xpt*233, *xpt*234, *ddl*271, *ddl*279 and *ddl*281) and 19 new STs, of which eight contained these alleles (STs 2925, 2983, 3110, 3112, 3116, 3193, 3194 and 3542) and 11 contained new allelic profiles of the known alleles (STs 2922, 2923, 2924, 3109, 3111, 3113, 3114, 3115, 3117, 3118 and 3543). The frequencies of the STs with multiple isolates did not differ in any of the participating hospitals. eBURST analysis revealed the presence of 27 CCs and three singletons, encompassing 10 PMEN CCs (92 isolates, 65.2%, Table 1).

Serotypes

Almost every ST was well correlated with one serotype except for ST236, ST242 and ST2924, indicating serotype switching. On the other hand, in numerous serotypes, isolates within the same serotype contained multiple STs (Fig. 1). Carriage serotypes were significantly associated with PMEN CCs (Table 2).

Antimicrobial susceptibility

Antimicrobial agent susceptibilities for each CC are shown in Table 1. PMEN clones among penicillin non-susceptible, erythromycin non-susceptible and multidrug-resistant isolates were found in 49 (76.6%), 87 (73.7%) and 84 (77.8%) isolates, respectively. Resistance to various antimicrobials

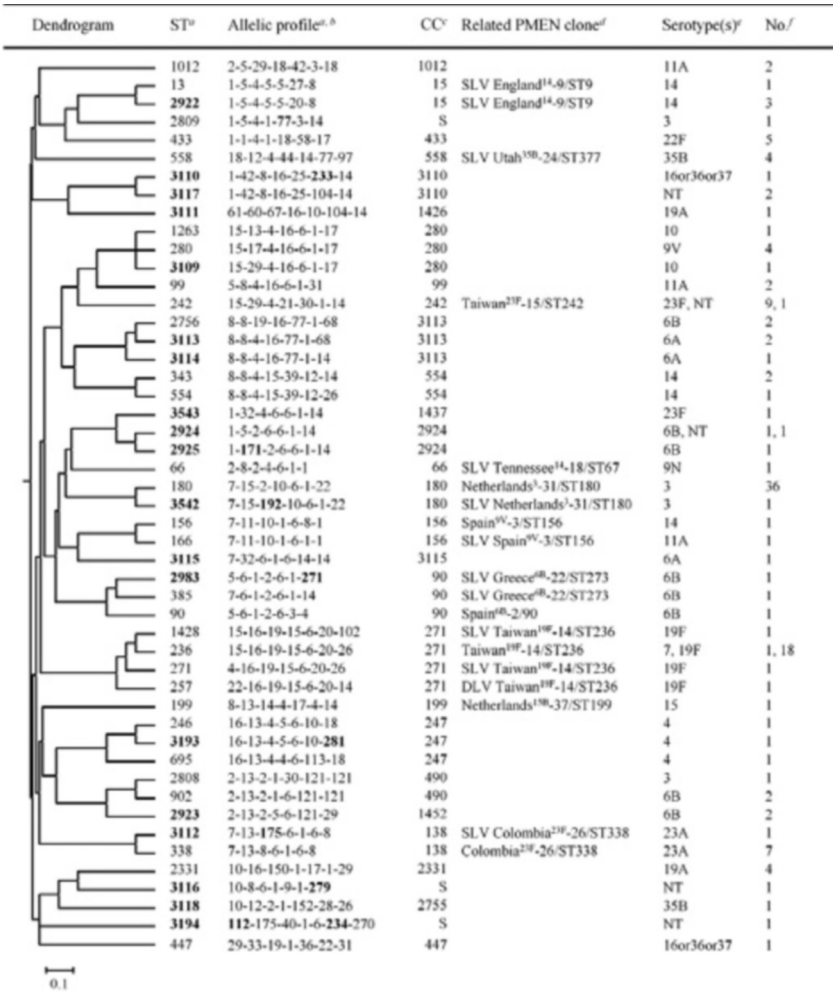

FIG. 1. Genetic relationships, multilocus sequence typing profiles and serotypes among the 49 sequence types (STs) of 141 *Streptococcus pneumoniae* isolates from community-acquired pneumonia patients, Japan, 2003–2004. The dendrogram was generated from a distance matrix between STs by using the unweighted pair group method with arithmetic averages. [a]New STs and alleles in bold. [b]In the order *aroE-gdh-gki-recP-spi-xpt-ddl*. [c]CC, clonal complex; S, singleton. [d]SLV, single locus variants; DLV, double locus variants. [e]NT, non-typeable. [f]Number of isolates.

TABLE 1. Non-susceptibility and presence of *rlrA* in each clonal complex among *Streptococcus pneumoniae*

Clonal complex	No. of isolates	Non-susceptible rate (%)							Prevalence of *rlrA* (%)
		PEN	ERY	CRO	CLI	MIN	SXT	MDR[a]	
PMEN clonal complex									
180 Netherlands[3]-180	37		95		95	89			
271 Taiwan[19F]-236	22	91	100	5	32	100	73	95	91
242 Taiwan[23F]-242	10	100	100		70	100	30	100	100
138 Colombia[23F]-338	8	100	100		13	100		100	
15 England[14]-9	4	75	100	25	50	75	25	75	
558 Utah[35B]-377	4	100	50		25	50	25	50	100
90 Spain[6B]-90	3	67	100		67	100	67	67	100
156 Spain[9V]-156	2	100	100	50	50	100	100	100	100
66 Tennessee[14]-67	1								
199 Netherlands[15B]-199	1		100		100	100			
Non-PMEN clonal complex									
280	6		83		50	50			
433	5		20		20	20			
3113	5	60	80	20	80	100	80	80	
2331	4		25		25	50			
247	3		33			33			100
490	3	100	67		33	100	100	100	
554	3	100	100	33	100	100		100	
2924	3	33	100		100	100	67	67	
3110	3	67	67			67	100	67	
99	2					50			
1012	2		100			100			
1452	2		100		50	100	50	50	
447	1								
1426	1	100	100		100	100		100	100
1437	1	100	100		100	100	100	100	
2755	1		100		100		100	100	
2809	1		100			100	100	100	
3115	1	100	100			100		100	
3116	1								
3194	1								

Data are presented as numbers or % of isolates. Clonal complex of each singleton is named in accordance with its sequence type number.
PMEN, Pneumococcal Molecular Epidemiology Network; PEN, penicillin; ERY, erythromycin; CRO, ceftriaxone; CLI, clindamycin; MIN, minocycline; SXT, trimethoprim-sulfamethoxazole.
[a]Multidrug resistance, including three or more antimicrobial non-susceptibilities except for combined ERY–CLI–MIN resistance.

and combinations of erythromycin, clindamycin and minocycline was significantly associated with PMEN clones (Table 2). Strains with resistance to combinations of erythromycin, clindamycin and minocycline were found in CC180 (33 isolates), CC90 (1), CC199 (1), CC271 (1), CC280 (1), CC433 (1), CC1452 (1) and CC2924 (1). Most of the multidrug-resistant isolates, including those with erythromycin, clindamycin and minocycline resistance, belonged to carriage serotypes (90/108 isolates, 83.3%). Among these carriage serotypes, multidrug resistance was found more frequently in PMEN CCs (75/79 isolates, 94.9%) than in non-PMEN CCs (15/20 isolates, 75%, p 0.02).

Prevalence of *rlrA*

A total of 43 (30.5%) isolates were positive for the *rlrA* gene, especially in PMEN CCs (Table 2). PCR analysis showed the *rlrA* islet consistently inserted into the same genomic region. Among isolates without *rlrA*, types A, B and C comprised 92, one and five isolates, respectively. The frequency of *rlrA* was correlated more often with MLST genotypes than with serotypes. Serotype 4 (three isolates) and 7 (one isolate) had *rlrA* in all serospecific isolates, whereas serotypes 6B, 11A, 14, 19A, 19F, 23F, 35B and non-typeable had *rlrA* only in some of the serospecific isolates. Among carriage serotypes, the *rlrA* gene was more common in PMEN CCs (39.2%) than in non-PMEN CCs (5.0%, p 0.003).

Factors associated with the PMEN clones

Factors associated with PMEN CCs compared with non-PMEN CCs upon univariate analysis are shown in Table 2. Multiple logistic regression analysis revealed that multidrug resistance including erythromycin, clindamycin and minocycline resistance (OR 6.3, 95% CI 2.0–22.9, p 0.0026), carriage serotypes (OR 7.2, 95% CI 2.5–23.7, p 0.0005), the presence of *rlrA* (OR 12.6, 95% CI 3.6–59.7, p 0.0003) and central nervous system (CNS) disorders (OR 7.7, 95% CI 1.8–48.4, p 0.014) were all significantly associated with the PMEN CCs.

Discussion

Several molecular epidemiological studies of *S. pneumoniae* have shown that the frequencies of antimicrobial-resistant

TABLE 2. Clinical and microbial characteristics and factors associated with Pneumococcal Molecular Epidemiology Network (PMEN) clones

		Infection due to				
		PMEN	Non-PMEN			
Characteristics	All cases *N* = 141	*n* = 92	*n* = 49	OR	95% CI	p
Age (years)	66.7 ± 15.1	65.7 ± 15.2	68.6 ± 14.9			0.28
Male gender	67.4	66.3	69.4	0.87	0.4–1.8	0.71
Mortality	5.8	5.6	6.2	0.90	0.2–3.9	1.00
Charlson index (point)	1.44 ± 1.6	1.4 ± 1.2	1.4 ± 2.1			0.99
PSI[a] score (point)	84.3 ± 36.3	84.0 ± 33.6	85.0 ± 41.4			0.88
Asthma	13.5	14.1	12.2	1.18	0.4–3.3	0.75
COPD[b]	19.9	20.7	18.4	1.16	0.5–2.8	0.75
Chronic heart disease	14.2	15.2	12.2	1.29	0.5–3.6	0.63
CNS[c] disorders	15.6	20.7	6.1	3.99	1.1–14.2	0.03
Previous antibiotic use	10.6	13.0	6.1	2.30	0.6–8.6	0.26
Previous macrolide use	5.0	7.6	0.0			0.10
Smoking status						
Current	24.4	23.5	26.2	0.87	0.4–2.0	0.74
Former	35.4	37.7	31.0	1.35	0.6–3.0	0.46
Sputum collection	93.6	93.5	93.9	0.93	0.2–3.9	1.00
Serogroup						
Invasive[d]	0.7	1.1	0.0			1.00
Medium[e]	11.4	6.5	20.4	0.27	0.1–0.8	0.01
Carriage[f]	70.2	85.9	40.8	8.81	3.9–20.0	<0.001
Presence of *rlrA*	30.5	42.4	8.2	8.28	2.7–24.9	<0.001
Antimicrobial non-susceptibility						
Penicillin	45.4	53.3	30.6	2.58	1.2–5.4	0.01
Erythromycin	83.7	94.6	63.3	10.10	3.5–29.5	<0.001
Ceftriaxone	3.6	3.3	4.1	0.79	0.1–4.9	1.00
Clindamycin	54.6	62.0	40.8	2.36	1.2–4.8	0.02
Minocycline	82.3	91.3	65.3	5.58	2.2–14.2	<0.001
SXT[g]	29.1	27.2	32.7	0.77	0.4–1.6	0.50
ERY-CLI-MIN[h]	28.4	39.1	8.2	7.23	2.4–21.8	<0.001
MDR[i]	48.2	52.2	40.8	1.58	0.8–3.2	0.20
Multidrug resistance	76.6	91.3	49.0	10.94	4.4–27.3	<0.001

Data are presented as mean ± SD or % of patients. Statistical analyses were performed using the non-PMEN CCs group as a comparison.
[a]Pneumonia severity index.
[b]Chronic obstructive pulmonary disease.
[c]Central nervous system.
[d]Serogroup 7.
[e]Serogroups 4, 9 and 14.
[f]Serogroups 3, 6, 15, 19, 23.
[g]Trimethoprim-sulfamethoxazole.
[h]Combined erythromycin–clindamycin–minocycline resistance.
[i]Multidrug resistance except for combined ERY–CLI–MIN resistance.

PMEN clones 1–26 were 55.5–71.8%, 51.2% and 69.2% among penicillin non-susceptible, erythromycin- and multidrug-resistant isolates, respectively [24–27]. Also in the present study, antimicrobial-resistant PMEN clones 1–26, excluding CC180 and CC199, were included in 76.6% of penicillin non-susceptible, 43.2% of erythromycin- and 38.3% of multidrug-resistant isolates.

Furthermore, the frequency of PMEN CCs was high (65.2%) overall in these *S. pneumoniae* isolates, which contributes to the high frequency of antimicrobial-resistant *S. pneumoniae* strains in the community in Japan.

CC180 was first described as a penicillin- and erythromycin-susceptible invasive isolate (http://www.sph.emory.edu/PMEN/).We previously reported that multidrug-resistant Netherlands^{3}-31/ST180 (CC180) was the most prevalent clone in this cohort study [6]. This is a variant of CC180 clone with erythromycin–clindamycin–minocycline resistance that is currently spreading in Japan.

Previous molecular epidemiological studies of *S. pneumoniae* clones have shown that penicillin- and erythromycin-resistant Taiwan^{19F}-14/ST236 (CC271) and Taiwan^{23F}-15/ ST242 (CC242) have been prevalent in Asia for some time [5,7,8]. These multidrug-resistant clones were also the second and the third most common isolates in the present study.

Colombia^{23F}-26/ST338 (CC138) was originally reported as an isolate with susceptibility to penicillin (MIC, 0.064 mg/L) (http://www.sph.emory.edu/PMEN/); in contrast, the CC138 isolates in the present study have acquired multidrug resistance (Table 1) and the serotype of all the CC138 isolates switched to 23A (Fig. 1). Recently, a new association of serotype 23A isolates with Colombia^{23F}-26/ST338 has emerged in the USA after introducing the seven-valent pneumococcal conjugate vaccine [28]. Although Colombia^{23F}-26/ST338 has not been reported in Asian countries so far, and the seven-valent pneumococcal conjugate vaccine has not yet been introduced in Japan, multidrug-resistant serotype 23A/

CC138 isolates were the fourth most prevalent in the present study. CC138 needs to be monitored closely, as it seems to be a very biologically successful clone in Japan as well as in the USA.

Other PMEN clones in the present study have been isolated predominantly in European countries and the USA [24,26,29]. Except for CC66, these CCs also developed multidrug-resistant isolates (Table 1). As a result, almost all PMEN CCs in the present study were resistant to multiple antimicrobial agents (Table 2).

In the present study, multidrug-resistant isolates were observed more frequently in most of the carriage serotypes (90.9%) than in medium carriage and invasive serotypes (52.9%). Among ten PMEN CCs, carriage serotypes were observed in six, three of which have already been reported to be multidrug-resistant clones (CC90, CC271 and CC242) and the remaining three CCs (CC180, CC138 and CC199) developed multidrug-resistant isolates. Four major PMEN CCs (CC180, CC271, CC242 and CC138) had carriage serotypes and multidrug resistance (Table 1).

As a result, the rates of carriage serotypes as well as multidrug resistance in the PMEN CCs were higher than in non-PMEN CCs (Table 2). Thus, PMEN clones with carriage serotypes probably acquire multidrug resistance easily in the community.

Our study has also shown that the presence of *rlrA* correlated to particular CCs, especially in PMEN clones (Table 1). Among carriage serotypes, *rlrA* was much more frequent in PMEN CCs (39.2%) than in non-PMEN CCs (5.0%), suggesting that *rlrA* confers an advantage for dissemination even among carriage-potential and antimicrobial-resistant isolates.

We expected that the CAP patients infected with PMEN clones were likely to have possessed risk factors for colonization and antimicrobial resistance, such as smoking, previous antimicrobial use, and reported comorbidities. However, these patients manifested only CNS disorders, which are likely to be associated with aspiration pneumonia. Even a small amount of nasal carriage of bacteria leads to aspiration pneumonia in a murine stroke model [30]. We speculate that patients with CNS disorders may not be especially frequently colonized by PMEN clones, but may more easily develop aspiration pneumonia after colonization.

This study has some limitations. First, our sample size was not sufficiently large to identify various risk factors. Second, some non-PMEN CCs may be included in the PMEN CCs in the future. For example, CC433/serotype 22F has been reported in meningitis patients from Poland [31], invasive isolates in the USA [29] and clinical isolates in Scotland [32]. Even when we included CC433 in the PMEN CCs, the microbiological and host factors associated with clonal spread remained significant in a multiple logistic regression model (data not shown). Third, we could not clarify why CC180 and CC138 without *rlrA* had any other selective advantages than carriage serotypes and multidrug resistance in the present study.

In conclusion, among *S. pneumoniae* isolates from adult patients with CAP in Japan, multidrug-resistant PMEN clones were highly prevalent, contributing to the high frequency of antimicrobial resistance in Japan. Carriage potential serotypes, the presence of *rlrA*, multidrug resistance and patients with CNS disorders were significantly associated with CAP patients infected with PMEN CCs. Continuous surveillance of phenotypic data, genotyping and clinical manifestations and investigations of pneumococcal virulence factors for prevalent clones will be necessary to understand the spread of successful clones.

Acknowledgements

We acknowledge the use of the pneumococcal MLST database, which is located at Imperial College London and is funded by the Wellcome Trust, with special thanks to C. Bishop for assigning numbers for new alleles and STs found in this study. The Kansai Community Acquired Pneumococcal Pneumonia Study Group includes the following investigators and centres: T. Ishida (Kurashiki Central Hospital), K. Tomii and I. Gohma (Kobe Japan Post Hospital), H. Tomioka (Nishi-Kobe Medical Centre), M. Hayashi (Kobe City Medical Centre General Hospital), H. Kagioka (Kitano Hospital), M. Hirabayashi (Hyogo Prefectural Tsukaguchi Hospital), K. Onaru (Sakai Municipal Hospital), K. Maniwa (Tenri Hospital), Y. Ito, Toyohiro Hirai, M. Mishima (Kyoto University Hospital) and I. Ito (Ono Municipal Hospital).

Transparency Declaration

Kansai Community Acquired Pneumococcal Pneumonia Study Group has received research funding from Pfizer, Inc., Abbott Japan Co., Ltd. and Taisho Toyama Pharmaceutical Co., Ltd. All other authors have no conflicts of interest.

References

1. Klugman KP. The successful clone: the vector of dissemination of resistance in *Streptococcus pneumoniae*. *J Antimicrob Chemother* 2002; 50 (suppl): 1–5.
2. Albrich WC, Monnet DL, Harbarth S. Antibiotic selection pressure and resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Emerg Infect Dis* 2004; 10: 514–517.

3. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae*: identification of clones associated with serious invasive disease. *Microbiology* 1998; 144: 3049–3060.
4. McGee L, McDougal L, Zhou J *et al.* Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J Clin Microbiol* 2001; 39: 2565–2571.
5. Ip M, Lyon DJ, Yung RW, Tsang L, Cheng AF. Introduction of new clones of penicillin-nonsusceptible *Streptococcus pneumoniae* in Hong Kong. *J Clin Microbiol* 2002; 40: 1522–1525.
6. Isozumi R, Ito Y, Ishida T *et al.* Molecular characteristics of serotype 3 *Streptococcus pneumoniae* isolates among community-acquired pneumonia patients in Japan. *J Infect Chemother* 2008; 14: 258–261.
7. Kasahara K, Maeda K, Mikasa K *et al.* Clonal dissemination of macrolide-resistant and penicillin-susceptible serotype 3 and penicillin-resistant Taiwan 19F-14 and 23F-15 *Streptococcus pneumoniae* isolates in Japan: a pilot surveillance study. *J Clin Microbiol* 2005; 43: 1640–1645.
8. Ko KS, Song JH. Evolution of erythromycin-resistant *Streptococcus pneumoniae* from Asian countries that contains *erm(B)* and *mef(A)* genes. *J Infect Dis* 2004; 190: 739–747.
9. Nuermberger EL, Bishai WR. Antibiotic resistance in *Streptococcus pneumoniae*: what does the future hold? *Clin Infect Dis* 2004; 38 (suppl): 363–371.
10. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonization: the key to pneumococcal disease. *Lancet Infect Dis* 2004; 4: 144–154.
11. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. *Nat Rev Microbiol* 2008; 6: 288–301.
12. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. *Lancet Infect Dis* 2005; 5: 83–93.
13. Brueggemann AB, Peto TE, Crook DW, Butler JC, Kristinsson KG, Spratt BG. Temporal and geographic stability of the serogroup-specific invasive disease potential of *Streptococcus pneumoniae* in children. *J Infect Dis* 2004; 190: 1203–1211.
14. Barocchi MA, Ries J, Zogaj X *et al.* A pneumococcal pilus influences virulence and host inflammatory responses. *Proc Natl Acad Sci USA* 2006; 103: 2857–2862.
15. Aguiar SI, Serrano I, Pinto FR, Melo-Cristino J, Ramirez M. The presence of the pilus locus is a clonal property among pneumococcal invasive isolates. *BMC Microbiol* 2008; 8: 41–51.
16. Moschioni M, Donati C, Muzzi A *et al. Streptococcus pneumoniae* contains 3 *rlrA* pilus variants that are clonally related. *J Infect Dis* 2008; 197: 888–896.
17. Sjöström K, Blomberg C, Fernebro J *et al.* Clonal success of piliated penicillin nonsusceptible pneumococci. *Proc Natl Acad Sci USA* 2007; 104: 12907–12912.
18. Cardozo DM, Nascimento-Carvalho CM, Andrade AL *et al.* Prevalence and risk factors for nasopharyngeal carriage of *Streptococcus pneumoniae* among adolescents. *J Med Microbiol* 2008; 57: 185–189.
19. Zalacain R, Sobradillo V, Amilibia J *et al.* Predisposing factors to bacterial colonization in chronic obstructive pulmonary disease. *Eur Respir J* 1999; 13: 343–348.
20. Ishida T, Maniwa K, Kagioka H *et al.* Antimicrobial susceptibilities of *Streptococcus pneumoniae* isolated from adult patients with community-acquired pneumonia in Japan. *Respirology* 2008; 13: 240–246.
21. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; 40: 373–383.
22. Fine MJ, Auble TE, Yealy DM *et al.* A prediction rule to identify low-risk patients with community-acquired pneumonia. *N Engl J Med* 1997; 336: 243–250.
23. Ito Y, Osawa M, Isozumi R *et al.* Pneumococcal surface protein A family types of *Streptococcus pneumoniae* from community-acquired pneumonia patients in Japan. *Eur J Clin Microbiol Infect Dis* 2007; 26: 739–742.
24. Gherardi G, Fallico L, Del GrossoM *et al.* Antibiotic-resistant invasive pneumococcal clones in Italy. *J Clin Microbiol* 2007; 45: 306–312.
25. Reinert RR, Ringelstein A, van der LindenM, Cil M, Al-Lahham A, Schmitz F. Molecular epidemiology of macrolide-resistant *Streptococcus pneumoniae* isolates in Europe. *J Clin Microbiol* 2005; 43: 1294–1300.
26. Sadowy E, Izdebski R, Skoczynska A, Grzesiowski P, Gniadkowski M, Hryniewicz W. Phenotypic and molecular analysis of penicillin-nonsusceptible *Streptococcus pneumoniae* isolates in Poland. *Antimicrob Agents Chemother* 2007; 51: 40–47.
27. Sogstad MK, Høiby EA, Caugant DA. Molecular characterization of non-penicillin-susceptible *Streptococcus pneumoniae* in Norway. *J Clin Microbiol* 2006; 44: 3225–3230.
28. Pai R, Gertz RE, Whitney CG, Beall B. Clonal association between *Streptococcus pneumoniae* serotype 23A, circulating within the United States, and an internationally dispersed clone of serotype 23F. *J Clin Microbiol* 2005; 43: 5440–5444.
29. Beall B, McEllistrem MC, Gertz RE Jr *et al.* Pre- and postvaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. *J Clin Microbiol* 2006; 44: 999–1017.
30. Prass K, Braun J, Dirnagl U, Meisel C, Meisel A. Stroke propagates bacterial aspiration to pneumonia in a model of cerebral ischemia. *Stroke* 2006; 37: 2607–2612.
31. Sadowy E, Skoczynska A, Fiett J, Gniadkowski M, Hryniewicz W. Multilocus sequence types, serotypes, and variants of the surface antigen *pspA* in *Streptococcus pneumoniae* isolates from meningitis patients in Poland. *Clin Vaccine Immunol* 2006; 13: 139–144.
32. Jefferies JM, Smith A, Clarke SC, Dowson C, Mitchell TJ. Genetic analysis of diverse disease-causing pneumococci indicates high levels of diversity within serotypes and capsule switching. *J Clin Microbiol* 2004; 42: 5681–5688.