Expansion and Evolution of *Streptococcus pneumoniae* Serotype 19A ST320 Clone as Compared to Its Ancestral Clone, Taiwan<sup>19F</sup>-14 (ST236)

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**Background.** The *Streptococcus pneumoniae* serotype 19A sequence type (ST) 320 clone, derived from an international Taiwan<sup>19F</sup>-14 (ST236) clone, has become prevalent in many countries.

**Methods.** The dynamics of invasive pneumococcal disease (IPD) were determined using the database of the National Notifiable Disease Surveillance System in Taiwan. The virulence of 19A ST320 and Taiwan<sup>19F</sup>-14 (ST236) were assessed in mice. By constructing an isogenic serotype 19F variant of the 19A ST320 strain (19F ST320), we analyzed the role of capsular type and genetic background on the difference in virulence between 19A ST320 and Taiwan<sup>19F</sup>-14 (ST236).

**Results.** Between 2008 and 2011, IPD due to serotype 19A increased from 2.1 to 10.2 cases per 100 000 population (P < .001); IPD due to any serotype also significantly increased (P = .01). Most serotype 19A isolates belonged to ST320. Using competition experiments in a murine model of colonization, we demonstrated that 19A ST320 out-competed Taiwan<sup>19F</sup>-14 (ST236; competitive index, 20.3; P = .001). 19F ST320 was 2-fold less competitive than the 19A ST320 parent (competitive index, 0.47; P = .04) but remained 14-fold more competitive than Taiwan<sup>19F</sup>-14 (ST236; competitive index, 14.7; P < .001).

**Conclusions.** Genetic evolution of pneumococcal clones from Taiwan<sup>19F</sup>-14 (ST236) to 19A ST320 has made this pneumococcus better able to colonize of the nasopharynx. This evolution reflects not only a switch in capsular serotype but also changes in other loci.

**Keywords.** *Streptococcus pneumoniae*; evolution; capsular switch.

*Streptococcus pneumoniae* serotype 19A is often resistant to multiple antibiotics and has emerged worldwide to be an important pathogen associated with invasive pneumococcal disease (IPD), pneumonia, acute otitis media, and hemolytic uremic syndrome[1–4]. In the United States, serotype 19A arose as the most common IPD-causing serotype following widespread use of the 7-valent pneumococcal conjugate vaccine (PCV7) in national immunization programs [5, 6]. A randomized controlled study in the Netherlands demonstrated that an increase in serotype 19A nasopharyngeal acquisition was detected among PCV7 recipients, compared with unvaccinated controls [7]. However, increases in serotype 19A diseases also occurred in countries without PCV7 implementation [8, 9]. Thus, the emergence of 19A likely is due to multiple factors, including the prevalence of 19A carriage, the properties of individual pneumococcal clones, and variations in antibiotic and vaccine use.

In the United States, most serotype 19A disease is due to proliferation of preexisting sequence type (ST)
Serotype 19A ST320 is prevalent in many countries [5, 8, 10, 11]. ST320 is a double-locus variant of ST236. ST236, an international Taiwan19F-14 (ST236) clone with high antibiotic resistance, had spread globally prior to the introduction of PCV7 [12]. Work by Moore et al. suggested that the serotype 19A ST320 strain was derived from a capsular switching event that occurred between an international Taiwan19F-14 (ST236) strain and a serotype 19A strain [5]. In Taiwan, serotype 19A had become, by 2007, one of the common serotypes in children with IPD, despite the low PCV7 vaccination rate [13]. Studies in Taiwan have shown the predominance of ST320 among serotype 19A isolates [14, 15]. In Taiwan and Korea, where PCV7 vaccination rates are low or even absent, the serotype 19A ST320 strain has replaced Taiwan19F-14 (ST236) as the cause of disease in children [8, 13]. Antibiotic resistance, not PCV7 use, was thought to be the strongest factor overall in serotype 19A ST320 emergence [8]. But, we observe that there are some differences between 19A and 19F in clinical terms. For example, although there are studies that have documented the invasive potential of serotype 19F strains, which can cause pneumonia and empyemas [16–18], serotype 19A strains were highly associated with necrotizing pneumonia and the development of bronchopleural fistula [14]. Serotype 19A ST320 seem to be more virulent clinically than its ancestral Taiwan19F-14 (ST236) clones.

Since October 2007, IPD has been a national notifiable disease in Taiwan. In this work, we report a follow-up study of the dynamics of serotype 19A prevalence in Taiwan between 2008 and 2011, using the database of the Centers for Disease Control and Prevention, Taiwan. To delineate the mechanism of expansion and evolution of serotype 19A ST320, we compared the virulence of serotype 19A ST320 and Taiwan19F-14 (ST236) strains in murine sepsis, pneumonia, and colonization models in the absence of PCV7 vaccination and antibiotic use. Furthermore, we constructed an isogenic serotype 19F variant of the serotype 19A ST320 strain (serotype 19F ST320) and used this mutant to analyze the role of capsular type and genetic background in the difference in virulence between Taiwan19F-14 (ST236) and serotype 19A ST320 clones.

METHODS

Surveillance
IPD cases must be reported and clinical specimens sent to Centers for Disease Control and Prevention, Taiwan, within 7 days of establishing the clinical diagnosis. IPD is defined as the recovery of pneumococcus from a normally sterile site of the human body, such as blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, or bone marrow. The numbers of IPD cases for analysis were obtained from the database of the Centers for Disease Control and Prevention, Taiwan (available at: http://nidss.cdc.gov.tw/). The yearly distributions of serotypes were also available on Centers for Disease Control and Prevention, Taiwan, website (available at: http://www.cdc.gov.tw/public/Attachment/237931971.pdf and http://www.cdc.gov.tw/public/Attachment/1691145571.jpg). The annual population figures provided by the Department of Household Registration Affairs of the Interior Ministry were used to calculate the IPD incidence. In Taiwan, the pneumococcal conjugate vaccine is available only on the private market. The vaccine manufacturer (Wyeth Pharmaceuticals, a subsidiary of Pfizer, Taiwan) estimates that 16.2%, 22.3%, 30.2%, and 33.6% of children <5 years of age received ≥1 dose of PCV7 or PCV13 during 2008, 2009, 2010, and 2011, respectively. PCV13 has been available on the Taiwan market since 2011.

S. pneumoniae Strains and Culture Conditions
Chang Gung Children’s Hospital (CGCH), a 495-bed facility, is the largest children’s hospital in Taiwan and has a 19-year history of operation. This hospital serves both as a primary care hospital and as a tertiary referral center, providing care for 350 pediatric inpatients and 900 outpatients per day. All pneumococcal isolates recovered from sterile sites from children at CGCH have been archived and frozen since 2000. The institutional review board of the Chang Gung Memorial Hospital approved this study (protocol 98–3451B). Pneumococcal isolates were grown at 35°C in Todd-Hewitt broth supplemented with 0.5% yeast extract (THY) in static culture in the presence of 5% CO₂. Bacterial stocks were frozen at –20°C in THY supplemented with 10% glycerol. The serotype 19A ST320 strain used in this study was isolated from the pleural fluid of a child with pneumococcal pneumonia. The Taiwan19F-14 (ST236) strain used in the study was recovered from the blood of a child with pneumococcal infection. The serotype 19A ST199 strain used in this study was kindly provided by Professor Donald E. Low (University of Toronto, Canada). The amoxicillin minimum inhibitory concentrations of the 19A ST320, Taiwan19F-14 (ST236), and serotype 19A ST199 strains were 8, 2, and 0.125 µg/mL, respectively. We used the different antibiotic susceptibilities to differentiate between ST320, ST236, and ST199 in the animal model.

Serotyping of Isolates
Polysaccharide capsule types were determined on the basis of the Quellung test with factor-specific sera (Statens Serum Institut, Copenhagen, Denmark).

Multilocus Sequence Typing
Multilocus sequence typing was performed as described previously [16]. The primers used for polymerase chain reaction (PCR) amplification have been described previously [19].

Construction of a Serotype 19A ST320 Capsular Polysaccharide Locus (cps) Deletion Mutant
A DNA fragment spanning the dexB locus was amplified by PCR, using chromosomal DNA from the serotype 19A ST320
strains as a template. The fragment was cloned into the pGEM-T Easy vector (Promega); the resulting plasmid was cleaved with PstI, and a cassette encoding spectinomycin resistance was ligated into the PstI site. The resulting construct was cleaved with SacI and then ligated with a DNA fragment containing the *aliA* locus, which had been amplified by PCR from chromosomal DNA of the serotype 19A ST320 strain. The pGEM::*dexB/spec/aliA* plasmid was used to transform the serotype 19A ST320 strain with selection for resistance to spectinomycin and create a serotype 19A ST320 *cps* deletion mutant. Primers used in the study are listed in Table 1.

### Construction of an Isogenic Serotype 19F Capsular Variant of Serotype 19A ST320 (Serotype 19F ST320)

Chromosomal DNA from the Taiwan19F-14 (ST236) strain was used to transform the serotype 19A ST320 *cps* deletion mutant to create an encapsulated strain. Notably, the colony morphologies of the encapsulated serotype 19A ST320 and Taiwan19F-14 (ST236) strains are larger and smoother than that of the 19A ST320 *cps* deletion mutant, which enabled us to screen for encapsulated strains among the transformants. Once we had identified an isolate with the serotype 19F capsule, we purified DNA from that isolate and used it to transform the serotype 19A ST320 *cps* deletion mutant into an encapsulated strain again. This backcross transformation procedure was repeated 3 times to construct triple-backcross (ie, 3× backcross) transformants [20]. The 3× backcross transformant was expected to have an extremely low probability of carrying any given gene from the original 19F donor, apart from the capsule gene cluster [20, 21]. The amoxicillin minimum inhibitory concentration of the isogenic serotype 19F capsular variant of serotype 19A ST320 (serotype 19F ST320) was 8 μg/mL.

### Mouse Challenge

Serotype 19A ST320, its isogenic serotype 19F variant (serotype 19F ST320), and Taiwan19F-14 (ST236) were grown in THY medium to mid-logarithmic phase (OD₆₂₀, 0.2–0.5) for intranasal and intravenous challenges. The bacterial cultures then were diluted (using phosphate-buffered saline [PBS]) to the desired cell density (measured in colony-forming units [CFU] per milliliter), using a previously determined standard curve (OD vs actual CFU) generated for pneumococci. The nominal size of the inoculating doses were confirmed by viable counts after plating on blood agar plates.

Animal studies were performed with 3-week-old female BALB/c mice, using groups of 5–10 mice. All experiments were approved by the local ethical committee for animal research. For the sepsis model, intravenous challenge of pneumococci were injected directly into the dorsal tail vein. For the colonization and pneumonia model, mice were anesthetized with 20 mg/kg ketamine and infected by intranasal challenge (instillation). At 24 and 48 hours after injection, the animals were euthanized. The trachea was exposed and cannulated for instillation of 500 μL of sterile PBS. The nasal lavage fluid exiting the nares was collected. The lungs were removed, rinsed in PBS, and homogenized. Nasal lavages and lung homogenates were subjected to serial dilution and plated on blood agar to determine the number of viable pneumococci. For the competition experiments, bacteria were combined at 1:1 ratios of CFU. A total of 20 μL (5 × 10⁵ CFU of each strain) was used for intranasal challenge, as described previously [22]. After 7 days, mice were killed, and nasal lavages were collected. The recovery of CFU was determined by dilution and plating on blood agar as described above. For competition experiments between serotype 19A ST320 and Taiwan19F-14 (ST236), between the isogenic 19F variant (serotype 19F ST320) and Taiwan19F-14 (ST236), and between serotype 19A ST320 and serotype 19A ST199, recovered organisms were distinguished by replica plating on blood agar containing 4 μg/mL amoxicillin. Briefly, replica plating was performed on agar containing 4 μg/mL amoxicillin to inhibit the ST236 and ST199 strains and allow quantitation of the ST320 colonies. The CFU for the ST320 strains subtracted from the total CFU recovered from plating nasal lavages onto blood agar without amoxicillin gave the CFU for the ST236 and ST199 strains. For competition between serotype 19A ST320 and its isogenic 19F variant (serotype 19F ST320), recovered organisms were distinguished by screening, using the Quellung test with factor-specific sera. The multilocus sequence type was not rechecked. Fifty randomly picked colonies were tested per mouse. A competitive index was calculated on the basis of the ratio of the competing bacterial strains recovered in nasal lavages normalized to the ratio of respective bacteria in the inoculum.

### Statistical Analysis

Statistical comparisons of incidence were performed using Poisson distribution with 95% confidence intervals; 95% confidence intervals for which the upper and lower bounds did not include 0 were interpreted as statistically significant. To test for significant differences between groups, the Mann-Whitney *U* test was used for continuous variables. The mouse competitive index assay was analyzed with a 1-sample, 1-tailed Student *t* test, using log-transformed competitive indexes to determine

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>dex F</td>
<td>ATG CAA GAA AAA TGG TGG CAC AAT GCC G</td>
<td>Generate <em>dexB</em> fragment for <em>cps</em> deletion mutation</td>
</tr>
<tr>
<td>dex R</td>
<td>TTA TAG TAA TTC CAC ACA GAA AGC ATC C</td>
<td>Generate <em>dexB</em> fragment for <em>cps</em> deletion mutation</td>
</tr>
<tr>
<td>ali F</td>
<td>ATG ATG AAA AGT TCA AGA CTA TTT GCC C</td>
<td>Generate <em>aliA</em> fragment for <em>cps</em> deletion mutation</td>
</tr>
<tr>
<td>ali R</td>
<td>TTA TTT CAC ATG TTT TGCC GAG ATC</td>
<td>Generate <em>aliA</em> fragment for <em>cps</em> deletion mutation</td>
</tr>
</tbody>
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### Table 1. DNA Primers Used in this Study

Serotype 19A ST320 Clones • JID 2013:208 (15 July) • 205
Table 2. Overall and Serotype-Specific Incidence of Invasive Pneumococcal Disease in Children <5 Years of Age Between 2008 and 2011

<table>
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<tr>
<td></td>
<td>Annual Incidence, by Period</td>
<td>Relative Change in Incidence</td>
<td></td>
<td>Percentage (95% CI)</td>
<td>P</td>
<td>Percentage (95% CI)</td>
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<tr>
<td></td>
<td>2008 vs 2010</td>
<td></td>
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<tr>
<td>All</td>
<td>16.6</td>
<td>14.4</td>
<td>20.7</td>
<td>21.4</td>
<td>25 (2–54)</td>
<td>.03</td>
</tr>
<tr>
<td>14</td>
<td>3.5</td>
<td>2</td>
<td>2.8</td>
<td>1.6</td>
<td>–20 (–51 to 32)</td>
<td>.4</td>
</tr>
<tr>
<td>6B</td>
<td>3.1</td>
<td>2.1</td>
<td>1.8</td>
<td>1.1</td>
<td>–43 (–69 to 2)</td>
<td>.06</td>
</tr>
<tr>
<td>23F</td>
<td>3.2</td>
<td>2.2</td>
<td>2.5</td>
<td>1.7</td>
<td>–23 (–54 to 31)</td>
<td>.3</td>
</tr>
<tr>
<td>19F</td>
<td>1.7</td>
<td>2.1</td>
<td>2.4</td>
<td>2.5</td>
<td>44 (–23 to 170)</td>
<td>.25</td>
</tr>
<tr>
<td>19A</td>
<td>2.1</td>
<td>3.3</td>
<td>8.4</td>
<td>10.2</td>
<td>292 (145–528)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Other</td>
<td>2.9</td>
<td>2.7</td>
<td>2.9</td>
<td>4.3</td>
<td>1 (–41 to 66)</td>
<td>.98</td>
</tr>
</tbody>
</table>

Statistically significant changes are in bold. Abbreviation: CI, confidence interval.

whether differences between the indexes were statistically significant. A P value of <.05 was considered statistically significant. Statistical analysis was performed using SPSS 15.0 for Windows (Statistical Package for Social Sciences, Chicago, IL).

RESULTS

Incidences of Serotype 19A–Attributable and Overall IPD Significantly Increased Among Children Aged <5 Years

During 2008–2010, among children aged <5 years, the incidence of IPD due to serotype 19A dramatically increased, from 2.1 cases per 100,000 population in 2008 to 8.4 cases per 100,000 population in 2010 (P < .001). The overall incidence of IPD also significantly increased between 2008 and 2010, from 16.6 cases per 100,000 population in 2008 to 20.7 cases per 100,000 population in 2010 (P = .03), but IPD due to serotypes 14, 6B, 23F, and 19F did not significantly change (P > .05; Table 2). In 2011, IPD due to serotype 19A continued to increase, with 10.2 cases per 100,000 population, and the overall incidence of IPD increased to 21.4 cases per 100,000 population. Compared with 2008, IPD due to serotypes 14, 6B, and 23F significantly decreased in 2011 (P < .05). By 2011, serotype 19A accounted for 48% of isolates in this group.

Clonal Expansion of Serotype 19A ST320

CGCH is the largest children’s hospital in Taiwan. On average, invasive pneumococcal isolates collected at CGCH accounted for 9% of all invasive pneumococcal isolates in children aged <5 years in Taiwan. Between 2008 and 2011, a total of 55 invasive pneumococcal isolates were collected in children aged <5 years at CGCH. Twenty-two of the 55 isolates (40%) were serotype 19A. Multilocus sequence typing analysis showed that 21 of these 22 serotype 19A isolates (95.5%) belonged to the ST320 clone. Thus, clonal expansion of serotype 19A ST320 clone accounted for the majority of the increase in serotype 19A disease.

Comparison of Serotype 19A ST320 and Taiwan19F-14 (ST236) in a Sepsis Model

To determine whether there was a difference in virulence between serotype 19A ST320 and Taiwan19F-14 (ST236), the 2 strains first were tested in a sepsis model, for which 3-week-old BALB/c mice were injected intravenously with 5 × 10⁶ CFU of pneumococci of the respective strain. At 10 days after infection, no difference in survival was seen (data not shown).

Comparison of Serotype 19A ST320 and Taiwan19F-14 (ST236) in a Colonization and Pneumonia Model

To determine whether there was a difference in virulence between serotype 19A ST320 and Taiwan19F-14 (ST236), the strains were next tested in a pneumonia and colonization model, in which 3-week-old BALB/c mice were inoculated intranasally with 5 × 10⁷ CFU of pneumococci of the respective strain. Animals were assessed for survival and for bacterial outgrowth over time in the nasopharynx and lungs. At 7 days after instillation, no significant difference was seen in the survival rates (data not shown). After 24 and 48 hours, the 2 strains exhibited similar bacterial outgrowth in the lung (P > .05; Figure 1A and 1B). However, nasal lavages revealed significantly higher densities in mice infected by serotype 19A ST320 ((P = .047 and P = .016 at 24 and 48 hours, respectively; Figure 1C and 1D). Together, the results of these in vivo studies suggest that serotype 19A ST320 and Taiwan19F-14 (ST236) strains differ in their ability to colonize host animals.

Nasopharyngeal Competition Between Serotype 19A ST320 and Taiwan19F-14 (ST236)

To mimic the natural situation in which pneumococci of different serotypes may compete for the same niche and to reduce
the variance caused by variation between individual mice, we performed a competition experiment in which mice were inoculated intranasally with equal CFU of bacteria of serotype 19A ST320 and Taiwan19F-14 (ST236). At 7 days after infection, the serotype 19A ST320 strain outcompeted the Taiwan19F-14 (ST236) strain by approximately 20-fold for colonization of the mouse nasopharynx (competitive index, 20.3; \( P = .001 \); Figure 2A).

**Construction of Isogenic Serotype 19F Capsular Variant of Serotype 19A ST320 (Serotype 19F ST320)**

The serotype 19A ST320 cps deletion mutant (Figure 3A) was transformed with chromosomal DNA of the Taiwan19F-14 (ST236) strain to create an encapsulated strain (Figure 3B). In each transformation/backcross experiment, we typically obtained 1 encapsulated transformant per approximately 500,000 CFU of recipient cells (serotype 19A ST320 cps deletion mutant).

**Nasopharyngeal Competition Between the Isogenic Serotype 19F Capsular Variant of Serotype 19A ST320 (Serotype 19F ST320) and Taiwan19F-14 (ST236)**

To assess the role of capsular type on virulence, the competition assay was repeated with the serotype 19A ST320 strain and its isogenic serotype 19F variant (serotype 19F ST320). At 7 days after instillation, the isogenic serotype 19F variant (serotype 19F ST320) was 2-fold less competitive than its parent serotype 19A ST320 strain for colonization of the mouse upper airway (competitive index, 0.47; \( P = .04 \); Figure 2B).

**Nasopharyngeal Competition Between Isogenic Serotype 19F Capsular Variant of Serotype 19A ST320 (Serotype 19F ST320) and Serotype 19F ST320**

To assess the role of genetic background on virulence (in the absence of serotype distinctions), the competition assay was repeated with the isogenic serotype 19F variant of serotype 19A ST320 (serotype 19F ST320) and the Taiwan19F-14 (ST236) strain. At 7 days after instillation, the isogenic serotype 19F variant of 19A ST320 (serotype 19F ST320) outcompeted the Taiwan19F-14 (ST236) strain by approximately 14-fold for colonization of the mouse upper airway (competitive index, 14.7; \( P < .001 \); Figure 2C).

**Nasopharyngeal Competition Between Serotype 19A ST320 and Serotype 19A ST199**

In the United States, ST199 was the predominant clone of serotype 19A prior to the introduction of PCV7, after which ST320

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**Figure 1.** Intranasal challenge of 3-weeks-old female BALB/c mice with serotype 19A ST320 and Taiwan19F-14 (ST236) strains separately. A and B, Bacterial counts in the lungs at 24 and 48 hours after challenge. \( P > .05 \) at 24 and 48 hours. C and D, Bacterial counts in the nasal lavages at 24 and 48 hours after challenge. \( P = .047 \) at 24 hours, and \( P = .016 \) at 48 hours.
became more common and, in some areas, the most common 19A clone. Thus, we compared serotype 19A ST320 isolates with serotype 19A ST199 strains in the murine colonization model by means of a competition assay. At 7 days after instillation, the serotype 19A ST320 strain did not significantly outcompete the serotype 19A ST199 strains (competitive index, 2.3; \(P = .3\); Figure 2D). Serotype 19A ST320 was comparable to serotype 19A ST199 for colonization of the mouse upper airway.

**DISCUSSION**

During 2008–2011, in a Taiwanese population in which one-third of children received PCV7, the overall rate of IPD among children <5 years did not decrease but significantly increased. The increase of IPD due to serotype 19A was a major contributor to the increase in IPD. Clonal expansion of the serotype 19A ST320 clone resulted in the increase in IPD due to serotype 19A. Taiwan is an epicenter of highly resistant bacteria [23] and was where Shi et al first identified Taiwan19F-14 (ST236) as a major clone of penicillin-resistant *S. pneumoniae* [24]. The Taiwan19F-14 (ST236) clone was presumed to have emerged in the Far East during the 1990s and then to have been disseminated internationally [25, 26]. In our previous studies, the Taiwan19F-14 (ST236) clone was one of the common strains...
causing IPD among children [27] and was responsible for the spread of high-level β-lactam resistance in Taiwan [12]. At the time of those reports, serotype 19A was not seen among the isolates associated with disease or elevated antibiotic resistance. However, since 2010, serotype 19A has emerged as the most common type causing IPD under a low rate of PCV7 coverage in Taiwan. Although the complete genome sequence of the serotype 19A ST320 clone has been decoded [11], the basis for serotype 19A ST320 displacement of Taiwan serotype 19F-14 (ST236) remains unclear. In the present study, we demonstrated that the serotype 19A ST320 and Taiwan 19F-14 (ST236) clones differ in their ability to cause colonization but not pneumonia or sepsis, as judged by a mouse colonization model in the absence of PCV7 vaccination and antibiotic use. The serotype 19A ST320 strain showed delayed clearance from the murine airway and outcompeted the Taiwan 19F-14 (ST236) strain in the nasopharynx of mice. Antibiotic stress, such as that resulting from the use of aminoglycosides or fluoroquinolones, can induce genetic transformability in S. pneumoniae, select for resistant clones, and promote the evolution of virulence [28]. It is likely that environmental pressures in Taiwan, including high antibiotic stress [23, 29], have driven the appearance and spread of the serotype 19A ST320 clone. Even in the absence of PCV7 vaccination, the serotype 19A ST320 clone might circulate and cause pneumococcal disease among children in Taiwan. In the mouse colonization model without antibiotic use, serotype 19A ST320 was not outcompeted by serotype 19A ST199. The result can explain why the ST320 background has been successful in the United States, especially under high-level antimicrobial resistance pressure [5, 30].

The pneumococci commonly reside on the mucosal surfaces of the upper respiratory tract as asymptomatic colonizers, with transmission occurring from this reservoir of commensal organisms. Colonization is the initial step in the pathogenesis of all pneumococcal disease [31]. Effective colonization by pneumococci would give the species increased access to normally sterile sites, contributing to the species’ virulence, pathogenicity, and prevalence in the community. In the present study, we demonstrated that the serotype 19A ST320 strain exhibited effective colonizing ability as compared to its ancestral Taiwan 19F-14 (ST236) strain. The evolution of Taiwan 19F-14 (ST236) to serotype 19A ST320 presumably provided the clone with increased capacity for host colonization. Although serotypes 19A and 19F belong to the same serogroup and have a similarly structured capsular polysaccharide, we found that 19A colonized more effectively than 19F in this study. More importantly, the genetic change from ST236 to ST320 confers a competitive advantage in nasopharyngeal colonization. We are trying to compare the transcriptomes between the 2 strains to identify whether there are differences between 19A ST320 and Taiwan 19F-14 (ST236) in some of the attachment proteins that can explain the differences in colonization effectiveness and virulence between them. The remarkable plasticity and heterogeneity of pneumococcal isolates reflect the ability of this species to take up DNA from the environment through natural competence [32]. Genetic exchanges via homologous recombination usually occur between pneumococci or closely related streptococci [33]. Thus, clone ST320 may have acquired effective colonization and virulence properties through horizontal gene transfer.

In conclusion, the virulence characteristics of serotype 19A ST320 clone are adaptations that increase the strain’s persistence within a host during colonization. In view of these findings, design of a vaccine that prevents S. pneumoniae colonization may be a better way to prevent pneumococcal disease [34].

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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