By use of molecular techniques, the genetic heterogeneity of 63 community pediatric pharyngeal group A streptococcal (GAS) isolates circulating within a 3-week period were compared with 17 contemporaneous invasive pediatric isolates. Pharyngitis isolates represented 16 pulsed-field gel electrophoresis (PFGE) patterns with 12 emm serotypes, and invasive isolates represented 10 PFGE patterns with 9 emm serotypes. One-fourth of the pharyngeal isolates (16/63) were identical to at least 1 invasive isolate; conversely, 10 (59%) of 17 invasive isolates were identical to at least 1 pharyngeal strain. sic allele analysis of emm1 strains demonstrated additional heterogeneity and overlap. More pharyngeal (71%) than invasive isolates (35%) were positive for both speA and speC (P < .02). Many pharyngitis GAS strains circulate simultaneously. Most invasive pediatric GAS strains are identical to acute pharyngitis strains; thus, childhood pharyngitis is a major reservoir for strains with invasive potential. Pharyngeal isolates were more likely to be speA and speC positive than were the invasive isolates.

The resurgence of invasive group A streptococcal (GAS) infections over the past 15 years has stimulated research into the molecular characteristics of invasive isolates [1–4]. Pharyngeal GAS isolates have also received increased attention in attempts to identify factors that determine the propensity of a particular strain to cause invasive disease [5–7].

Traditionally, M and T serotyping have been used in epidemiologic studies of group A streptococcus, but there are limitations to this methodology [8]. Serotyping is available in only a few laboratories worldwide, and many strains cannot be typed with available antisera. Sequencing the emm gene that encodes M protein is more widely available and identifies M types for which antisera do not exist [9, 10]. Pulsed-field gel electrophoresis (PFGE) of bacterial DNA provides genotypic information with a resulting pattern of DNA fragments. The utility of this method to assess strain similarity among group A streptococcus has been demonstrated elsewhere [8, 11].

In 1998, we used PFGE, M and T serotyping, and emm typing and assessed the presence of the genes speA, speB, and speC that encode for streptococcal pyrogenic exotoxin (SPE) A, SPE B, and SPE C. We also determined the sic alleles of M1 strains so that we could study the molecular epidemiology of pharyngeal and invasive isolates of GAS from children in the greater Chicago area. sic encodes streptococcal inhibitor of complement, a virulence factor in M1 group A streptococcus, and exhibits marked allelic variability. Our goals were to assess the degree of heterogeneity of endemic pharyngeal GAS isolates circulating in a community simultaneously, to compare pharyngeal strains to GAS isolates that caused invasive infections, during the same season and in the same metropolitan area, by using molecular characteristics to assess the degree to which pediatric pharyngitis serves as a reservoir for GAS strains with invasive potential, and to study the frequency of speA, speB, and speC among pharyngeal and invasive isolates.

Materials and Methods

GAS strains. We studied 63 pharyngeal GAS isolates that had been collected at 3 large (multiphysician) unrelated geographical-multicenter institutions; 21 isolates were from pharyngitis and 42 isolates were from invasive infections.
ly separate Chicago area community pediatric offices during winter 1998, as part of a study of diagnostic tests for streptococcal pharyngitis in 18 offices (figure 1) [12]. These 3 pediatric offices were located in the northern part of Chicago (office 1), a far northern suburb (office 2), and a western suburb (office 3); they provided 23, 21, and 19 pharyngeal isolates, respectively. The offices are separated by 30.6–67.6 km and are 12.9–57.9 km from the hospital. GAS strains from each office were collected over periods of 19 days (office 1), 7 days (office 2), and 10 days (office 3) in late February and early March 1998.

In addition, 17 invasive GAS isolates cultured from normally sterile sites of pediatric patients treated in the greater Chicago metropolitan area from January to August 1998 were studied. Of these 17 isolates, 13 were from patients treated at Children’s Memorial Hospital with intravenous antibiotics for the following GAS infections: empyema (3), cervical abscess (2), cellulitis associated with varicella infection (2), lymphangitis (2), bacteremia (2), retropharyngeal abscess (1), and chronic supplicative otitis media (1). The remaining 4 invasive isolates were obtained from a cluster of deaths due to GAS infection (not varicella associated) during February 1998 in a Chicago pediatric nursing home located ~16 km from the hospital. These isolates were obtained from blood (2), endotracheal aspirate (1), and pleural fluid (1). The endotracheal aspirate sample was obtained from an intubated patient with pneumonia. Figure 1 shows the time course of the invasive infections. All isolates were confirmed as GAS by Lancefield serogrouping by use of Wellcogen (Burroughs Wellcome) latex agglutination reagents.

Laboratory methods and PFGE. PFGE was done on each isolate by use of the Gene Path System (BioRad) universal and enzyme modules as described elsewhere [11]. An overnight subculture from a single GAS colony was pelleted, resuspended, and heated to 50°C for 10 min; lysozyme, mutanolysin, and liquefied embedding agarose at 50°C were added and then immediately poured into plug molds. Plugs were treated with mutanolysin and lysostaphin and with proteinase K and washed and stored at 4°C. Plugs were digested by Smal overnight and loaded into wells of a 1% agarose gel with a λ ladder for DNA size standards. PFGE was done in a contour-clamped homogenous electric field apparatus at 14°C by using the enterococcus program with a total run time of 20 h [11]. The gels were stained with ethidium bromide and photographed under UV light.

PFGE interpretation. PFGE shows that single genetic events occur unpredictably, even within a well-defined outbreak of infections lasting several months. A 1–3 band difference in PFGE patterns can result from a single genetic event. Published guidelines suggest that isolates in an outbreak that differ by 1–3 bands should be considered “closely related,” those that differ by 4–6 bands “possibly related,” and those that have >6 differing bands are distinct strains [13, 14].

PFGE banding patterns (pulsotypes) were analyzed by visual inspection (figure 2). We considered 2 isolates to have identical pulsotypes when their PFGE banding patterns were exactly the same. We considered 2 isolates to have distinct pulsotypes when their PFGE patterns differed by >3 bands (i.e., >1 genetic event has occurred). We considered 2 isolates to have clonally related pulsotypes when their banding patterns differed by 1–3 bands [15]. We arbitrarily designated PFGE patterns by their M/emm type in Roman numerals followed by a lowercase letter to indicate variations within M/emm types.

Other laboratory methods. All isolates were M and T serotyped by standard serologic methods at the World Health Organization Collaborating Center for Reference and Research on Streptococci at the University of Minnesota [16] and were emm typed at the Cen-

Figure 1. Time course of isolation of group A streptococcal strains included in this study. Strains were isolated in 1998.

Figure 2. Pulsed-field gel electrophoresis patterns of representative pairs of identical (left pair), clonally related (middle pair), and distinct (right pair) strains of group A streptococci.
Table 1. Heterogeneity of pediatric pharyngeal group A streptococcal isolates.

<table>
<thead>
<tr>
<th>Site where isolates were obtained</th>
<th>No. of isolates</th>
<th>Dates isolated in 1998</th>
<th>No. of isolates with distinct PFGE patterns*</th>
<th>No. of isolates with distinct emm/M and T serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office 1</td>
<td>23</td>
<td>16 Feb to 6 Mar</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Office 2</td>
<td>21</td>
<td>2 Mar to 8 Mar</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Office 3</td>
<td>19</td>
<td>16 Feb to 25 Feb</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>16 Feb to 8 Mar</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

NOTE. PFGE, pulsed-field gel electrophoresis.

*Distinct is >3 band difference.

†Total of all 3 offices. Two M28 isolates from separate offices had distinct PFGE patterns.

The 63 pharyngeal isolates, collected over 21 days in winter 1998 from the 3 community pediatric offices, represented 16 distinct pulsotypes as determined by PFGE patterns (table 1). Two isolates, 1 invasive and 1 pharyngeal, are not included in these data because they did not generate a PFGE pattern with Smal despite repeated attempts. Both serotyped as M75/T8/25. Office 1 had 14 distinct pulsotypes among 23 isolates collected over 19 days, office 2 had 9 distinct pulsotypes among 21 isolates collected over 7 days, and office 3 had 12 distinct pulsotypes among 19 isolates collected over 10 days. M serotyping and emm genotyping were concordant for all isolates. M and T serotyping and emm genotyping of the isolates also demonstrated heterogeneity, with 12 distinct types (table 1). Isolates of serotypes M3, M12, M18, and M28 manifested 2 distinct pulsotypes each, therefore accounting for 16 pulsotypes among 12 M/T or emm types.

Ten distinct pulsotypes were identified among the 17 invasive isolates (table 2). Three of 4 pediatric nursing home isolates and 3 of 13 hospital isolates were M1/T1 (emm1) strains with the same PFGE pattern. The 3 emm1 nursing home strains shared the same sic allele and were identical by all criteria. The fourth nursing home isolate was unique and serotyped as M22\T12/B (emm22). Thus, 3 of the 4 nursing home cases were epidemiologically related. There were 8 distinct pulsotypes (7 different M/emm types) among the remaining 10 invasive hospital isolates. Again, there was slightly more diversity among PFGE patterns than M/T serotypes and emm genotypes, with 2 distinct PFGE patterns among M3 strains. One invasive strain obtained very late in the study period (August 1998) was distinct from all other strains and was the only M59/ennm59 isolate in the study.

The 64% of 80 GAS isolates were speC positive; a significantly higher proportion of pharyngeal (89%) than invasive (71%) isolates were speC positive (P < .05). speA was present in 78% of pharyngeal and 65% of invasive isolates (P not significant). There was a significant difference in toxin gene profile between the 4 locally invasive isolates (cervical adenitis, retropharyngeal abscess, and suppurative otitis) and those associated with more overt invasive infections (bacteremia, empyema, and lymphangitis). Overall, 64% of all strains were positive for both speA and speC. Unexpectedly, a significantly higher portion of pharyngeal isolates were positive for both speA and speC (71%) compared with the invasive isolates (35%) (P < .02). In addition, only 3 (4%) of 80 study iso-

Table 2. Heterogeneity of invasive pediatric group A streptococcal isolates.

<table>
<thead>
<tr>
<th>Site where isolates were obtained</th>
<th>No. of isolates</th>
<th>Dates isolated in 1998</th>
<th>No. of isolates with distinct PFGE patterns*</th>
<th>No. of isolates with distinct emm/M and T serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>13</td>
<td>26 Jan to 30 Aug</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Nursing home</td>
<td>4</td>
<td>9 Feb to 17 Feb</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>26 Jan to 30 Aug</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

NOTE. PFGE, pulsed-field gel electrophoresis.

*Distinct is >3 band difference.
period, 16 different strains characterized by PFGE pulsotypes, uncomplicated pharyngitis in children. During the 3-week study in the Chicago metropolitan area simultaneously as agents of acute pharyngeal strains from a single office. The 2 strain heterogeneity. All were M12/emm12 pharyngeal strains from a single office.

**Discussion**

We found a large number of distinct GAS strains circulated in the Chicago metropolitan area simultaneously as agents of acute uncomplicated pharyngitis in children. During the 3-week study period, 16 different strains characterized by PFGE pulsortypes, representing 12 different emm or M and T types (emm/M/T), were identified among the 63 study pharyngeal strains. The 2 most common pulsortypes (M12/emm12 and M1/emm1) accounted for 19 (30%) of 63 pharyngeal isolates; the 5 most common pulsortypes included only 33 (53%) of 63 pharyngeal isolates; the other 47% comprised 11 other pulsortypes.

The present data demonstrate more marked diversity among pharyngeal GAS strains from one geographic area within a very brief period than reported previously. Others found diverse pharyngeal strains collected over longer time periods or in larger areas. Nguyen et al. [20] reported heterogeneity among adolescent and adult pharyngeal GAS isolates in a French colony (Reunion Island) in 1997; however, M1 and M12 serotypes accounted for 35 (67%) of 52 isolates. Perea Mejia et al. [21] identified 11 emm types among 54 pediatric pharyngitis GAS isolates in Mexico City collected over several years in the 1990s; 3 emm types (1, 3, and 6) accounted for 67% of these isolates. Musser et al. [6] found 10 different T types and 15 additional T nontypeable GAS pharyngeal strains among 149 isolates from 9 US cities collected over 1.5 years. Earlier studies that used only classic serologic testing documented that as many as 4–6 different GAS serotypes caused pharyngitis during a specific season in a geographic area [22, 23] but were limited by large numbers of nontypeable strains as a result of limited serologic tools, leading to underestimates of strain heterogeneity.

The invasive isolates in this study were also quite heterogeneous. Serotypes M1/emm1 and M3/emm3 accounted for 6 (46%) of 13 invasive hospital and 3 of 4 pediatric nursing home isolates, including 1 M1 and 2 M3 pulsortypes. Seven additional emm/M/T types and pulsortypes made up the remaining 8 invasive isolates. These results are similar to those in a recent study over 6 months of 16 consecutive invasive GAS isolates at a referral hospital, which showed predominance of 1 pulsortype but 6 different strains [24]. The same group reported isolates of 36 different pulsortypes responsible for 77 invasive infections in Minnesota over 9 months [25]. Chaussee et al. [26] and Nakashima et al. [27] each also showed heterogeneity among invasive isolates, but isolates in the former study were obtained from 14 countries and those in the latter were from throughout Japan and were obtained over 3 years.

A substantial fraction (25%) of our pharyngitis GAS isolates were identical by pulsortype, emm, M/T, and toxin gene profile to invasive infection isolates obtained during the same season; the majority of invasive isolates (56%) were identical to pharyngitis strains. Seven of the invasive strains studied were obtained from respiratory foci. When we exclude the 2 invasive isolates from July and August, 10 of 15 invasive isolates were identical to pharyngeal isolate, including M1, M3, M4, and M6 strains. This confirms and extends earlier observations [25, 27, 28]. For example, Muotiala et al. [29] found a great degree of genetic identity among M1 GAS isolates from invasive and pharyngeal infections in Finland during 1988–1995. Johnson et al. [7] reported identical restriction-enzyme analysis patterns of M1, M3, and M28 isolates from severe systemic infections and from uncomplicated pharyngitis. These data and ours emphasize that children with endemic pharyngitis serve as a major community reservoir for GAS strains with invasive potential. Study of sic allelic variability among our 7 pharyngeal and 6 invasive M1/emm1 strains lends further support to this concept in that only 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Total</th>
<th>By source</th>
<th>SpecA positive</th>
<th>SpecC positive</th>
<th>PFGE patternsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1/T1 emm1</td>
<td>13</td>
<td>H, 3; N, 3; O, 7</td>
<td>13</td>
<td>10</td>
<td>Ia, 12; Ib, 1</td>
</tr>
<tr>
<td>M2/T2 emm2</td>
<td>5</td>
<td>H, 1; O, 4</td>
<td>3</td>
<td>5</td>
<td>Ia2, Ib; Iic; 1, Ild, 1</td>
</tr>
<tr>
<td>M3/T3 emm3</td>
<td>8</td>
<td>H, 3; O, 5</td>
<td>8</td>
<td>5</td>
<td>Ila4; Iib, 1; Iic, 1; IId, 1; Ile, 1</td>
</tr>
<tr>
<td>M4/T4 emm4</td>
<td>7</td>
<td>H, 1; O, 6</td>
<td>6</td>
<td>7</td>
<td>Iva; 6; Ivb, 1</td>
</tr>
<tr>
<td>M5/T5/T6/T2/T12 emm5</td>
<td>6</td>
<td>H, 2; O, 4</td>
<td>3</td>
<td>6</td>
<td>Va; 4; Vb, 2</td>
</tr>
<tr>
<td>M6/T6 emm6</td>
<td>6</td>
<td>H, 1; O, 5</td>
<td>6</td>
<td>5</td>
<td>Vla, 4; Vlb, 2</td>
</tr>
<tr>
<td>M12/T12 emm12</td>
<td>12</td>
<td>O, 12</td>
<td>6</td>
<td>8</td>
<td>XIIa; XIIb, 4; XIIc, 1</td>
</tr>
<tr>
<td>M18/NT</td>
<td>5</td>
<td>H, 1; O, 4</td>
<td>2</td>
<td>5</td>
<td>XVIIa, 2; XVIIIb, 1; XVIIc, 1; XVIIId, 1</td>
</tr>
<tr>
<td>M22/T12/B</td>
<td>3</td>
<td>N, 1; O, 2</td>
<td>2</td>
<td>2</td>
<td>XXIIa, 1; XXIIb, 1; XXIIc, 1</td>
</tr>
<tr>
<td>M28/T28</td>
<td>5</td>
<td>O, 5</td>
<td>5</td>
<td>5</td>
<td>XXVIIIa, 3; XXVIIIb, 1; XXVIIIc, 1</td>
</tr>
<tr>
<td>M59/T12</td>
<td>1</td>
<td>H, 1</td>
<td>0</td>
<td>1</td>
<td>LIXa, 1</td>
</tr>
<tr>
<td>M75/T8/25</td>
<td>2</td>
<td>H, 1; O, 1</td>
<td>1</td>
<td>2</td>
<td>No PFGE pattern observed</td>
</tr>
<tr>
<td>M77/T13/28</td>
<td>4</td>
<td>O, 4</td>
<td>4</td>
<td>4</td>
<td>LXXVIIa, 3; LXXVIIb, 1</td>
</tr>
<tr>
<td>M89/T11/12</td>
<td>5</td>
<td>O, 5</td>
<td>2</td>
<td>5</td>
<td>LXXXIXa, 4; LXXXb, 1</td>
</tr>
</tbody>
</table>

NOTE. H, hospital (invasive) isolates; N, nursing home isolates; O, office (pharyngitis) isolates; PFGE, pulsed-field gel electrophoresis.

* * *

*a* Arbitrary designation of PFGE pattern followed by no. of isolates exhibiting that pattern.
invasive strain had a *sic* allele (1.302) that was not also identified in at least 1 M1 pharyngeal strain. *sic* demonstrates very marked allelic hypervariability that arises very rapidly by natural selection on mucosal surfaces [18].

In the present study, *speA* and *speC* genes were detected with very high frequency in both pharyngeal and invasive isolates. Toxin production by our strains was not studied. The observation that significantly more pharyngeal strains than invasive isolates were positive for both *speA* and *speC* (71% and 35%, respectively; *P* < .02) is striking and unexplained. All strains possessed *speB*, which is present in most group A streptococci. Previous efforts to distinguish invasive and noninvasive GAS strains on the basis of *speA* and/or *speC* positivity or SPE A and/or SPE C toxin production have yielded mixed findings. Reports indicate that 43%–64% of pharyngeal isolates are *speA* positive (particularly serotypes M1, M3, and M6) and 44%–69% of invasive isolates are *speA* positive. All 27 of our *emm*1, 3, and 6 isolates but only 33 (62%) of 53 other types were *speA* positive (*P* < .01). Similarly, 20% of pharyngeal and 27%–34% of invasive GAS are *speC* positive [6, 21, 26, 27, 30]. Factors responsible for development of invasive GAS infections by strains usually associated with uncomplicated pharyngitis remain to be identified.

We found complete agreement between *emm* type and M/T serotype among our 80 study isolates. PFGE patterns were fairly consistent within homogenous M/T and *emm* types and distinctive from those of different *emm* and M/T types. PFGE enabled demonstration of related pulotypes within an *emm* or M/T group, further demonstrating isolate heterogeneity, particularly with M3, M5, M12, M18, and M22 isolates.

Several candidate GAS vaccines are under development, including a multivalent M protein–based vaccine and other protein-based vaccines [31]. Our data suggest that even though considerable strain heterogeneity exists, an effective multivalent vaccine targeting the most prevalent serotypes may be an attractive vaccine strategy. Prospective monitoring of community pharyngitis would likely also protect against serious invasive GAS infections.

**Acknowledgment**

We thank Pamela Diaz for providing the nursing home strains.

**References**


21. Perea Mejia LM, Stockbauer KE, Pan X, Cravioto A, Musser JM. Charac-