Clonal Analysis of Neisseria meningitidis Serogroup B Strains in South Africa, 2002 to 2006: Emergence of New Clone ST-4240/6688

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From August 1999 through July 2002, hyperinvasive Neisseria meningitidis serogroup B (MenB) clonal complexes (CCs), namely, ST-32/ET-5 (CC32) and ST-41/44/lineage 3 (CC41/44), were predominant in the Western Cape Province of South Africa. This study analyzed MenB invasive isolates from a national laboratory-based surveillance system that were collected from January 2002 through December 2006. Isolates were characterized by pulsed-field gel electrophoresis (PFGE) (n = 302), and multilocus sequence typing (MLST) and PorA and FetA typing were performed on randomly selected isolates (34/302, 11%). In total, 2,400 cases were reported, with the highest numbers from Gauteng Province (1,307/2,400, 54%) and Western Cape Province (393/2,400, 16%). 67% (1,617/2,400) had isolates and 19% (307/1,617) were identified as serogroup B. MenB incidence remained stable over time (P = 0.77) (average incidence, 0.13/100,000 population [range, 0.10 to 0.16/100,000 population]). PFGE (302/307, 98%) divided isolates (206/302, 68%) into 13 clusters and 96 outliers. The largest cluster, B1, accounted for 25% of isolates (76/302) over the study period; its prevalence decreased from 43% (20/47) in 2002 to 13% (8/62) in 2006 (P < 0.001), and it was common in the Western Cape (58/76, 76%). Clusters B2 and B3 accounted for 10% (31/302) and 6% (19/302), respectively, and showed no significant change over time and were predominant in Gauteng. Randomly selected isolates from clusters B1, B2, and B3 belonged to CC32, CC41/44, and the new CC4240/6688, respectively. Overall, 15 PorA and 12 FetA types were identified. MenB isolates were mostly diverse with no single dominant clone; however, CC32 and CC41/44 accounted for 35% and the new CC4240/6688 was the third most prevalent clone.

Among meningococci, antigenic properties of the polysaccharide capsule are used to divide the organism into serogroups. Globally, six meningococcal serogroups, A, B, C, Y, W135, and, more recently, X, are responsible for most cases of invasive meningococcal disease (27). Neisseria meningitidis serogroup B is a major cause of meningococcal disease in industrialized and developing countries, contributing to at least 30% to 80% of the disease (27, 46, 50, 57). Serogroup B disease occurs mostly as sporadic cases and is endemic to many regions of the world (42, 45, 48, 52, 53). However, prolonged outbreaks have occurred in Brazil (49), Chile (11), Cuba (51), and Oregon (14). Epidemics can span several years, as in the case of New Zealand (19), Norway (1), and the Netherlands (42, 50). In South Africa, serogroups A, B, C, Y, and W135 cause invasive disease (10, 37, 63). From August 1999 through July 2002, serogroup B caused 41% (251/615) of invasive cases (10). More recently, serogroups B and W135 caused the majority of cases and are prevalent in two geographically different regions, the Western Cape Province and Gauteng Province, respectively.

The bacterium’s polysaccharide capsule also serves as a vaccine antigen (54). While polysaccharide and polysaccharide-protein conjugate vaccines are available against serogroups A, C, Y, and W135 (41), the development of a universal vaccine against serogroup B has been extremely challenging (25). Unlike serogroups A, C, Y, and W135, serogroup B is unique because its capsule is structurally similar to human brain components (22), a characteristic shared with the neonatal pathogen Escherichia coli serotype K1, which renders it poorly immunogenic. Vaccine development against serogroup B meningococci has therefore sought to investigate meningococcal outer membrane proteins (OMPs) as vaccine antigens (24). Several OMPs have been used as vaccine targets, including PorA and FetA (9, 38, 65). PorA is a major porin while FetA is an iron receptor, and both are used to characterize meningococci.

One of the first meningococcal OMP-based vaccines to be developed and licensed (1989) was VA-MENGOC-BC (9). The vaccine was developed to control a Cuban epidemic and was based on OMPs (including PorA and FetA) of the epidemic strain. Serogroup C was also incorporated into the vaccine. The vaccine successfully controlled the Cuban epidemic and was used in Brazil (Sao Paulo), Uruguay, and Colombia. This led to the development of other tailor-made OMP vaccines to potentially control epidemics in Norway (PorA based) and New Zealand (PorA based) (21, 38, 65). However, a vaccine with much broader coverage is required for global use. Through reverse vaccinology, a number of potential protein vaccine targets were identified (24, 25). Currently, two protein-based vaccines have shown broad coverage, safety, and immunogenicity. One vaccine targets meningococcal factor H binding protein (fHbp) (28), while the other is a 4-component vaccine that includes fHbp (32).
The majority of meningococcal disease cases worldwide are caused by strains that belong to hyperinvasive clonal complexes (CCs) (27). ST-32/ET-5 and ST-41/44/lineage 3 are responsible for the majority of serogroup B cases (6). In South Africa, limited molecular epidemiological data exist for serogroup B strains. From 1985 to 1990, 22 of 124 (18%) routinely collected invasive meningococcal isolates from the Western Cape Province were characterized as CC ST-32/ET-5 by multilocus enzyme electrophoresis (39). Coulson et al. (10) confirmed the continued circulation of the ST-32/ET-5 clone in the country and its prevalence in the Western Cape Province from August 1999 through July 2002. The study also identified ST-41/44/lineage 3 strains.

Vaccination is not routinely carried out in South Africa but may be used in response to an outbreak. The meningococcal quadrivalent polysaccharide vaccines which target serogroups A, C, Y, and W135 are currently licensed for use in South Africa.

This study aimed to update serogroup B molecular epidemiology data in South Africa by investigating the molecular diversity of circulating invasive N. meningitidis serogroup B isolates collected from January 2002 through December 2006 by pulsed-field gel electrophoresis (PFGE) and selected multilocus sequence typing (MLST) and PorA and FetA typing.

**MATERIALS AND METHODS**

**Bacterial isolates.** Invasive N. meningitidis serogroup B isolates sent to the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS), Johannesburg, South Africa, were used. The NICD conducts ongoing national laboratory-based surveillance for invasive disease caused by N. meningitidis. Viable isolates, together with patient and specimen details, are routinely submitted from approximately 180 private, public, military, and mining hospital laboratories countrywide. Laboratory-confirmed meningococcal cases are defined as patients with N. meningitidis identified from normally sterile body fluids (e.g., blood, cerebrospinal fluid [CSF], joint fluid, or pleural fluid) by culture or PCR. Since 2003, at least one site in each of the nine provinces performed enhanced surveillance, with a local surveillance officer obtaining additional demographic and clinical data.

Once received, isolates were cultured from Dorset egg transport media (64) (Diagnostic Media Products, NHLS, Johannesburg, South Africa) onto Columbia agar (Oxoid Ltd., Basingtoke, United Kingdom) supplemented with 5% horse blood (South African Vaccine Producers, NHLS, Johannesburg, South Africa) and incubated at 37°C in 5% CO2. Isolates were serogrouped by slide agglutination using polyclonal and monoclonal antisera (Remel Europe Ltd., Dartford, England) against serogroups A, B, C, Y, W135, and X. Pure cultures were prepared for long-term storage by suspension in 1 ml of 10% skim milk (Oxoid Ltd., Basingtoke, United Kingdom) and stored at ~70°C.

**PFGE.** PFGE was carried out as described previously (23). Of the 307 N. meningitidis serogroup B (MenB) cases reported, 305 viable isolates were available for PFGE and 302 produced clear, readable PFGE patterns. PFGE images were captured using a gel documentation system (Vacutec, Johannesburg, South Africa). Fingerprints were analyzed using BioNumerics v6.1 software (Applied Maths, Saint-Martens-Latem, Belgium). Genetic relatedness was determined by creating dendrograms using the unweighted pair group method with arithmetic averages (UPGMA) and the Dice coefficient with an optimization of 1.5% and position tolerance of 1.5%. A cluster was defined as five or more isolates that shared 80% similarity on the dendrogram (10, 43, 55). Isolates that did not meet the cluster definition were referred to as outliers.

**MLST and PorA and FetA typing.** A random sample of 11% (34/302) of isolates with PFGE patterns was selected for multilocus sequence typing (MLST) and PorA and FetA typing. A Web-based random number generator ([http://www.random.org/sequences/](http://www.random.org/sequences/)) was used to select the isolates. The MLST procedure was based on the method described on the PubMLST website ([http://pubmlst.org/neisseria/](http://pubmlst.org/neisseria/)). PorA typing was done as described by Sacchi and colleagues (47). FetA typing was performed as described on the PubMLST website ([http://pubmlst.org/neisseria/info/FetA.shtml](http://pubmlst.org/neisseria/info/FetA.shtml)). PCR products were purified using the MSB spin PCRApice purification kit (Invitek, Berlin, Germany). Sequencing of each gene was carried out using the BigDye v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and an ABI 3130 genetic analyzer (Applied Biosystems). In order to remove unincorporated dye terminators from the sequencing reaction, cycle sequencing products were cleaned using one of the following two kits: Dyex 2.0 spin kit (Qiagen Inc., CA) or NucleoSEQ (Macherey-Nagel, Düren, Germany). Nucleotide sequence data were analyzed using DNAStar Lasergene v7 software (Madison, WI). Sequences were submitted to the PubMLST database to obtain a PorA type, FetA type, or sequence type and clonal complex for MLST. Diagrams depicting genetic relatedness by ST were generated using eBURST software (available at [http://eburst.mlst.net](http://eburst.mlst.net)). The default setting of 6/7 identical loci for the group definition was used.

**Data analysis.** Incidence rates were calculated using the total number of reported laboratory-confirmed cases divided by midyear total population estimates provided by Statistics South Africa ([http://www.statssa.gov.za/](http://www.statssa.gov.za/)). Age-specific rates were only calculated for the last year, which was 2006. Comparisons by year, geographic area (province), and PFGE cluster were made. Differences in proportions were calculated using the χ² test (Mantel-Haenszel test or Fisher’s exact test), while the trend over time was evaluated using the χ² test for the trend. P values of less than 0.05 were considered significant. Statistical tests were performed using Epi Info v6.04d ([Centers for Disease Control and Prevention, Atlanta, GA]).

**Ethics.** Ethics approval and clearance for national surveillance (protocol no. M081117) and this project (protocol no. M080701) were obtained from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg, South Africa.

**RESULTS**

**Epidemiology of meningococcal disease in South Africa.** During the 5-year study period (January 2002 through December 2006), 2,400 cases of laboratory-confirmed meningococcal disease were reported: 269, 445, 414, 605, and 667 cases for each year, respectively. Gauteng (1,307/2,400, 54%) and the Western Cape (393/ 2,400, 16%) recorded the highest number of cases among the 9 provinces. Of 2,400 cases, 1,617 (67%) had viable isolates that were serogrouped. Serogroup B (307/1,617, 19%) and serogroup W135 (694/1,617, 43%) were collectively responsible for 62% of cases in South Africa. Nationally, serogroup B incidence remained stable over the 5-year period (P = 0.77) (average incidence, 0.13/100,000 population [range, 0.10 to 0.16/100,000 population]) (Fig. 1). Serogroup B cases were reported in all provinces but were most common in the Western Cape Province (144/307, 47%).

Age was known for 93% (2,232/2,400) of all cases and 97% (299/307) of serogroup B cases. Children of <5 years of age were at the highest risk for meningococcal disease (Fig. 2). Disease was more common in males, and this was true for serogroup B (all cases, 1,333/2,338 [57%], and serogroup B cases, 169/298 [57%]; P = 0.92). Overall, 68% (1,624/2,400) of all meningococcal disease cases were diagnosed by identification of the organism from CSF specimens alone, 495 (21%) from blood specimens, and 271 (11%) from CSF and blood specimens. Additionally, 1 case was identified from peritoneal fluid, 2 cases from pleural fluid, and 7 cases from synovial fluid or joint tissue. Serogroup B isolates were predominantly cultured from CSF specimens (193/307, 63%), 23% (70/307) were cultured from blood specimens alone, and 14% (44/307) were cultured from both CSF and blood specimens. The proportion...
of cases of serogroup B identified from CSF specimens (with or without other positive specimens) was not different from the proportion of non-serogroup B isolates from the same specimens (237/307 [77%] versus 1,024/1,310 [78%]; P = 0.71). Of the 307 MenB cases, 121 were reported from enhanced surveillance sites and therefore had clinical details. Of these 121 cases, 108 (89%) were diagnosed with meningitis, 9 (7%) with generalized sepsis without meningitis, and 4 with pneumonia. The outcome of serogroup B disease was known for 103 cases (85%) from enhanced sites, and the case-fatality ratio was 10% (10/103).

Molecular characterization of *N. meningitidis* serogroup B isolates. Isolates were grouped into 13 PFGE clusters (206/302, 68%) and 96 outliers (96/302, 32%) (Fig. 3). Clusters were designated B1 to B13 in order of size, with B1 being the largest. Half of all isolates belonged to 5 PFGE clusters, B1 to B5 (156/302, 52%) (Fig. 3). Selected isolates from these clusters belonged to CCs ST-32/ET-5 (B1) and ST-41/44/lineage 3 (B2 and B4) and the new CC ST-4240/6688 (B3).

The largest cluster, B1, accounted for 25% (76/302) of all isolates, and its prevalence decreased over time (43% [20/47] in 2002 to 13% [8/62] in 2006; P < 0.001) (Fig. 4). B1 isolates were most prevalent in the Western Cape (58/76, 76%). MLST of six randomly selected isolates identified ST-33 (5) and ST-6589 (1), and both STs belong to CC ST-32/ET-5. Cluster B2 accounted for 10% (31/302) of isolates (Fig. 3), and its prevalence showed no significant change over time (6% [3/47] in 2002 to 16% [10/62] in 2006; P = 0.4) (Fig. 4). B2 isolates were most common to Gauteng (18/31, 58%). Selected isolates from B2 (n = 4) belonged to clonal complex ST-41/44/lineage 3. Three STs were identified, ST-154...
Selected isolates from clusters B4, B7, and B8 also belonged to CC ST-41/44/lineage 3 (Fig. 3). Cluster B3 accounted for 6% (19/302), and its prevalence appeared to increase over time from 2% (1/47) in 2002 to 8% (5/62) in 2006; however, this was not significant ($P = 0.06$) (Fig. 3 and Fig. 4). Isolates were most common to Gauteng (11/19, 58%).

MLST was done on 4 isolates, of which 3, all ST-6688, belonged to the new CC ST-4240/6688. The remaining isolate was identified as ST-2437 and belonged to CC ST-35. ST-6688 and ST-2437 differ by 7 alleles.

Approximately one-third of the isolates (96/302, 32%) did not meet the cluster definition and were classified as outliers (Fig. 3). There was no significant change in outliers from 2002 (17/47, 36%) through 2006 (20/62, 32%) ($P = 0.88$) (Fig. 4). MLST was done on 11 randomly selected outliers. An MLST result was not available for 1 isolate, which did not yield a PCR product for 4/7 MLST genes. Ten STs were identified among the 10 isolates, of which 5/10 (50%) (ST-7392, ST-7223, ST-3982, ST-7946, and ST-6992) were not assigned to a CC in the MLST database. The remaining isolates belonged to CC ST-41/44/lineage 3 ($n = 1$; ST-7945), the new CC ST-4240/6688 ($n = 3$; ST-4240, ST-7222, and ST-6990), and ST-1157 ($n = 1$; ST-1157).

Overall, MLST results were obtained for 33/34 isolates (2002,
n = 6; 2003, n = 7; 2004, n = 5; 2005, n = 5; and 2006, n = 10) and 24 STs were identified. No data were available for one isolate, as PCR failed to amplify some of the housekeeping genes. The eBURST algorithm divided the isolates into 6 groups (n = 23) and 10 outliers (Table 1). A population snapshot was used for representation of all groups (Fig. 5). The diagram was edited to show the possible relation of singletons and the association of groups with CC. The most common CCs were ST-41/44/lineage 3 (9/33, 27%), ST-32/ET-5 (6/33, 18%), and ST-4240/6688 (6/33, 18%).

Fifteen PorA types were identified among the 34 isolates. PorA types P1.5,2 (n = 6), P1.7,9 (n = 5), P1.19,15 (n = 4), and P1.7-2,4 (n = 4) accounted for 56% (19/34) of isolates. PorA type P1.19,15 (4/34, 12%) was mostly associated with CC ST-32/ET-5. PorA types P1.7,9 (5/34, 15%) and P1.7-2,4 (4/34, 12%) were mostly associated with ST-41/44/lineage 3. P1.5,2 (6/34, 18%) was exclusively associated with ST-4240/6688 (Table 2). Twelve FetA types were obtained for 94% (32/34) of isolates. Two isolates failed to produce FetA PCR products. Four FetA types, F5-1 (n = 6), F1-5 (n = 5), F5-8 (n = 5), and F3-20 (n = 5), accounted for 66% (21/32) of isolates. F5-1 (6/32, 19%) was mostly associated with ST-32/ET-5, whereas F1-5 (5/32, 16%) and F3-20 (5/32, 16%) were mostly associated with ST-41/44/lineage 3. F5-8 (5/32, 16%) was mostly associated with ST-41/44/lineage 3.

**FIG 4** Meningococcal serogroup B PFGE clusters in South Africa from 2002 to 2006 (n = 302). By inference, B1 represents ST-32/ET-5; B2, ST-41/44/lineage 3; B3, ST-4240/6688; B4, ST-41/44/lineage 3; B5, ST-865; B7, ST-41/44/lineage 3; B8, ST-41/44/lineage 3; B10, ST-269; and B12, ST-334. No isolates were selected for MLST from cluster B6, B9, B11, or B13.

**FIG 5** Population snapshot of N. meningitidis serogroup B isolates in South Africa from 2002 to 2006 (n = 33). The size of the circle is proportional to the number of isolates. Pale blue lines connect double locus variants. eBURST groups are associated with the following clonal complexes: 1, ST-41/44/lineage 3; 2, ST-4240/6688; 3, ST32/ET-5; 4, ST-41/44/lineage 3; 5, not assigned to a clonal complex; and 6, ST-865.

**TABLE 1** eBURST analysis groups of 23 randomly selected meningococcal serogroup B isolates (South Africa, 2002 to 2006)

<table>
<thead>
<tr>
<th>eBURST group</th>
<th>No. of isolates</th>
<th>ST (no. of isolates)</th>
<th>Allele&lt;sup&gt;abc&lt;/sup&gt;</th>
<th>abc</th>
<th>adk</th>
<th>aroE</th>
<th>fumC</th>
<th>gdh</th>
<th>pdhC</th>
<th>pgm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>154 (2)</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>11</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6698</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>11</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>41</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>5</td>
<td>9</td>
<td>6</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>6688 (3)</td>
<td>15</td>
<td>5</td>
<td>18</td>
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<td>8</td>
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<td>62</td>
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<td>5</td>
<td>9</td>
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<td>8</td>
<td>19</td>
<td>62</td>
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</tr>
<tr>
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<td>6</td>
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<td>10</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>8</td>
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<tr>
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<td></td>
<td>6589</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>26</td>
<td>6</td>
<td>3</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>6590 (3)</td>
<td>9</td>
<td>6</td>
<td>351</td>
<td>9</td>
<td>9</td>
<td>6</td>
<td>311</td>
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<td>26</td>
<td>6</td>
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</tr>
<tr>
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<td>11</td>
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<td>6687</td>
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<td>8</td>
<td>8</td>
<td>21</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Ten out of 33 isolates with MLST results were outliers, and these data have been excluded from the table, as they do not form an eBURST group. eBURST groups are associated with the following clonal complexes: 1, ST-41/44/lineage 3; 2, ST-4240/6688; 3, ST32/ET-5; 4, ST-41/44/lineage 3; 5, not assigned to a clonal complex; and 6, ST-865.

<sup>b</sup> The number of isolates that belong to each ST (if more than one).

<sup>c</sup> Allele differences are indicated in bold.
TABLE 2 Genotypes of randomly selected invasive meningococcal serogroup B isolates by clonal complex (CC) and PorA and FetA type (South Africa, 2002 to 2006)

<table>
<thead>
<tr>
<th>CC(s) (no. of isolates of indicated ST) and genotype profile</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-41/44/lineage 3, ST-6590 (3), ST-154 (2), ST-7945, ST-6689, ST-41, and ST-43</td>
<td></td>
</tr>
<tr>
<td>P1.7,9:F3-20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
</tr>
<tr>
<td>P1.7-2:A:F1-5</td>
<td>4</td>
</tr>
<tr>
<td>P1.19,15-1:F1-5</td>
<td>1</td>
</tr>
<tr>
<td>ST-32/ET-5, ST-33 (5), and ST-6589</td>
<td></td>
</tr>
<tr>
<td>P1.12-1,13-1:F3-1</td>
<td>3</td>
</tr>
<tr>
<td>P1.19,15:F3-7</td>
<td>1</td>
</tr>
<tr>
<td>P1.19,15:F5-1</td>
<td>2</td>
</tr>
<tr>
<td>ST-4240/6688, ST-6688 (3), ST-4240, ST-7222&lt;sup&gt;a&lt;/sup&gt; and ST-6990</td>
<td></td>
</tr>
<tr>
<td>P1.5,2:F5-8</td>
<td>5</td>
</tr>
<tr>
<td>P1.5,2:F3-16</td>
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</tr>
<tr>
<td>ST-865, ST-6687, and ST-7195</td>
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</tr>
<tr>
<td>P1.7-1,1:F1-6</td>
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<tr>
<td>ST-35 and ST-2437</td>
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<tr>
<td>P1.22-1,14: not determined</td>
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<td>ST-334 and ST-7978&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>P1.12-1,16-8:F5-2</td>
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</tr>
<tr>
<td>ST-269 and ST-283</td>
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<tr>
<td>P1.19-1,30-2:F3-1</td>
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</tr>
<tr>
<td>ST-1157 and ST-1157</td>
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</tr>
<tr>
<td>P1.21-7,16:F5-5</td>
<td>1</td>
</tr>
<tr>
<td>Not assigned to a CC: ST-4243, ST-7392,&lt;sup&gt;a&lt;/sup&gt; ST-7223,&lt;sup&gt;a&lt;/sup&gt; ST-7946,&lt;sup&gt;a&lt;/sup&gt; ST-3982, and ST-6992</td>
<td></td>
</tr>
<tr>
<td>P1.7,9:F3-20&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>P1.5-1,2-2:F3-27</td>
<td>1</td>
</tr>
<tr>
<td>P1.17,16-4:F3-34</td>
<td>1</td>
</tr>
<tr>
<td>P1.5-3,10-24:F3-5</td>
<td>1</td>
</tr>
<tr>
<td>P1.19,15:F5-5</td>
<td>1</td>
</tr>
<tr>
<td>P1.17,16-4:F4-1</td>
<td>1</td>
</tr>
</tbody>
</table>
| Total                                                        | 33              

<sup>a</sup> New STs.

was exclusively associated with CC ST-4240/6688 (Table 2). Isolates were assigned an MLST CC PorA:FetA genotype (Table 2).

DISCUSSION

The incidence of serogroup B disease remained stable over the 5-year period. The clones ST-32/ET-5 and ST-41/44/lineage 3 and the new clone ST-4240/6688, as imputed by PFGE clusters, accounted for 42% of serogroup B invasive disease. A proportion of isolates belonged to STs that were initially not assigned to a known clonal complex. Some of these STs were related to each other on the PFGE dendrogram, as well as by eBURST, and on the basis of these data, together with published and unpublished serogroup B MLST data, a new clonal complex, ST-4240/6688, was subsequently designated for these isolates. ST-32/ET-5 and ST-41/44/lineage 3 were previously identified as the most prevalent serogroup B clones from August 1999 through July 2002 (10). However, in this study, there was a decline in the prevalence of ST-32/ET-5 compared to the previous period (10). A more recent study by our group, which analyzed (by MLST) all invasive MenB strains collected during a later period (January 2005 through December 2008), showed the increase of ST-32/ET-5 in a particular geographic region of South Africa where it was previously not very common (17). In addition, newly assigned clonal complex ST-4240/6688 also increased in prevalence from 6% (2002 to 2006) to 16% (2005 to 2008) and was shown to be associated with disease in young children.

As expected, PFGE showed substantial diversity among invasive isolates, which is characteristic of sporadic serogroup B (46, 52). Sporadic serogroup A, C, Y, and W135 strains are generally more clonal than serogroup B (3, 18, 43). Coulson et al. also demonstrated high levels of diversity among South African MenB isolates by PFGE (10). This diversity is attributed to high rates of recombination and phase variation within the naturally transformable meningococcal population (33). However, despite the high degree of diversity among serogroup B isolates, the majority of isolates collected over the 5-year period could be grouped into clusters representative of MLST clones.

Both ST-4240 and ST-6688, belonging to the new CC ST-4240/6688, were first identified in South Africa. ST-4240 was first identified in 2000 and again in 2003 (10, 18). ST-6688 was identified in 2005 (37). ST-4240 and ST-6688 are single locus variants (SLV) of each other. The earliest recorded ST from this CC, ST-16, was from an invasive serogroup B case in Denmark in 1940 and was submitted in 2001 (34). While this particular isolate had a PorA deletion, 10/11 South African ST4240/6688 strains from 2005 displayed the same PorA:FetA genotype, P1.5,2:F5-8 (37). At the time of writing this article, there were 52 records of ST-4240/6688 strains in the global MLST database. In our study, one isolate was identified as ST-2437, belonging to CC ST-35, and clustered with ST-4240/6688 isolates on the PFGE dendrogram. PFGE was repeated on this isolate to ensure that the fingerprint pattern was correct. ST-2437 is a SLV of ST-35 but differs by 7 alleles from ST-4240 and ST-6688. It is possible that recombination events led to substantial changes in the genome of the ST-2437 isolate, causing it to cluster with ST-4240/6688 isolates (33).

From August 1999 through July 2002, ST-32/ET-5 was responsible for 37% (90/245) of serogroup B disease in mostly one geographical region, Western Cape Province (10). Although this clone continues to predominate in the same geographical region, it declined in prevalence over the 5-year period reviewed in our study, followed by an increase during the period from 2007 to 2008 (17). This is most likely due to natural fluctuations and waxing and waning of particular STs, a phenomenon typical of meningococci. ST-32/ET-5 strains have spread between continents since the 1970s, causing outbreaks of various magnitudes (5). Among our isolates, ST-33 was the predicted primary founder of ST-32/ET-5 strains by eBURST. ST33 is a SLV of ST-35 but differs by 7 alleles from ST-4240 and ST-6688. It is possible that recombination events led to substantial changes in the genome of the ST-2437 isolate, causing it to cluster with ST-4240/6688 isolates (33).

From August 1999 through July 2002, ST-32/ET-5 was responsible for 37% (90/245) of serogroup B disease in mostly one geographical region, Western Cape Province (10). Although this clone continues to predominate in the same geographical region, it declined in prevalence over the 5-year period reviewed in our study, followed by an increase during the period from 2007 to 2008 (17). This is most likely due to natural fluctuations and waxing and waning of particular STs, a phenomenon typical of meningococci. ST-32/ET-5 strains have spread between continents since the 1970s, causing outbreaks of various magnitudes (5). Among our isolates, ST-33 was the predicted primary founder of ST-32/ET-5 strains by eBURST. ST33 is a SLV of ST-32 and a subgroup founder within the ST-32/ET-5 complex (44). A substantial amount of invasive meningococcal disease in Europe is due to ST-32/ET-5 strains (32, 57). The epidemic in Norway, which spanned at least 20 years (1970s to 1990s) (1), was caused by a single ST-32/ET-5 clone (4, 42). In the United States, the state of Oregon continues to experience an ST-32/ET-5 outbreak, which began in 1993 (14, 46).
The proportion of the ST-41/44/lineage 3 clone has increased from 12% (29/242) during the period from 1999 to 2002 (10) to 20% (63/302) during our study period. Unlike ST-32/ET-5, the ST-41/44/lineage 3 CC was predominant in a different geographical region, Gauteng Province, where it was previously identified by Coulson et al. (10). Strains of the ST-41/44/lineage 3 complex are highly diverse (66). Two central STs have been defined: ST-41 is largely associated with invasive disease, while ST-44 tends to be associated with meningococcal carriage (29). In this study, eBURST identified ST-154 and ST-6590 as predicted primary founders of ST-41/44/lineage 3 strains. ST-154 is a SLV of ST-41 and a subgroup founder within the CC (44). ST-6590 is a double locus variant (DLV) of ST-110, which is a SLV of ST-44 and a subgroup founder within the ST-41/44/lineage 3 complex. In New Zealand, ST-41/44/lineage 3 strains were responsible for a 10-year epidemic (19). ST-154 was responsible for a substantial amount of disease at this time. Strains of ST-41/44/lineage 3 cause substantial disease in Europe (32, 57), the Netherlands (42, 50), Japan (53), and Taiwan (7), more so than ST-32/ET-5.

Clonal complexes ST-865, ST-269, and ST-334 were also identified in this study. Coulson et al. (10) identified ST-865 as the second most predominant complex among serogroup C isolates (9/49, 18%), preceded only by the ST-11/ET-37 clone (13/49, 27%). A study of invasive meningococcal isolates from South Africa in 2005 showed that ST-865 has expanded, as the majority of serogroup C isolates (16/20, 80%) belonged to ST-865 (37). Capsular switching among ST-865 strains was indicated by serogroup C isolates from 2005 having the same genotype (P1.7-1:F1-6:ST865) as a serogroup B strain isolated in the same year (26, 37). Since ST-865 is usually associated with serogroup B (according to the central MLST database), it is likely that the direction of the switch was from serogroup B to C.

ST-269 has expanded in Europe in recent years and is responsible for a substantial portion of invasive disease (2, 15, 31, 57). There have been reports of an increase in ST-269 following the introduction of the meningococcal C vaccine in Canada (30) and Scotland (15), with suggestions of possible capsule switching (between C and B) in Canada. ST-269 strains have also been identified in the United States (26), Cuba (9), and Europe (66). The identification of serogroup B isolates belonging to ST-334 indicates possible capsule switching from serogroup C to B since strains of ST-334 are mostly associated with serogroup C (26). ST-334 strains have been described in the United States (26).

We have PorA and FetA data for our meningococcal strains from one previous study which extensively characterized all available serogroup B strains (n = 58) collected in 2005 (37). Although only 11% of serogroup B strains were selected for PorA and FetA typing for this study, the results were similar to what we observed for the 2005 strains and therefore adequately represent the most important PorA and FetA types circulating among our serogroup B strains. The most common PorA types throughout the 5-year period included P1.7-2, P1.19,15, P1.7-2,4, P1.7-2,4, and P1.7-2,4. These serotypes have been identified in the United States (56), Cuba (9), Brazil (12, 48), and Spain (62). P1.19,15 has been associated with high disease incidence in Cuba, Brazil, and Spain. Based on data from the central MLST database, P1.19,15 is the dominant PorA type of the ST-32/ET-5 CC (6). P1.7-2,4 is associated with the ST-41/44/lineage 3 clone responsible for the New Zealand epidemic. Devoy et al. (13) showed that this PorA type was relatively stable throughout the epidemic and therefore could be used in an OMP-based vaccine. Based on data from the central MLST database, P1.7-2,4 is the dominant PorA type of the ST-41/44/lineage 3 CC (6). A multicomponent serogroup B vaccine, currently in late stages of clinical development, comprises 4 targets including outer membrane vesicles derived from the New Zealand epidemic strain (24). Our PorA data indicate poor coverage, given that the New Zealand PorA type is not very common among our serogroup B strains. Multiple mechanisms of phase variation, which lead to a high degree of diversity, have been described for PorA (59). However, PorA-deficient isolates have been reported to cause invasive disease (60). Isolates were also diverse by FetA typing, with the most common FetA types being the global F5-1, F5-8, F1-5, and F3-20 (9, 20, 26, 58). In this study, F5-1 and F1-5 were the dominant types associated with ST-32/ET-5 and ST-41/44/lineage 3, respectively. FetA type could not be determined for two isolates. One of these belonged to CC ST-35. FetA gene deletions have been reported in different clones but mostly among isolates of the ST-35 complex and in both invasive and carriage isolates (8, 35).

Common genotypes included P1.5,2:F5-8:ST-4240/6688, P1.19,15:F5-1:ST-32/ET-5, P1.7-2,4:F1-5:ST-41/44/lineage 3, and P1.7,9:F3-20:ST-41/44/lineage 3. P1.5,2:F5-8 has been reported in Germany but among serogroup C isolates (20). P1.11,15:F5-1:ST-32/ET-5 was frequently identified in Cuba (9). P1.7-2,4:F1-5:ST-41/44/lineage 3 was prevalent among isolates from Germany (20) and the United States (26). Using a global collection of isolates representative of hyperinvasive lineages, Urwin et al. (58) identified P1.7-2,4:F1-5 as the most common PorA:FetA genotype among ST-41/44/lineage 3 strains (58). The genotype causing the ongoing epidemic in Oregon (P1.7,16:F3-3:ST-32/ET-5) was not identified among the South African isolates (26).

Due to limited resources, PFGE was used to screen isolates to get a sense of the overall diversity of the population, followed by the selection of a small subset of random isolates for further characterization by MLST and PorA and FetA typing. Isolates that cluster together on a PFGE dendrogram are genetically related (40, 55). Although isolates within a PFGE cluster may have some variation in their fingerprint patterns, they are still considered to be related if the band differences are few (55). In general, isolates from the same PFGE cluster belong to the same clonal complex (7, 36). Thus, MLST of a few randomly selected isolates within a cluster may be used to infer the clonal complex for the majority of (if not all) isolates within the cluster. We have PFGE and MLST data for all available serogroup B isolates from 2005 (n = 58), and careful analyses of these data verified the correlation (37). Since PFGE has been shown to be more discriminatory than MLST (61), this may explain why the highly diverse ST-41/44/lineage 3 strains are not clustered together on the dendrogram.

Continued molecular surveillance using multiple typing methods is crucial for monitoring outbreak-associated and emerging clones. Currently, our serogroup B fHbp (37) and PorA data indicate poor vaccine coverage of the multicomponent serogroup B vaccine. Nevertheless, further characterization of the other vaccine antigens (NadA and NHBA) may show improved coverage.

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16. Reference deleted.


