Population Snapshot of Invasive Serogroup B Meningococci in South Africa from 2005 to 2008

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In South Africa, serogroup B meningococcal disease is sporadic. The aim of this study was to characterize serogroup B strains causing invasive meningococcal disease (IMD) in South Africa from 2005 to 2008. Isolates, collected through a national, laboratory-based surveillance program for IMD, were characterized by multilocus sequence typing (MLST). Two thousand two hundred thirty-four cases were reported, of which 1,447 had viable isolates. Intermediate resistance to penicillin was observed in 2.8% (41/1,447) of all strains. Serogroup B was the second most common serogroup (17%, 251/1,447) and increased from 14% (58/414) in 2005 to 25% (72/290) in 2008 ($P < 0.001$); however, incidence remained stable during the study period (average incidence, 0.13/100,000 population) ($P = 0.54$). Serogroup B was predominantly characterized by three clonal complexes, namely, ST-41/44/lineage 3, ST-32/ET-5, and the new complex ST-4240/6688, which accounted for 27% (65/242), 23% (55/242), and 16% (38/242) of isolates, respectively. ST-4240/6688 was more prevalent among young children (<5 years) than other clonal complexes (27/37 [73%] versus 108/196 [55%]; $P = 0.04$). In the most densely populated province of South Africa, Gauteng, the prevalence of ST-32/ET-5 increased from 8% (2/24) in 2005 to 38% (9/24) in 2008 ($P = 0.04$). Capsular switching was observed in 8/242 (3%) strains. The newly assigned clonal complex ST-4240/6688 was more common in young children.

Serogroup B meningococcal disease remains a concern due to the absence of a universal licensed vaccine. Vaccine development has been hampered by the fact that the B polysaccharide capsule mimics human neural tissue and may therefore have the potential to act as an autoantigen (16). Serogroup B is mostly associated with sporadic cases and localized outbreaks. Nevertheless, prolonged epidemics caused by a single strain have occurred in several parts of the world, including Norway in the 1970s (4), Brazil and Cuba in the 1980s (36), and New Zealand and Oregon State in the United States in the 1990s (1, 11). Strain-specific vaccines based on outer membrane proteins and/or vesicles were developed in response to some of these epidemics with various efficacies (2, 3, 31, 38); however, no broadly effective vaccine is currently available for protection against all strains of serogroup B. Bacterial surface proteins are extremely variable, which complicates vaccine development. Two vaccines, based on outer membrane proteins, are currently in late stages of clinical development (19, 29).

Since the introduction of the meningococcal serogroup C conjugate vaccine in the United Kingdom and Europe, serogroup B meningococcus now accounts for the majority (70% to 90%) of cases in these countries (10, 20). In the United States, serogroup B accounts for approximately 45% of cases (22). In South Africa, in 2000, serogroup B was the most common serogroup, accounting for 52% of cases, which declined to 14% in 2005, causing it to become the second most prevalent serogroup (43). Simultaneously, serogroup W135 had increased in prevalence to become the most dominant serogroup, representing 62% of cases in 2005. Similar to what occurs in other countries, the incidence of serogroup B disease is highest in infants less than 1 year of age (21, 37).

Meningococcal disease in South Africa is mostly sporadic, and only a few localized outbreaks have been reported during the last 50 years. Vaccination is not given routinely but may be initiated in

response to an outbreak. Historically, outbreaks occurred mostly among young adult miners who were living in hostels accommodating up to 18 men per room (39). A retrospective analysis of meningitis cases, conducted over a 25-year period (1972 to 1996) from a hospital serving four mines near Johannesburg in Gauteng Province, showed that during this period four outbreaks occurred, in 1972, 1975, 1990, and 1996 (39). The 1996 outbreak was caused predominantly by serogroup A multilocus enzyme electrophoresis (MLEE) clone I-I (27), the same clone that was previously described in South Africa 3 decades earlier.

In the 1970s there was a shift in meningococcal disease epidemiology in South Africa, both geographically and in the at-risk population: disease became more prevalent among young, mixed-race children less than 5 years old in the Western Cape region (25). Since then, meningococcal disease and, in particular, serogroup B disease have remained prevalent in this region of the country (9, 25, 33). An epidemic caused predominantly by serogroup B meningococcus occurred in Cape Town between 1976 and 1986 (13, 35), with the peak of the epidemic occurring in 1979. Mortality was 4.4%, and the majority of infected children were younger than 2 years of age.

Sporadic serogroup B strains are genetically diverse, and two

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hypervirulent clonal complexes, ST-41/44/lineage 3 and ST-32/ET-5, are responsible for the majority of serogroup B disease in several countries, including South Africa (4, 9, 22, 32, 36). In South Africa, two serogroup B molecular epidemiology studies have been conducted previously (9, 28), using invasive strains collected through our national, laboratory-based surveillance system. In these studies, strains collected from 1999 to 2002 and from 2002 to 2006 were characterized by pulsed-field gel electrophoresis (PFGE), with only a few randomly selected isolates characterized by multilocus sequence typing (MLST). The aim of the present study was to determine the circulating serogroup B clonal complexes in South Africa over a period of 4 years (2005 to 2008) by MLST analysis of all available serogroup B strains.

MATERIALS AND METHODS

National meningococcal surveillance. Bacterial isolates, sent from public, private, and military laboratories throughout South Africa, were collected as part of a national, laboratory-based surveillance for invasive meningococcal disease in South Africa (23). A case of meningococcal disease was defined as caused by *Neisseria meningitidis*, with the pathogen isolated from a normally sterile body fluid, e.g., cerebrospinal fluid (CSF), blood, or joint or pleural fluid. Culture-negative samples that were positive by latex agglutination and/or Gram stain microscopy and confirmed by PCR were also included as cases. Quarterly laboratory audits identified previously unreported cases.

Strain identification. Identification of cultures was confirmed using standardized microbiological procedures (24). Serogroup was determined by slide agglutination with polyclonal antisera to capsular polysaccharides A, C, X, Y, Z, and W135 and monoclonal antiserum to capsular polysaccharide B (Remel Biotech Limited, Dartford, England). For culture-negative cases and transport medium isolates that were no longer viable upon receipt at the reference laboratory, meningococcal identification was determined by PCR. Initially, conventional PCR was used (34, 42), subsequently followed by real-time PCR from September 2008 (8) using *ctrA* as a target. Serogroup was determined by real-time PCR amplification of the capsule-specific *siaD* gene for serogroups B, C, W135, and Y and *sacB* for serogroup A (42).

MLST. Two hundred fifty-one of 276 (91%) serogroup B cases, reported from January 2005 to December 2008, had viable isolates available for further testing, of which 249 (99%) were genetically characterized. MLST was performed as described by Maiden et al. (26), using primers listed on the *Neisseria* MLST website (<http://pubmlst.org/neisseria/>). Isolates were sequenced in both forward and reverse directions using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems), and products were separated using an ABI 3100 genetic analyzer (Applied Biosystems). Allele sequences were assembled and edited using the DNASTar Lasergene v7 software. Allele numbers, sequence types (STs), and clonal complexes were assigned by submitting sequence data to the global MLST database (<http://neisseria.org>). STs were considered to belong to the same clonal complex if they shared 5 or more alleles. The eBURST v3 algorithm was applied to determine founding genotypes and patterns of evolutionary descent between related STs (15). Population snapshots for our serogroup B population were generated by entering the allele numbers for all isolates that were characterized in this study and setting the group definition to 0/7 alleles. Single-locus variants (SLV) and double-locus variants (DLV) were defined as STs differing from each other by one and two alleles, respectively. Since ST is largely associated with serogroup, any isolate with an MLST genotype normally associated with a different capsular type was considered to demonstrate capsular switching.

Antimicrobial susceptibility. Susceptibility testing was performed using the Clinical and Laboratory Standards Institute (CLSI) guidelines using disc screening and MIC by Etest (AB Biodisk, Solna, Sweden) (7).

Data and statistical analyses. Incidence rates were calculated using population estimates from Statistics South Africa (40, 41). The χ^2 test for

trend was used to determine linear trends over time. Only those cases for which a viable isolate was available were included in the trend analyses, as PCR testing methods were not consistently applied throughout the 4-year period. Univariate assessment of characteristics associated with the major clonal complexes was performed using Fisher's exact or the Mantel-Haenszel test. Variables evaluated included age group, sex, syndrome (laboratory-confirmed meningitis versus bacteremia), HIV infection, and outcome. Analyses were performed with Epi Info software, version 6.04d (CDC). A *P* value of less than 0.05 was considered to be statistically significant.

Ethics. The surveillance study and molecular characterization of isolates were approved by the Human Research Ethics Committee, University of the Witwatersrand, South Africa (protocol numbers, M081117 and M060950, respectively).

RESULTS

National meningococcal surveillance. During the 4-year period, 2,234 cases were reported: 605, 666, 503, and 460 for each respective year. Viable isolates and serogroup were available for 1,447 cases. Serogroup W135 accounted for the majority (902/1,447; 62%) of cases, and incidence decreased over time from 0.54 per 100,000 population in 2005 to 0.34 in 2008 ($P < 0.001$) (Fig. 1). Serogroup B was the second most prevalent (251/1,447; 17%), and incidence remained stable during the study period (average incidence, 0.13/100,000 population) ($P = 0.54$). Serogroups Y and C accounted for 10% (143/1,447) and 8% (112/1,447), respectively. Serogroup Y incidence decreased from 0.11/100,000 population in 2005 to 0.04 in 2008 ($P < 0.001$), while serogroup C incidence remained stable. Serogroup A incidence declined from 0.05/100,000 population to 0.01 (24 cases in 2005 versus 2 cases in 2008; $P < 0.001$). Serogroups X ($n = 3$) and Z ($n = 1$) together with a nongroupable isolate represented 0.3%. In addition, PCR assigned a serogroup for 153 meningococci from culture-negative cases and cases where isolates lost viability during transport to the reference laboratory as follows: A, 2 isolates; B, 25 isolates; C, 20 isolates; W135, 102 isolates; and Y, 4 isolates. There were 634 cases for which a serogroup was not assigned, and this was due to one of the following reasons: cases were identified retrospectively on audit ($n = 263$), isolates lost viability during transit to the reference laboratory ($n = 166$), isolates were never sent from the source laboratory to the reference laboratory ($n = 85$), cases identified by PCR were PCR negative for serogroup ($n = 77$), cases were identified on the basis of latex agglutination ($n = 31$), and isolates were contaminated upon arrival and subsequent subculturing at the reference laboratory ($n = 12$).

For serogroup B, age was known for 242 of 251 (96%) cases, and incidence was highest in children less than 1 year of age and remained stable during the study period (average incidence, 1.9/100,000 population) ($P = 0.46$). The distribution of serogroup B cases by presentation was as follows: organism identified from CSF only, 180/276, 65%; organism identified from blood cultures only, 60/276, 22%; and organism identified from CSF and blood cultures, 36/276, 13%.

Serogroup B MLST. Complete data were available for 236 of 249 (95%) serogroup B isolates. Insufficient data were available for 13 isolates because either one or more alleles yielded no amplification product or the sequence data for one or more alleles were poor, and therefore sequence type could not be assigned. However, for 6 of these isolates, data for only one or two alleles were missing in each and a clonal complex was assigned on the basis of 5 or 6 of 7 matching alleles. Therefore, a sequence type was assigned for 236 isolates, a clonal complex was assigned for

FIG 1 Incidence of reported invasive meningococcal disease cases in South Africa, by serogroup, 2005 to 2008 ($n = 2,234$).

218 isolates, and 24 isolates were not associated with any clonal complex (singletons). Overall, 106 STs (including 17 new STs) were grouped into one of 16 clonal complexes (Fig. 2).

Clonal complexes ST-41/44/lineage 3, ST-32/ET-5, and new ST-4240/6688 were most common and accounted for 27% (65/242), 23% (55/242), and 16% (38/242) of isolates, respectively (Fig. 2). Nationally, ST-32/ET-5 fluctuated over time (Fig. 3); however, in Gauteng, ST-32/ET-5 increased 7-fold from 8% (2/24) in 2005 to 58% (15/26) in 2007 ($P = 0.02$) (Fig. 4). In 2008, it started to decline (38%, 9/24), although this decline was not significant ($P = 0.17$). ST-32/ET-5 comprised 15 STs with ST-33 ($n = 25$) as the founder genotype (Fig. 5). ST-33 had 10 SLVs and 4 DLVs. ST-7212 ($n = 14$) emerged as a subgroup founder of ST-33 in 2007 (Fig. 6). Similarly, ST-41/44/lineage 3 fluctuated over the 4-year period (Fig. 3). ST-41/44/lineage 3 comprised 2 groups of 13 and 11 STs each, of which ST-154 ($n = 12$) and ST-6590 ($n = 11$) were the founding STs, respectively (Fig. 5). ST-6688 ($n = 8$) was assigned the founding ST for new clonal

complex ST-4240/6688 and had 8 SLVs, of which ST-4240 was a subgroup founder ($n = 9$) with 4 SLVs and 1 DLV (Fig. 5).

Univariate analysis. The three most prevalent clonal complexes (ST-32/ET-5, ST-41/44/lineage 3, and ST-4240/6688) showed no statistically significant associations with age, gender, increased mortality, HIV infection, or clinical syndrome, with the exception of ST-4240/6688, which was more prevalent among young children (<5 years) than other clonal complexes (27/37 [73%] versus 108/196 [55%]; $P = 0.04$; odds ratio, 2.20; 95% confidence interval, 1.01 to 4.79). ST-32/ET-5 (4/6 [67%] versus 9/30 [30%]) was more prevalent in HIV-infected patients, but this was not significant ($P = 0.16$), presumably due to the small numbers.

Capsular switching. Capsular switching was evident in 3% (8/242) of serogroup B strains. Clonal complexes ST-103 and ST-334, which are usually associated with serogroup C, were observed in 1 and 3 strains, respectively. ST-174 and ST-175 clonal complexes were found in 1 strain each, both of which largely correlate with serogroup Y or W135.

FIG 2 Clonal complexes among invasive serogroup B meningococci, South Africa, 2005 to 2008 ($n = 242$). "Not assigned*" indicates that some alleles are missing due to poor or no sequence data ($n = 7$).

FIG 3 Distribution of clonal complexes of serogroup B meningococci causing invasive disease in South Africa by year, 2005 to 2008 ($n = 242$). “Not assigned” ($n = 7$) indicates those isolates for which sequence data for some alleles were poor or inadequate.

Antimicrobial susceptibility. Intermediate resistance to penicillin was detected in 2.8% (41/1,447) of isolates ($MIC_{50} = 0.19 \mu\text{g/ml}$, $MIC_{90} = 0.25 \mu\text{g/ml}$) and belonged to serogroup W135 ($n = 29$), B ($n = 7$), Y ($n = 3$), or C ($n = 2$). Nonsusceptibility to trimethoprim-sulfamethoxazole and rifampin was detected in 87% (1,266/1,447) (MIC_{50} and $MIC_{90} = 32 \mu\text{g/ml}$) and 0.3% (4/1,447) of isolates, respectively. Intermediate resistance to penicillin was observed in 2.4% (6/251) of serogroup B isolates ($MIC_{50} = 0.125 \mu\text{g/ml}$). Three of the isolates were ST-41/44/lineage 3 strains originating from the same geographic region. The three remaining isolates were ST-35 ($n = 1$) and ST-4240/6688

($n = 2$). Trimethoprim-sulfamethoxazole nonsusceptibility was observed in 198/251 (79%) serogroup B strains; 100%, 85%, and 51% nonsusceptibility was observed in ST-32/ET-5, ST-41/44/lineage 3, and ST-4240/6688 strains, respectively. Rifampin nonsusceptibility was observed in one serogroup B isolate.

DISCUSSION

This study presents a detailed analysis of serogroup B genotypes circulating in South Africa during a 4-year period, from 2005 to 2008. The incidence of serogroup B disease remained stable throughout the study period, with some variation in the preva-

FIG 4 Distribution of clonal complexes among invasive serogroup B meningococci in Gauteng Province, South Africa, by year, 2005 to 2008 ($n = 93$).

FIG 5 eBURST diagram depicting a population snapshot of invasive serogroup B meningococci, South Africa, 2005 to 2008 ($n = 236$). Each circle represents a sequence type (ST). Blue circles indicate the founding genotype, and yellow circles denote subgroup founders. The size of each circle is proportional to the number of isolates for that ST. Relationships between STs are depicted by the lines connecting the STs.

lence and geographic distribution of hypervirulent clones. This is presumably due to natural fluctuations characteristic of this organism. Almost two-thirds of serogroup B disease was characterized by three clones, namely, ST-41/44/lineage 3, ST-32/ET-5, and ST-4240/6688. ST-32/ET-5 and ST-41/44/lineage 3 strains have been described previously in South Africa (9) and continue to circulate, except that the geographic distribution of ST-32/ET-5 appears to have shifted. Nationally, ST-32/ET-5 remained relatively stable with minor fluctuations over time; however, in Gauteng Province this clonal complex increased 7-fold. The reasons for this are unclear, except that the emergence of ST-7212 in 2007, an SLV of ST-33 (which belongs to clonal complex ST-32/ET-5), appears to be responsible for the increase of ST-32/ET-5 in Gauteng. Intermediate resistance to penicillin remains low in South Africa and has declined from an overall prevalence of 6% in 2001 to 2005 (14) to 2.8% in 2005 to 2008.

The *N. meningitidis* MLST database shows that ST-41/44/lineage 3 and ST-32/ET-5 currently represent the vast majority of serogroup B isolates in the database, accounting for 32% (1,820/5,715) and 21% (1,208/5,715) of submitted serogroup B isolates, respectively. Six African countries—Morocco, Tunisia, Egypt, Cameroon, The Gambia, and South Africa—and the neighboring islands of Malta, Reunion, Madagascar, and Mayotte have some form of representation in the global database, although most (with the exception of Morocco and South Africa) have submitted data for fewer than five serogroup B isolates. ST-41/44/lineage 3 and ST-32/ET-5 strains have been described in all of these countries, with the exception of Egypt, Mayotte, and Reunion, which show

no record of ST-32/ET-5, and Madagascar, which shows no record of ST-41/44/lineage 3.

ST-32/ET-5 strains were first reported from South Africa in 1980 when MLEE of a selection of serogroup B outbreak isolates identified two strains of the ST-32/ET-5 complex (B:NT:P1.15) (5, 6, 9). Subsequently, this clonal complex was identified retrospectively among 18% of serogroup B strains isolated from specimens submitted for routine meningitis investigation of patients; specimens were collected between 1985 and 1990 from one geographic region of the country (Western Cape Province). Coulson et al. (9) analyzed surveillance invasive meningococcal cases reported between August 1999 and July 2002. Serogroup B disease represented 41% of all cases, and 70% of these were reported from a single geographic region (Western Cape Province). PFGE analysis divided the majority of serogroup B strains (72%) into five distinct clusters with clusters 1 and 2 representing 50% of all isolates. ST-32/ET-5 and ST-41/44/lineage 3 were present among isolates randomly selected for MLST from PFGE clusters 1 and 2, respectively. Clonal complex ST-35 was also identified in this study.

The assigning of clonal complex ST-4240/6688 was a consequence of the relatively large number of isolates from South Africa for which data were submitted to the global database. Data for 42 of 51 (82%) currently submitted serogroup B isolates belonging to this clonal complex are from South Africa. There is also documentation of a single nongroupable isolate from the United Kingdom that belongs to clonal complex ST-4240/6688. Although the assigning of the clonal complex is new, strains of this clonal complex

FIG 6 eBURST diagram of clonal complex ST-32/ET-5 among invasive serogroup B meningococci, South Africa, by year, 2005 to 2008 ($n = 54$). Each circle represents a sequence type (ST). ST-7212 in panel C is the founding genotype, and ST-33 denotes the subgroup founder for this cluster of isolates. The size of each circle is proportional to the number of isolates for that ST. Relationships between STs are depicted by the lines connecting the STs.

have been documented as early as 1940, mainly in Europe. The MLST database documents one strain each isolated in Denmark in 1940 (ST-16), The Netherlands in 1963 (ST-6840), the Czech Republic in 1976 (ST-707), Sweden in 1978 (ST-2875), New Zealand in 1997 (ST-2346), and Germany in 2000 (ST-764) and three strains from the United Kingdom in 2001 (ST-1408, -2311, and -3009). Strains of this clonal complex have been in South Africa at least since 2000, but the clone appears to have expanded in 2007 and 2008. This clone was also more likely to cause disease in young children, whereas ST-32/ET-5 and ST-41/44/lineage 3 strains were equally prevalent among children and adults. Since very few strains of this clonal complex have been described elsewhere in the world and limited age data are available for patients infected with this clone, it is not certain whether this observation is specific to South Africa.

The MLST global database shows that the overwhelming majority (93%) of ST-32/ET-5 strains are associated with serogroup B; however, a small proportion of strains (2%) also express serogroup C capsules or are nongroupable (4%). Similarly, 83% of ST-41/44/lineage 3 strains are associated with serogroup B but have also been shown to express one of eight other serogroups (A, C, H, W135, X, Y, Z, and 29E) or are nongroupable (10%). Our study showed some evidence of capsular switching among serogroup B with strains arising mostly from serogroup C strains and, to a lesser degree, serogroups Y and W135. The Harrison et al. study (22), which describes the molecular epidemiology of *N. meningitidis* over a 6-year period (2000 to 2005) in the United States, demonstrated capsular switching as a naturally occurring phenomenon prior to routine use of any meningococcal vaccine.

Capsular switching was evident in serogroup B, C, X, and Y strains. Four ST-103 strains and one ST-334 strain expressed serogroup B capsules—these clonal complexes are usually associated with serogroup C. This correlates with our study, which also demonstrated switching in ST-103 and ST-334 strains, i.e., from serogroup C to B. The low frequency of capsular switching among serogroup B strains (1.5%) in the U.S. study was also consistent with our findings.

Large recurring outbreaks, mostly due to serogroup A and more recently serogroup W135, are characteristic of meningococcal disease observed in the sub-Saharan countries of the “meningitis belt,” which extends from Ethiopia in the East to Senegal in the West. During the last decade, serogroup X also emerged as a cause of both localized and larger epidemics (12, 17). Limited published data describing meningococcal disease outside the meningitis belt are available. Apart from South Africa, serogroup B is rarely reported from the African continent and appears to have been described only in Egypt, Morocco, and Angola (18, 30, 44). In Egypt, a bivalent A/C polysaccharide vaccine was implemented in schools in 1992. Prior to that, outbreaks of serogroup A were common; however, following implementation of the vaccination program no outbreaks have been reported, and a shift in epidemiology from serogroup A to serogroup B has occurred. In Morocco, serogroup B was detected most frequently (76%) among 163 strains collected over a 9-year period (1992 to 2000). Six outbreaks caused by serogroup A were described in Angola between 1994 and 2000; however, sporadic cases of serogroup B were also reported during the same period.

In South Africa, serogroup B disease remains sporadic and stable. Nevertheless, we describe some variation related to the prevalence and distribution of global hypervirulent clonal complexes. Capsular switching was evident in the absence of routine meningococcal vaccine use in the country. The new clonal complex ST4240/6688 was mostly associated with disease in young children. Hypervirulent clones ST-32/ET-5 and ST-41/44/lineage 3 continue to predominate, although changes were evident in terms of geographic prevalence and distribution in different regions of the country.

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