Molecular Epidemiology of Serogroup A Meningitis in Moscow, 1969–1997

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Molecular analysis of 103 serogroup A Neisseria meningitidis strains isolated in Moscow from 1969 to 1997 showed that four independent clonal groupings were responsible for successive waves of meningococcal disease. An epidemic from 1969 to the mid-1970s was caused by genocloud 2 of subgroup III, possibly imported from China. Subsequent endemic disease through the early 1990s was caused by subgroup X and then by subgroup VI, which has also caused endemic disease elsewhere in eastern Europe. A 1996 epidemic was part of the pandemic spread from Asia of genocloud 8 of subgroup III. Recent genocloud 8 epidemic disease in Moscow may represent an early warning for spread of these bacteria to other countries in Europe.

Until the mid-1970s, Neisseria meningitidis expressing the serogroup A capsular polysaccharide caused numerous epidemics of meningitis and septicaemia, with annual incidence rates >100/100,000 (1). During serogroup A epidemics, these bacteria can also be isolated from the nasopharynx of a considerable proportion of the healthy population (2). After the mid-1970s, large serogroup A epidemics became rare, except in the so-called Meningitis Belt in the Sahel region of Africa (3,4). In 1997, N. meningitidis caused approximately 500,000 cases of disease and 50,000 deaths worldwide, half of them in Africa (5). Serogroup A meningococci are currently isolated only rarely in western Europe and the United States, and a rise in either the healthy carriage rate or the proportion of serogroup A cases would be cause for concern.

Epidemic isolates of N. meningitidis have been assigned to a limited number of clonal groupings, historically by multilocus enzyme electrophoresis (MLEE) (4) and currently by multilocus sequence typing (MLST) (6). For MLST, sequences of housekeeping gene fragments are assigned different numeric allele designations, even if they differ by only one nucleotide. The combination of alleles from seven housekeeping gene fragments is called the sequence type (ST). Serogroup A bacteria have also been assigned to clonal groupings by random amplification of polymorphic DNA (RAPD) (7). These three methods yield largely concordant results, except that MLST is the most conservative and RAPD differentiates the largest number of isolates. The clonal groupings among serogroup A meningococci have been designated subgroups I through IX according to MLEE (7,8). Some of these subgroups have not been differentiated by RAPD analysis (I vs. II, III vs. VIII) (7) or MLST.

Recent epidemic serogroup A disease in Europe has largely reflected the pandemic patterns of spread of subgroup III meningococci. After a large subgroup III epidemic in China in the mid-1960s (8), these bacteria caused an outbreak of meningococcal disease in 1969 in western Norway (9), followed in the mid-1970s by a major epidemic in Finland (10). During the 1970s, subgroup III meningococci were isolated throughout western and northern Europe (11). In 1987, serogroup A, subgroup III meningococci caused a meningitis epidemic during the Hajj pilgrimage to Mecca, Saudi Arabia (12), and healthy pilgrims carried these bacteria throughout the world. Localized serogroup A disease was associated with former pilgrims for several years in the United Kingdom (13) and France (14), but these meningococci have since largely disappeared from western Europe. Rare endemic serogroup A disease was associated with subgroup VI in East Germany in the 1980s (8) and with subgroup IX in the Netherlands in the early 1990s (7).

Meningococci are naturally transformable and can import novel alleles from neisseriae that colonize the nasopharynx (15,16). However, these recombinant strains are usually lost during the bottlenecks caused by limited numbers of bacteria being transmitted during epidemic spread (17). Occasionally, variants pass through these bottlenecks first, and all subsequent progeny have a common genetic background different from that of the parental bacteria (17). Most subgroup III bacteria isolated from the mid-1960s to the mid-1980s (“pre-Mecca”) had particular alleles of the hypervariable opaB and opaD loci encoding Opa (opacity) proteins and the igA locus encoding IgA1 protease. In contrast, subgroup III bacteria isolated after the 1987 Mecca epidemic (“post-Mecca”) had other alleles at these three loci (17). Exceptional isolates with still other alleles at one or more of these loci were also found in several countries, but these variants all disappeared during subsequent epidemic spread (17).
Data in the English literature about meningococci from Russia and other areas of eastern Europe are scarce. In recent decades, most patients with meningitis in Moscow (population 8.6 million during 1990 to 1999) have been treated at the Second Moscow Hospital for Infectious Diseases. Our review of their records shows that from 1980 to 1999, the annual incidence rates of meningitis caused by *Haemophilus influenzae* and *Streptococcus pneumoniae* were approximately 0.2/100,000 and 0.4/100,000, respectively. In contrast, several epidemics of meningococcal disease with much higher incidence rates occurred in Moscow from 1924 to 1998 (Figure 1). Large serogroup A epidemics occurred in 1931 and 1940, followed by a decline in disease incidence until the late 1960s. In 1968, a serogroup A epidemic began in Lipezk, Russia, where factory workers had recently arrived from Vietnam. These immigrants had traveled by rail through China, where subgroup III disease was prevalent (8). The incidence rate of meningococcal disease in Moscow and the proportion of serogroup A isolates remained high until the 1980s. In 1996, a new outbreak of serogroup A disease began, again initially associated with the Vietnamese community. During the outbreak peak in early 1996, 50 (34%) of 147 patients with meningococcal disease were Vietnamese, and all their disease isolates were serogroup A. During the rest of 1996, only 8 (6%) of 145 patients with meningococcal disease belonged to the Vietnamese community. During 1997 to 1999, hundreds of thousands of Muscovites were immunized with A polysaccharide vaccine. The incidence rate and the proportion of serogroup A isolates decreased and have remained stable through 1999.

We present data on the molecular epidemiology and genetic relationships of serogroup A meningococci isolated in Moscow from 1969 to 1997. The data show that four clonal groupings have been responsible for successive waves of disease and that epidemic disease in Moscow since 1994 is associated with a new subgroup III pandemic.

### Materials and Methods

#### Serogroup A Isolates from Moscow

One hundred three strains of *N. meningitidis* were isolated in Moscow from the cerebrospinal fluid or blood of patients with systemic meningococcal disease or from throat swabs of their close contacts (seven subgroup VI strains, 1988-89). Strains were serogrouped by immune precipitation with specific sera and lyophilized. The bacteria we describe represent almost all the viable serogroup A bacteria still available in the laboratory collections at the Central Research Institute of Epidemiology and the Gamaleya Institute of Epidemiology and Microbiology, with the following exceptions: Only one third of the bacteria available from 1969 to 1972 and half the bacteria from 1996 were tested to avoid bias toward these two periods. The lyophilized bacteria were purified again by single colony isolation. These bacterial cultures were then serogrouped, serotyped, and subtyped by using whole-cell enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (8). DNA was prepared as described (21) and used for molecular fine typing and MLST.

#### MLST

Both strands of fragments of *abcZ*, *adh*, *aroE*, *gdh*, *pdhC*, *pgm*, and *fumC* were sequenced as described (http://www.mlst.net), except that primers O1101 (5'TCCGGCT-GTGGCGTTTTGTCA) and O1102 (5'TTGAGCCTTTTTTGGGAC) were used for *fumC*. At the beginning of this project, the MLST database inadequately represented the diversity of serogroup A meningococci because it contained data for only 36 strains, predominantly from MLEE subgroups I, III, and IV-1 (6). To enlarge the database, MLST was performed with 66 serogroup A isolates from diverse sources that had previously been assigned to subgroups II through IX by MLEE (7,8) and in some cases by RAPD analysis (7). MLST was also performed with 42 serogroup A isolates from Moscow chosen from the most diverse branches of the RAPD tree. Other laboratories had deposited data for eight serogroup A strains in six novel STs, resulting in 152 serogroup A strains in 31 STs (Table 1). The results, including strain description, source, and MLEE assignments, are publicly available (http://www.mlst.net).

#### Molecular Fine Typing of Subgroup III

Alleles of the four chromosomal *opa* genes were determined by restriction analysis of polymerase chain reaction (PCR) products generated by using one primer in the upstream conserved flanking DNA and a second HV2-specific primer, as described (17). The complete *opa* genes were also sequenced from selected representative strains.

A fragment of *iga* was amplified from chromosomal DNA by using primers O103 and O115 and tested for a *Bfa*I site that is present in *iga*1 but not in *iga*2 or *iga*3 (17). A different fragment of *iga* was amplified with primers O113 and O151 and tested for a *Dde*I site that is present in *iga*3 but not in *iga*2 or *iga*1 (17). In addition, the entire *iga* gene was amplified with primers O103 and O152 and sequenced from one ST5 and one ST7 strain.
The IS1106A locus near opcA was amplified by using primers in conserved DNA flanking this locus (22). Two sizes of PCR products (4.8 and 3.5 kb) were obtained among the subgroup III strains. (One strain yielded a 12-kb product that was not investigated further.) An internal, polymorphic primer located near the ATG start codon (3’-Met2) and a primer located in the intergenic region between \( \text{tbpB} \) and \( \text{tbpA} \) (5’-Inter1), as described (16). Different alleles of \( \text{tbpB} \) were recognized by digesting the 2.1-kb fragment with \( \text{HaeIII} \), \( \text{CiaI} \), and \( \text{BclI} \). The allele in the 4.8-kb fragment was named IS1106A10, and the allele in the 3.5-kb fragment was named IS1106A7. PCR products from all the subgroup III strains were tested for the four distinctive restriction sites, but no additional diversity was identified. Details on alleles at this locus in subgroup III bacteria from sources other than Moscow have been described (23).

The 2.1-kb \( \text{tbpB} \) gene was amplified by using an internal primer located near the ATG start codon (3’-Met2) and a primer located in the intergenic region between \( \text{tbpB} \) and \( \text{tbpA} \) (5’-Inter1), as described (16). Different alleles of \( \text{tbpB} \) were recognized by digesting the 2.1-kb fragment with \( \text{HaeIII} \), \( \text{MspI} \), and \( \text{SspI} \) and by sequencing the entire fragment from two ST5 strains and one ST7 strain. The \( \text{tbpB} \) allele designations are congruent with those described for a 600-bp 5’-terminal region that has been examined from numerous clonal groupings by comparison with previous data (7). The third cluster (24 isolates) differs from previously defined subgroups and was therefore tentatively designated subgroup X. Two singleton strains were labeled IV (a strain formerly assigned to subgroup IV-2 by MLEE) and ST79.

Forty-two strains that represent the diversity revealed by RAPD were analyzed by MLST. These data were combined with data from other strains as described in Materials and Methods, resulting in a database containing 152 serogroup A isolates (Table 1, Figure 3). MLST did not distinguish several subgroups defined by MLEE (I and II, IV-1 and IV-2, III and VIII, V and VII). Otherwise, the subgroup structure in a neighbor-joining (NJ) tree of the fraction of alleles differing between STs (Figure 3) was largely concordant with prior subgroup assignments from MLEE (8) or RAPD (7). Only STs 1 and 57 clustered inappropriately in the NJ tree (Figure 3).

The MLST results confirmed the assignments of isolates from Moscow to subgroups III and VI. They also demonstrated that subgroup X is distinct from previously defined subgroups and is closely related to subgroups I and II. Individual STs of
these three subgroups share identical alleles for up to six of the seven gene fragments sequenced (Table 1).

**Inconsistent Fine Typing by RAPD and MLST**

Subgroup III isolates from Moscow were isolated during more than one epidemic wave, raising the question whether these isolates shared a common source. However, the concordance between RAPD and MLST did not extend below the subgroup level. The two major RAPD branches in subgroup III initially seemed to correspond to MLST ST5 and ST7. These STs are identical except for their pgm alleles (ST5, pgm3; ST7, pgm19,) which differ by 19 (4%) of 450 bp, including a distinctive restriction site. Genotyping of pgm from all subgroup III isolates by PCR-restriction fragment length polymorphism (PCR-RFLP) showed that both major RAPD branches contained both pgm alleles (Figure 2). Therefore, additional powerful fine typing methods, such as

Figure 2. UPGMA tree of band differences between random amplified polymorphic DNA (RAPD) patterns from 103 serogroup A strains from Moscow. RAPD tests were performed with primers 1254, 1281, NM03, and NM04 as described (7), generating four patterns for each strain. The combination of the differences between these patterns was used to generate a UPGMA tree with the program GelCompar (Applied Maths, Kortrijk, Belgium), as described (7). Subgroup designations are indicated within the tree. The strain designations and the sequence type (ST) assignments from MLST analysis are shown at the right. For subgroup III bacteria, dark gray rectangles indicate pgm3 and light gray rectangles indicate pgm19. Strain Z4714 is indicated as belonging to subgroup IV because RAPD does not distinguish between subgroups IV-1 and IV-2.
multilocus PCR-RFLP, were needed to resolve whether all bacteria within a subgroup originated from the same sources.

**Serologic Properties**

In some cases, serologic serotyping and serosubtyping can be used to enable rapid recognition of clonal groupings. The serologic properties of all the serogroup A bacteria from Moscow were determined by ELISA with monoclonal typing antibodies. The results confirmed previous results for subgroups III and VI (8) and showed that subgroup X is 4,21:P1.10 or 21:P1.10 (Table 2). Subgroup X is therefore not distinguishable by serologic testing from serogroup A subgroups I, II, IV-2 and VI, which are also 4,21:P1.10. The subgroup III strains uniformly expressed the class I and IIa pilin epitopes (8); however, the pilin epitopes of subgroups VI and X were not uniform: half of each group of strains expressed class I and IIa epitopes and the other half the class IIb epitope. Thus, except for subgroup III, serologic typing would not be useful for distinguishing these various subgroups.

<table>
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<th>Year</th>
<th>III (ST5a)</th>
<th>X</th>
<th>VI</th>
<th>III (ST7)</th>
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<th>Total</th>
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<td>17</td>
<td>23</td>
<td>40</td>
<td>21</td>
<td>2</td>
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*aST = sequence type
Subgroup assignments are according to data from random amplification of polymorphic DNA (Figure 2). For strains of subgroup III where a complete multilocus sequence typing analysis was not performed, bacteria were assigned to ST5 and ST7 on the basis of results with pgm polymerase chain reaction/restriction analysis. Other: 1971, subgroup IV-2; 1995, ST79.

Asia in the early 1990s. Genocloud 8 bacteria differ from other subgroup III meningococci in having the tbpB55, pgm19, and IS1106A7 alleles (Table 4). All 21 subgroup III bacteria isolated in Moscow from 1994 to 1997 also have these alleles (Table 4) and thus represent an extension of the third pandemic wave.

The subgroup III ST5 meningococci isolated in Moscow from 1969 to 1977 are distinct from all other subgroup III meningococci and define genocloud 2 (Table 4). The most closely related genocloud is genocloud 1, isolated in China in the mid-1960s, which contains the same IS1106A10 element but differs at tbpB and opaB. The genocloud 3 bacteria that caused outbreaks in northern Europe concurrently with the epidemic in Moscow differed in IS1106A (IS1106A4) as well as tbpB and opaB. Other subgroup III genoclouds, including genocloud 5 (post-Mecca), differed at even more loci (Table 4).

**Phylogenetic Ancestry of Genocloud 2**

The unique tbpB allele in ST5 subgroup III bacteria from Moscow is not informative for phylogenetic descent: It was not found in any other genocloud and was probably imported by DNA transformation (16) shortly before or after these bacteria arrived in Moscow. However, the phylogenetic history of the unique opaB alleles in the subgroup III bacteria from Moscow can be reconstructed from their sequences. Genocloud 1 bacteria from China have the opa132, opa92, opa131, and opa101 alleles at the opaA, B, D, and J loci, respectively. The novel alleles at the opaB and opaD loci in genocloud 2 bacteria from Moscow probably represent gene conversion with the opaJ101 and opaA132 alleles (Figure 4). The opaB140 allele, which was present in all but one strain from Moscow (Table 4), differs from opaJ101 by only one nucleotide (Figure 4). The opaB113 allele (one strain) can be explained by gene conversion of opaB140 with DNA from the opaA132 allele. opaD131 is present in genocloud 1 and was
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Table 4. Hypervariable alleles among subgroup III meningococci isolated in Moscow

<table>
<thead>
<tr>
<th>Strains</th>
<th>No.</th>
<th>Locus</th>
<th>tpB</th>
<th>iga</th>
<th>pgm</th>
<th>IS1106</th>
<th>opaA</th>
<th>opaB</th>
<th>opaD</th>
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<td>1</td>
<td>38</td>
<td>2</td>
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<td>131</td>
</tr>
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<td>A10</td>
<td>132</td>
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aData for the genoclouds at the top of the table are from a separate manuscript (23). Sources of genoclouds: 1, China, 1966; 3, pre-Mecca isolates (1969-1984) from Europe, Brazil and China; 5, post-Mecca isolates (1987-1998) from Africa and pilgrims returning from Mecca in 1987 to various countries; and 8, China, Mongolia, and Africa, 1992-2000. All serogroup A isolates from Moscow that were assigned to subgroup III by random amplification of polymorphic DNA are included in this table. The opaA and opaD alleles have not been tested in all bacteria from the various genoclouds because they are otherwise so uniform.

Discussion

Since the 1920s, serogroup A meningococci have been responsible for a large proportion of the bacterial meningitis in Moscow (Figure 1). Serogroup A meningococci isolated in Moscow from 1969 to 1997 (103 isolates) were analyzed by RAPD. Strains representing the diversity revealed by the RAPD data were analyzed by MLST (44 isolates), and all 38 subgroup III strains were subjected to molecular fine typing by multilocus PCR-RFLP. The results illustrate the limitations of these methods and yield important information about the phylogenetic relationships among serogroup A meningococci. They also demonstrate that serogroup A meningococcal disease in Moscow since 1969 has been caused by four successive, independent waves of bacterial colonization, most recently by the spread of a third subgroup III pandemic wave from East Asia.

Typing Methods

RAPD analysis was used to provide a quick overview of the genetic diversity in the 103 strains investigated (Figure 2). This information was used to select a smaller group of bacteria to be tested by more expensive and labor-intensive methods such as MLST. As in previous comparisons between RAPD and MLEE (7), the subgroup assignments were concordant between MLST and RAPD. The RAPD data allowed the initial recognition of a new subgroup, subgroup X, and the MLST data confirmed that subgroup IX is not genetically related to the epidemic serogroup A subgroups as originally demonstrated by RAPD data (7). Thus, RAPD analysis can be a highly reliable, quick method for recognizing genetically related bacterial groups.

Finer subdivisions in subgroups III and X, suggested by the RAPD data, were contradicted by MLST results. ST76 was distributed among both primary RAPD subdivisions of the subgroup X cluster, and the two primary RAPD subdivisions of the subgroup III cluster did not correlate with presence of pgm3 versus pgm19 (Figure 2) or with epidemiologic data. Such random noise will probably be most extreme for bacteria such as N. meningitidis, which frequently import DNA from unrelated neisseriae (15). Thus, putative subdivisions within phylogenetically related groups suggested by RAPD data should be confirmed by independent methods.

MLST is based on sequences of conserved housekeeping genes and sequence variants are rarer than with most other methods used in molecular epidemiology, thus facilitating global, long-term epidemiology (6). For subgroup III, the distinction between STs 5 and 7 based on pgm is a very useful molecular marker for recognizing bacteria from the third pandemic wave. However, the epidemiologic data do not suggest that other STs are useful markers for genetic variants that have spread extensively, and MLST does not necessarily provide the discrimination desired for fine typing.

Unfortunately, the fine structure of bacteria that frequently import foreign DNA is very difficult to resolve, and we know of no method that is eminently suitable for this task. Our data argue against the use of RAPD and MLST. Although pulsed-field gel electrophoresis is highly suitable for differentiating almost identical bacteria in limited numbers of strains (17,24,25), it is poorly suited for elucidating the relationships among large numbers of less strongly related bacteria. Fine typing was therefore attempted based on multilocus RFLP of PCR fragments of pgm plus the variable genes opa, iga, and tpB and the IS1106A element. Multilocus PCR-RFLP of variable genes allowed molecular fine typing within subgroup III meningococci, but the data are also limited by considerable random noise, resulting in genoclouds of related variants (23). Alternative strategies involving IS
elements (26), amplified fragment-length polymorphism (27),
or variable nucleotide repeat sequences (28) have been used
successfully for fine typing of highly uniform microorganisms.
Such methods have not yet been extensively tested with
*N. meningitidis* but seem likely to be limited by at least as
much random noise as the methods we used. Thus, reliable
fine typing remains difficult to achieve.

**Phylogeny of Serogroup A Meningococci**

The MLST data on 152 serogroup A strains allow
inferences about the evolution of these bacteria. The STs in
subgroups I through VIII and X (the epidemic subgroups)
probably stem from a common ancestor. They form a
monophyletic clade in phylogenetic trees (Figure 3), and many
of these STs share the *fumC1, abcZ1, adk3, gdh1, pdhC1,* and
*pgm3* alleles (Table 1). The epidemic subgroups include
meningococci isolated since the beginning of this century
(ST4, subgroup IV-2, 1917; ST57, subgroup II, 1930) (8) and
must have evolved before then.

Older isolates of subgroups III (1960s), IV-1 (1960s), and
IV-2 (1917) had identical or nearly identical *opa, iga,* and
*tbpB* alleles (16,17), whereas more recent isolates have
imported novel alleles at these loci. These data indicate that
the common ancestor of these three subgroups existed
approximately 100 years ago (17).

The branch lengths (Figure 3) and common alleles indicate that the epidemic subgroups are probably not much
older than the split between subgroups III, IV-1, and IV-2.
The epidemic serogroup A subgroups may therefore have
descended from a common ancestor that existed within the
last few hundred years. Epidemic meningitis was first
observed in Geneva in 1805 (29), and that epidemic may have
marked the evolution of serogroup A after the import of the
*sacA-D* genes encoding the A polysaccharide. Horizontal
exchange between serogroups B and C of genes encoding
capsular polysaccharides has been documented (30), and
more than one capsular polysaccharide serogroup is present
in several of the otherwise uniform STs in the complete MLST
database. Furthermore, the *sacA-D* genes are characterized
by an unusually low GC content (31), as if these genes were
recently imported from an unrelated species. If this
especulation is correct, the other serogroup A bacteria (Figure
3, bottom) may represent unrelated *N. meningitidis* that have
imported *sacA-D* genes from the epidemic subgroups.
Alternatively, the *sacA-D* genes may have been imported from
an unrelated species more than once, or the epidemic
subgroups may have imported the *sacA-D* from one of the
nonepidemic STs (Figure 3, bottom). Analysis of the sequence
diversity of the *sacA-D* genes in various STs might resolve
these alternative explanations.

**Molecular Epidemiology**

Evaluation of the long-term molecular epidemiology of
bacterial pathogens within a global context is complex,
requiring large, representative strain collections and reliable,
preferably phylogenetically based, typing schemes. Our
results illustrate both strengths and weaknesses in our
knowledge of serogroup A epidemiology.

The assignment of genocloud 8 bacteria isolated in the
mid-1990s in Moscow to the third subgroup III pandemic
wave is straightforward and indisputable. It rests on three
unusual markers (*pgm19, tbpB55,* and *IS1106A7,* and no
differences in these three markers were detected among
recent subgroup III isolates from China, Mongolia, Africa,
and Russia. The initial association of genocloud 8 disease in
Moscow with the Vietnamese community remains unexplained.

The genocloud 2 subgroup III bacteria from the early
1970s differ at the *opaB* and *tbpB* loci from all known
subgroup III meningococci. We argue that genocloud 2 is
probably derived from genocloud 1 in China because of the
shared IS1106A10 allele, which was not found in any other
subgroup III bacteria. In support of this interpretation based
on molecular data, this epidemic first broke out among
Vietnamese immigrants who had traveled through China en
route to Russia. However, few subgroup III strains exist that
were isolated before 1969, and our knowledge about the
diversity and patterns of serogroup A disease before 1969 is
only fragmentary.

Subgroup VI bacteria of serogroups A, B, and C were
occasionally isolated from endemic disease in East Germany
in the 1980s (8). Our data show that most of the serogroup A
meningococci isolated during 1986 to 1994 in Moscow were
also subgroup VI, and these bacteria may have been widely
distributed in eastern Europe in the 1980s. In contrast,
subgroup X, common in Moscow during 1983 to 1985, has not
been described previously. Various STs (Figure 3, bottom)
have also been isolated only from single countries (subgroup
IX in the Netherlands during 1989 to 1993 [7]; STs 299, 300,
388, and 400 in Czechoslovakia in 1972 [K. Jolley and P. Kriz,
pers. comm.]; ST79 in Moscow in 1995; and ST103 in Greece
in 1996 [R. Urwin, pers. comm.]). These bacteria have
apparently not spread extensively, unlike the epidemic
subgroups. In this respect, they resemble most endemic
serogroup B meningococci, such as those identified by MLEE
among endemic disease isolates from the Netherlands (32)
and carrier isolates from Norway (33). Additional serogroup A
STs specific to certain countries or regions probably remain to
be identified. Current meningococcal strain collections
containing a few thousand isolates are probably one or two
orders of magnitude too small to yield reliable epidemiologic
data for unusual STs that have not caused larger disease
outbreaks.

Subgroup III has caused large disease outbreaks, and its
molecular epidemiology has been extensively investigated
(11,17,23). As a result, the available information on genetic
changes during pandemic spread of these bacteria is among
the most extensive for any bacterial pathogen. Our data show
that the extensive pandemic spread of these bacteria is likely
to continue. Although Moscow and western Europe differ
somewhat in epidemiologic patterns, the recent extension of
this third pandemic wave to Moscow warns us that we should
be prepared for new, large serogroup A outbreaks in countries
in both eastern and western Europe.

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Dr. Achtman is a scientific group leader at the Max-Planck Institut für Infektionsbiologie, Berlin. His group investigates the population structures of bacterial pathogens, including *N. meningitidis*, *Helicobacter pylori*, and *Yersinia pestis*. These analyses integrate results from epidemiologic studies of global disease patterns.

References