Group A streptococcus (GAS), *Streptococcus pyogenes*, is a highly prevalent Gram-positive human pathogen with a worldwide distribution. Most often, it causes superficial infections of the upper respiratory tract and of the skin, leading to pharyngitis and impetigo, respectively. Invasive GAS infections, on the other hand, can be life-threatening due to conditions such as bacteremia, cellulitis, erysipelas, meningitis, and pneumonia, including the severe manifestations of necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) (8). In a historical perspective, GAS has been associated with high fatality rates due to severe scarlet fever, puerperal sepsis, and systemic disease (24). With the introduction of antibiotics in the 1940s, incidence rates of severe GAS infections dropped in developed countries and stayed low until the 1980s. Increased virulence and invasiveness then resulted in an increased number of reported septicemia, NF, and STSS cases in previously developed countries, including the severe manifestations of necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) (8). In association with *emm* types 28, 77, and 87, the serotype T-28 comprised 24.8% of the strains. *emm* types 28, 1, and 82 were dominating. In 2007, a sharp increase in the number of *emm*-6 strains was noted. All strains were sensitive to penicillin and quinupristin-dalfopristin, while 3.4% and 6.1% of the strains were resistant to macrolides and tetracycline, respectively. Furthermore, the *emm*-6 strains had intermediate susceptibility to ofloxacin. Isolates displayed a wide variety of gene profiles, as shown by the presence or absence of the Spe genes, *smeZ*, and *ssa*, but 48% of the isolates fell into one of three profiles. In most cases, an *emm* type was restricted to one gene profile. Although the incidence decreased during this study, invasive GAS disease still has a high endemic rate, with involvement of both established and emerging *emm* types displaying variability in virulence gene profiles as well as differences in gender and age group preferences.

To investigate the epidemiological patterns and genetic characteristics of disease caused by group A *Streptococcus* (GAS), all available isolates from invasive cases in Norway during 2006 to 2007 (262 isolates) were subjected to antimicrobial susceptibility testing, T serotyping, *emm* typing, and multilocus sequence typing and screened for known streptococcal pyrogenic exotoxin (Spe) genes, *smeZ*, and *ssa*. The average incidence rate was 3.1 cases per 100,000 individuals. The most prevalent sequence types (STs) were STs 52, 28, and 334. In association with *emm* types 28, 77, and 87, the serotype T-28 comprised 24.8% of the strains. *emm* types 28, 1, and 82 were dominating. In 2007, a sharp increase in the number of *emm*-6 strains was noted. All strains were sensitive to penicillin and quinupristin-dalfopristin, while 3.4% and 6.1% of the strains were resistant to macrolides and tetracycline, respectively. Furthermore, the *emm*-6 strains had intermediate susceptibility to ofloxacin. Isolates displayed a wide variety of gene profiles, as shown by the presence or absence of the Spe genes, *smeZ*, and *ssa*, but 48% of the isolates fell into one of three profiles. In most cases, an *emm* type was restricted to one gene profile. Although the incidence decreased during this study, invasive GAS disease still has a high endemic rate, with involvement of both established and emerging *emm* types displaying variability in virulence gene profiles as well as differences in gender and age group preferences.

*Streptococcus pyogenes* Isolates Causing Severe Infections in Norway in 2006 to 2007: *emm* Types, Multilocus Sequence Types, and Superantigen Profiles

Roger Meisal,1,2 Ida K. G. Andreasson,1 E. Arne Høiby,1 Ingeborg S. Aaberge,1 Terje E. Michaelsen,1,3 and Dominique A. Caugant1,2*

Department of Bacteriology and Immunology, Division of Infectious Disease Control, Norwegian Institute of Public Health,1 and Department of Oral Biology2 and School of Pharmacy,3 University of Oslo, Oslo, Norway

Received 6 July 2009/Returned for modification 21 August 2009/Accepted 17 December 2009

The M protein has traditionally been targeted for serotyping of GAS strains because of its importance as a virulence determinant. However, sequencing of the *emm* gene (3) is now becoming the standard method, and to date, more than 150 *emm* types have been described (36). Another method, which has been used for the last 50 years and is still an important alternative to serological M typing, is T typing using slide

---

842

* Corresponding author. Mailing address: Department of Bacteriology and Immunology, Division of Infectious Disease Control, Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, NO-0403 Oslo, Norway. Phone: 47 21 07 63 11. Fax: 47 21 07 65 18. E-mail: dominique.caugant@fhi.no.

† Published ahead of print on 30 December 2009.
agglutination tests (39). Multilocus sequence typing (MLST), a widely used method for genetic characterization of organisms of a bacterial species, which is based on the nucleotide sequence variation in seven housekeeping genes, provides unambiguous results that are easily comparable between laboratories (15). Although geographical and temporal variation has been described for GAS populations (15, 35, 47), strains with the same emm type isolated as much as 50 years apart may harbor identical allelic profiles (15) and share the same T type (23). Due to the clonal population structure of S. pyogenes strains, results obtained by T typing, emm typing, and MLST correlate with each other (15, 23, 53).

Norway experienced relatively low incidence rates of severe GAS disease after the introduction of antibiotics, but in the mid-1980s there was an increased occurrence of severe invasive disease, especially in otherwise healthy young adults, largely caused by M-1 strains (9, 33). Thereafter, until the early 2000s, there was a significant decrease in the frequency of emm-1 strains and, at the same time, an increase in diversity among the Norwegian GAS strains (37). Antibiotic resistance levels were generally very low in Norway during this period (37).

In more recent reports from the United States (2000 to 2004) and the United Kingdom (2003 to 2004), emm-1 and emm-3 strains were still among the most frequent strains found in invasive GAS disease (29, 43). The overall distributions of the most prevalent emm types in Europe and the United States during this period were in congruence, but there were marked differences in the emm type distributions between countries such as Norway’s neighbors, Denmark, Finland, and Sweden (29). The most prominent difference was seen in Finland, where 45% of the strains were emm-28 strains (29). Recently, Finland also reported a rapid change in genotype prevalence caused by the previously uncommon emm type 84 during 2005 to 2007 (50).

The distribution of GAS strains and the virulence factors associated with the different strains are not stable over time. Therefore, epidemiological studies targeting genetic types, important virulence factors, and the antimicrobial susceptibility status of these microorganisms are of basic importance for detection of new emerging clones, determination of their potential to cause disease, and development and refinement of vaccines. To provide better insight into the current epidemiological status of severe GAS infections in Norway, we characterized all available isolates from invasive GAS disease obtained in 2006 to 2007, using emm typing, MLST, spe gene profiling, including smeZ and ssa, and antibiotic resistance screening using selected antibiotics.

MATERIALS AND METHODS

Bacterial isolates. All cases of invasive GAS occurring in Norway are notified to the Norwegian Surveillance System for Communicable Diseases (MSIS), and the isolates should be sent to the national reference laboratory at The Norwegian Institute of Public Health (NIPH). For the 292 cases reported to MSIS, 160 cases were from 2006 and 124 were from 2007. For 15 of 19 counties, 50 to 100% of the isolates were obtained, while for the remaining 4 counties the coverage was 0 to 16%. All 262 isolates were included in this study. GAS invasive disease was defined by isolation of S. pyogenes from blood or other normally sterile body sites. However, isolates from pus (11 isolates) were included when the cases were diagnosed as NF (Table 1).

T typing and SOF testing. Isolates were T typed using T sera (Trans Europe Chemicals, S.R.O., Prague, Czech Republic) and tested for serum opacity factor (SOF), using in-house serum, as described by Moody et al. (39) and Maxted et al. (34), respectively.

Antibiotic susceptibility testing. All bacterial isolates were examined for sensitivity to antibiotics, using the BD BBL Sensi-Disc agar diffusion system (BD Diagnostics, Sparks, MD), on Mueller-Hinton agar supplemented with 5% defibrinated horse blood. Fresh overnight cultures grown at 35°C in 5% CO2 were suspended in Mueller-Hinton broth to a density of a 0.5 McFarland standard, diluted 1:20 to a final volume of 3 ml. Agar plates were flooded with bacterial suspensions, followed by removal of all excess fluid and drying. Antibiotics used were penicillin-G (10 U), clindamycin (2 μg), erythromycin (15 μg), ofloxacin (5 μg), quinupristin-dalfopristin (15 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and tetracycline (30 μg). Inhibitory zones were measured as recommended by the manufacturer, after overnight growth in 5% CO2 at 35°C. To detect the inducible macrolide-lincosamide-streptogramin B (MLSB) type of resistance, erythromycin-resistant isolates were tested using the D test, with interdisk distances of 10, 15, and 20 mm (55). Isolates were classified as susceptible, intermediate, or resistant according to the 2009 standards given by the Clinical and Laboratory Standards Institute (CLSI) (9a), except for trimethoprim-sulfamethoxazole, for which the criteria given by CA-SFM (Comité de l’Antibiogramme de la Société Française de Microbiologie [http://www.sfmasso.fr]) were used (critical zone diameters, 16 mm for susceptible [S] isolates and ≤16 mm for resistant [R] isolates). Isolates that were resistant or had intermediate susceptibility by the agar diffusion method were further tested using Etest (AB Biodisk, Solna, Sweden), as previously described (37). CLSI breakpoints were used, except for trimethoprim-sulfamethoxazole, for which the criteria given by CA-SFM were used (concentration ranges were ≤1/19 mg/liter for S strains and ≥2/38 mg/liter for R strains). For quality control, S. pneumoniae ATCC 49619 was used.

Isolation of DNA. Chromosomal DNA was isolated from fresh overnight cultures grown on Columbia agar with 5% defibrinated horse blood in 5% CO2 at 35°C. Using a Zymo Research fungal/bacterial DNA kit (Zymo Research Corporation, Orange, CA), most of the bacterial growth on the plates was suspended in 200 ml of water in BashingBead lysis tubes, and DNA was purified according to the kit protocol. Raw high-quality chromosomal DNAs were transferred to 96-well plates and diluted to a final concentration of 10 ng/μl for positive template DNA in PCR. DNA transfer and all downstream procedures were performed in 96-well plates, with the help of an Eppendorf 5075 pipetting robotic platform (Eppendorf, Hamburg, Germany).

MLST. The MLST procedure was described as performed by Enright et al. (15), using primers synthesized by MWG-Biotech AG, Ebersberg, Germany. Amplified DNA fragments were enzymatically purified using ExoSAP-IT (USB Corporation, Cleveland, OH). Using the PCR primers and BigDye chemistry, purified PCR fragments were sequenced as recommended by the manufacturer, with a reaction volume of 10 μl. Removal of excess primers and fluorochromes after the sequencing reaction was performed by filtration, using a Montage SEQ10 sequencing reaction cleanup kit (Millipore, Billerica, MA). An ABI3730 DNA analyzer (Applied Biosystems, Foster City, CA) was used for sequencing. Nucleotide sequences were analyzed using SeqScape v2.5 software (Applied DNA analyzer (Applied Biosystems, Foster City, CA). Allele numbers and sequence types (STs) were identified using the MLST database (http://Spyogenes.mlst.net). New alleles and STs were assigned by the curator of the database.

emm typing. The 5′-end fragment of the M protein gene used for emm typing was amplified as described by Beall et al. (3), using the primers emm1-for and emm2-rev for PCR and emmSeq1 (MWG-Biotech AG, Ebersberg, Germany) for sequencing (Table 2). PCR products were purified and sequenced as described above, and the emm type was obtained as described at http://www.cdc.gov/neidod/biotech/strep/strepblast.htm, by BLAST comparison to emm sequences in the database.

Detection and profiling of spe genes, smeZ, and ssa. PCR, run separately for each gene, was used to amplify speA to –C, speF to –M, smeZ and ssa, using the primers listed in Table 2. After an initial denaturation at 94°C for 5 min, amplification was performed by 30 cycles of 95°C for 40 s, hybridization for 1 min, and elongation at 72°C for 1 min 30 s, with finalization at 72°C for 5 min. As positive controls, the sequenced strains MGAS315 (serotype M3), MGAS6708 (serotype M1 [also known as strain SF370]), and MGAS8232 (serotype M18), kindly provided by James M. Musser, were used for speB, speF, speK, and ssa, for speG to –O and semeZ, and for speA, speC, speE, and speM, respectively. PCR products were examined using a standardised E-Gel electronic electrophoresis system and E-Gel 96 with SYBR Safe (Eli Lilly Biotechnologies, Tel-Hai, Israel). The presence or absence of different spe genes, smeZ, and ssa produced an exotoxin gene profile for each GAS isolate. To confirm cases where the presence or absence of genes produced new profiles for any particular emm type, repeat testing was performed.

Downloaded from http://jcm.asm.org on September 12, 2017 by guest
Statistical analysis. Statistically significant differences in proportions of emm types and genders were detected using two-tailed Fisher's exact test, in which all of the analyses were performed separately for each emm type or age group compared to the group consisting of cases caused by all the other emm types or age groups. Ninety-five percent confidence intervals (CI) for gender proportion were calculated using GraphPad software.

RESULTS

From the 292 reported cases of invasive GAS disease, isolates for 86.3% of the cases in 2006 and 93.9% of the cases in 2007 were submitted to our institute. Available clinical information was, however, limited.

T types and SOF testing. In total, 21 different T agglutination patterns were recognized, among which T-28 (24.8%), T-1 (14.1%), T-5/27/44 (13.1%), T-12 (12.2%), and T-4 (6.9%) were the five most common T types. A total of 5.3% of the isolates were not typeable, and in total, 61.1% of the isolates were SOF positive (Table 1). For
most of the emm types comprised more than one emm subtype, one subtype was always dominant, except for emm-6, which was represented by almost the same proportions of emm-6.0 and emm-6.4. From 2006 to 2007, there was a significant increase in the prevalence of emm-6 (0.8% to 9.3%; P < 0.005). The 10 cases from 2007 occurred in six different counties.

MLST. A total of 41 distinct STs were identified (Table 1). Twenty-four STs were represented by single isolates, including 10 STs new to the MLST database. The most prevalent STs were ST-52 (14.5%), ST-28 (13.7%), ST-334 (13.7%), ST-36 (11.5%), ST-15 (6.1%), and ST-62 (5.3%). The only significant change between 2006 and 2007 was the emergence of ST-382, which corresponded to the increase of emm-6 isolates reported above.

T type/emm type/ST combinations. Of the 56 T type/emm type/ST combinations, 38 were represented by single isolates (Table 1). The most commonly occurring combinations were T-1/emm-1/ST-28 (13.7%), T-28/emm-28/ST-52 (13.7%), T-5/27/44/emm-82/ST-334 (13.0%), and T-12/emm-12/ST-36 (10.7%). All 65 strains with T type 28 (24.8%) were associated with the vast majority of strains with emm-28, emm-77, and emm-87. Ten emm types were associated with two or three different STs, while four STs were associated with two different emm subtypes each. In most cases, one type was dominant, except for emm-4, where both ST-38 and ST-39 were present in the same proportions, and for ST-382, where both emm-6.0 and emm-6.4 were present in the same proportions. The T patterns of these strains were identical.

Antibiotic susceptibility. Intermediate resistance or resistance to one or more of the antimicrobials was detected in a total of 42 (16%) GAS strains by the disk diffusion method. Upon further testing using Etest, 24 (14.5%) isolates were confirmed as resistant. All isolates were sensitive to penicillin and oxacillin-dalfopristin. Nine strains were erythromycin resistant, and these had emm types 4 (two isolates), 11, 12 (four isolates), 28, and 94. Six of them were also resistant to clindamycin, one of which (emm-94) was of the iMLSBLB type. Thus, the total macrolide resistance rate in invasive GAS strains was 3.4%. Resistance to tetracycline was detected in 16 (6.1%) isolates, displaying emm types 4, 5, 9, 11, 12, 69, 80, 82, 90, 94, 104, and 109 (one each) and including all four emm-77 isolates. Four isolates were resistant to ofloxacin, and these were of emm types 6 (two isolates), 11, and 75. In addition, eight strains of emm-6 and one strain of emm-75 were of intermediate susceptibility. Ten of 11 emm-6 isolates were resistant toward ofloxacin or had intermediate susceptibility. Only three isolates showed decreased susceptibility to trimethoprim-sulfamethoxazole, including one emm-4 strain and one emm-11 strain, which were fully resistant, and one emm-12 strain of intermediate susceptibility.

Profiling of spe genes, smeZ, and ssa. The occurrence of chromosomally encoded Spe genes was high: speB was present in all but one emm-82 isolate, and speF, speG, speI, and smeZ were found in 98%, 89%, 41%, and 99% of the isolates, respectively (Table 3). speG was present in all strains, except for the emm-4 and emm-77 (P < 0.0001 for both strains); speI was found in all of the emm-1 and emm-28 strains and in 93% of the emm-87 isolates (P < 0.0001 for all); and smeZ was found in all strains, except for the emm-2, emm-44, and emm-49 strains. Among genes known to be carried by prophages, speA was detected primarily in emm-1 and emm-3 strains (P < 0.0001 for both). The remainder of speA-positive strains (23%) included all emm-4, 2 strains, all emm-6 strains, and 6% of emm-28 strains. Isolates of 18 emm types harbored speC, including all of the emm-4, 20 strains, 87% of the emm-12 strains, 92% of the emm-28 strains, 97% of the emm-82 strains, and 93% of the emm-87 strains (P < 0.05 for all). Strains of nine different emm types were positive for one or both of the speH and speI genes, including all of the emm-12 strains and 97% of the emm-82.0 strains (P < 0.0001). With a few exceptions, speH and speI were codetected, and 94% of the speH- and speI-positive strains were negative for speI to -M and ssa. Strains of 13 different emm types were speM positive, including all of the emm-3 strains, 91% of the emm-6 strains, and 39% of the emm-28 strains (P < 0.05 for all). Except for three isolates, speM was codetected either with speK or with speL. Seven different emm types were positive for the ssa gene, including all of the emm-3 and emm-4.0 strains and 93% of the emm-87 strains (P < 0.0001 for all).
<table>
<thead>
<tr>
<th>enm type</th>
<th>No. of isolates</th>
<th>% of isolates</th>
<th>speA</th>
<th>speB</th>
<th>speC</th>
<th>speF</th>
<th>speG</th>
<th>speH</th>
<th>speI</th>
<th>speJ</th>
<th>speK</th>
<th>speL</th>
<th>smeZ</th>
<th>ssa</th>
</tr>
</thead>
<tbody>
<tr>
<td>82 (33), 12 (26), 11</td>
<td>60</td>
<td>22.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 (33), 28</td>
<td>34</td>
<td>13.0</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28 (29), 89 (2)</td>
<td>31</td>
<td>11.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>7.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>15</td>
<td>5.7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>87</td>
<td>14</td>
<td>5.3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
<td>3.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>89 (5), 5, 11, 82</td>
<td>8</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>89 (4), 55, 69, 87</td>
<td>7</td>
<td>2.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12 (4), 82</td>
<td>5</td>
<td>1.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11 (2), 94</td>
<td>3</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>3</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>75 (2), 80</td>
<td>3</td>
<td>1.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>2</td>
<td>0.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>77</td>
<td>2</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>77, 109</td>
<td>2</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>122, 124</td>
<td>2</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>49</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>68</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>75</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>77</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>82</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>82</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>89</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>104</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>enm type</td>
<td>262 (100)</td>
<td>69 (26.3)</td>
<td>261 (99.6)</td>
<td>180 (68.7)</td>
<td>257 (98.1)</td>
<td>234 (89.3)</td>
<td>76 (29.0)</td>
<td>73 (27.9)</td>
<td>108 (41.2)</td>
<td>52 (19.8)</td>
<td>9 (3.4)</td>
<td>60 (22.9)</td>
<td>258 (98.5)</td>
<td>54 (20.6)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses represent the number of isolates with the particular enm type, if >1.
The 262 invasive GAS strains displayed as many as 45 different profiles for the spe genes, smeZ, and ssa (Table 3), 10 of which were represented by strains of two to four different emm types. The three major profiles accounted for 48% of the strains, whereas the top 10 profiles accounted for 80% of the total number of strains. Most of the strains of a given emm type were usually associated with one gene profile, with only one or a few strains differing due to loss or acquisition of one or a few genes. Two of the exceptions involved the emm-28 and emm-89 strains, which were divided into two groups each due to loss or acquisition of speK-speM and speC. Strains with identical gene profiles but with different emm types were not related to each other by MLST.

Incidence, age, gender, and emm types. The yearly incidence rate of invasive GAS disease in Norway during 2006 and 2007 decreased from 3.4 to 2.8 cases per 100,000 individuals, but the difference was not significant. Incidences in different age groups followed a typical pattern, with elevated incidences in children 0 to 9 years of age and adults 30 to 39 years of age (Fig. 1). In adults over the age of 60, the incidence increased sharply and was highest in 80- to 89-year-old individuals. Overall, cases were more frequent in females (58.6%; 95% CI = 0.53 to 0.64) than in males (41.4%; 95% CI = 0.36 to 0.47). This was true for cases caused by most emm types, except for those due to emm-6, which were overrepresented in males (63.7% of cases). Typically, 70% of the cases caused by each emm type occurred in individuals above 50 years of age. An exception was seen with the emm-82 strains, for which 73.0% of the cases were in patients under 50 years of age (P < 0.0001). Strains of emm-12 were overrepresented in children and young adults (0 to 19 years) and in older adults (50 to 79 years) (P < 0.001 and P < 0.01, respectively), causing 44% and 18% of the cases in the respective age groups. In the age group of 20 to 49 years of age, there was a significantly higher prevalence of emm-82 strains (P < 0.0001), which caused 34% of the cases in this group. Uncommon emm types (emm-2, -11, -68, -75, -77, and -104) were overrepresented in patients above 80 years of age, causing 22% of the cases in this group (P < 0.05). emm-28, which is associated with puerperal sepsis (10, 16, 57), was significantly overrepresented not only in females in the age group of 20 to 49 years of age but also in males in the age group of 50 to 79 years of age (P < 0.05 for both). The emm-28 and emm-82 strains caused 52% of the cases in females of 20 to 49 years of age and accounted for 53% of the total number of cases in young adults. Nearly 50% of the cases in patients over 50 years of age were caused by three emm types, emm-1 (15.6%), emm-12 (13.7%), and emm-28 (20.4%).

Disease manifestations. Bacteremia was recorded for 236 (90%) cases. Meningitis was recorded in five (2%) cases and was caused by emm-1 and emm-12 strains. The ages of these patients ranged from 4 to 68 years. There were seven (3%) cases of pneumonia caused by strains of six different emm types. The ages of these patients ranged from 52 to 79 years. Clinical data indicated that there were nine cases of puerperal sepsis; emm-28 strains were responsible for four cases, in the 20- to 49-year-old age group, and the remaining cases were
caused by emm-3 (three cases), emm-87, and emm-89 strains. There were also 14 (5.2%) cases of NF, caused by seven different emm types, in nine females and five males from 9 to 86 years of age. In 2006, of the nine cases of NF, five were caused by emm-28 strains, including strains of both ST-52 and ST-456. There were no reported cases of STSS in the years 2006 and 2007.

There were clinical records of five injecting drug users (IDUs), two females and three males, of 36 to 54 years of age, with an average age of 43 years. These five patients were from four different counties, and the two from the same county were isolated in different years. All were infected by the same emm-82 strain, displaying the T-agglutination pattern T-5/27/44.

**DISCUSSION**

This study included all GAS isolates from invasive and NF cases sent to the NIPH in 2006 to 2007, covering approximately 90% of the cases reported to MSIS. Strain coverage was 50 to 100% for 15 of 19 counties. The remaining four counties were poorly represented in our collection, two of which included a small fraction (4.9%) of the Norwegian population. Except for two counties on the west coast, we believe that this collection should be representative of the GAS strains causing severe disease in the Norwegian population during this time.

Antibiotic susceptibility testing revealed that only a few emm types had decreased susceptibility to antimicrobials; the greatest variation in emm type was found among the 16 tetracycline-resistant strains. Resistance to both clindamycin and erythromycin was found in 2.3% of the isolates, while some macrolide resistance was found for 3.4% of the isolates. Susceptibility results correlated well with those from our previous study of 100 strains from 1988 to 2003 (37) and from reports of the Norwegian Surveillance Program for Antimicrobial Resistance in Human Pathogens (41, 42), indicating that macrolide and tetracycline resistance in Norway is still low. Four emm-77 isolates were resistant to tetracycline in this study. This was also the most frequent emm type resistant to tetracycline in Norway in 1988 to 2003. Other emm types that were resistant to tetracycline in 1988 to 2003 were either absent in 2006 to 2007 or were susceptible. The link between emm-77 and tetracycline resistance was also found in a Danish report by Luca-Harari et al. (30), who suggested a clonal spread of these strains. Recent publications from Greece and Portugal (38, 51) showed that the majority of macrolide-resistant strains in these countries belonged to the same emm types as those in our study, suggesting that macrolide resistance in Norway reflects the macrolide-resistant strains circulating elsewhere in Europe. Among them are emm-4 strains persistent in Finland, Sweden, and other European countries since the 1990s. Macrolide-resistant emm-4, emm-75, and emm-94 strains were also identified in a study of macrolide resistance in Norway between 1993 and 2002 by Littauer et al. (28). However, our emm-75 strains were resistant or of intermediate susceptibility to fluoroquinolones, which may be explained by the loss of macrolide resistance and acquisition of fluoroquinolone resistance, as described by Billal et al. (5, 6), suggesting that emm-75 strains presently circulating in Norway have changed genetically since 2002. Most of the fluoroquinolone-resistant strains in both this study and our previous study (37), however, were emm-6 strains.

T-agglutination patterns and the SOF test can be used as quick, cost-effective typing methods for GAS strains before M serotyping or emm typing. However, strains of different emm types may display the same T patterns and strains of the same M/emm type may display more than one T pattern. Although T typing is of limited value when used alone, it can reveal valuable supplementary information. Most of the T patterns and all of the SOF results for our strains were in accordance with the respective emm types, as described in the last comprehensive summary of the M protein by Johnson et al. (23). However, the agglutination pattern of the emm-3 strains in that study, T-3/13/B3264, was found for only one of our emm-3 strains. T-3 alone, which was the most frequent T pattern for our emm-3 strains, was found in only a few strains of emm types other than emm-3 by Johnson et al. (23). Four other T patterns found herein (T-25, T-13/5, T-1/5/Imp19, and T-8/25/Imp19/11) differed from those reported by Johnson et al. (23). The observed differences may reflect differences in conditions, methods, or reagents, such as the batch of T-antisera used.

MLST provides unambiguous characterization of strains via databases on the Internet and is crucial for addressing questions related to the epidemiology of the studied organism. In our material, all combinations of STs and emm types (Table 1), except for those produced by new STs found herein, correlated with what has previously been reported (15; http://spyogenes.mlst.net). Interestingly, the newly assigned ST-544, identified in late 2006 in Norway, was reported to the MLST database from the Vellore region in India, where it was isolated from a child in 2001. Four of the 11 new STs (STs 540, 542, 543, and 546) differed by only one allele from other known STs (single-locus variants), three of which were prevalent in this study (STs 46, 28, and 36). Two additional new STs (STs 536 and 538) also differed by one allele from known STs, but these (STs 71 and 420) were not represented in our collection. One strain, the ST-371 (emm-49) strain, has, to our knowledge, been reported previously only in a study of macrolide resistance of GAS in Norway by Littauer et al. (28), where it was isolated from a patient with pharyngitis in 2002.

The most prevalent emm types from invasive disease in Norway in 2006 to 2007 were similar to those reported from the United States in a 4-year study from 2000 to 2004 (43) and to those in a recent report from 11 European countries in 2003 and 2004, including our neighboring countries Denmark, Finland, Sweden, and the United Kingdom (29). The most noticeable difference was the lack of emm-82 in the other Nordic countries and the high level of emm-28 (45%) experienced by Finland. The prevalence of bacterial strains can change quickly, as shown by the sudden increase of emm-6 strains between 2006 and 2007 observed in our study. New successful clones can emerge and rapidly spread through the population. This was also reflected in a recently published study from Finland, where a rapid emergence of emm-84 strains and a decrease of the highly prevalent emm-28 strains were experienced (50).

The average annual incidence of invasive GAS in Norway in 2006 to 2007 was 3.1 cases per 100,000 individuals, which was comparable to the average incidence rates reported from our neighboring countries and from the United States pre-2004.
(13, 25, 26, 30, 43). Incidences in most of the age groups were comparable, as shown in Fig. 1. However, the incidence of neonatal (<1 year) infections was very low, at <1 case per 100,000 individuals, in comparison to incidences of up to 9.7 cases per 100,000 individuals reported from the United Kingdom and the United States (26, 43) for this age group.

In 2006 and 2007, there were more female cases than male cases in Norway, in contrast to what has been reported for most European countries (25). However, in 2005 and 2008, cases were more frequent in males than in females (www.msis.no). Temporal variation in the male-to-female ratio among patients with invasive GAS disease is unclear but may depend on the prevalence of the different circulating strains. The emm-28 type, associated with females of 20 to 49 years of age, is known for its association with puerperal sepsis (13, 29, 30), and the emm-82 strain which has been associated strongly with IDUs in the United Kingdom (27) caused 29% of the invasive cases in females aged 20 to 49 years.

Different emm types predominated in different age groups: emm-12 was common in 1- to 9-year-old patients and in patients more than 50 years old, while emm-4 was overrepresented in patients over 60 years of age. These two emm types are predominant as a cause of invasive infections in children (0 to 17 years) in Europe (29) and are also known to be common in noninvasive infections in children (13, 22).

Although both emm-1 and emm-3 strains are historically known to cause NF and STSS at higher frequencies than those for other strains, none of the reported NF cases in our data were caused by emm-1 strains. The frequency of NF caused by emm-3 (12.5%) was not significantly different from the frequencies of NF caused by other emm types, suggesting no particular propensity of these strains to cause NF in Norway. It is noteworthy that all cases of meningitis caused by the emm-1 strain were in patients older than 40 years of age, while cases caused by the emm-12 strain all occurred in children. Pneumonia, on the other hand, was found mainly in persons older than 50 years of age, which is in accordance with previous findings (37). Puerperal sepsis was associated with emm-28, whose role in puerperal sepsis has long been recognized. It is speculated that horizontally acquired genetic elements from the group B streptococci, the major cause of neonatal infections, enable emm-28 strains to more frequently cause puerperal sepsis (19).

The same emm type was also found to be significantly associated with puerperal sepsis in our neighboring countries and in a recent European survey (13, 29, 30).

There were only five records of invasive GAS infections related to IDUs, all of which were caused by a single strain, the emm-82 strain. These represented 13.5% of our emm-82 cases. It was striking that the emm-82 type was overrepresented in the age group of 20- to 49-year-olds in Norway, whereas many of the IDUs would be expected to be found. In contrast, 70% of the total emm-82 cases in the United Kingdom were IDU related (27, 29). The emm-83 type, which was the most common among IDUs in the United Kingdom, was not represented in our data.

The chromosomally carried spe genes speB and speF were present in close to 100% of the strains. Repeated PCR analysis on fresh lysates failed to detect speB in one emm-82, ST-334 isolate that was also speF negative (Table 3). An emm-11 isolate negative for speB has previously been reported by Tyler et al. (56). The chromosomally encoded superantigens (speG, speJ, and smeZ) were emm type restricted. Most of the strains carried speG and smeZ, but only 41% of the strains carried speJ. These findings are in contrast to those of Proft et al. (45), who found a 100% prevalence for all three genes in a study of 51 S. pyogenes isolates from New Zealand. However, except for smeZ, which was present in all of our emm-3 strains, our findings for the chromosomally encoded superantigens are in agreement with those of Commons et al. (11) in a study of 107 Australian S. pyogenes isolates and their relationship with emm type and with those of Schmitz et al. (48), who looked at the toxin gene profiles of 239 European isolates. This suggests that the low level of speJ and the high levels of speG and smeZ may be linked to the present emm type distribution.

Most of the superantigen genes in GAS reside on prophages. The association of speA with emm-1 and emm-3 is in agreement with what has been found previously. Although most of our emm-1 strains harbored speA, four strains also harbored the speC gene. Usually, emm-1 and emm-3 strains lack the speC gene, or it is infrequently found in this group (11). However, in a Swedish study of clinical GAS isolates obtained between 1986 and 2001, speC was found in 60% of the emm-1 strains (32). The ssa gene was detected in the same emm types as those recently described by Commons et al. (11). Superantigen gene pairs speH-speI and speL-speM were detected together almost exclusively. Similarly, speK and speM were codetected when speL was absent. These observations are in agreement with the adjacent locations of these gene pairs on each of their prophages (17, 20, 45, 54). However, the phages themselves seem to display a great deal of variability, since speH, speJ, speK, and speM were also found as single genes in our material. In the case of speH and speI, it has been suggested that speI is lost during integration of the phage into the genome (11). It is also possible that other phages carry other repertoires of virulence genes and thus may harbor single superantigen genes.

The most common gene profile for the smeZ and ssa superantigen genes was shared by the emm-12 and emm-82 strains, which were nonrelated strains by MLST. Most of the strains from a given emm type had the same gene profile. However, loss or acquisition of superantigens divided both the emm-28 and emm-89 strains into separate gene profile groups. The large number of gene profiles, the existence of strains of different emm types sharing the same gene profile, and the grouping of strains sharing the same emm type among different gene profiles reflect the ongoing diversification and strain emergence of GAS, where loss or acquisition of virulence genes is crucial for its survival and dissemination.

In conclusion, the overall strain distribution found in this study, based on emm type and MLST, was similar to previous reports from the United States and Europe. The incidence of GAS was most comparable to those for our neighboring countries, with a low incidence rate for neonatal infections and a high incidence rate for females aged 30 to 39 years and for the elderly. Antibiotic susceptibility was high, and the resistant strains, in most cases, were the same resistant strains found circulating in other countries. The presence of Spe genes, smeZ, and ssa was, in most cases, restricted to specific emm types and comparable to that found in other geographical areas.
REFERENCES


