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# Clinical and Epidemiological Aspects of Invasive *Streptococcus pyogenes* Infections in Denmark during 2003 and 2004▽

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**Active surveillance of invasive group A streptococcal (GAS) infections was conducted in Denmark during 2003 and 2004 as a part of the Strep-EURO initiative. The main objective was to improve understanding of the epidemiology of invasive GAS disease in Denmark. During the 2 years, 278 cases were reported, corresponding to a mean annual incidence of 2.6 cases per 100,000 inhabitants. The vast majority of isolates, 253 (91%), were from blood, with the remaining 25 (9%) being from cerebrospinal fluid, joints, or other normally sterile sites. The mean case fatality rate (CFR) was 20%, with the rate being higher in patients more than 70 years of age (36.5%). For streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis the CFRs were 53% and 25%, respectively. Out of 16 T types recorded, three predominated: T28 (23%), T1 (22%), and the cluster T3/13/B3264 (14%). Among 29 different *emm* types, *emm*28 and *emm*1 accounted for 51% of strains, followed by *emm*3 (11%), *emm*89 (7%), and *emm*12 (5.5%). Low resistance rates were detected for macrolide-lincosamide-streptogramin B ($MLS_B$) antibiotics (3%) and tetracycline (8%); two isolates exhibited coresistance to tetracycline and macrolides. Of nine pyrogenic exotoxin (superantigen) genes examined, *speA* and *speC* were identified in 58% and 40% of the strains, respectively; either of the genes was present in all strains causing STSS. Most strains harbored *speG* (99%). *ssa* was present in 14% of the isolates only. In Denmark, as in comparable countries, GAS invasive disease shows a sustained, high endemicity, with involvement of both established and emerging streptococcal *emm* and T types.**

*Streptococcus pyogenes* (group A streptococci [GAS]) is a gram-positive, exclusively human pathogen causing common throat and skin infections but also severe invasive disease and the nonsuppurative complications acute rheumatic fever and poststreptococcal glomerulonephritis. In particular, the spectrum of acute invasive disease includes erysipelas, cellulitis, endometritis, pneumonia, septicemia, meningitis, and the severe manifestations necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) (50).

The late 1980s marked a sudden rise of the severe infections and consequently an increased interest in this pathogen. Recent epidemiological studies from the United States and Europe have shown a sustained, high incidence of severe streptococcal infections (7, 14, 22, 42, 52) accounting for hundreds of thousands of cases each year (4) and thus currently a global concern.

Various surface structures of GAS, including M proteins, hyaluronic acid capsule, and matrix-binding proteins, play important roles in virulence by mediating adherence, colonization, and invasion of human skin and mucus membranes (3). In addition, a number of extracellular toxins and enzymes are produced by this organism. The antigenically variable M protein has been used since 1928 in a serotyping system, now comprising about 80 known types. Based on variability of the N-terminal end of the *emm* gene (encoding the M protein), about 250 defined *emm* types (31) and an increasing number of subtypes are recognized (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). This is in contrast to traditional serological T typing, which comprises fewer than 30 different types (27). Of the extracellular factors produced by GAS, several superantigens (SAg), among these the pyrogenic exotoxins A and C (SpeA, and SpeC, respectively), have received particular attention in the context of invasive disease (6, 33). The number of described GAS SAgs as well as knowledge of their involvement in GAS pathogenicity is increasing continuously (3, 47). Use of the SAg genes, combined with more conventional methods (T typing, M/*emm* typing, and pulsed-field gel electrophoresis), may also be useful as an epidemiological tool (10).

In spite of more than half a century of extensive use of β-lactams, no resistance against these drugs among GAS has been reported; however, resistance to macrolide-lincosamide-streptogramin B ($MLS_B$) antibiotics is common in many countries. A combination of a β-lactam and clindamycin is the recommended treatment for severe GAS disease (59). Addi-

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TABLE 1. Primers used for detection of SAg genes

| Gene | Primer sequence (5′→3′) | | Amplicon size (bp) |
|---|---|---|---|
| | Forward | Reverse | |
| *speA* | CTT AAG AAC CAA GAG ATG GC | ATA GGC TTT GGA TAC CAT C | 200 |
| *speB* | TTC TAG GAT ACT CTA CCA GC | ATT TGA GCA GTT GCA GTA GC | 300 |
| *speC* | CAT CTA TGG AGG AAT TAC GC | TGT GCC AAT TTC GAT TCT GC | 246 |
| *speF* | GCG AAA TTA GAA AAG AGG AC | GCT GAG CAA AAG TGT GTG | 1,193 |
| *speG* | TAT AAT ATT ACC CCA TGC GA | AAG GCT CCC CGA TG | 447 |
| *speH* | AAG CAA ATT CTT ATA ATA CAA CC | TTA GCT GAT TGA CAC ATC TAC A | 630 |
| *speI* | ATG AGT AGT GTG GGA GTT ATT AA | TTA TTT ATT AAA TTT AAC TAA GTA TAT ATC AAT A | 678 |
| *speJ* | AAA TCA GAT AGT GAA AAT ATT AAA GAC | TTA TTT AGT CCA AAG GTA AAT ATC | 629 |
| *ssa* | AGT AGT CAG CCT GAC CCT AC | TTT GGT AAG GTG AAC CTC TAT | 691 |

tionally, though tetracycline is not used in the treatment of GAS disease, the susceptibility of GAS to tetracycline seems to be highly variable between countries (26, 32, 38).

Since invasive GAS disease is not notifiable by law in most countries, national incidence data have generally been obtained through enhanced surveillance during limited periods. The present paper, a part of the EU-funded action Strep-EURO (http://www.strep-euro.lu.se), concerns severe GAS disease in Denmark during 2003 and 2004, with special focus on epidemiological trends compared to previous national or regional reports. The results demonstrate a continued, high incidence and a potential need for prevention of invasive GAS disease, especially soft tissue infections, puerperal sepsis, and nosocomial infections.

## MATERIALS AND METHODS

**Case definition.** The study was conducted during 2003 and 2004 and included GAS isolates recovered from hospitalized patients with invasive disease. The case definition was that of the Working Group on Severe Streptococcal Infections, published in 1993 (57). The criteria for inclusion of patients in the study were based on the isolation of GAS from blood or another normally sterile body site or from nonsterile sites in the presence of a clinical diagnosis of STSS or NF.

**Collection system.** GAS isolates were received at the Statens Serum Institut (SSI) from all 15 Danish clinical microbiological departments (15 different regions), with a national catchment area of ~5.5 million inhabitants (http://www.dst.dk/HomeUK/Statistics/Key_indicators/Population/pop.aspx; accessed 10 July 2007). The reporting system from the departments to the Streptococcus Unit, SSI, has been the same since 1988, and since 1996 the unit has distributed a detailed questionnaire to the clinicians treating the patients, which is intended to collect basic and clinical patient data, e.g., age, gender, and site of bacterial isolation, as well as diagnosis, predisposing factors, and treatment. Dates of death or a confirmation that the person was alive was obtained from the Central Office of Civil Registration. The annual numbers of inhabitants in the respective areas were obtained from Statistics Denmark.

**Bacterial isolates.** The clinical microbiology departments identified the GAS isolates by agglutination tests from 5% horse blood agar plates (SSI) that were incubated overnight, sent to SSI in Stuart transport medium, and subsequently cultured on 5% horse blood agar plates (SSI) and incubated overnight at 37°C in a 5% $CO_2$ atmosphere. Bacterial strains were stored at −80°C on filtered broth with glycerin.

**Typing procedures.** The isolates were T typed by slide agglutination using mono- and polyspecific sera (SSI, Copenhagen, Denmark, for isolates from 2003 and Sevapharma, Czech Republic, for isolates from 2004). Tests were performed at two laboratories in order to assess the concordance of results using the two brands of antisera. For *emm* typing the hypervariable region of the *emm* gene was amplified and sequenced as described previously (39). Sequence alignment and *emm* type assignment were performed using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) (available at http://www.ncbi.nlm.nih.gov/BLAST/) and sequences listed at the Centers for Disease Control and Prevention (CDC) (http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm). Uncommon T/*emm* combinations were retested at Department of Laboratory Medicine, Lund University, Lund, Sweden.

**Detection of SAg genes.** For detection of nine SAg genes (Table 1), an initial multiplex PCR was developed. Bacterial DNA was isolated as described before (25). The PCR was performed in a 50-μl final volume containing 5 μl 10× PCR buffer, 1.25 μl deoxynucleoside triphosphates (10 mM), 2.5U *Taq* DNA polymerase (New England Biolabs), 5pmol/μl of each primer (Invitrogen, Sweden), 1 μl supernatant, and distilled water to 50 μl. The thermal scheme was as follows: denaturing for 2 min at 95°C; 35 cycles of denaturing for 1 min at 94°C, annealing for 50 s at 50°C, and elongation for 1.5 min at 72°C; and a final cycle of 5 min at 72°C. As noted from Table 1, some of the primers generated amplicons of similar size (*speH* and *speJ* [approximately 630 bp] and *speI* and *ssa* [approximately 680 bp]); for this reason, the isolates with positive bands of these sizes were subjected to single PCRs with corresponding primers.

**Antimicrobial susceptibility testing.** Susceptibility to tetracycline, erythromycin, and clindamycin was determined using disk diffusion according to Swedish Reference Group for Antibiotics guidelines (http://www.srga.org; accessed 17 February 2005). MICs for resistant isolates were determined by Etest (AB Biodisk, Solna, Sweden). Macrolide resistance phenotypes, i.e., macrolide (M), constitutive $MLS_B$ ($cMLS_B$), and inducible $MLS_B$ ($iMLS_B$) resistance, were determined by the double-disk test using erythromycin and clindamycin, as described previously (44).

**Identification of antibiotic resistance genes.** Macrolide-resistant strains were subjected to a multiplex PCR for detection of the resistance determinants *erm*(A), *erm*(B), and *mef*(A), using primer sequences in accordance with previously described methodologies (12, 53). A pair of primers giving an 180-bp amplicon of the chromosomally encoded 16S RNA was introduced as an internal positive control (34). Tetracycline-resistant isolates were screened for presence of resistance determinants *tet*(M) and *tet*(O), using published primers (1).

**Statistics.** For nominal data, the chi-square or Fisher's exact test was used when appropriate (significance, $P < 0.05$).

## RESULTS

During the active surveillance period, a total of 278 GAS isolates from invasive infections were received at SSI. Completed questionnaires were received for 253 cases (91%).

**Incidence.** The values reported indicated a mean incidence of 2.6 cases per 100,000 inhabitants per year (2.9 in 2003 and 2.3 in 2004). The incidence increased with age, reaching 12/100,000 for those over 80 years of age (Fig. 1). The median and the mean ages of patients were 61 years (range, 0 to 100 years) and 56 years, respectively, with no differences between males and females. During 2003 a higher incidence was reported among the group comprising ages 30 to 39 compared to 2004 (3.6 and 1.2, respectively; $P < 0.005$). There were marked regional differences between incidence rates, ranging from 0.25/100,000 (Ribe County) to 5.4/100,000 (Copenhagen City). Among 13 cases in children between 0 and 4 years, 4 (31%) were from Storström County (total reported cases, 16). A seasonal fluctuation could be observed, with an increasing number of cases during the cold months (January to April), but for

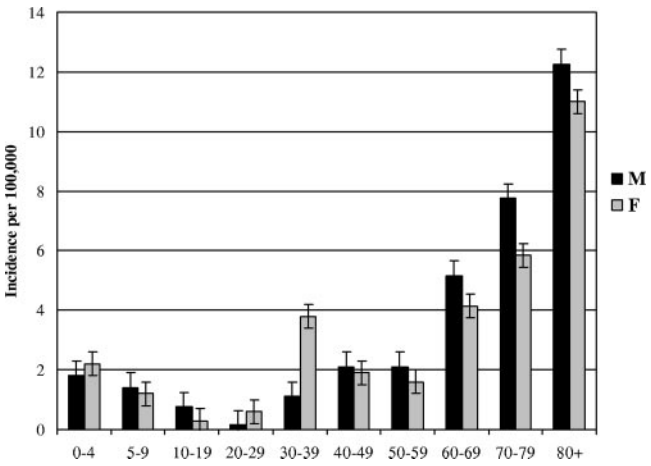


FIG. 1. Incidence of invasive GAS infections in Denmark during 2003 and 2004 as related to gender and age. Error bars indicate standard deviations.

puerperal fever, most cases were reported during May to August of the first year.

**Clinical presentations.** The majority of isolates (91%) were recovered from blood, with the remaining 9% being from cerebrospinal fluid (4 isolates), joints (3 isolates), and other normally sterile sites (18 isolates). Skin and soft tissue infections accounted for 26% of the cases, arthritis for 5.5%, puerperal sepsis for 5%, and meningitis for 3%. Eleven percent developed STSS, and 6% had NF. Finally, 19% had no focal symptoms, and 34% were reported as having other clinical presentations. According to the questionnaire, "other clinical presentations" included a variety of infections, e.g., wound sepsis, peritonitis and miscellaneous orthopedic diagnoses. As many as 55% of the patients had more than one clinical presentation (i.e., STSS and NF, skin/soft tissue infection and NF, meningitis and other clinical presentation, etc.).

**Risk factors.** Seventy-five percent of all patients were reported to have at least one risk factor, of which skin lesions (42 cases) were registered as the most prevalent. Diabetes accounted for 13 cases, immunosuppression for 16, and injecting drug use for 6. Among 15 children aged 0 to 16 years, three had chicken pox; of those, two cases occurred in the same area (Århus) within 10 days. All three patients underwent surgery; two had NF (one developed STSS), and the third had septic arthritis. In 64% of the cases, risk factors other than those listed in the questionnaire were recorded, i.e., inflammatory bowel disease, pregnancy, and disseminated sclerosis. Hospital-acquired infections accounted for 11% of all the cases; of those, 17 were from 2003 and 9 were from 2004.

**Outcome.** During the entire study, 35 patients were admitted to intensive care. A total of 64 patients underwent surgical interventions; among those 18 had STSS (case fatality rate [CFR], 44%), and 11 had NF (CFR, 9%).

The overall CFR within 7 days after hospital admission was 16%, compared to 20% within 30 days. As expected, the CFR was significantly higher among patients with STSS and NF (47% and 25%, respectively) than in the remaining patients (13%) ($P < 0.00004$). The CFR also varied among age groups, with 95% of fatal cases occurring among patients over 50 years of age ($P < 0.0013$) (31% among those 50 to 69 years, 33% among those 70 to 79 years, and 31% among those 80 years or older).

**Type distribution of GAS.** A broad range of 29 *emm* types was recorded (Fig. 2), with the five most common types being *emm*28 (26%), *emm*1 (24%), *emm*3 (11%), *emm*89 (7%), and *emm*12 (5.5%). The frequency of *emm*28 isolates was fairly constant, whereas that of *emm*1 decreased over time (from 47 in 2003 to 19 in 2004; $P < 0.003$). In contrast, 24 (80%) of the 30 *emm*3 isolates were registered in the second year ($P < 0.005$). Out of 16 different T types, T28, T1, and T3/13/B3264 accounted for 60% of all isolates, whereas 17% of strains were nontypeable (NT).

**T/*emm* type combinations.** As shown in Fig. 2B, of the total number of *emm*28 isolates, 78% (56 strains) were T28, 21% (15 strains) were NT, and 1 strain was T12. Almost all *emm*1 isolates (92%) were T1, with the remaining being NT (5 strains). The *emm*3 isolates were evenly distributed between T3, the cluster T3/13/B3264, and NT (37%, 33%, and 30%, respectively). The cluster T3/13/B3264 was present in 80% of *emm*89 strains (the remaining were T11, T13, T28, and TB3264 [one isolate each]), whereas *emm*12 strains were mainly T12 (93%), (the remaining were NT). Among the 48 NT isolates, 15 different *emm* types were found. Four unusual T/*emm* combinations were detected (T12/*emm*28, T12/*emm*6, T23/*emm*76, and T11/*emm*118), all of which were confirmed in two laboratories.

**Disease manifestation and *emm* type.** Out of 253 patients for whom complete clinical data were available, 67 (26%) had skin or soft tissue infections (Table 2). The corresponding GAS isolates belonged to a range of 16 *emm* types, with the five leading types mentioned above accounting for 73% within this group. Most cases of cellulitis/erysipelas were caused by *emm*1, followed by *emm*3 and -28. STSS and NF were mostly (66% and 81%, respectively) caused by *emm*1 or -3. Nine of 16 NF patients developed STSS; the implicated strains were of *emm*3 (five strains) and 1 (four strains). Bacteremia cases ($n = 51$) belonged to 17 different *emm* types, with *emm*28 accounting for 33%. Three bacteremia cases (two *emm*3 and one *emm*77) were complicated by STSS.

The puerperal sepsis cases ($n = 12$) were caused primarily by *emm*28 (75%), with the remaining strains being *emm*1, -3, and -9 (one case each). STSS occurred in the case caused by the *emm*3 strain; this was the only fatal case.

In terms of CFR, three *emm* types were overrepresented; the first was *emm*28 (31% of total fatal cases after 30 days), followed by *emm*1 and *emm*3 (27 and 18%, respectively).

**SAg gene distribution.** The multiplex PCR revealed the presence of three to six SAg genes in the various isolates. As expected, *speB*, *speF*, and *speG* were detected in most or all tested isolates. The phage-associated genes *speC* and *speA*, and *ssa*, were detected in 58%, 40%, 14% of the isolates, respectively (Table 3). In 78% of the strains, four of the nine SAg genes studied were detected, whereas in 14% of the strains five genes were found and in 4% only three genes were found. No strain exhibited *speI* or *speJ*, and *speH* was detected in two strains only.

***emm* types and SAg genes.** Among *emm*28 strains, 68% were *speA* negative and *speC* positive, whereas 30% were *speA* positive and *speC* negative. Among *emm*1 strains, 67% were *speA*

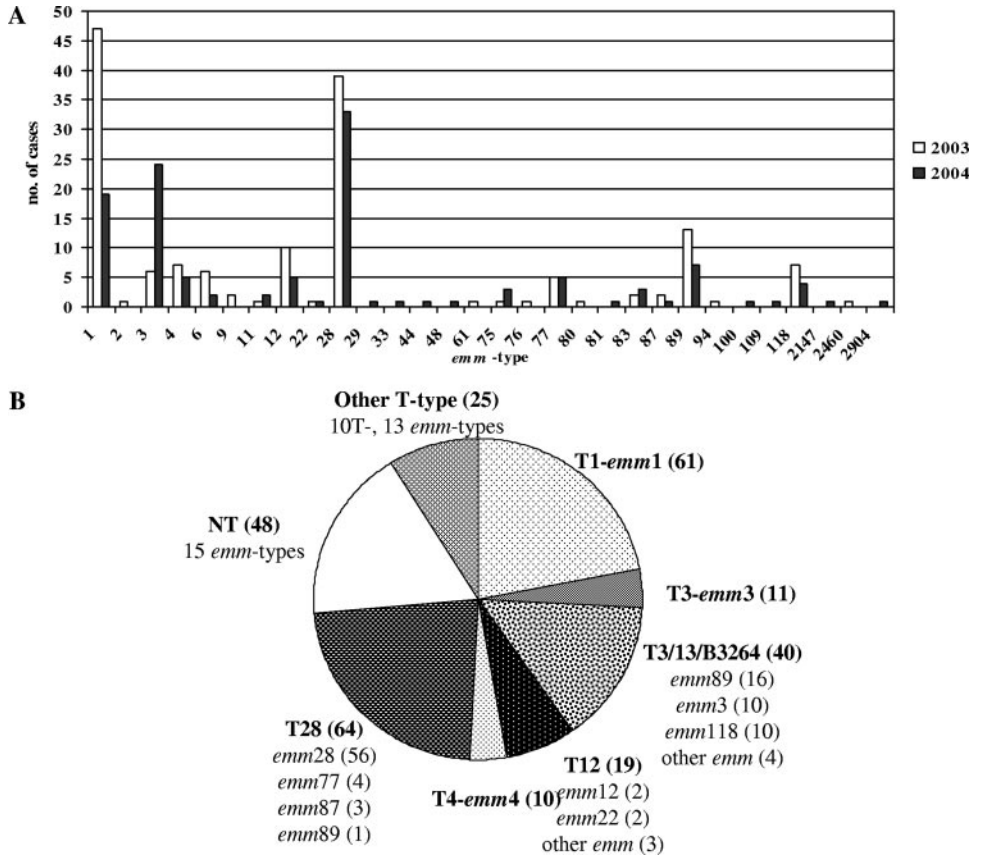


FIG. 2. Invasive GAS isolated during 2003 and 2004. (A) *emm* type distribution; (B) *emm* type combinations for the most common T types.

positive and *speC* negative, whereas 24% were *speA* negative and *speC* positive. Among *emm*3 strains, 47% were *speA* negative and *speC* positive, and 47% were *speA* positive and *speC* negative. Of the remaining types, *speA* was detected in 25% and *speC* in 70%. Only six strains were *speA* positive and *speC* positive (*emm*1, four strains; *emm*3 and *emm*77, one strain each).

**Antibiotic resistance.** The rate of resistance to erythromycin was 3%, with all eight strains being recovered from blood. The strains exhibited the following $MLS_B$ phenotype distribution: four showed the $iMLS_B$, one the $cMLS_B$, and three the M phenotype. The genotypes detected were *erm*(A) (five strains, with either $iMLS_B$ or $cMLS_B$) and *mef*(A) (three strains with the M phenotype). No strain harbored *erm*(B). All *erm*(A)-positive strains were T28; four of them were *emm*28 and one was *emm*77, showing coresistance to tetracycline and harboring the *tet*(O) gene. *mef*(A)-positive isolates were T11/*emm*61, T4/*emm*4, and NT/*emm*75.

Resistance to tetracycline was encountered in 8% of the strains, with MICs ranging between 4 and 48 mg/liter. Out of the 23 tetracycline-resistant strains, 16 harbored *tet*(M) and the remaining 7 harbored *tet*(O). Notably, all strains harboring

TABLE 2. *emm* types and clinical presentation of invasive GAS infections in Denmark in 2003 and 2004

| Clinical manifestation | No. of cases/ CFR (%) | No (%) of STSS cases/CFR (%) | No. of cases of *emm* type/CFR (%): | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *emm*1 | *emm*3 | *emm*12 | *emm*28 | *emm*89 | Other |
| Bacteremia (with no focal symptom) | 48/15 | 3 (6)/33 | 5/40 | 6/25 | 2/0 | 17/18 | 4/0 | 17/6 |
| Necrotizing fasciitis | 16/27 | 9 (56)/33 | 8/29 | 5/40 | 0/0 | 0/0 | 0/0 | 3/0 |
| Skin and soft tissue infections | 67/19 | 8 (12)/88 | 20/10 | 10/20 | 4/25 | 12/42 | 3/0 | 18/17 |
| Arthritis | 14/21 | 3 (21)/67 | 3/33 | 1/100 | 2/0 | 3/33 | 1/0 | 4/0 |
| Puerperal sepsis | 12/8 | 1 (8)/100 | 1/0 | 1/100 | 0/0 | 9/0 | 0/0 | 1/0 |
| Meningitis | 8/0 | 0 | 1/0 | 1/0 | 1/0 | 1/0 | 1/0 | 3/0 |
| Other | 85/20 | 0 | 24/0 | 4/0 | 7/0 | 26/0 | 8/0 | 16/0 |
| Clinical data not available | 25/24 | | 5/40 | 3/67 | 0/0 | 4/25 | 4/25 | 9/11 |
| Non-STSS | 223/14 | | 51/16 | 18/17 | 13/0 | 62/21 | 16/6 | 72/10 |
| STSS | 30/53 | | 10/50 | 9/56 | 2/50 | 6/50 | 0/0 | 3/66 |
| Total | | | 66/23 | 30/33 | 15/7 | 72/24 | 20/10 | 75/13 |

TABLE 3. Presence of SAg genes among the most represented *emm* types

| *emm* type | Total | | *speA* | | *speC* | | *speG* | | *speH* | | *ssa* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of isolates | % | No. of isolates | % | No. of isolates | % | No. of isolates | % | No. of isolates | % | No. of isolates | % |
| 1 | 66 | 24 | 48 | 73 | 20 | 30 | 66 | 100 | | | 2 | 3 |
| 3 | 30 | 11 | 15 | 50 | 15 | 50 | 30 | 100 | | | 15 | 50 |
| 4 | 12 | 4 | 4 | 33 | 8 | 67 | 12 | 100 | | | 8 | 67 |
| 12 | 15 | 5 | 6 | 40 | 9 | 60 | 15 | 100 | | | 1 | 7 |
| 28 | 72 | 26 | 21 | 29 | 49 | 68 | 72 | 100 | | | 1 | 1 |
| 89 | 20 | 7 | 6 | 30 | 12 | 60 | 19 | 95 | 1 | 5 | 1 | 5 |
| 118 | 11 | 4 | 2 | 18 | 8 | 73 | 11 | 100 | | | 2 | 18 |
| 77 | 10 | 4 | 3 | 30 | 8 | 80 | 10 | 100 | | | | |
| 6 | 8 | 3 | | | 7 | 88 | 8 | 100 | 1 | 13 | 1 | 13 |
| Other ($n < 5$) | 34 | 12 | 6 | 18 | 25 | 74 | 32 | 94 | | | 7 | 19 |
| Total | 278 | 100 | 111 | 40 | 161 | 58 | 275 | 99 | 2 | 0.7 | 38 | 14 |

*tet*(O) were *emm*77, whereas the *tet*(M)-positive strains exhibited 12 different *emm* types.

Of the 23 tetracycline-resistant isolates, questionnaires were available for 18 cases. Six isolates were from patients with no focal symptoms: two were *emm*77 (T28 and T13/28), and the other four were T11/*emm*61, NT/*emm*83, NT/*emm*33, and NT/*emm*st2904. Eight isolates were from cases of cellulitis, with one complicated by NF: three isolates were T28/*emm*77 (one of them was the NF case), and the remaining isolates (one each) were of types T13/*emm*77, T13/13/B3264/*emm*2460, T5/27/44/*emm*2147, NT/*emm*83, and T6/*emm*109. Two isolates were from patients with arthritis (T13/*emm*77 and T11/*emm*44), whereas two were from patients with clinical manifestations other than those listed in the questionnaire (NT/*emm*9 and -1). Erythromycin-resistant isolates were from two patients with cellulitis (one without focal symptoms), whereas five had other clinical presentations.

## DISCUSSION

We present here results of 2 years of surveillance of invasive GAS disease in Denmark. The mean incidence during the study period was 2.6 cases per 100,000 inhabitants, which was similar to that during the last two decades in Denmark (16) and to other reported Scandinavian data (10, 16, 19, 54). Population-based surveillance studies from the United States, Canada, and The Netherlands have shown rates ranging between 1.5 and 5 cases per 100,000 inhabitants per year (30, 36, 37, 56). A seasonal fluctuation could be observed, with an elevated number of cases during January to April, which could be explained by the well-known influence of indoor overcrowding on transmission of streptococci (46). Regional differences in incidence rates were observed; in particular, more densely populated regions had higher incidence rates (e.g., Copenhagen city, 5.0 and 5.8/100,000, compared to 2.6 and 1.9/100,000 in suburban Copenhagen areas) during the 2 years, which is conceivably due to population density determining incidence rates. Finally, in the rural district of Bornholm (43,956 inhabitants), the only four reported cases were from 2003, yielding a misleadingly high incidence of 9.1; however, this did not represent an outbreak (three different *emm* types), though three infections were hospital acquired.

Skin and soft tissue infections accounted for 26% of all cases. These infections are reported to be the most common focal manifestations of invasive GAS disease (16, 19, 60). However, this rate was much lower than that in the close neighbor Sweden (63% during 2002 to 2004) (10).

Three of the puerperal fever cases appeared to be nosocomial, showing a need for a constant focus on hospital-acquired infections, which were unexpectedly common in our study (11%). This number is similar to that in a report from the GAS study group in Ontario (9). However, a European perspective on this problem is still missing.

The majority of fatalities (95%) occurred in patients older than 50 years. However, 33% were in the group comprising ages 50 to 69, which was not considered a risk group for invasive GAS infection. It is interesting to note that bacteremia with *emm*1, NF with *emm*3, and soft tissue infections with *emm*28 were more often fatal than other *emm* type/disease combinations (Table 2).

There were no significant differences regarding gender, but during 2003 a peak in incidence was noted among patients between 30 and 39 years of age, and of those (29 cases), 23 patients (80%) were female. Puerperal sepsis was the most common disease (35%); interestingly, five of these cases were from Copenhagen area, accounting for 20% of invasive cases from this region in 2003. Elevated rates of invasive GAS infections were previously reported among this age group, due mainly to puerperal sepsis among females and arthritis among males (11). However, the gender difference in 2003 was not only due to puerperal sepsis, since of 23 females in this age group, only 8 had puerperal fever. Thus, excluding puerperal sepsis from this age group, the proportion of females to males (15/6) still was more than double, and no significant difference in specific disease manifestations and gender was observed.

Fifty percent of all cases were caused by *emm*28 and *emm*1, followed in frequency by *emm*3, -89, -12, and -4. These same types were the most prevalent during 1999 to 2002. Previous reports of invasive infections from Denmark (15) showed a gradual increase in frequency of *emm*1 from 1999 to 2002 (15% to 40%). During 2003, *emm*1 declined to 30%, and during 2004, it declined to 15%. In the same time, *emm*3 increased from 2% in 2002 to 20% in 2004. Thus, the distribution of *emm*1 and *emm*3 was changing over time, which could be at least in part related to epidemic waves (2) and to

type substitution due to herd immunity or population mobility (5). Most *emm*1 isolates (62%) were reported from Jutland (41 out of 66 *emm*1 strains). Unexpectedly, there was only partial agreement between the present types and those recorded in Sweden during 2002 to 2004, where, besides *emm*89 (the most prevalent type), *emm*81 and -77 were common (10).

During the study period, there was a sustained, high prevalence of *emm*28 (24% in 2003 and 26% in 2004), a type showing a steady increase from 15% in 1999 to 24% in 2002 (17). A prominent role of *emm*28 strains in invasive GAS disease was also reported in Sweden and Finland, as well as in the United States (19, 36, 40, 45). Of the present *emm*28 strains, as many as nine were isolated from cases of puerperal fever. These isolates are under investigation for clonal relatedness. Also in France, type *emm*28 strains were implicated in a puerperal sepsis outbreak (40). Notably, it has been shown that M/*emm*28 strains exhibit a surface protein (R protein) closely related to the group B streptococcal Rib protein, which confers adhesion to vaginal epithelial cells (48). Recently it was shown that M28 GAS strains contain a 37.4-kb region (region of difference 2) that is similar in gene content and organization to a genomic island from group B streptococci and encodes Rib and some other putative virulence factors. It was suggested that this element, acquired by horizontal gene transfer, has enabled type M28 to expand its pathogenic features and be overrepresented among puerperal sepsis cases (21, 58).

In addition to the predominant types mentioned, a broad spectrum of other *emm* types was found, e.g., *emm*118. Since this uncommon type, in particular, affected only subjects over 30 years of age, it is interesting to know its prevalence in throat infections of children, raising immunity to this type. Our finding of new T/*emm* type combinations is in line with reports from others; e.g., in a recent paper reviewing more than 40,000 GAS isolates, many uncommon T/*emm* combinations were reported (28). Obviously, the surveillance of circulating *emm* types is important in the ongoing attempts to develop an M protein-based GAS vaccine (24).

Several studies described the potential involvement of streptococcal pyrogenic exotoxin SpeA in severe streptococcal disease (18, 33, 51, 55), while others reported an association with SpeC (13, 22). Nevertheless, some cases of STSS were reportedly not associated with either of these (23). In the present study, *speA* was detected in 17 and *speC* in 13 out of 30 strains causing STSS, whereas the combination of both was not found in any of those isolates. Similarly, out of 51 isolates from bacteremia with no focal symptoms, one had both *speA* and *speC*, 20 had *speA*, and 28 had *speC*, thus not differing from STSS isolates. Either one of these two factors, though also common in noninvasive isolates (10), could hypothetically be required in order for severe GAS disease to be manifested. The occurrence of *ssa* among tested isolates was in line with other reports (41).

In the present study, a low level of macrolide resistance was found, possibly related to low macrolide consumption in Denmark (around two defined daily doses per 1,000 inhabitants per day in the last few years [49]). In the beginning of the 1990s, high macrolide resistance rates in GAS occurred in several European countries (i.e., Finland, Italy, Spain, and France) (8, 20, 29, 43, 49), whereas in the last years high rates have been reported in Asia (26). Though resistance against clindamycin, a potential threat in the treatment of invasive GAS disease, is still uncommon, surveillance of $MLS_B$ phenotypes is important for prevention of resistance.

Prior to this study, high rates of tetracycline resistance among GAS (approximately 30%) were reported in Denmark (35) and were attributed to a high use of tetracycline in livestock. In our study only 8% of tested isolates were tetracycline resistant. The present tetracycline-resistant strains harbored either the *tet*(M) or the *tet*(O) determinant. As many as 7 out of 10 *emm*77 strains were tetracycline resistant, all of which harbored *tet*(O), suggesting a clonal spread. In contrast, *tet*(M) strains were more heterogeneous, with 16 isolates belonging to 12 different *emm* types.

In conclusion, the present national Danish study demonstrated a high incidence of invasive diseases, in particular among the elderly, who showed a high fatality rate. Furthermore, many cases of hospital-acquired GAS infections and puerperal sepsis were noted. In addition to a wide range of T/*emm* types recorded, minor changes of predominant types compared to those in previous Danish reports were found. Characterization of GAS by different typing methods helps to improve understanding of the epidemiology of invasive disease, with impact on disease control, notification of outbreaks, detection of changes in population immunity, and vaccine development.

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