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Epidemiology of invasive group A streptococcal infections in Norway 2010–2014: A retrospective cohort study

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Abstract *Streptococcus pyogenes* or group A streptococcus (GAS) causes mild to severe infections in humans. GAS genotype *emm1* is the leading cause of invasive disease worldwide. In the Nordic countries *emm28* has been the dominant type since the 1980s. Recently, a resurgence of genotype *emm1* was reported from Sweden. Here we present the epidemiology of invasive GAS (iGAS) infections and their association with emm-types in Norway from 2010–2014. We retrospectively collected surveillance data on antimicrobial susceptibility, multilocus sequence type and emm-type, and linked them with demographic and clinical manifestation data to calculate age and sex distributions, major emm- and sequence types and prevalence ratios (PR) on associations between emm-types and clinical manifestations. We analysed 756 iGAS cases and corresponding isolates, with overall incidence of 3.0 per 100000, median age of 59 years (range, 0–102), and male 56 %. Most frequent clinical manifestation was sepsis (49 %) followed by necrotizing fasciitis (9 %). Fifty-two different emm-types and 67 sequence types were identified, distributed into five evolutionary clusters. The most prevalent genotype was *emm1* (ST28) in all years (range, 20–33 %) followed by 15 % *emm28* in 2014. All isolates were susceptible to penicillin, 15 % resistant to tetracycline and <4 % resistant to erythromycin. A PR of 4.5 (95 % CI, 2.3–8.9) was calculated for *emm2* and necrotizing fasciitis. All *emm22* isolates were resistant to tetracycline PR 7.5 (95 % CI, 5.8–9.9). This study documented the dominance of *emm1*, emergence of *emm89* and probable import of tetracycline resistant *emm112.2* into Norway (2010–2014). Genotype fluctuations between years suggested a mutual exclusive dominance of evolutionary clades.

Introduction

Streptococcus pyogenes or group A streptococcus (GAS) is an exclusively human pathogen that causes mild infections of the throat and skin, as well as severe infections and life threatening complications. While most common clinical manifestations are non-invasive, GAS may cause invasive infections including bacteraemia, septicaemia, pneumonia, puerperal sepsis, necrotising fasciitis (NF) and osteomyelitis. The inflammatory response of the body to the exotoxins produced by GAS may lead to streptococcal toxic shock syndrome (STSS) [1].

GAS strains have been characterized by serological identification of the surface protein M, an important GAS virulence factor. With time, serology has been replaced by emm-typing as a predictor of the M serotype [2]. To date more than 200 different emm-types have been reported and associated with various types of disease manifestations and prevalence rates worldwide. In most high-income countries emm-types 1, 3, 12 and 28 have traditionally been associated with invasive GAS (iGAS) disease [3].

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Many countries including Norway witnessed a change in iGAS epidemiology in the 1980s and 1990s, with a sudden increase in both incidence and severity of infections caused by genotypes *emm1* and *emm3* [4, 5]. Data collected from 1998 to 2007 document a gradual change in the epidemiology of circulating iGAS genotypes in Norway, with *emm1* on a steady decline and *emm28* on a steady incline [6–8]. By 2006 the most frequently reported iGAS genotypes in Norway were *emm28* (20 %) followed by *emm1* (14 %). This change was also reported from neighbouring countries, with Denmark, Sweden and Finland reporting *emm28* as the dominant genotype in their respective countries [9–11], while *emm1* remained the dominant genotypes in most other high-income countries [3, 12].

A correlation between emm-types and specific clinical manifestations has been suggested. In particular, genotypes *emm1*, *emm3* and *emm18* have all been associated with outbreaks of NF and STSS in Europe and the United States [13, 14]. However, this association has not been perpetual and many interplaying and regulating factors are involved [12, 15]. Resistance to antimicrobials may increase the severity of an iGAS infection. Although resistance to penicillin (the antimicrobial of choice) is absent in naturally occurring GAS isolates, resistance to macrolides and tetracyclines have been documented in many emm-types, including *emm1*, *emm3* and *emm28* [16].

Recently, Sweden reported a shift in the iGAS epidemiology with a sudden rise of *emm1* following a rapid increase in the incidence of iGAS infections. This change has not been reported from any of the neighbouring countries [17, 18]. We performed this study to provide a better insight into the current epidemiological situation of iGAS infections in Norway. Here we describe iGAS cases in terms of age, sex, demographics and clinical manifestations, and iGAS isolates in terms of emm-type and sequence type distribution in the five-year period from 2010 to 2014. Furthermore, we explore the associations between different emm-types and the most frequently reported clinical manifestations of iGAS infections.

Methods

Case definition, setting and data collection

An iGAS infection was defined as the detection of a pathogen from a normally sterile body site. Since 1993, all iGAS infections in Norway have been mandatorily notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS) and isolates have been sent to the National Reference Laboratory (NRL) at The Norwegian Institute of Public Health (NIPH). From 2010 to 2014 the NRL received a total of 808 isolates associated with iGAS infections. Isolates that were received from the same patient with an identical molecular type less than three months apart from each other (n = 17),

and isolates for which molecular typing results were inconclusive (n = 3), were excluded from the dataset. The remaining isolates (n = 788) were searched against the MSIS national data registry for linked iGAS cases. A total of 756 isolates (89 % of the notified cases) were matched in MSIS, and data on demographics, clinical manifestations and outcomes were linked with the molecular data for further analyses.

Multilocus sequence typing (MLST)

MLST was performed as described by Enright et al. [19]. Briefly, primers were synthesized by Eurofins Genomics (Ebersberg, Germany), amplified DNA was purified and sequenced using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA) and nucleotide sequences were analysed using SeqScape v2.5 (Applied Biosystems). Allele numbers and sequence types (STs) were recovered from the MLST database (http://pubmlst.org/spyogenes), with the new alleles and STs assigned by the curator of the database.

Emm typing

The 5′-end fragment of the M protein gene was used for emm-typing, amplified as described by Beall et al. [2]. Briefly, DNA from all isolates was extracted by boiling, and an 180 bp fragment of the emm gene was amplified using primers emm1-fwd and emm2-rev as published by CDC (http://www.cdc.gov/streplab/protocol-emm-type.html), with attaching an M13-tail on the primers to facilitate sequencing [20]. Amplicons were purified using ExoSAP-IT before sequencing on ABI 3730 DNA analyzer (Applied Biosystems). Sequenced reads were inspected in Sequencer 4.8 (Gene Codes, USA) and the emm-sequence database of CDC was searched for homology. Sequences with less than 100 % similarity were re-sequenced and submitted to the curator to obtain a new emm-type or subtype.

Phylogeny

Relatedness of all isolates was investigated using the MLST data on allelic profiles. All recovered STs from the dataset were included only once in the construction of an unrooted optimized maximum parsimony tree (simulated annealing). Allele numbers were used as unordered character data values with equal cost. A phylogenetic tree was constructed with highest resampling support after 500 bootstraps in BioNumerics v.7.5 (Applied Maths, Belgium).

Antibiotic susceptibility

Antibiotic susceptibility testing for iGAS isolates was introduced in 2013, and all isolates from 2013–2014 (n = 346) were tested using E-tests according to the manufacturer's

instructions (bioMérieux SA, Marcy l'Etoile, France). Briefly, fresh overnight cultures were grown at 35 °C in 5 % CO_2, suspended in Mueller-Hinton (MH) broth to a density of 0.5 McFarland. Bacteria were evenly spread on the surface of an MH-F agar plate using a cotton swab and allowed to dry for 10 min before the E-test strips were applied. Antibiotics tested were: penicillin G, erythromycin, clindamycin, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol. Interpretation of results was according to clinical breakpoints set by the European Committee for Antimicrobial Susceptibility Testing (EUCAST).

Statistics

Incidence was calculated using population data as of 1st October each year from Statistics Norway (www.ssb.no). The discriminatory power of emm-typing and MLST was estimated by the Simpsons Diversity Index (SDI) with 95 % confidence intervals (CI), and the adjusted Wallace coefficient (AW) with 95 % CI was used for measuring agreement between partitions of the two typing methodologies (http://darwin.phyloviz.net). Statistically significant differences in proportions of genders in different age groups were detected using a two-tailed Fisher's exact test and associations between variables were estimated using prevalence ratios (PR) and 95% CI in Stata version 13.1 (StataCorpLP, USA). Exposure variables used were age groups, sex, health regions and emm-types, with clinical manifestations (n > 10), emm-types (n > 15) or antimicrobial resistance (n > 10) as outcome measures.

Results

Descriptive epidemiology

A total of 756 cases of iGAS and corresponding bacterial isolates from Norway were analysed from a 5-year period starting in 2010 (Table 1). Isolates were submitted to the NRL from all four health regions of Norway (South-East, West, Central and North) averaging 151 per year (range,

122–173). The overall incidence of iGAS was calculated to 3.0 per 100,000 population, ranging from 2.4 to 3.4 during the study period. Incidence per 100,000 population was highest in the South-Eastern and Western health regions (3.4) compared to the Northern health region (1.7).

Cases were slightly overrepresented by males (56 %) with an incidence of 3.4 per 100,000 males compared to 2.6 in females. The median age was 59 years (range, 0–102) and the age distribution of cases displayed three peaks in the 0–9, 40–49 and 60–69 age groups. Incidence of iGAS was seen increasing with increasing age groups (Fig. 1). Males were overrepresented among the <20 year olds and between 40 and 80 year olds, most profoundly in age group 0–9 years, where 79 % were male (n = 45, p-value 0.0002).

Data on clinical manifestation were complete for 664 (88 %) cases and these were grouped into the following manifestations (Fig. 2): sepsis (n = 323), necrotizing fasciitis (n = 71), pneumonia (n = 46), erysipelas (n = 19), arthritis/osteomyelitis (n = 23), meningitis (n = 11), abscess (n = 5), pyelonephritis (n = 9), endocarditis (n = 2) and other (n = 155). No cases of STSS were reported during the study period. The clinical manifestations were evenly distributed among the different health regions and between all years. However, arthritis/osteomyelitis was more frequently described for cases in age group 40–49 years (40 %), and NF was more frequently described for cases in age group 60–69 years (30 %) compared to other age groups.

Data on outcome were available for 382 (51 %) cases. Death was recorded in 19 % (n = 72) of the cases with 64 % of them attributed to sepsis (n = 46) and 21 % to NF (n = 15).

Molecular epidemiology

The 756 iGAS isolates were typed into 52 distinct emm-types (80 emm-subtypes) and 67 STs. The most frequent emm-types were *emm1* (n = 208; 28 %), *emm89* (n = 89; 12 %) and *emm12* (n = 65; 9 %) (Fig. 3), and the most frequent STs were ST28 (n = 205; 27 %), ST101 (n = 83; 11 %) and ST36 (n = 65; 9 %).

The SDI with 95%CI was calculated for emm-type, emm-subtype and ST, scoring the highest for emm-subtypes (0.902)

Table 1 Annual cases and incidence of invasive group A streptococcal infections by health regions and sex in Norway 2010 to 2014

Year	Cases	Incidence[a]	South-East (%)	West (%)	Central (%)	North (%)	Male (%)	Female (%)
2010	130	2.7	77 (59)	38 (29)	8 (6)	7 (5)	79 (19)	51 (16)
2011	158	3.2	94 (60)	31 (20)	27 (17)	6 (4)	87 (20)	71 (22)
2012	122	2.4	83 (68)	28 (23)	9 (8)	2 (2)	63 (15)	58 (18)
2013	173	3.4	93 (55)	50 (29)	16 (9)	11 (6)	93 (22)	78 (24)
2014	173	3.4	108 (62)	28 (16)	23 (13)	14 (8)	105 (25)	68 (21)
Total	756	3.0	455 (60)	175 (23)	83 (11)	40 (5)	427 (56)	326 (43)

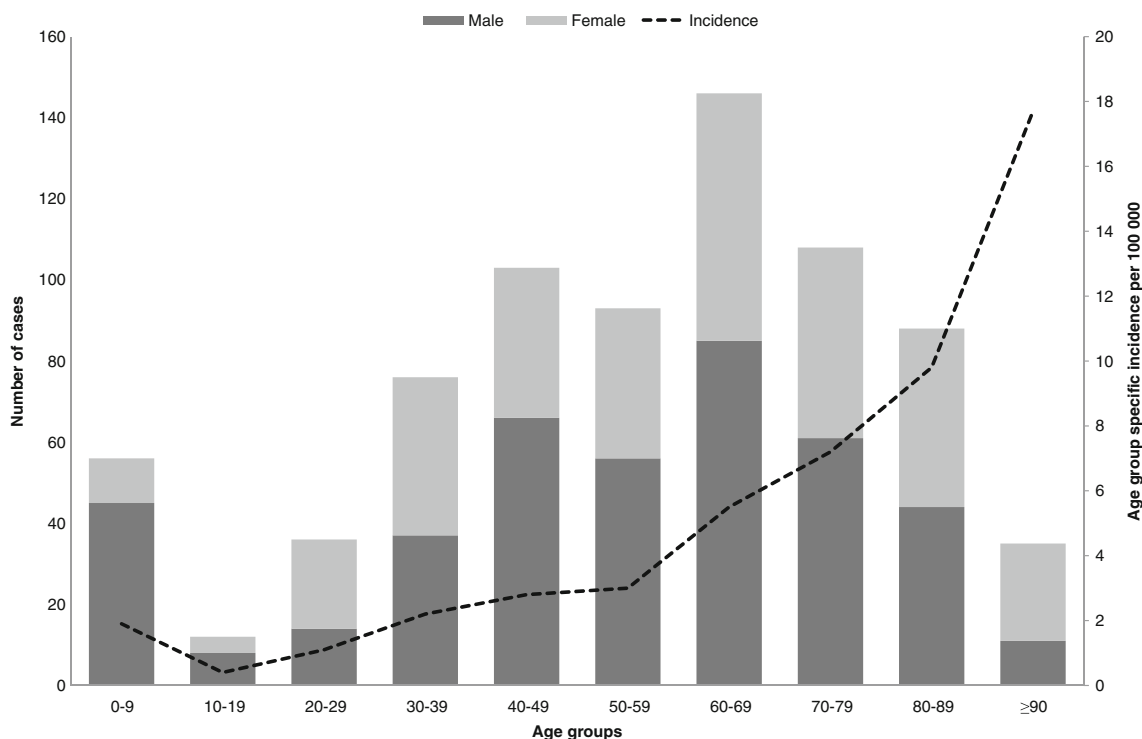[a] Incidence is calculated per 100,000 population in Norway

Fig. 1 Age and sex distribution of invasive group A streptococcal cases and age group specific incidence per 100,000 in Norway, 2010–2014. Different *shaded bars* represent the two sexes (see legend), distributed into age groups of 10 years from left to right on the x-axis. The absolute numbers of cases are given on the primary y-axis. The *dotted line* represents the age group specific incidence given per 100,000 population on the secondary y-axis

compared to ST (0.880) and emm-type (0.874) with overlapping 95%CI. AW measurement of agreement between the typing methods estimated high concordance of ST partitions included within emm-types (p-value <0. 001). For the most frequent STs (n > 20) the level of concordance with emm-types was 92–100 %, e.g., ST28 and *emm1* (99 %), ST101 and *emm89* (99 %), ST36 and *emm12* (100 %), ST15 and *emm3.1* (97 %), ST52 and *emm28* (98 %), ST44 and *emm66* (100 %), ST39 and *emm4* (92 %), ST655 and *emm112.2* (100 %).

The annual distribution of the most common emm-types showed major variations in relative frequency. Noticeably, *emm1* was seen as the dominant emm-type during the entire study period, although a drop in frequency was recorded in 2011 and 2012, largely attributed to the South-Eastern and Western health regions of Norway. The decrease in *emm1* was mirrored by an increase of *emm12* in the same health regions. For *emm28* we observed an increasing trend in all regions except in the Central health region of Norway, resulting in an increase from 4 % in 2010 to 15 % in 2014. The increase of *emm28* was mirrored by a decrease in *emm89* in the South-Eastern and Central health regions of Norway (Fig. 4, regional level data not shown).

With regards to clinical manifestations, *emm1* was the most frequently recovered emm-type from cases presenting with meningitis (73 %), pneumonia (46 %), erysipelas (37 %), necrotizing fasciitis (32 %) and sepsis (27 %). Also *emm1* was the

most frequently recovered emm-type from all age groups with the exception of 20–29, 40–49 and ≥90 age groups.

Investigations into the potential evolutionary relationship between the genotypes circulating in Norway showed the existence of five distinct clusters (resampling score 0.74) (Fig. 5). The five most frequently recovered emm-types, namely, *emm1*, *emm89*, *emm12*, *emm3* and *emm28*, all seemed to originate from evolutionary distinct clusters indicating divergent evolutionary paths.

Antimicrobial susceptibility

All tested isolates (n = 346) were susceptible to penicillin and chloramphenicol, 15 % were resistant to tetracycline (n = 52), 3 % were resistant to erythromycin (n = 10) and trimethoprim-sulfamethoxazole (n = 12) and 2 % were resistant to clindamycin (n = 5). Tetracycline resistance was seen in isolates of 23 different emm-types, of which 13 (25 %) were *emm112.2*, seven were *emm22*, four were *emm11* and three were *emm73*. Tetracycline resistance among *emm112.2*, *emm22* and *emm73* isolates accounted for 87 %, 41 % and 75 %, respectively.

Isolates typed as *emm11* were the largest contributors to the observed clindamycin and erythromycin resistance, accounting for 4/5 and 4/10 of the resistant isolates, respectively. Only a single *emm42* isolate was identified with a MDR phenotype, which was defined as resistance to three or more classes of
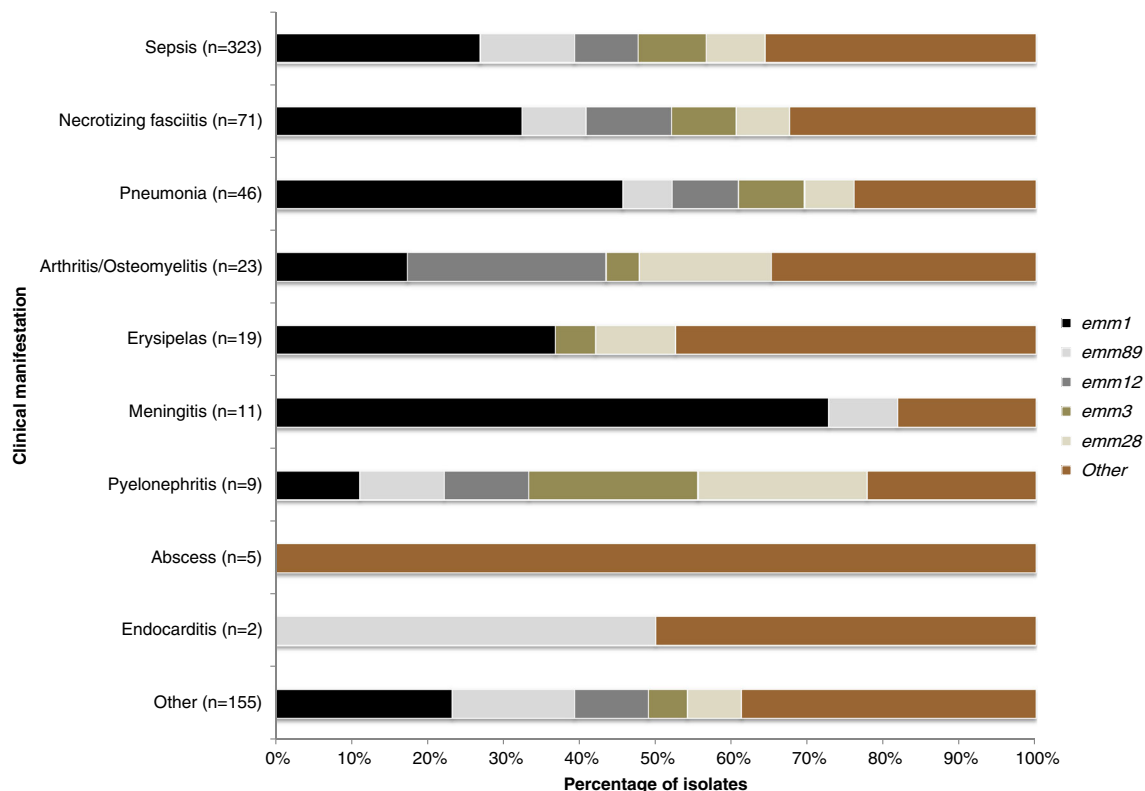
Fig. 2 Notified clinical manifestations of invasive GAS infections by percentage of the five most common emm-types in Norway, 2010–2014. Clinical manifestations are given on the y-axis and the percentage of isolates belonging to the five most common emm-types is given on the x-axis, coloured according to legend. Clinical manifestation data were complete for 664 (88 %) isolates; the number of isolates within each manifestation is in parentheses

antibiotics. The MDR isolate was from a patient with sepsis in the Northern health region in 2014, displaying resistance to erythromycin, trimethoprim-sulfamethoxazole and tetracycline.

Associations

Exploring potential associations between age groups of iGAS cases and implicated emm-types, we found *emm66* to be more frequently associated with infections in age group 40–49 years (PR 7.3, 95 % CI 4.2–12.8) compared to other age groups. In addition, *emm22* had a more frequent association to age group 30–39 years (PR 6.3, 95 % CI 2.5–16.0) compared to other age groups, while *emm112.2* had a more frequent association to age group 50–59 years (PR 5.5, 95 % CI 2.5–12.2).

A significant association was observed for age groups 0–9 and 10–19 years with meningitis, PR 17.7 (95 % CI 4.8–64.8). No significant association was observed between sex and clinical manifestation or emm-type. With regards to association of emm-types with clinical manifestations of iGAS, we found that *emm2* had a PR of 4.5 (95 % CI 2.3–8.9) for necrotizing fasciitis. Similarly, *emm12* had a PR of 3.5 (95 % CI 1.4–8.5) for arthritis/osteomyelitis. All *emm22* isolates were resistant to tetracycline with a calculated PR of 7.5 (95 % CI 5.8–9.9), and

a PR of 7.4 (95 % CI 5.2–10.5) was calculated for *emm112.2* and tetracycline resistance.

Discussion

We present the epidemiology of iGAS infections in Norway from 2010 to 2014, exploring the isolates submitted to NRL and their associated cases. The isolate collection was recovered consistently for the entire study period from laboratories all across Norway and matched against MSIS notification data to give the best possible representation of iGAS epidemiology in Norway.

Calculated annual and overall incidences were comparable to incidence rates calculated from iGAS notification data in MSIS. For 2014 MSIS reported an iGAS incidence of 3.7 per 100,000 in Norway, a rate which has been stable since 2006 and comparable to neighbouring countries, with the exception of the sudden increase reported from Sweden in 2012 and 2013 [17]. The South-Eastern and Western health regions are the two most populated health regions in Norway covering about 77 % of the total population. Incidence of iGAS cases in these regions were relatively stable compared to the Central and Northern health regions, where there seemed to be on an upwards trend since 2012. However, the increase in the central and northern part of the country did not affect the national
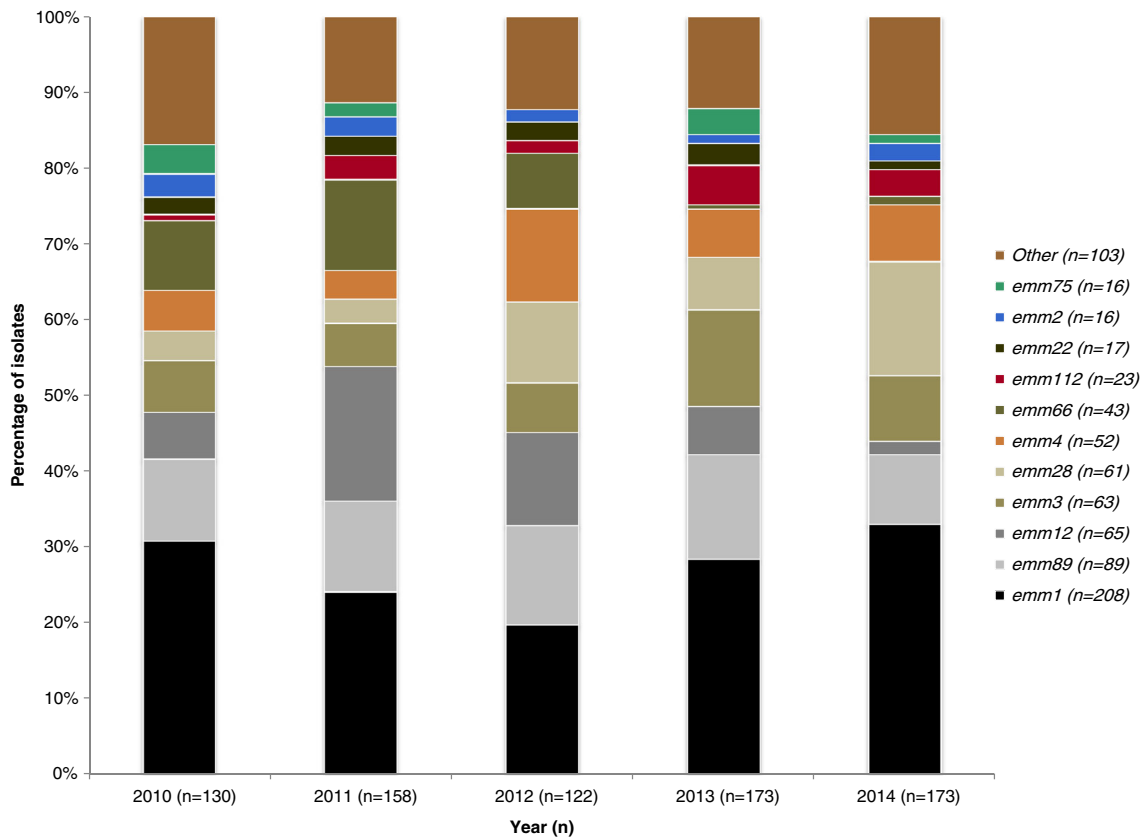
Fig. 3 Proportions of the most common emm-types (for $n > 15$) in Norway, 2010–2014 ($n = 756$). Each year of isolation is represented by a separate bar on the x-axis. Percentages of isolates with specific emm-types are given according to legend, with emm-types arranged from high to low bottom up. Crude numbers of isolates per year for each emm-type is in parentheses

incidence level. The reported increase in Sweden was noticeably marked in the older population (≥ 80 years) while in Norway the incidence in all age groups has remained stable.

Typically iGAS cases follow an age and sex distribution where both sexes are equally affected and an increasing incidence rate is seen with age. During the study period we documented a slight overrepresentation of males (56 %), compared to studies performed earlier (2006–2007) [6]. Among cases <10 years of age we uncovered a significantly higher proportion of males (79 %, p-value 0.0002). The skew in this age group was recorded in all years of the study period, highest in 2013–2014 (89 %, 24/27). Cases were reported from across Norway with no apparent change in reporting patterns or identified clusters that would suggest a sudden increase in this group. Data on clinical manifestation for this age group were either missing or unspecified for 35 % of the cases; for cases with a known clinical manifestation, no significant difference was observed between the two sexes. The underlying reasons for this pronounced skew towards males in the younger age group are unknown, but warrant investigation and close monitoring in the future.

The dominating emm-type among iGAS isolates in Norway was *emm1*, which has been the most prevalent emm-type since 2010 (Table 1). The shift from *emm28* to

emm1 seemed to have occurred somewhere between 2007 and 2010. The top five emm-types from 2010 to 2014 accounted for 64 % of the isolates and included *emm1* (27 %) followed by *emm89* (12 %), *emm12* (9 %), *emm3* (8 %) and *emm28* (8 %). Comparing emm-type distribution between 2007 and 2014, *emm28* the dominating type in 2007 (20 %) had declined to 15 %, and *emm1* had increased from 14 % to 33 % in 2014 (Fig. 3). Five of the six most frequent emm-types in 2007, *emm1*, *emm28*, *emm12*, *emm4* and *emm3*, remained among the top six emm-types in 2010 to 2014. However, *emm82*, the third most frequent emm-type in 2007 (14 %) was completely absent in 2010–2014, and was replaced by *emm89* which had increased from 5 % in 2007. Isolates typed as *emm89* were reported consistently in all years from 2010 to 2014, and from all health regions in Norway. Also in the UK and Canada a recent upsurge of *emm89* and associated iGAS disease has been reported [13, 21]. Among high-income countries *emm89* is now reported among the top five emm-types [12, 13, 18, 21–24]. Whole genome sequencing data on *emm89* attribute its upsurge to the development of a successful clade within *emm89* genotype which has incorporated a prophage-like element similar to that of *emm1* [21].
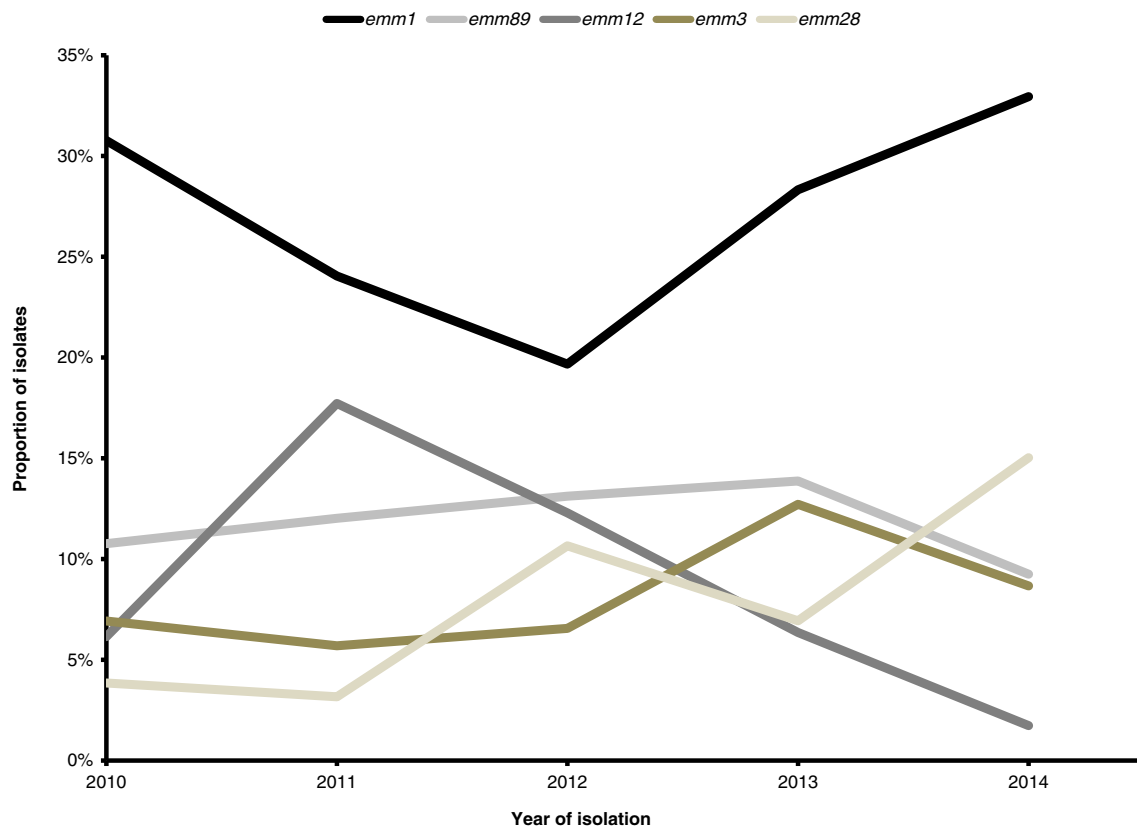
Fig. 4 Trends in proportions of the five most frequently isolated emm-types in Norway, 2010–2014

Overall *emm1* was the most frequently isolated emm-type from males and females in most of the age groups and from most clinical manifestations of iGAS infections (Table 1). However, no specific associations were seen between *emm1* and a particular disease manifestation, a defined age group, or a resistance phenotype. A significant association was seen for *emm22* in the age group 30–39 years and for *emm112.2* in the age group 50–59 years. These particular emm-types had not been associated with disease in specific age groups before in Norway. In China, *emm22* has been implicated in severe iGAS disease in children [25]. Internationally, resistance to both macrolide and tetracycline has been associated with *emm22* [26, 27]. In our study, *emm22* also showed a positive association with tetracycline resistance (PR 7.5, 95%CI 5.8–9.9), although this association was not present in 2007 [6].

emm112.2 is an unusual emm-type. First reported in isolates obtained in the 1990s from Ethiopian children with various streptococcal diseases, it has thus far not been recorded as an important emm-type from any high-income country [28]. Interestingly, it was now among the top ten genotypes recovered in Norway (Fig. 3). It seemed to be significantly associated with the age group 50–59 years and also significantly associated with tetracycline resistance. All *emm112.2* isolates were ST655 and submitted from the South-Eastern health region of Norway. Reports from India have suggested *emm112*

to be frequently associated with skin infections [29]. Absence of *emm112.2* from the other Nordic countries, its association with a particular age group, association with tetracycline resistance and recovery only from the South-Eastern health region suggested it to be a recently imported iGAS genotype, although we did not have data on travel and the ethnicity of cases to confirm this assumption.

In our data set *emm2* had a positive association with NF (PR 4.5, 95 % CI 2.3–8.9) with five NF cases out of a total of 71. These five cases represented 31 % of all infections caused by *emm2*. Although NF caused by *emm2* has been reported earlier, no significant association has been documented [30]. For NF a strong correlation has been observed with *emm18*, *emm3*, *emm5* and *emm12* [12]. In our study the most frequently recovered genotypes associated with NF cases were *emm1* (n = 23), *emm12* (n = 8), *emm89* (n = 6) and *emm3* (n = 6).

Data on the trends of the most frequently recovered emm-types suggested that *emm12* mirrored the development of *emm1*, and *emm28* was mirrored by *emm3* (Fig. 4). The evolutionary relationship between the circulating genotypes in Norway displayed the presence of five distinct branches (Fig. 5). Thus, the increase of *emm1* and corresponding decrease of *emm12* may have indicated a shift in not only a single emm-type, but an evolutionary distinct cluster. A similar shift in dominant cluster could be proposed for *emm28* contra *emm3*. It was also
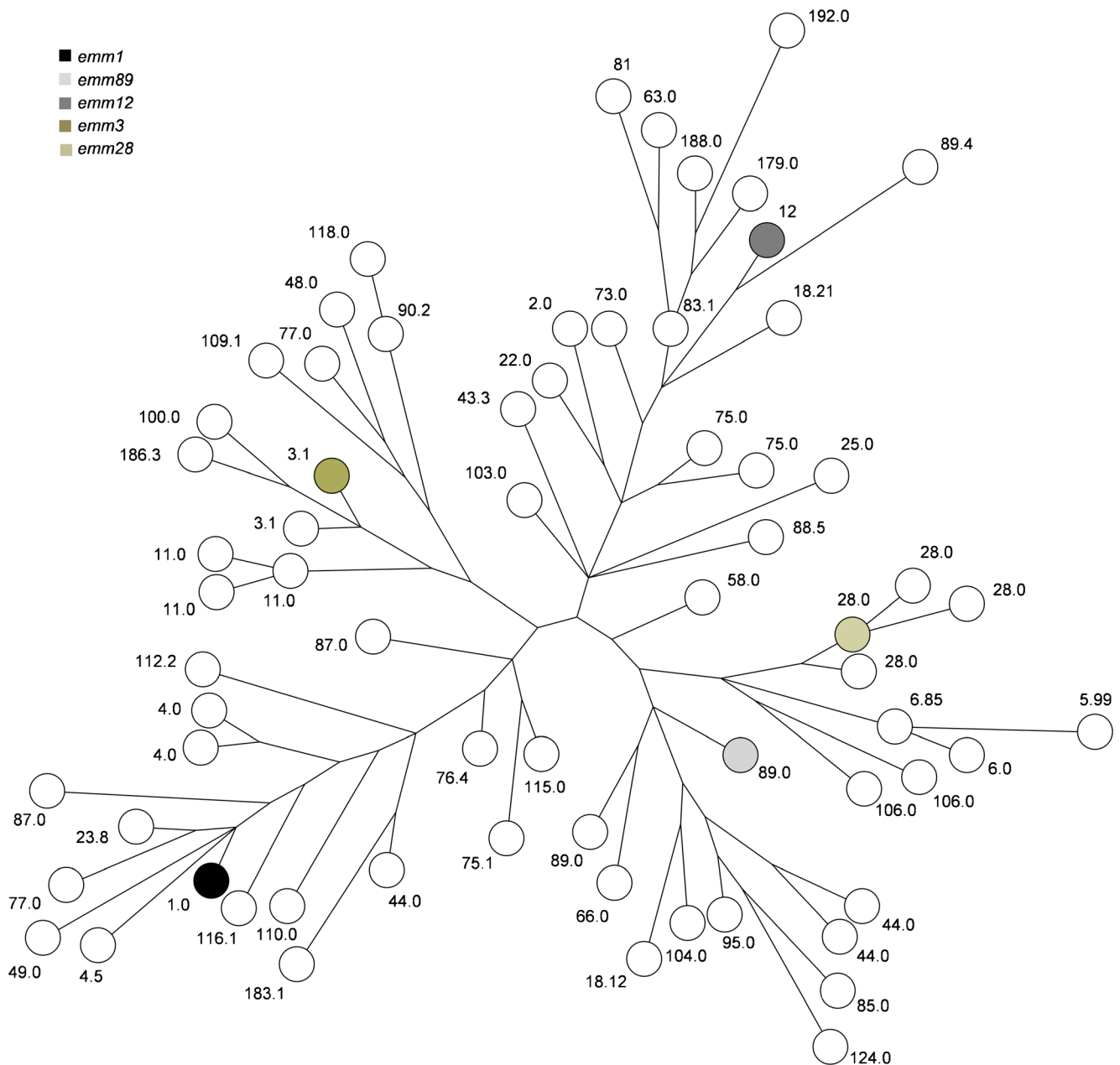
Fig. 5 Unrooted maximum parsimony tree based on MLST profiles of estimated potential relationship between all invasive GAS isolates in Norway, 2010–2014. All nodes represent unique sequence types ($n = 67$), with the corresponding emm-types labelled. Nodes (most frequent) of the five major emm-types are coloured according to the legend

interesting to note that *emm89*, the second most prevalent emm-type, seemed to be more closely related to *emm28*, which might have given way for the increasing prevalence of *emm89* (Fig. 4).

When looking into associations between emm-types and clinical manifestations, it should be taken into account that data on clinical manifestations were recovered from MSIS, which primarily serves as a notification tool and thus has its limitations with regards to completeness of the reported clinical data. Also for some of the clinical manifestations the numbers of cases were low and may thus lead to an overestimation of the associations. Therefore only the most frequent manifestations and most frequent emm-types were analysed for statistical associations. Our data are strengthened by high notification completeness and isolate submitting compliance across Norway and throughout the study period. Overall we believe the data gives a good overview of the most common clinical manifestations of iGAS infections and the emm-type distribution in Norway.

We conclude that *emm1* was the dominant emm-type in Norway, recovered in all years from 2010 to 2014, from all age groups and from most clinical iGAS manifestations. We

documented *emm89* as an emerging emm-type in Norway, along with probable import of tetracycline resistant *emm112.2* type ST655, both of which will require close monitoring in the future. ST distributions of the fluctuating emm-types suggested a mutually exclusive dominance of distinct evolutionary clades.

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