Novel *emm*4 lineage associated with an upsurge in invasive group A streptococcal disease in the Netherlands, 2022

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Running title: Emerging *S. pyogenes emm*4 lineage in outbreak

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Abstract

Invasive group A streptococcal (iGAS) disease cases increased in the first half year of 2022 in the Netherlands with a remarkably high proportion of *emm4* isolates. Whole-genome sequence analysis of *emm*4 isolates from 2009-2022 identified a novel GAS lineage, which accounted for 85% *emm*4 iGAS cases in 2022.

Main text

Increase in *emm*4 iGAS disease predominantly among young children

In 2022, an upsurge in invasive group A streptococcal (iGAS) infections was observed in the Netherlands compared to pre-COVID-19 levels shortly after restrictions were lifted. Alarmingly, this increase disproportionally affected children under 5 years of age (*1*). Bacteriological surveillance of *Streptococcus pyogenes*, the causative agent of iGAS, is based on *emm* typing; changes or shifts in dominance of *emm*-types may indicate changed virulence or transmission potential of *S. pyogenes*.

Bacteriological surveillance for *S. pyogenes* was initiated as part of a research project in 2019. *S. pyogenes* isolates cultured from normally-sterile compartments are voluntarily submitted to the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) for *emm*-typing by medical microbiology laboratories (see appendix for details). Between 1 January to 13 May 2022, 25 isolates were received from children under the age of 5 years; *emm*4 and *emm*12 were the most dominant *emm* types (28% each), followed by *emm*1.0 (16%). Among isolates from all ages (n=134), *emm*4 ranked third (13%). The dominance of *emm*4 among iGAS cases was unexpected, since data from a retrospective study (2009-2019)

indicated that this *emm*-type was associated with carriage rather than invasive disease in the Netherlands (L.W. Rümke, unpub. data).

Recently, a hypervirulent *emm*4 clone (referred to as "hypervirulent clone") was described, which has replaced nearly all other *emm*4 *S. pyogenes* strains in the USA and UK (*2*). We hypothesized that this hypervirulent *emm*4 clone was responsible for the increase of *emm*4 iGAS cases in the Netherlands.

Sample inclusion, sequencing and analysis

We included all 26 *emm*4 *S. pyogenes* isolates from iGAS patients that were received by the NRLBM between January 1st and May 13th 2022 (see Table 1 and Appendix for details). Additionally, we included 40 *emm*4 *S. pyogenes* isolates between 2009-2019 (pre-COVID samples), which were from invasive disease or from asymptomatic carriage (Table 1). All 66 isolates were subjected to whole-genome sequencing (WGS) on an Illumina HiSeq platform. Bioinformatic analysis included phylogenetic analysis and single nucleotide variants (SNV) comparisons (see Appendix for details).

Patient characteristics

For one patient no information was available on age or sex. For the remaining 25 iGAS cases from 2022, median patient age was 34 years (IQR: 5-43 years; Table 1) and 15 (60%) were male. Site of isolation is summarized in Table 1. Of the pre-COVID isolates, 26 out-of-40 (65%) were from 0-5 years old, while the remaining 14 isolates were from adults.

Bacterial characteristics: new *emm*4 clone

In contrast to the strong emergence of hypervirulent clone in USA and UK, this clone represented less than 10% among recent and older *S. pyogenes* isolates (Table 1). In contrast, 22 out-of-26 (84.6%) of 2022 iGAS isolates clustered together, representing a new *emm*4 lineage $M4_{NL22}$ (Figure). Fifteen out-of-22 (68%) $M4_{NL22}$ isolates showed very limited genetic variation with a median of 6 SNVs between them (range 0-16). This suggests an emergence beginning of 2021 and potentially a high transmission rate. In contrast, only two out-of-40 (5%) isolates from 2009-2019 were identified as $M4_{NL22}$ strains (isolated in 2017 and 2018). Analysis of public surveillance databases, identified one isolate (NCBI BioSample SAMEA111521236) from 2022 from Norway (Norwegian Public Institute of Health surveillance, NCBI BioProject PRJEB42599) that clustered in the clonal part of the $M4_{NL22}$ lineage and differed 9 SNVs from the closest Dutch isolate. No $M4_{NL22}$ isolates were identified from USA surveillance data from 2021-2022 (CDC Active Bacterial Core surveillance, NCBI BioProject PRJNA395240).

Characteristics of $M4_{NL22}$ lineage strains

The $M4_{NL22}$ lineage displayed several genetic variations compared to other *S. pyogenes emm*4 strains, including six unique SNVs and a 306 bp deletion in the *proA* gene, which is involved in amino acid biosynthesis (see Table S2 in the Appendix for details). Characteristically, $M4_{NL22}$ lacked the *hasABC* genes for synthesis of a hyaluronic acid capsule (*3*). Further characterisation through *in vitro* and *in vivo* experiments should reveal whether $M4_{NL22}$ is more transmissible than other *emm*4 *S. pyogenes*. No changes in known virulence determinants were observed (*nga-ifs-slo* region, superantigens, known virulence genes) in isolates from this $M4_{NL22}$ lineage.

Discussion

Several novel *S. pyogenes* clones have emerged over the past years, including *emm*4 (*2*), *emm*89 (*4*) and *emm*1 lineages (*5*). Although most emerged lineages seem endowed with increased capacity for transmission or invasiveness, there may be additional explanations for the emergence of $M4_{NL22}$ reported in the current study. As with many other infectious diseases (*6*), we have observed a marked shift in the epidemiology of *S. pyogenes* infections after COVID-19 pandemic restrictions were lifted, with a surprising absolute and proportional increase of *emm*4 isolates among iGAS cases. Based on routine surveillance of invasive cases alone, we cannot distinguish between increased transmission of $M4_{NL22}$, increased invasiveness of $M4_{NL22}$, or both as potential causes for the increase in iGAS cases. A new bacterial carriage study running from September 2022 until February 2023 as well as continued surveillance of invasive disease isolates will help to unravel this further.

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Table 1. *S. pyogenes* isolate characteristics from 2009-2019 (pre-COVID) and 2022 periods.

	2009-2019 (pre-COVID)		**2022**
	Carriage	**Invasive**	**Invasive**
Number of isolates	29	11	26
Median year of isolation (IQR)	2012 (2009-2016)	2013 (2013-2015)	2022
Cultured from adult*, no. (%)	6 (21%)	8 (72%)	17 (65%)
Site of isolation			
Blood, no. (%)	0 (0%)	11 (100%)	15 (58%)
Nose, no. (%)	23 (79%)	0 (0%)	0 (0%)
Throat, no. (%)	6 (21%)	0 (0%)	0 (0%)
Female genital tract, no. (%)	0 (0%)	0 (0%)	5 (19%)
Other[#], no. (%)	0 (0%)	0 (0%)	6 (23%)
$M4_{NL22}$ lineage, no. (%)	1 (3%)	1 (3%)	22 (85%)
Hypervirulent clone, no. (%)	3 (10%)	1 (9%)	2 (8%)

*For one group we only have age categories available instead of exact age

[#]Other sources include wound, abscess, general tissue and a dialysis machine

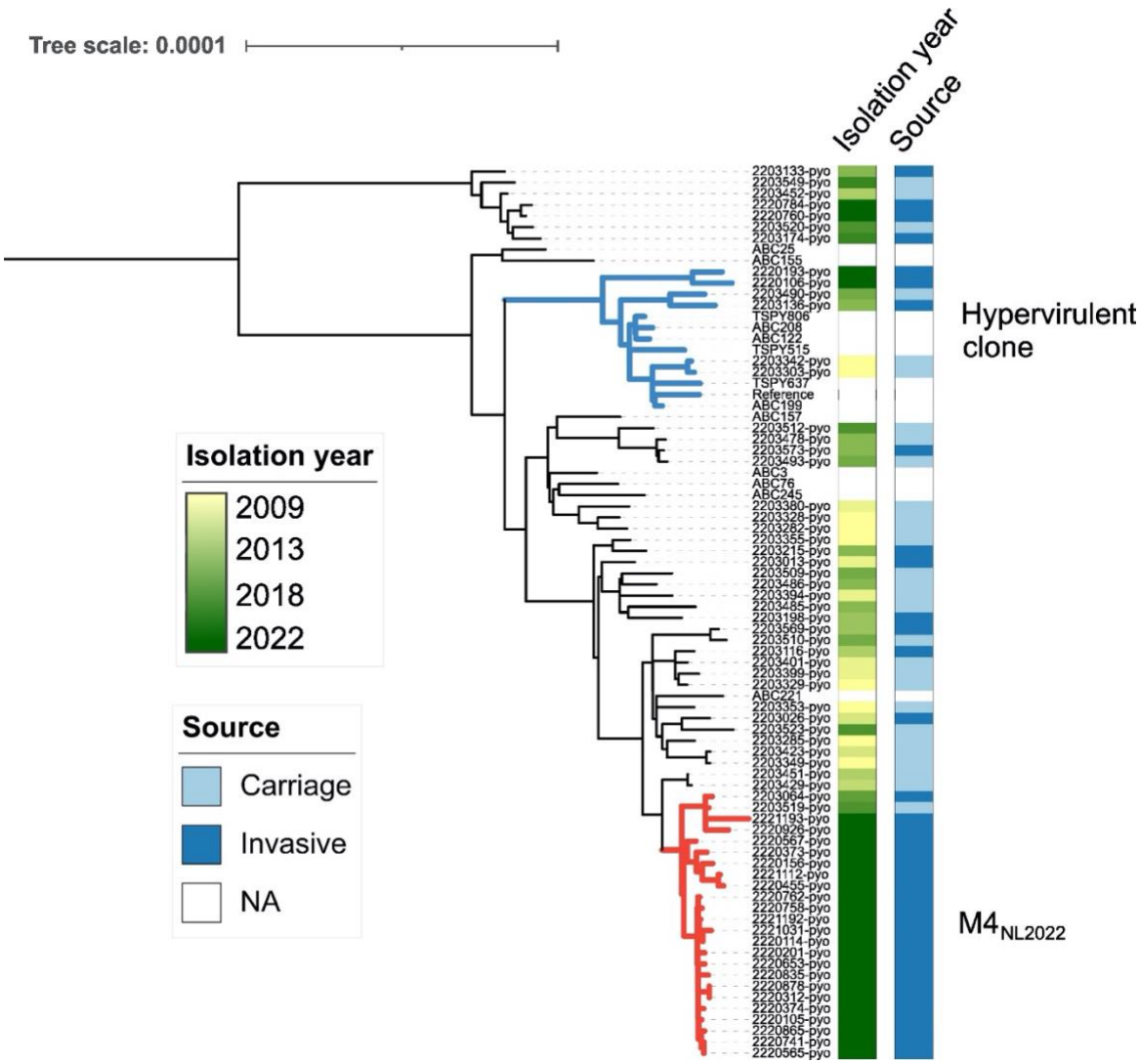

Figure 1. Phylogeny of 66 Dutch *emm*4 isolates and 14 reference genomes. Reference genomes originate from Debroy *et al*. (2021) (*2*) and are shown without metadata. The hypervirulent clone lineage is colored blue and the $M4_{NL22}$ lineage is colored red.