NATIONAL REFERENCE CENTRE FOR INVASIVE B-HEMOLYTIC STREPTOCOCCI NON GROUP B

Report 2012-2024

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1. INTRODUCTION

This report describes the activities performed by the National Reference Centre (NRC) for invasive β-hemolytic streptococci non group B from 2012 until 2024, including species identification via MALDI-TOF MS, detection of virulence genes and/or macrolide/tetracycline/quinolone resistance by Whole Genome Sequencing (WGS) and *emm* typing using Sanger sequencing or WGS. This report aims to follow up on the epidemiological trends of invasive β-hemolytic streptococci over the past decade, highlighting changes in strain distribution, resistance patterns, and the emergence of new virulence factors.

Streptococcus pyogenes (group A *Streptococcus*, GAS) and *Streptococcus dysgalactiae* (groups C and G streptococci, GCS and GGS respectively) are capable of causing a wide range of infections. These range from superficial infections such as pharyngitis and impetigo, to severe invasive infections like necrotizing fasciitis, bacteremia, and streptococcal toxic shock syndrome. Invasive infections are associated with significant morbidity and mortality, particularly in vulnerable populations.The virulence of these organisms is largely attributed to a variety of factors, including the M proteins (encoded by the *emm* or *stG* genes), which help the bacteria evade the immune system. Given the significant burden of invasive streptococcal infections, vaccine development has been a subject of interest, particularly targeting the M protein due to its role in pathogenesis and surface accessibility. Although no vaccine is currently available, ongoing research explores the potential for vaccine strategies to prevent infections by *S. pyogenes* and *S. dysgalactiae*.

2. STRAIN IDENTIFICATION AND DEMOGRAPHICS

The strains received by the NRC for invasive β-hemolytic streptococci non group B are mostly sent to be typed for epidemiological reasons. At the NRC, identification is performed on all isolates and *emm* typing on all invasive *S. pyogenes* and *S. dysgalactiae* strains. The total number of strains received per year is shown in Figure 1, with a differentiation between *S. pyogenes*, *S. dysgalactiae* and other streptococcal species (*S. canis* and *S. equi*).

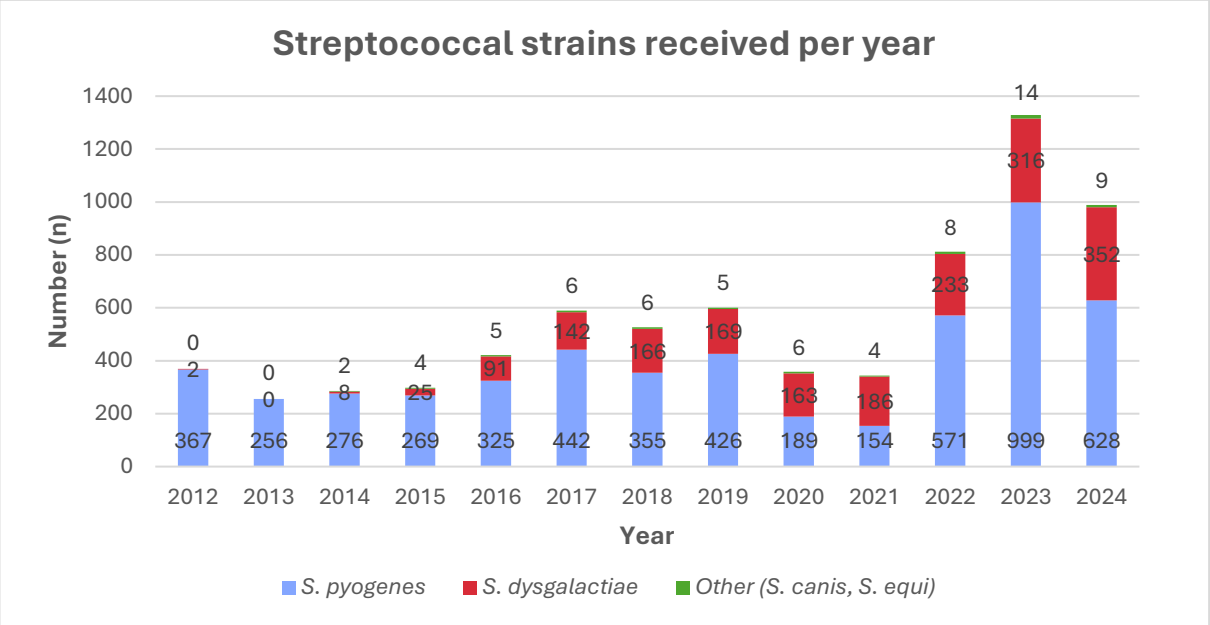


Figure 1: Number of invasive β-hemolytic streptococci non group B received per year. Colours differentiate between *Streptococcus pyogenes* (blue), *Streptococcus dysgalactiae* (red) and other species (*Streptococcus canis* and *Streptococcus equi*) (green).

Since 2012, the number of received strains increased every year with a notable drop of invasive *S. pyogenes* strains in 2020 and 2021 due to the COVID-19 pandemic, which is in line with observations of decreased incidence of other invasive bacterial infections due to containment measures [1]. A steep incline is observed from 2022 onwards. This could be explained by an increased incidence in iGAS (invasive GAS) infections which is in line with reported increased incidence in Europe [2], [3] and the USA [4].

To follow up on seasonal changes, the number of monthly received *S. pyogenes* strains during 2020-2024 is shown in Figure 2, with the pre-COVID mean (and standard deviation) of received strains in 2017 – 2019 as reference. The large increase of invasive *S. pyogenes* strains in 2022 (Figure 1) is observed from November 2022 through the summer of 2023 (Figure 2). Furthermore, on the 12th of December in 2022, the WHO sent out a press release to raise awareness of the increase of iGAS infections [5]. In total, the number of invasive ß-hemolytic strains received by the NRC in 2023 was 1334, which is 65% higher compared to 2022 (n=812). Since 2012, the highest number of invasive strains were received in 2023. Specifically, this increase seems to be accompanied by an increase in the proportion of the toxigenic M1UK lineage [6], further elaborated on in Chapter 3: Strain typing. After seasonal declines in iGAS infections incidence during the summer of 2023, simultaneous, rapid expansion is observed from December 2023 to May 2024. This increase is due to the emergence of the previously rare *emm* type 3.93. In the Netherlands and England this increase has also been reported [3].

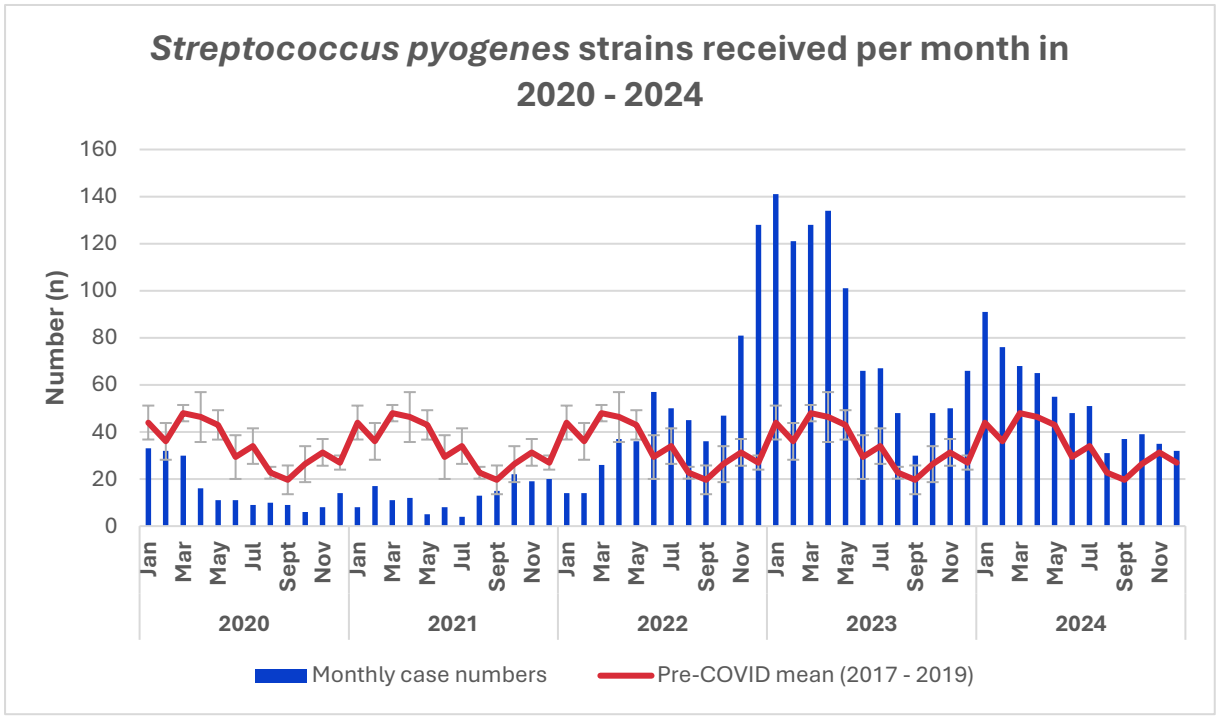


Figure 2: *Streptococcus pyogenes* strains received per month in 2020 – 2024. The pre-COVID mean (and standard deviation) of received strains in 2017 – 2019 is shown in red to indicate the seasonal variaton over the year.

From 2016 onwards, the number of invasive *S. dysgalactiae* sent to the NRC increased as the NRC activity was expanded from solely group A streptococci to all β-hemolytic streptococci non group B. The highest absolute number of *S. dysgalactiae* strains was observed in 2024 (n=352). Increased rates of invasive infections with *S. dysgalactiae* have been reported in several countries [7]. During COVID-19 pandemic years 2020 and 2021 its proportion was the highest (45% and 54% respectively), suggesting that its incidence was less affected by the containment measures (Figure 1). The number of monthly received *S. dysgalactiae* strains during 2020-2024 is shown in Figure 3, with the pre-COVID mean (and standard deviation) of received strains in 2017 – 2019 as reference. Figure 3 shows also that during COVID-19 pandemic years the numbers of invasive infections with *S. dysgalactiae* remained the same as before COVID-19 pandemic years, while the absolute numbers of invasive infections with *S. dysgalactiae* increased from May 2022 onwards.

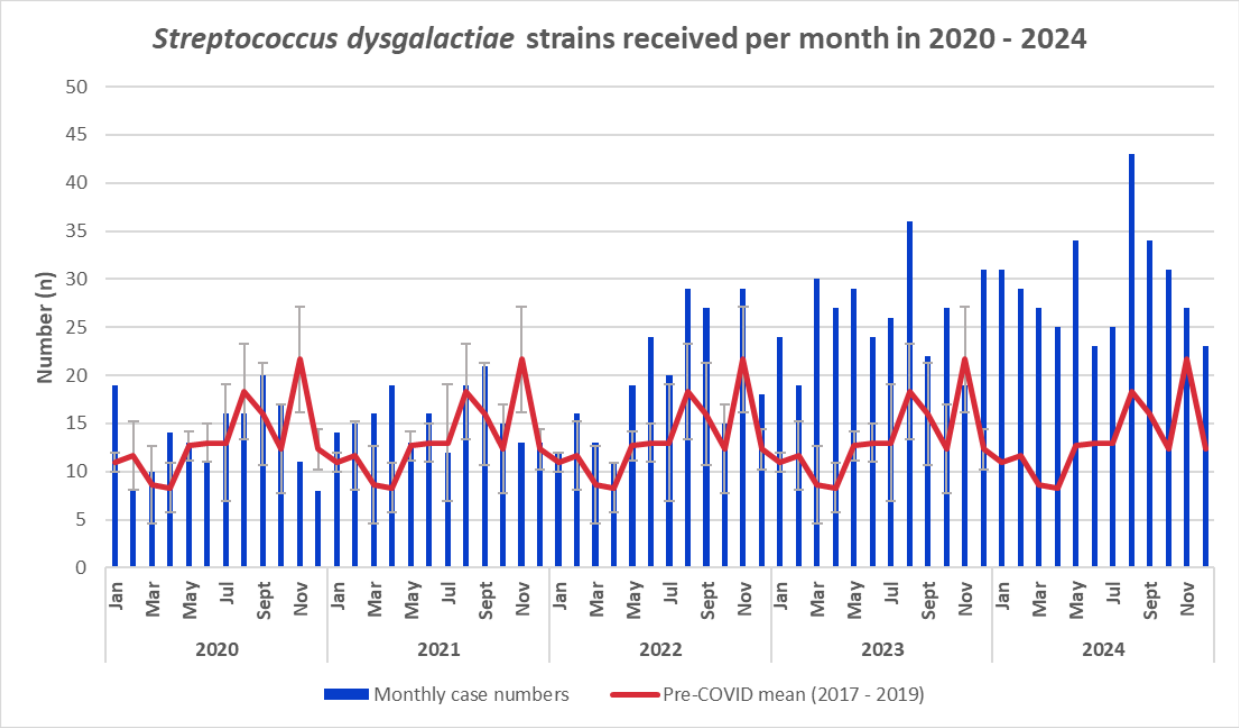


Figure 3: *Streptococcus dysgalactiae* strains received per month in 2020 – 2024. The pre-COVID mean (and standard deviation) of received strains in 2017 – 2019 is shown in red to indicate the seasonal variaton over the year.

The streptococcal invasive strains (*S. pyogenes*, *S. dysgalactiae* and other) received in 2024 by age group are shown in Figure 4. iGAS strains were mainly observed in children up to 5 years of age (n = 76, 12%) and in aldults aged 60+ (n = 264, 42%). The incidence was highest in elderly patients (80+ years old) (n = 80, 13%). The proportion of *S. dysgalactiae* invasive isolates relative to *S. pyogenes* increased with age. Notably, in individuals over 70 years of age, the proportion of *S. dysgalactiae* even exceeded that of *S. pyogenes*, with a clearly ascending trend across the three oldest age categories. For instance, the proportions of *S. pyogenes* and *S. dysgalactiae* in the 76–80 age group are nearly equal, whereas in the 6–10 age group, only *S. pyogenes* was observed. The age distribution is comparable to the previous years.

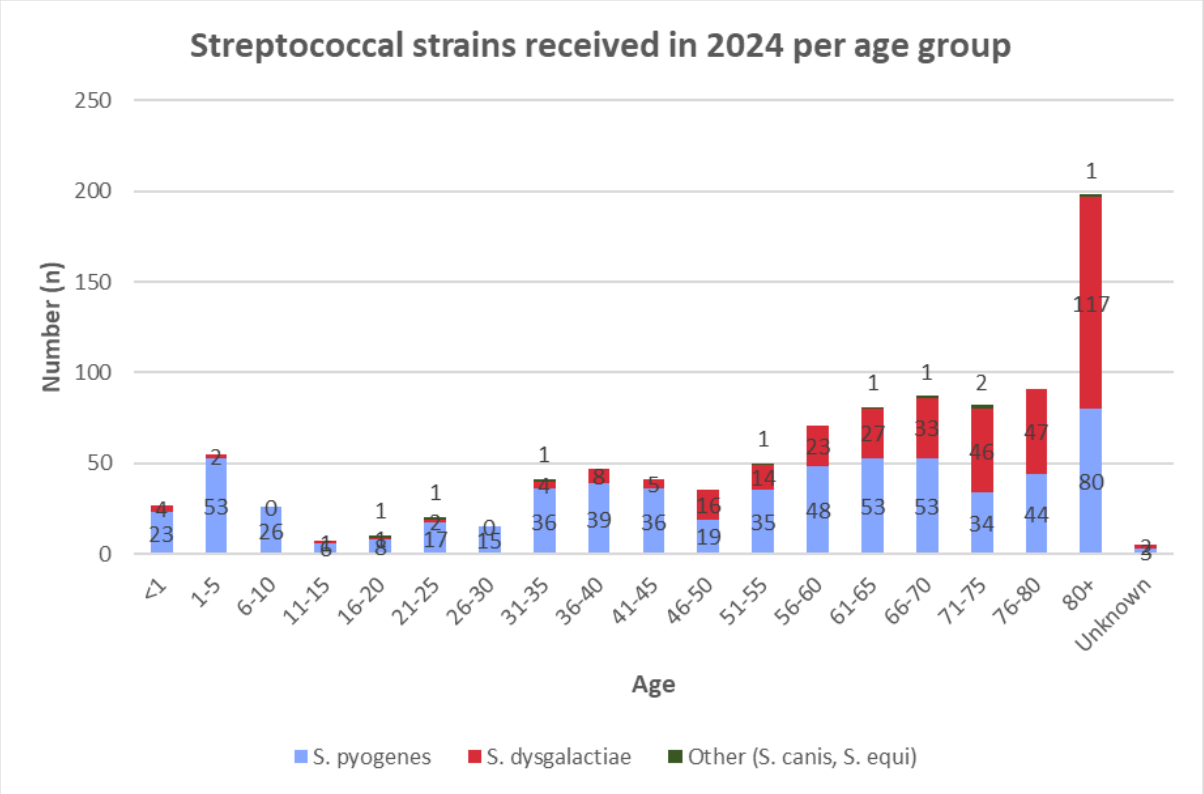


Figure 4: Streptococcal strains received in 2024 per age group. Colours differentiate *Streptococcus pyogenes* (blue), *Streptococcus dysgalactiae* (red) and other species (*Streptococcus canis* and *Streptococcus equi*) (green).

Figure 5 shows the geographical distribution of strains received in 2024 based on the patient's postal code. The strains originate from all over Belgium with the highest prevalence in urban areas (Antwerp, Brussels, Charleroi), which correlates with the population density in these regions.

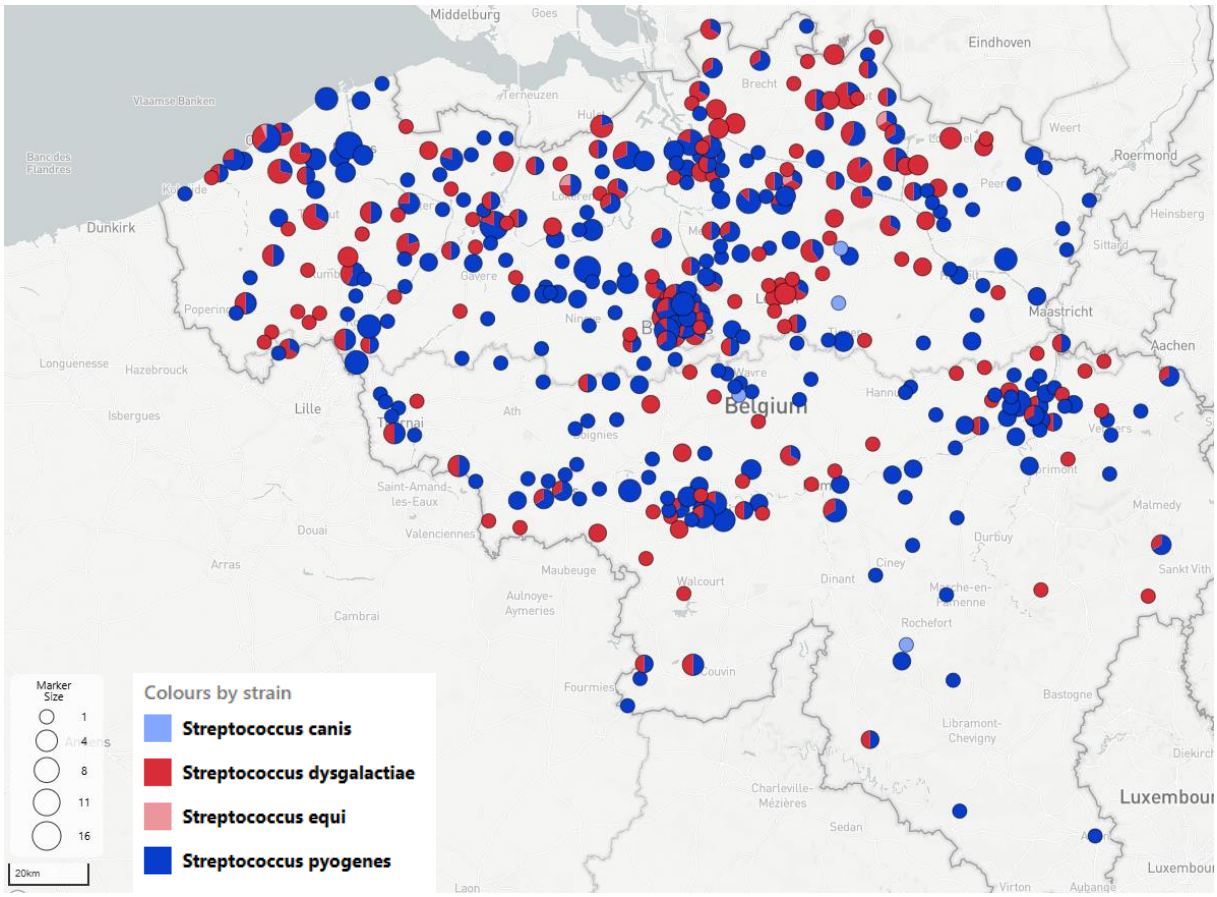


Figure 5: Geographical distribution of strains received in 2024 based on patient's postal code. Colours differentiate *Streptococcus pyogenes* (blue), *Streptococcus dysgalactiae* (red) and other species (*Streptococcus canis* (light blue) and *Streptococcus equi* (light red)). Each dot represents a number of strains as defined by the size of the circle.

The sample types from which the *S. pyogenes* and *S. dysgalactiae* strains orginated are shown in Figures 6. Strains were isolated from both sterile and non-sterile body sites. The primary source of invasive *S. pyogenes* and *S. dysgalactiae* isolates was blood, with approximately 70% of *S. pyogenes* isolates and over 80% of *S. dysgalactiae* isolates being derived from this sample type. The second most common source is tissue (wounds and biopsies), accounting for about 10% of *S. pyogenes* isolates and 5% of *S. dysgalactiae* isolates. The category "fluid" consists of pleural, synovial, cerebrospinal and ascites fluid. *S. dysgalactiae* isolates were not isolated from respiratory samples. 'Other' consist mainly of urine and vaginal samples. Despite the fact that the activity of the NRC primarily focuses on investigating invasive strains, strains originating from throats swabs were also received in low numbers, often associated with a non-invasive clinical presentation.

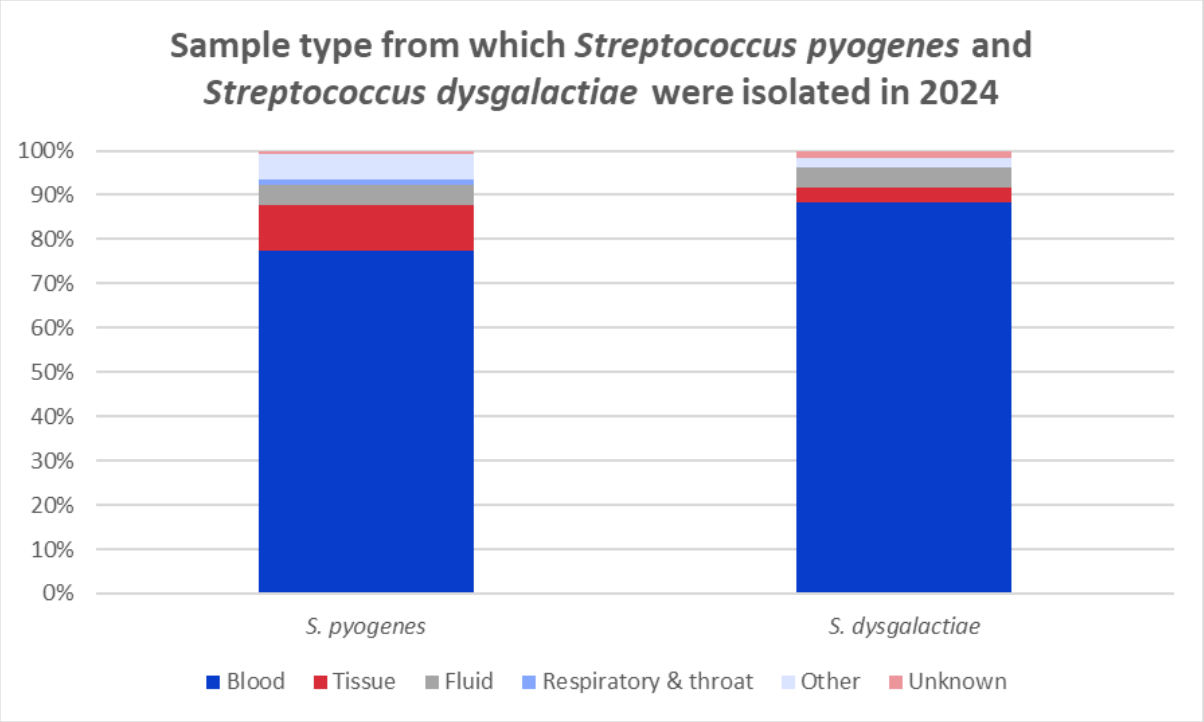


Figure 6: Sample type from which invasive *S. pyogenes* and *S. dysgalactiae* were isolated. The category "fluid" consists of pleural, synovial, cerebrospinal and ascites fluid. *S. dysgalactiae* isolates were not isolated from respiratory samples. 'Other' consist mainly of urine and vaginal samples.

The most common clinical presentation of infection with invasive β-hemolytic streptococci non group B was septicemia, followed by numerous other infection types or syndromes (Figure 7). Some examples of 'other' clinical presentations are abscesses, mastitis and adenitis. Sometimes multiple clinical presentations were provided for a single strain, most common was a combination of septicemia with any other clinical presentation. In such case, the most severe/invasive syndrome was counted. Although there are similarities in clinical presentation between *S. pyogenes* and *S. dysgalactiae* infections, some were more often linked to iGAS infection, such as fasciitis, puerperal sepsis and septic toxic shock syndrome. Pneumonia was also more common for *S. pyogenes* infections. Furthermore, an increase in the number of GAS-associated pneumonia cases was observed in 2024 which is in line with observations in the Netherlands and England with the *emm*3.93 type [3]. *S. dysgalactiae* is increasingly recognized as a cause of various human infections. Its manifestations range from non-invasive superficial skin and soft tissue infections to life-threatening conditions such as streptococcal toxic shock syndrome and necrotizing fasciitis. The crude incidence of invasive *S. dysgalactiae* disease is approaching that of the closely related pathogen *S. pyogenes* [8].

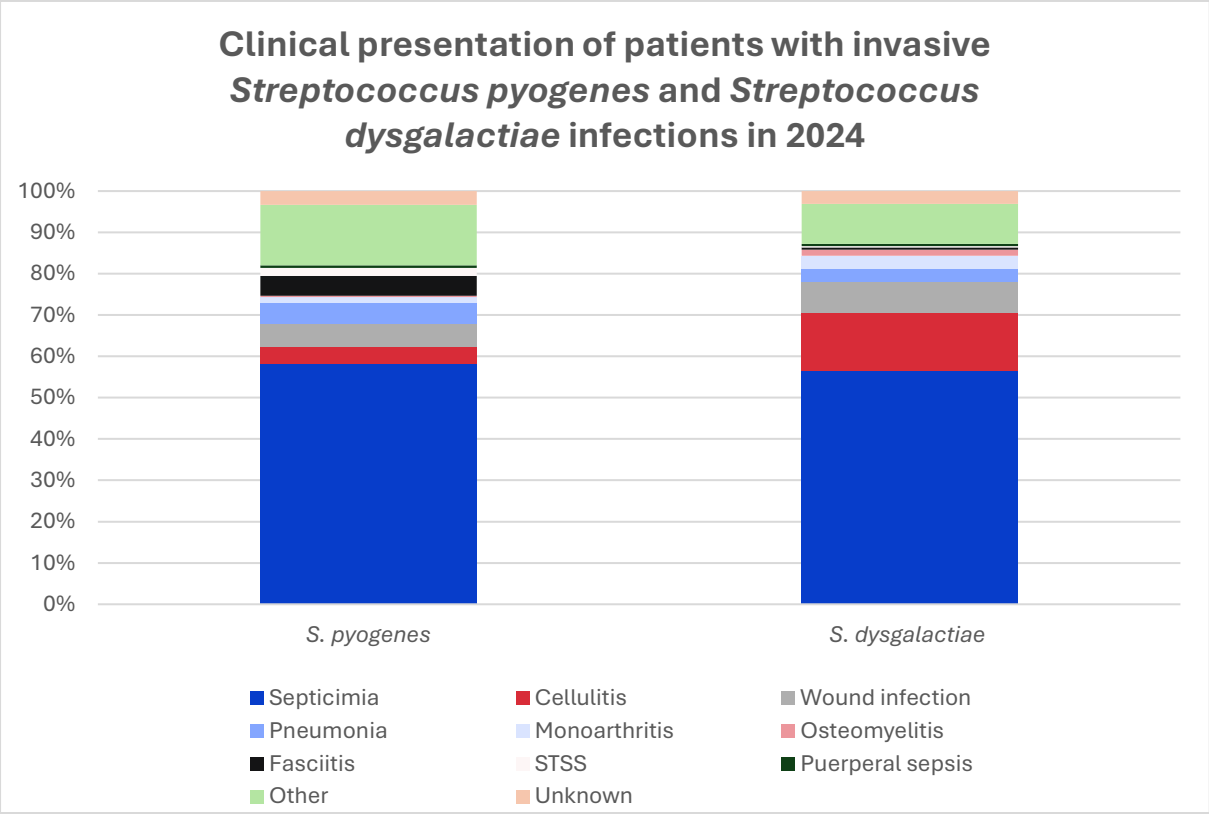


Figure 7: Clinical presentation of invasive *S. pyogenes* and *S. dysgalactiae* infections in 2024. The category 'other' clinical presentations consist mainly of abscesses, mastitis and adenitis.

3. STRAIN TYPING

One of the key methods used to classify and differentiate strains of *S. pyogenes* and *S. dysgalactiae* strains is by typing genes that encode M-like proteins, which are major virulence factors in both species.The *emm* and *stG* genes, present in *S. pyogenes* and *S. dysgalactiae* respectively, encode these M-like surface proteins that play a crucial role in helping the bacteria evade the host immune system by inhibiting phagocytosis. Both *emm* and *stG* genes display significant variability among strains, making them useful targets for strain typing.These M-like proteins not only contribute to the virulence of the bacteria, but are also considered potential targets for vaccine development due to their surface exposure and immunogenic properties. Targeting these proteins could provide a pathway for vaccines aimed at preventing infections caused by *S. pyogenes* and *S. dysgalactiae*. In the following sections, we will discuss the epidemiological surveillance of the different *emm* types for *S. pyogenes* and *stG* types for *S. dysgalactiae*.

3.1. *Emm* typing of *S. pyogenes*

Emm typing is performed on all *S. pyogenes* strains with Sanger Sequencing or WGS. Figure 8 shows the yearly distribution of the most prevalent *S. pyogenes emm* types from 2012 until 2024. *Emm* types can also be classified in *emm* clusters containing closely related *emm* proteins according Sanderson-Smith et al. [9] (see Figure 9). Classifying closely related *emm* types can facilitate the epidemiological surveillance with regard to vaccine development. There is an enormous diversity of *emm* types in the Belgian strains. *Emm*1 (cluster A-C3) and *emm*3 (cluster A-C5) were the most prevalent *emm* types in the early years of surveillance with a combined proportion of 28% (2012) to up to 42% (2013) but their prevalence decreased from 2018 onwards to 9% in 2021. High prevalence of *emm*1 and *emm*3 was also detected in Spain between 2007 -2019 [10]. The seven most prevalent *emm* types identified between 2000 - 2017 in Europe and North America are *emm*1 (cluster A-C3), *emm*28 (cluster E4), *emm*89 (cluster E4), *emm*3 (cluster A-C5), *emm*12 (cluster A-C4), *emm*4 (cluster E1), and *emm*6 (Single protein emm-cluster clade Y) [11]. These *emm* types are also frequently isolated in Belgium. Additionally, *emm* types *emm*11 (cluster E6), *emm*22 (cluster E4), *emm*75 (cluster E6) and *emm*87 (cluster E3), were also regularly detected by the NRC in Belgium. Furthermore, the prevalence of *emm*11, *emm*22, and *emm*87 increased in relative numbers in 2020 and 2021. However, in 2022 the proportion of *emm*11, *emm*22 and *emm*87 has decreased again, while the proportion of *emm*1 exceeded 30% and *emm*12 has become more common (16%). In 2023, the *emm*1 proportion increased further to 39% of all the received *S. pyogenes* isolates. Interestingly, a new subtype of *emm*1 was described in UK in 2019. This subtype is named the M1UK clone and is characterized by 27 SNPs in the *S. pyogenes* genome [12]. This clone shows an increased *spe*A production explaining its more toxigenic behavior translated in an unusually high number of admissions for paediatric pleural empyema. This specific clone also emerged in Belgium. The first Belgian M1UK variants were detected in January 2020 with WGS data. The observed increase in *emm*1 in 2022 was accompanied by a shift towards a higher proportion of M1UK variants (about 2/3 of the *emm*1 strains in Belgium were M1UK variants). After seasonal declines, described by figure 2, rapid expansion of a previously rare *emm* type 3.93 was seen in 2024. The contemporary *emm*3.93 strain disproportionately affected individuals under 18 and was associated with increased risk of sepsis, pneumosepsis, pneumonia and meningitis in Belgium. Genomic analysis of both contemporary and historical *emm*3.93 strains in Belgium, the Netherlands and England revealed the presence of distinct clades, which may be associated with potentially improved DNA configurations, suggesting evolutionary adaptations with possible implications for virulence [3]. Grouped cases, clusters or outbreaks were not reported to the NRC in Belgium. Geographic differences of clustering patterns of different *emm* types were not observed in Belgium.

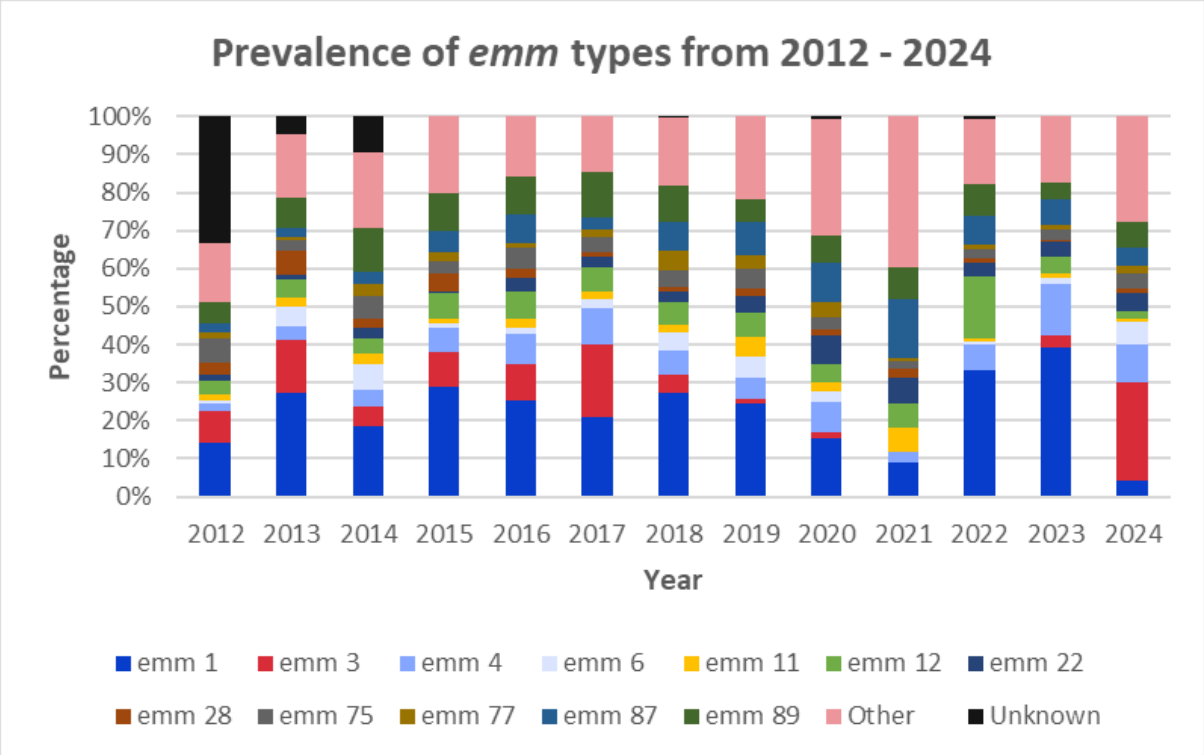


Figure 8: Prevalence of most common *emm* types of invasive *S. pyogenes* isolates.

The yearly distribution of the most prevalent *S. pyogenes emm* clusters was stable between 2012 and 2019 (Figure 9). During the COVID-19 pandemic, a shift in the distribution *of emm* clusters was observed. In 2021, cluster E3 saw an increase (n=46, 30%), predominantly driven by *emm87*, while in 2022 and 2023, a notable rise in A-C3 was observed, primarily driven by an increase in *emm1*. Additionally, in 2024, a significant rise in A-C5 (n=161, 26%) was noted, with a marked prevalence of *emm3.93*, as previously discussed.

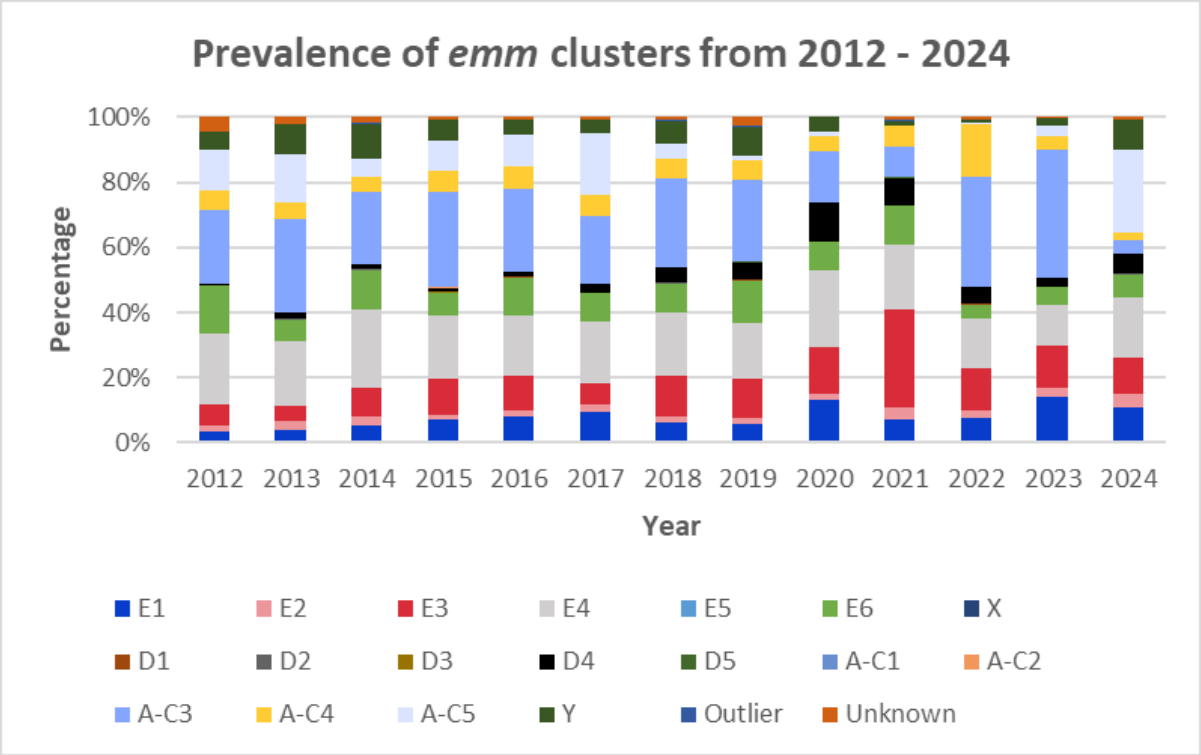


Figure 9: Prevalence of *emm* clusters of invasive *S. pyogenes* isolates.

In Figure 10, the percentages of the age groups 0-5 years and 80+ are presented for the most common *emm* types from 2019 to 2024. An increase in *emm*3 can be observed in both age groups in 2024. Sililarly, in 2022 and 2023, the same trend was observed for *emm*1. *Emm*12 was a prevalent *emm* type in 2022, while *emm*87 dominated among the 0-5 age group in 2021. *Emm*89 is more frequently isolated in the 80+ age group.

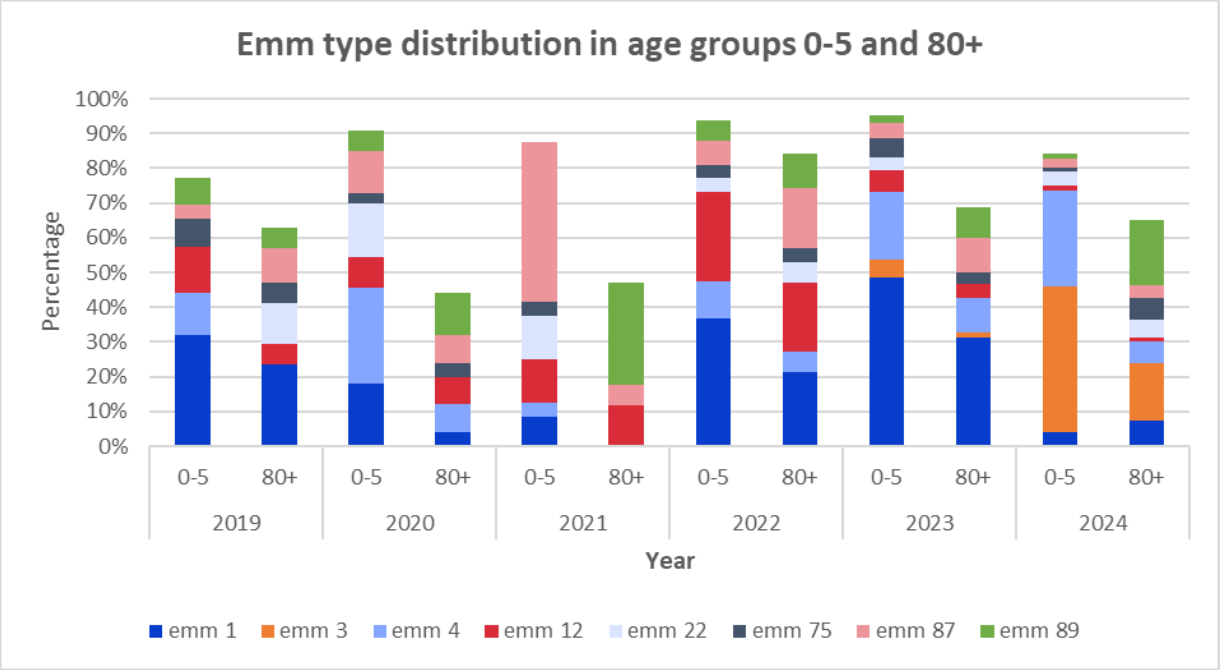


Figure 10: *Emm* type distribution in the age groups 0-5 years and 80+ from 2019 – 2024 for the most common *emm* types.

3.2. STG typing of *S. dysgalactiae*

STG typing is performed on all *S. dysgalactiae* strains with Sanger Sequencing or WGS. Figure 11 presents the 11 most prevalent STG types of *S. dysgalactiae* from 2016 to 2024. In absolute numbers, the STG62647 type showed the greatest increase (n=32 in 2016 and n=113 in 2024), with STG485 being the second most common type. In relative numbers, variation in the distribution of STG types was limited over the years. *S. dysgalactiae* is an increasingly recognized cause of human disease. Other countries have also observed an increase in *S. dysgalactiae* infections [8]. In Western Norway, invasive infections by the *S. dysgalactiae* STG62647 type have been increasing since 2013 [13]. These infections are often associated with streptococcal toxic shock syndrome, necrotizing infections and endocarditis. However, in our laboratory based surveillance data, no association of *S. dysgalactiae* infection with these clinical syndromes could be observed (Figure 7).

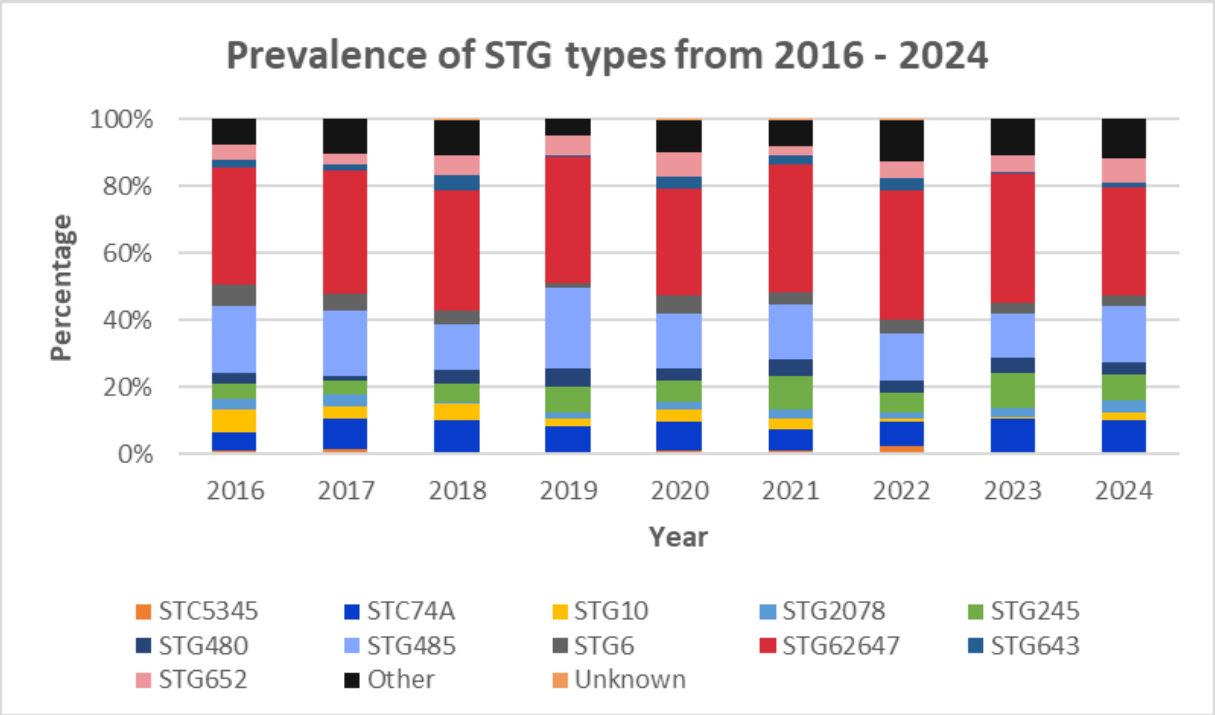
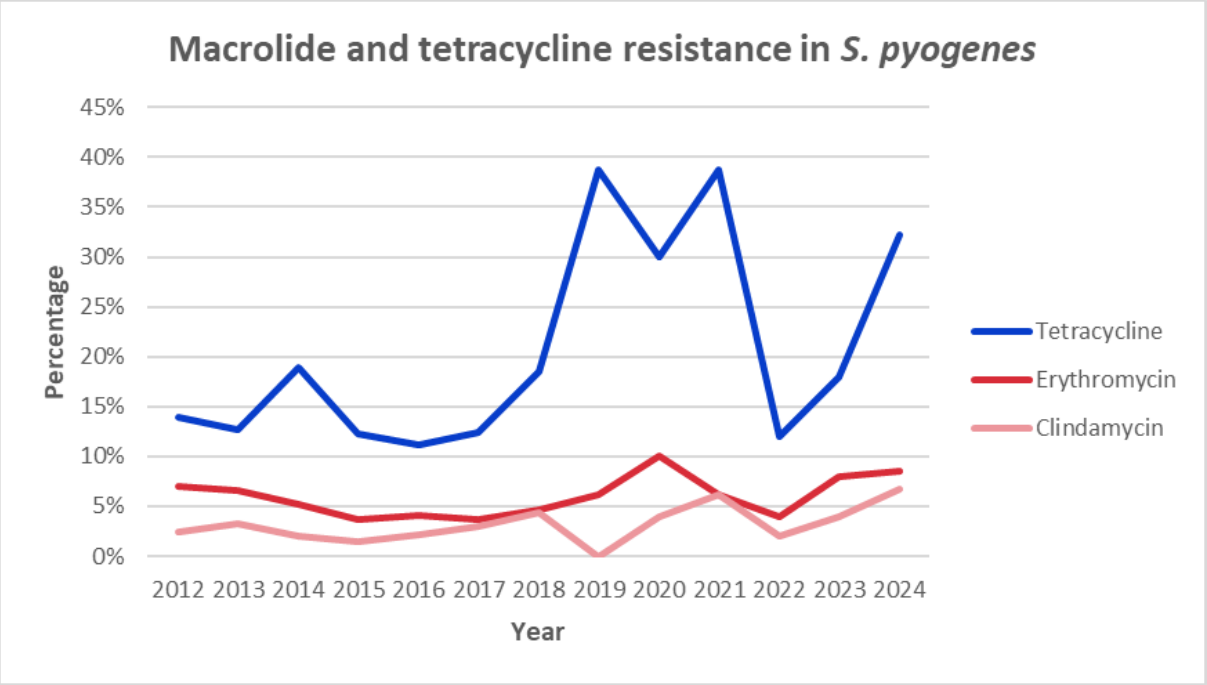


Figure 11: Prevalence of the 11 most common *S. dysgalactiae* STG types.

4. ANTIBIOTIC SUSCEPTIBILITY

4.1. Macrolide and tetracycline resistance

From 2012 until 2018, the *in vitro* susceptibility to penicillin, tetracycline, erythromycin and clindamycin was determined by disk agar diffusion for all submitted *S. pyogenes* strains. From 2019 onwards, a yearly random selection of 50 strains (with wide geographical distribution) was made for susceptibility testing. No resistance to penicillin has been observed in *S. pyogenes* during the entire surveillance period. The percentage of resistant strains of the other tested antibiotic is shown in Figure 12. The resistance of *S. pyogenes* to tetracyclines has increased up to 38% in 2021. In 2022, the tetracycline resistance decreased drastically to 12%. However, in 2024, resistance levels began to rise again, reaching 32%. It is uncertain if this drop in tetracycline resistance can be extrapolated to all strains as only a tenth of the total number of strains was phenotypically tested but it is in line with genetic data (see figure 13). The phenotypic sensitivity to tetracycline in 2024 is 32% and also in line with the genetic data of 28% resistance of 65 strains. Resistance to erythromycin and clindamycin remained rather stable



over the years, with 8% resistance for erythromycin in 2024 and 7% for clindamycin.

Figure 12: Percentage of macrolide and tetracycline resistance in *S. pyogenes* by phenotypical susceptibility testing.

Upon request by the sending laboratories, multiplex PCR or WGS for the detection of tetracycline (*tet(K), tet(L), tet(M), tet(O)*) (Figure 13) and macrolide resistance genes (*erm(A), erm(B), mef*) (Figure 14) was performed. Since the number of tested strains per year varies significantly, identifying trends over time is difficult. However, it is clear that *tet*(M) is the most prevalent gene conferring tetracycline resistance (protection of the 30S ribosomal subunit against tetracycline binding) while *erm*(A) (ribosomal methylation of adenine base on the 23s rRNA of the 50S subunit) used to be the most prevalent macrolide conferring resistance gene. In recent years, a decrease in the detection of macrolide resistance genes have been observed, which may be attributable to a more unbiased selection of strains for typing, rather than a targeted selection of phenotypically resistant isolates.

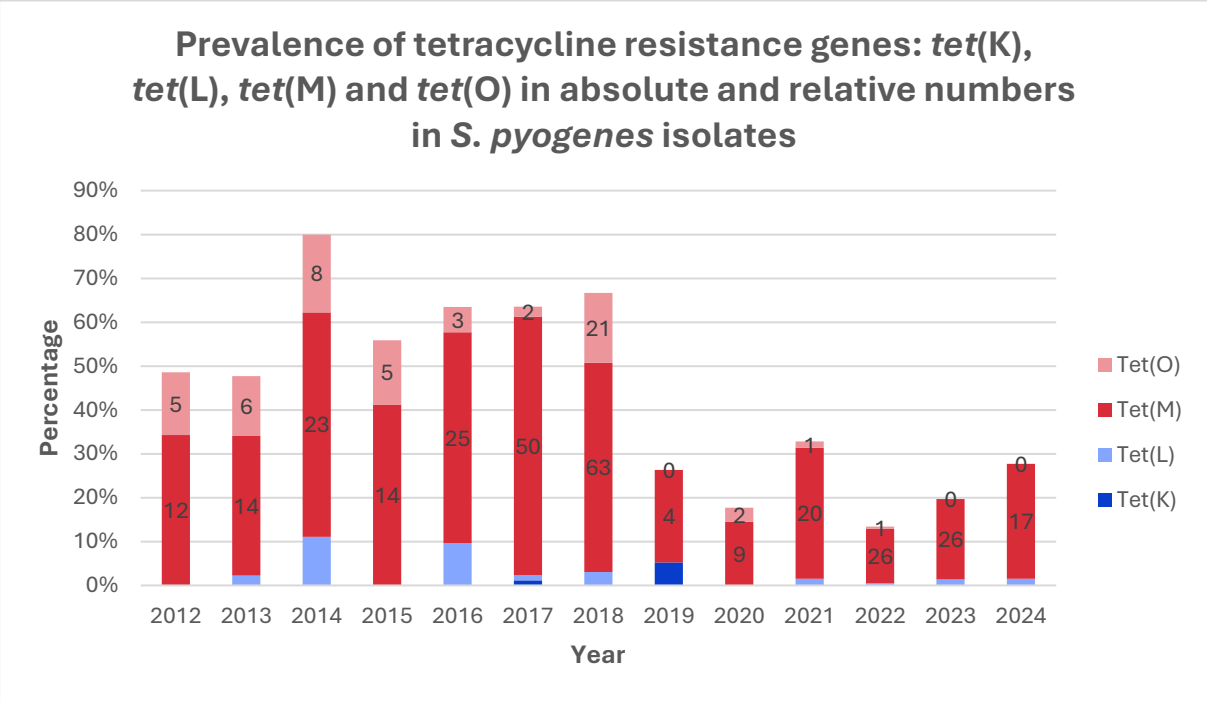


Figure 13: Prevalence of tetracycline resistance genes: *tet*(K), *tet*(L), *tet*(M) and *tet*(O) in absolute and relative numbers in *S. pyogenes* isolates.

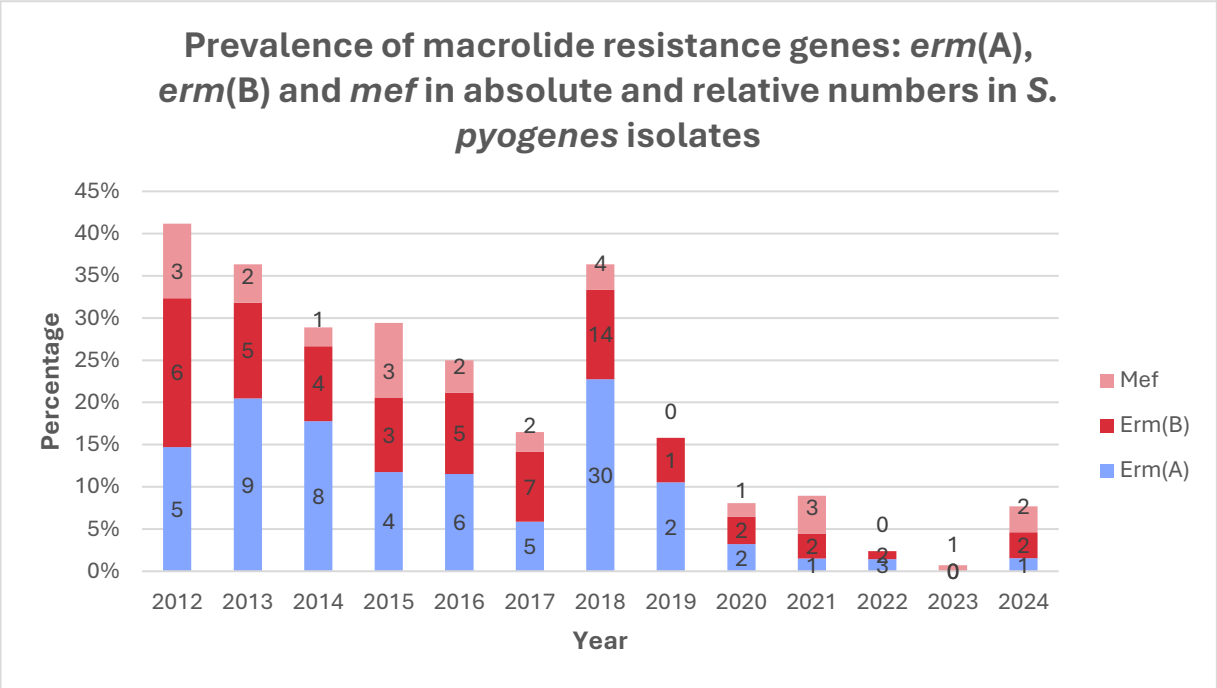


Figure 14: Prevalence of macrolide resistance genes: *erm*(A), *erm*(B) and *mef* in absolute and relative numbers in *S. pyogenes* isolates.

The presence of certain resistance genes seems to correlate with specific *emm* types (Table 1). Certain *emm* types (*emm*1, *emm*28, *emm*3 *emm*75, *emm*82, *emm*87 and *emm*89) lack resistance genes, in others (*emm*11, *emm*22, *emm*4, *emm*43 and *emm*77) resistance genes are rarely encountered and certain types (*emm*108, *emm*33, *emm*5 and *emm*83) always carry the *tet(M)* gene. Michos et al. reported that the most common *emm* types among macrolide-resistant isolates in Greece between 2007 and 2014 were *emm*77 and *emm*4 [14]. In Belgium, macrolide resistance genes have been identified in these same *emm* types, as well as in *emm*11, *emm*22 and *emm*27. Michos et al. also described macrolide-resistant isolates for these types [14].

Table 1: The prevalence of resistance genes in different *emm* types of *S. pyogenes* isolates in 2012 – 2024. Colours of the cells indicate the prevalence of resistance genes, green cells is 0%, light orange is <10%, orange is >=10% - <30%, dark orange is >=30 - <50%, red >=50%.

Emm type	Strains tested *n*	*erm*(A)	*erm*(B)	*mef*	*tet*(K)	*tet*(L)	*tet*(M)	*tet*(O)
***Emm*1**	185	0%	0%	0%	0%	0%	0%	0%
***Emm*108**	4	0%	0%	0%	0%	0%	100%	0%
***Emm*11**	9	0%	11%	0%	0%	0%	11%	0%
***Emm*22**	36	8%	0%	3%	0%	0%	83%	0%
***Emm*27**	4	0%	50%	0%	0%	0%	100%	0%
***Emm*28**	3	0%	0%	0%	0%	0%	0%	0%
***Emm*3**	49	0%	0%	0%	0%	0%	0%	0%
***Emm*33**	6	0%	0%	0%	0%	0%	100%	0%
***Emm*4**	47	0%	0%	2%	0%	0%	0%	0%
***Emm*43**	10	0%	0%	0%	0%	0%	10%	0%
***Emm*5**	4	0%	0%	0%	0%	0%	100%	0%
***Emm*75**	7	0%	0%	0%	0%	0%	0%	0%
***Emm*77**	7	71%	0%	0%	0%	0%	14%	71%
***Emm*82**	11	0%	0%	0%	0%	0%	0%	0%
***Emm*83**	8	0%	0%	0%	0%	0%	100%	0%
***Emm*87**	44	0%	0%	0%	0%	0%	0%	0%
***Emm*89**	54	0%	0%	0%	0%	0%	0%	0%

We have conducted limited monitoring of the phenotypic susceptibility of *S. dysgalactiae* to tetracycline and macrolide antibiotics. From 2017 to 2024, resistance genes were identified in 12 *S. dysgalactiae* strains using WGS. The *tet(M)* resistance gene was detected in 15% (n=4) of the strains, while macrolide resistance genes were present in 37% (n=10) of the strains, specifically including six *erm(A)*, two *erm(B)*, and two *mef(A)* genes.

4.2 Quinolone resistance

Quinolone resistance in *S. pyogenes* was determined using WGS data but was not phenotypically tested. Fluoroquinolone resistance is mediated mainly by point mutations in the targetenzymes, especially in *gyrA* and *parC* [15]. The prevalence of quinolone resistance has shown significant variation over the years, ranging from 13% in 2023 to 36% in 2022 and 17% in 2024 (Figure 15). This trend mirrors previous observations, where the proportion of fluoroquinolone-non-susceptible *S. pyogenes* has steadily increased over time. For instance, between 2008 and 2010, the resistance rate rose from 4.3% in 2008 to 10.9% in 2009 and further to 21.6% in 2010 in Belgium [16]. This increase in fluoroquinolone resistance was notably associated with a significant rise in the prevalence of *emm*6

strains among the fluoroquinolone-non-susceptible *S. pyogenes* isolates [16]. Similar patterns of fluctuating quinolone resistance highlight the complex relationship between resistance development and the distribution of specific *emm*-types. In 2022, a high prevalence of *emm*12 strains was observed (Figure 8), and 100% of these *emm*12 strains harbored the *parC*_A121V mutation amino acid change in *parC*, a known marker of quinolone resistance (Table 2). In contrast, in 2023, *emm*1 strains predominated (Figure 8), and no quinolone resistance was detected in these *emm*1 strains (Table 2). The most common mutations associated with quinolone resistance in invasive Group A Streptococcus (iGAS) strains were *parC*_S140P (n=79, 13%) and *parC*_A121V (n=63, 10%), sometimes in combination with other mutations (Figure 15).

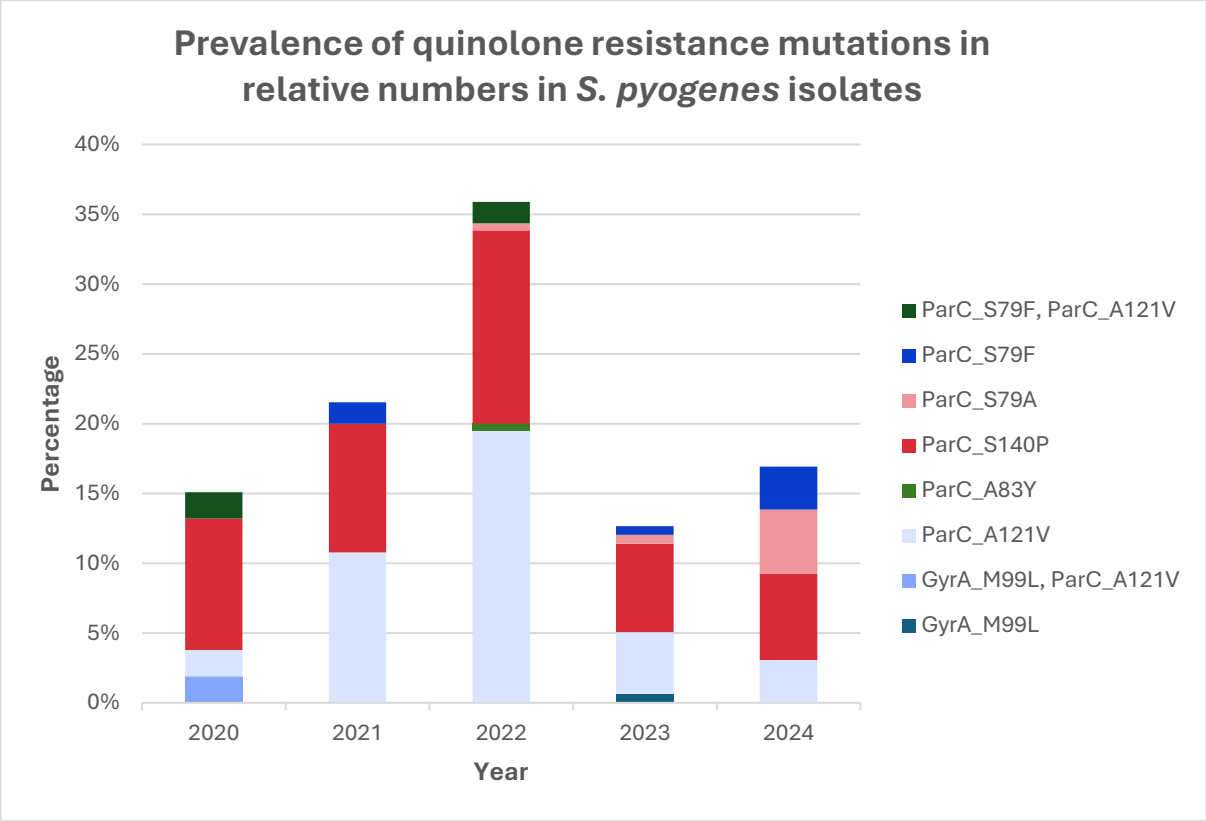


Figure 15: Prevalence of quinolone resistance amino acid changes: *gyrA*_M99L, *parC*_A121V, *parC*_A83Y, *parC*_D83Y, *parC*_S140P and *parC*_S79F in relative numbers in *S. pyogenes* isolates in 2020 - 2024.

Table 2: The prevalence of quinolone resistance mutations in different *emm* types of *S. pyogenes* isolates in 2012 – 2024. Colours of the cells indicate the prevalence of resistance genes, green cells is 0%, light orange is <10%, orange is >=10% - <30%, dark orange is >=30 - <50%, red >=50%.

Emm type	Strains tested *n*	*gyrA*_M99 L	*parC*_S79F	*parC*_A83 Y	*parC*_D83 Y	*parC*_A121 V	*parC*_S140 P
Emm1	185	0%	0%	0%	0%	0%	0%
Emm12	54	2%	7%	0%	0%	100%	0%
Emm22	36	0%	0%	3%	0%	0%	0%
Emm3	49	0%	6%	0%	0%	0%	0%
Emm33	6	0%	0%	0%	0%	0%	100%
Emm4	47	0%	0%	0%	0%	0%	0%
Emm43	10	0%	0%	0%	0%	0%	10%
Emm77	7	0%	0%	0%	0%	0%	71%
Emm82	11	0%	0%	0%	0%	9%	0%
Emm83	8	0%	0%	0%	0%	0%	100%
Emm87	44	0%	0%	0%	0%	0%	0%
Emm89	54	0%	0%	0%	2%	0%	100%

5. VIRULENCE GENES

The virulence genes *speA*, *speC*, and *ssa* (Figure 16) have been part of the routine virulence gene detection via PCR since 2012 for *S. pyogenes* strains only. The presence of *speC* has been stable (~45%) over the years, with an increase during the COVID-19 pandemic to 69%. In contrast, a remarkable decrease in the prevalence of *speA* was observed in 2020-2021. Subsequently, in 2022 - 2024, *speA* prevalence increased to higher levels comparable to pre-COVID years (42-63%). This phenomenon can be explained by the increased prevalence of *emm*1 types, especially the M1UK variant which is associated with *speA* [6]. In 2024, a rise in the prevalence of the *ssa* gene was observed, reaching 69%. This increase is associated with the high prevalence of *emm*3.93, as *emm*3 is linked to the *ssa* gene, as further detailed in Table 3.

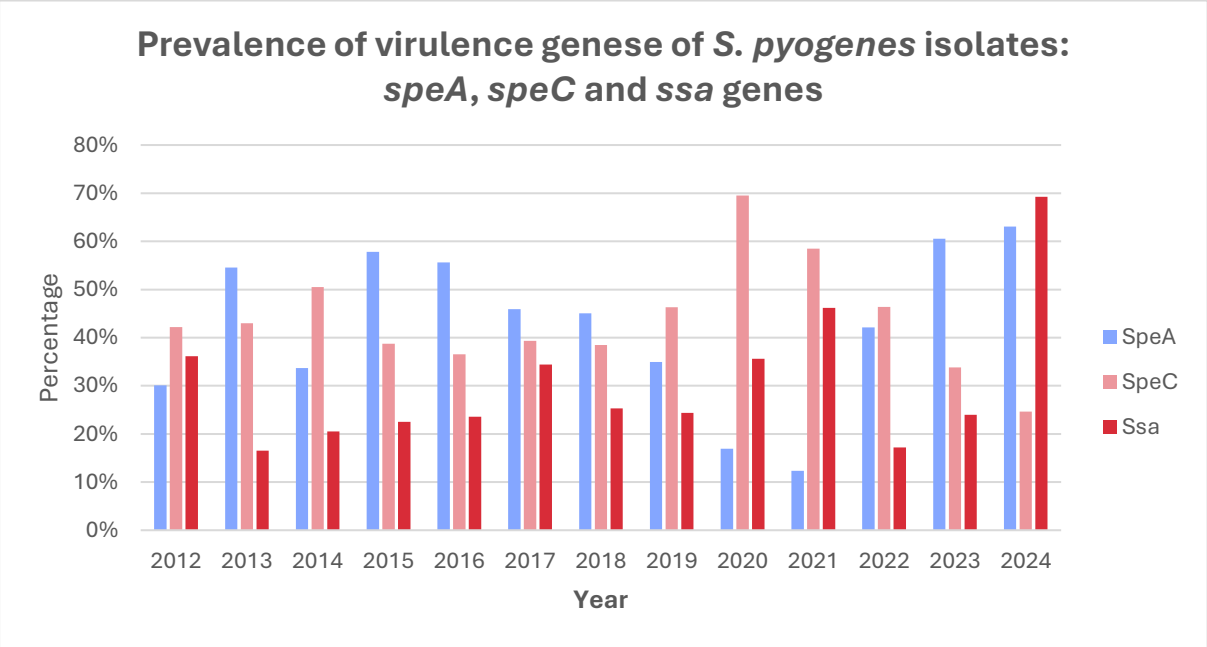


Figure 16: Prevalence of virulence genes of *S. pyogenes* isolates: *speA*, *speC* and *ssa* genes.

Since 2020, an increasing number of *S. pyogenes* strains were sequenced by the NRC. From 2022 onwards, we have shifted to detecting virulence genes exclusively through WGS rather than multiplex PCR to capture a broader spectrum of virulence genes. This approach was applied only to highly invasive strains associated with clinical manifestations such as toxic shock syndrome, puerperal sepsis, necrotizing fasciitis, or meningitis. Virulence genes that were detected are multiple streptococcal pyrogenic exotoxins (*spe*), streptococcal superantigen (*ssa*) and streptococcal mitogenic exotoxin Z (*smeZ*). These virulence genes are recognized for their potential to alter and disturb the normal function of the immune system. Figure 17 shows an increasing prevalence of *speA* in 2022 - 2024, as previously discussed. *speH* and *speI* were more prevalent in 2022 compared to other years. *speH* and *speI* were associated with *emm*12 (Table 3), which was common in 2022 (Figure 8). In 2021, we observed a high prevalence of *speQ* and *speR*, which may be linked to *emm*87 (Table 2), frequently detected in 2021 (Figure 8). *speG* and *smeZ* are common virulence genes, present in nearly all isolates. As of 2017, 31 *S. dysgalactiae* strains have been sequenced of which 16 (52%) contain *speG*. Other superantigens have not been detected in *S. dysgalactiae* strains. These results are consistent with a study by Kaci et al., where only *speG* was detected in 61% of the *S. dysgalactiae* strains from isolates collected in Norway in 2018 [17].

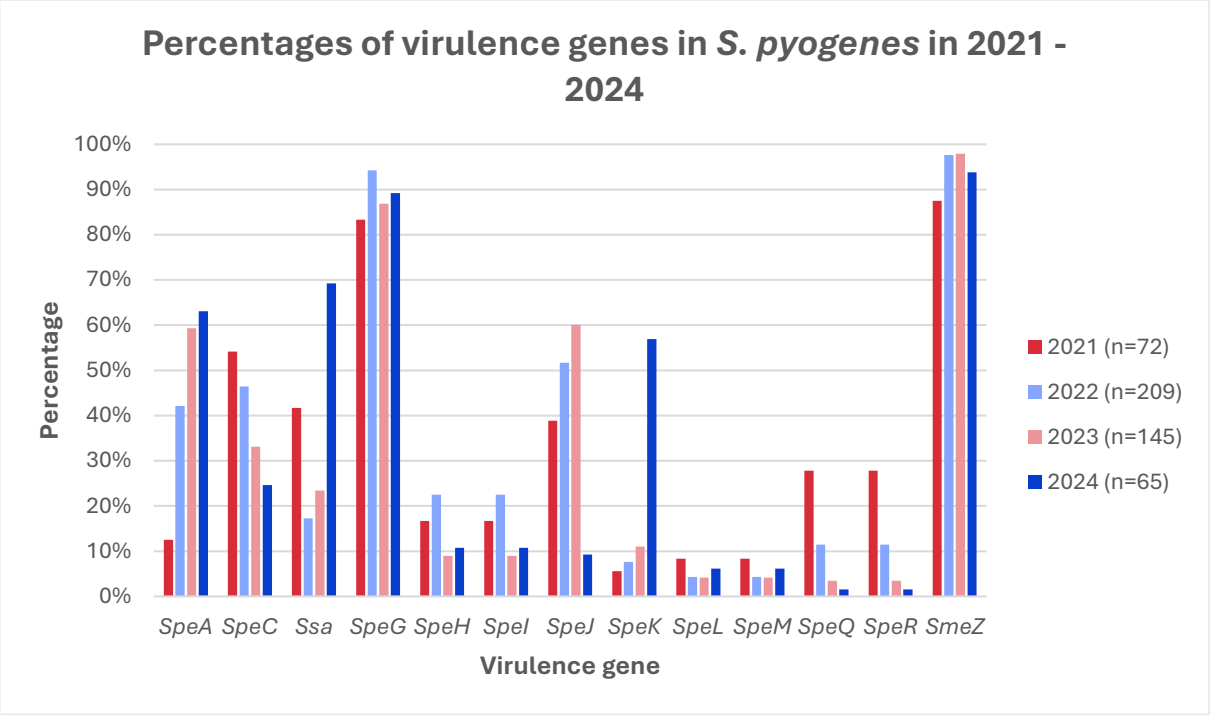


Figure 17: Percentage of virulence genes found in *S. pyogenes* strains in 2021 – 2024.

Table 3 shows the frequency of virulence genes in the six most prevalent *emm* types: *emm*1, *emm*3, *emm*4, *emm*12, *emm*87 and *emm*89. Certain virulence genes can be linked to specific *emm* types. *Emm*1 strains harbor virulence genes *speA*, *speG* and *speJ*. *SpeG* is a common virulence gene that can be found in almost every *emm* type, except for *emm*4 strains [18]. Other virulence factors are only detected in specific *emm* types: such as *ssa* in *emm*3, *emm*4 and *emm*87, *speH* and *speI* in *emm*12 or *speQ* and *speR* in *emm*87.

Table 3: Prevalence of virulence genes in the five most common *emm* types: *emm*1, *emm*4, *emm*12, *emm*87 and *emm*89. Colours of the cells indicate the prevalence of virulence genes, green cells is 0%, light orange is <10%, orange is >=10% - <30%, dark orange is >=30 - <50%, red >=50%.

Emm type	*speA*	*speC*	*ssa*	*speG*	*speH*	*speI*	*speJ*	*speK*	*speL*	*speM*	*speQ*	*speR*
*Emm*1 n=185)	99%	7%	0%	100%	0%	0%	100%	0%	0%	0%	0%	0%
*Emm*3 n=49)	100%	0%	100%	100%	0%	0%	0%	90%	0%	0%	0%	0%
*Emm*4 (n=47)	0%	91%	100%	0%	0%	0%	0%	2%	2%	2%	0%	0%
*Emm*12 (n=54)	2%	89%	4%	100%	100%	100%	0%	0%	0%	0%	0%	0%
*Emm*87 (n=44)	0%	91%	100%	100%	0%	0%	93%	2%	0%	0%	100%	100%
*Emm*89 (n=54)	2%	87%	0%	100%	0%	0%	0%	20%	0%	0%	0%	0%

6. SUMMARY

The results presented in this report are based on the data of the National Reference Centre (NRC) for invasive β-hemolytic streptococci non group B during the period 2012 – 2024.

In 2024, the NRC received a total of 628 *S. pyogenes* strains. A steep incline in the number of received strains was observed compared to the COVID-19 pandemic years 2020-2021 and even pre-pandemic years. The increased incidence in invasive Group A Streptoccal (iGAS) infections was observed in the summer and autumn months (July – December) of 2022 with a very steep increase from November 2022 onwards. Previous years did not show such pronounced seasonal variation. This increase in 2023 is cased by the M1UK clone and in 2024 by the previous rare *emm* type 3.93. In 2024, a rise in the prevalence of the *ssa* gene was observed, reaching 69%. This increase was associated with the high prevalence of *emm*3.93, as *emm*3 is linked to the *ssa* gene. The *emm*3.93 strains disproportinonately affected individuals under 18 and is associated with increased risk of sepsis, pneumosepsis, pneumonia and meningitis in Belgium. The highest incidence of iGAS strains was observed in children up to 5 years of age (n = 76, 12%) and in adults aged 60+ (n=264, 42%). The incidence generally increased with age and was highest in the 80+ elderly (n = 80, 13%). Resistance rates to erythromycin and clindamycin remained stable and ≤10% between 2012 and 2024, while tetracycline resistance rates varied between 10 and 39% over the years. However, these variations need further investigation. *tet(M)* was the most prevalent tetracycline resistance gene, *erm(A)* and *erm(B)* were the most prevalent genes conferring resistance to macrolides. Quinolone resistance in *S. pyogenes* has fluctuated over the years, ranging from 13% in 2023 to 36% in 2022, and is primarily linked to mutations in *parC*. This variation is influenced by changes in the prevalence of specific *emm*-types, with *emm*12 strains in 2022 exhibiting high resistance, while *emm*1 strains in 2023 showed no resistance.

In 2024, the NRC received 352 *S. dysgalactiae* strains. In contrast to *S. pyogenes* isolates, *S. dysgalactiae* strains were almost exclusively isolated from invasive infections in adults with the highest incidence in elderly patients aged 80+. During the COVID-19 pandemic years the number of invasive infections with *S. dysgalactiae* was stable and similar to pre-COVID-19 pandemic years. However, the absolute numbers of invasive infections with *S. dysgalactiae* increased from May 2022 onwards. The most common *S. dysgalactiae* types between 2016 and 2024 were STG6247 and STG485. The main infection sites of invasive β-hemolytic isolates are blood (> 80%) and tissue (> 5%) and the most common clinical presentation is septicemia. Although there are similarities in clinical presentation between *S. pyogenes* and *S. dysgalactiae* infections, fasciitis, puerperal sepsis and septic toxic shock syndrome are more linked to iGAS infection. *speG* is the only streptococcal pyrogenic exotoxin detected in *S. dysgalactiae* isolates.

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