REPORT 2012 - 2022

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

Antwerp University Hospital
Drie Eikenstraat 655
2650 Edegem, België
referentiecentrum@uza.be
+32 3 821 3672

In collaboration with the Laboratory of Medical Microbiology (LMM) at the University of Antwerp led by prof. dr. Surbhi Malhotra-Kumar and Laboratoire de bactériologie moléculaire de l'Université Libre de Bruxelles led by prof. Dr. Pierre Smeesters

Kennis / Ervaring / Zorg

This report describes the activities performed by the National Reference Centre (NRC) for invasive β-hemolytic Streptococci non group B up until 2022, including species identification via MALDI-TOF MS, antibiotic susceptibility testing for penicillin, tetracycline, erythromycin and clindamycin by disk diffusion, detection of virulence genes and/or macrolide/tetracycline resistance genes by PCR or Whole Genome Sequencing (WGS) and *emm* typing using Sanger sequencing or WGS.

1. 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.equi, S.canis, S. uberis*).

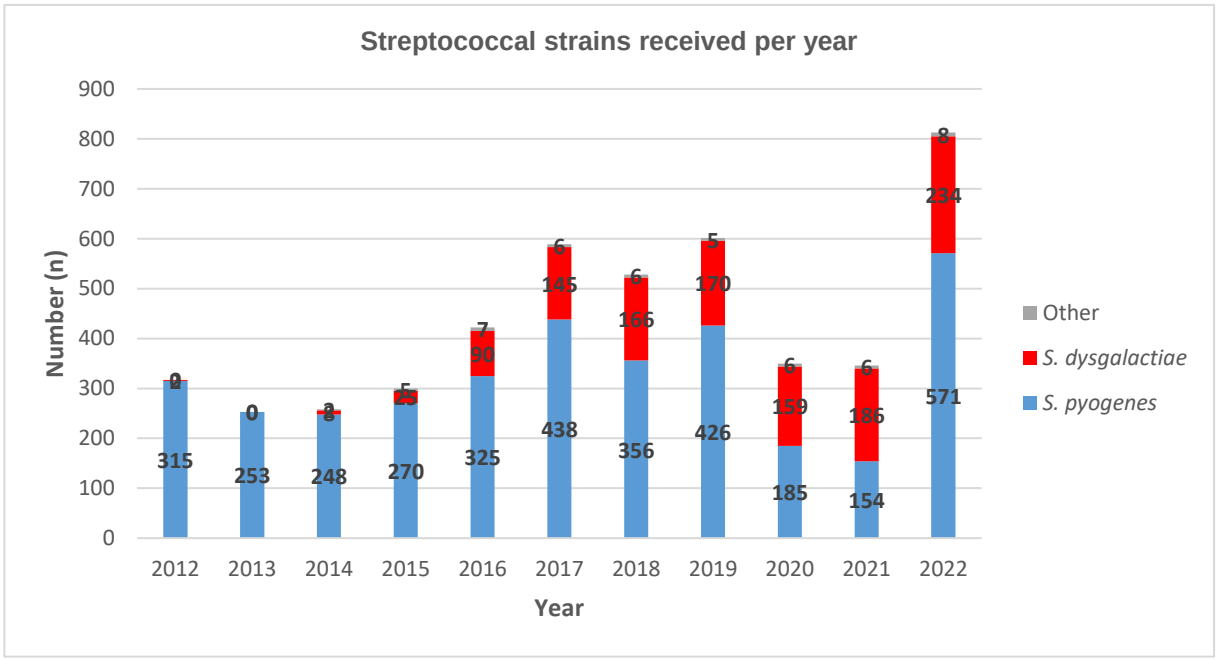

Figure 1: Strains received per year with differentiation between S. pyogenes, S. dysgalactiae and other species (S.equi, S.canis, S. uberis)

Since the start of the NRC, the number of strains increased every year with a remarkable 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 in 2022. This could be explained by an increased ability of laboratories to send samples again after the COVID-19 pandemic but an increased incidence in iGAS (invasive group A Streptococcal) infections is more likely and in line with reported increased incidence in Europe [2] and the USA [3]. 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 2022 but 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.

To follow up on seasonal changes, the number of monthly received *S. pyogenes* strains during 2019-2022 is shown in Figure 2. The large increase of invasive *S. pyogenes* strains in 2022 is stemming from the increase in the summer and autumn months (July – December) with a very steep increase from November onwards. Previous years did not show such pronounced seasonal variation. This increase is mainly caused by *emm*1, further elaborated on in Chapter 2: Strain typing.

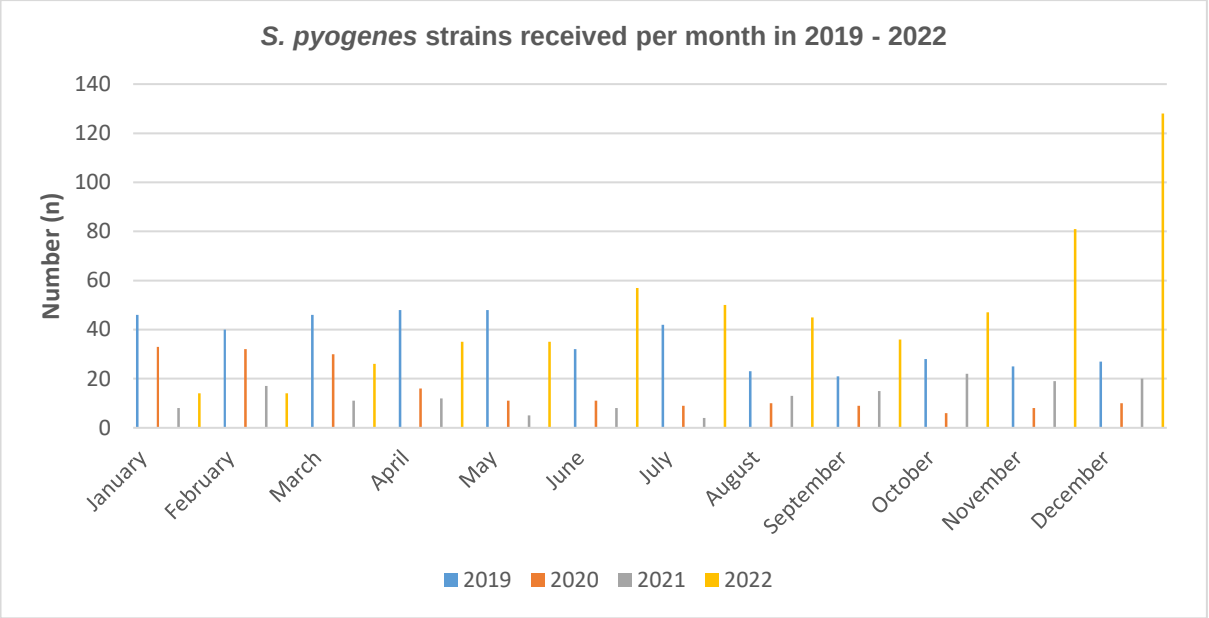

Figure 2: S. pyogenes strains received per month in 2019 - 2022

Figure 3 shows the streptococcal strains (*S. pyogenes*, *S. dysgalactiae* and others) received in 2022 by age group. The highest incidence of invasive GAS strains was observed in children up to 5 years of age (n = 142, 17.5%) and in adults aged 50+. The incidence generally increases with age with the 80+ elderly representing almost a fifth of all strains (n = 145, 18%). The distribution of *S. pyogenes* and *S. dysgalactiae* in the 80+ age group is equal, however in the age group 1-5, only *S. pyogenes* was found.

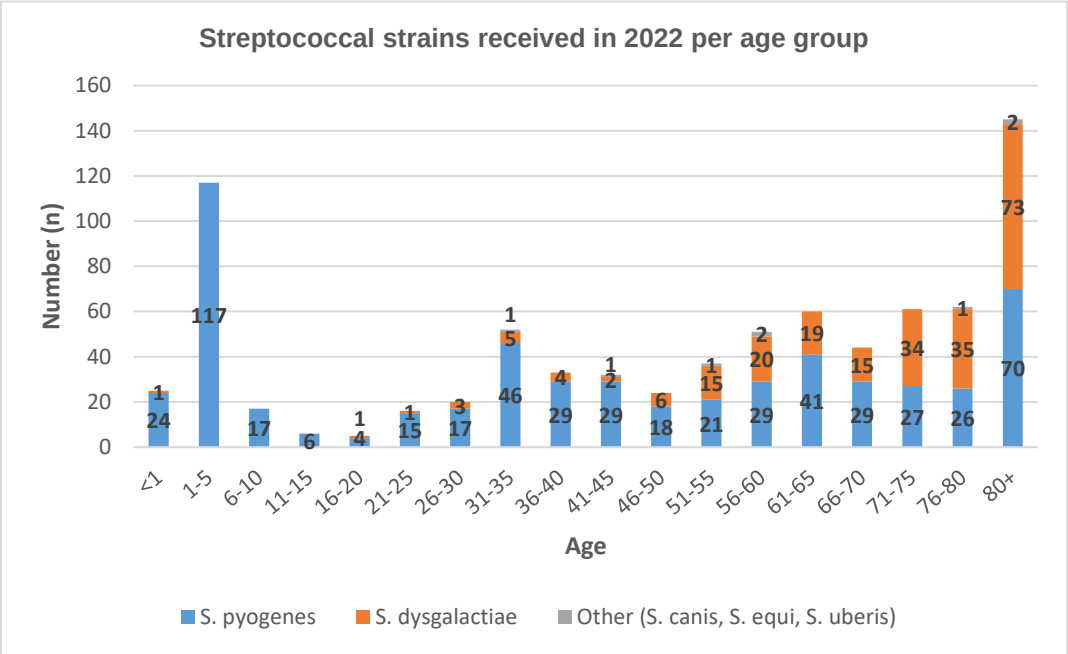

Figure 3: Number of streptococcal strains (S. pyogenes, S. dysgalactiae and S. canis, S. equi & S. uberis) received in 2022 per age group

The age distribution is comparable to the previous years, with the exception of the age group 1 – 5 years. In 2022 there are 117 children between the ages of 1 to 5 with an invasive GAS infection (which is 21% of all *S. pyogenes* infections in 2022), compared to 16 in 2021 (which is 10% of *S. pyogenes* infections in 2021). Noteworthy, the proportion of *S. dysgalactiae* invasive isolates relative to *S. pyogenes* isolates is higher from age +70.

Figure 4 shows the geographical distribution of strains received in 2022 based on the patient's postal code. The strains originate from all over Belgium with the highest prevalence in urban areas, which correlates with the population density in these regions.

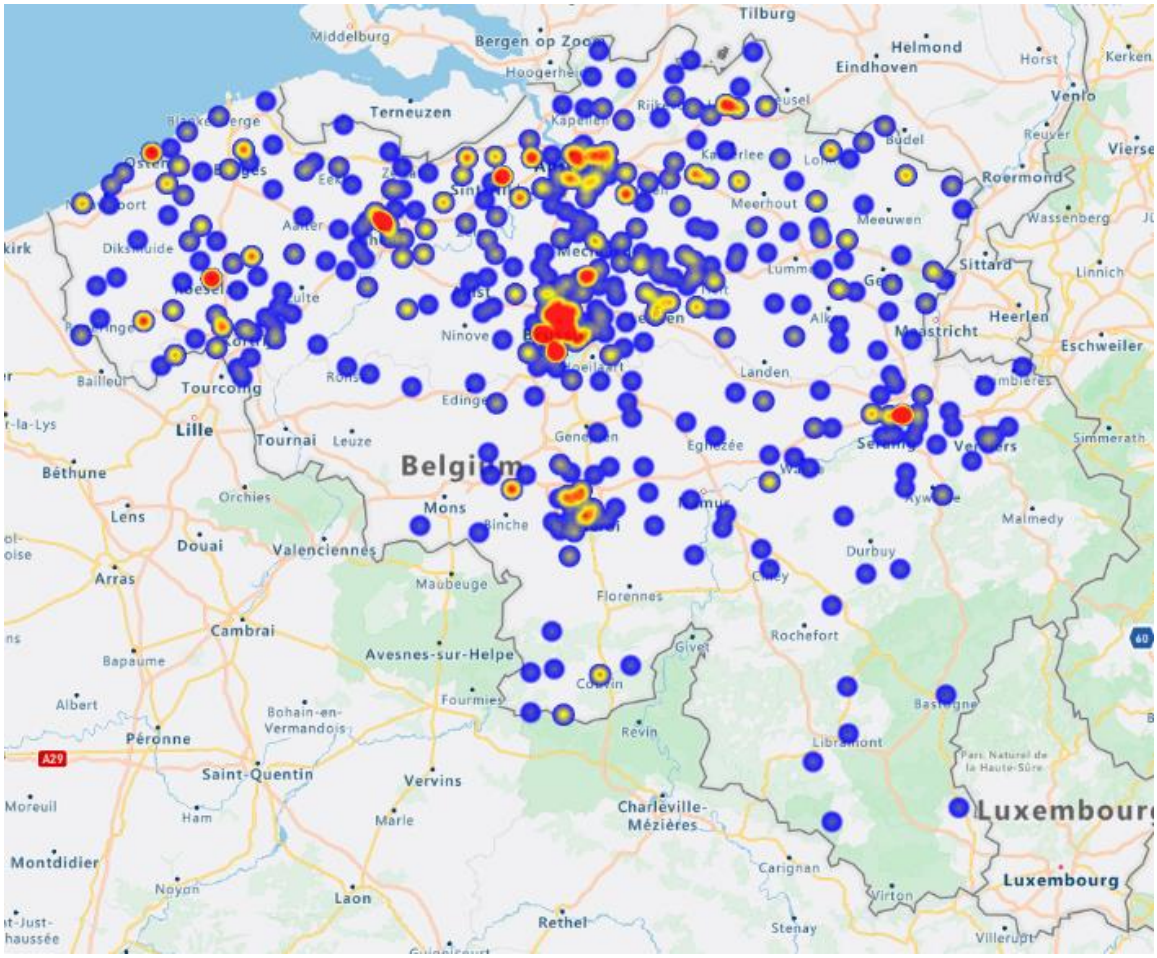

Figure 4: Geographical distribution of strains received in 2022 based on patient's postal code. Blue shows one received strain, yellow two to five received strains and red more than five received strains

The sample types from which the strains originate are shown in Figure 5. Strains have been isolated from both sterile and non-sterile samples. The main source of invasive β-hemolytic isolates is blood (> 75%), followed by tissue (> 9%). The category “fluid” consists of pleural, synovial, cerebrospinal and ascites fluid. ‘Other’ consist mainly of urine and vaginal samples. Despite the fact that the NRC tends to investigate only invasive strains, strains originating from throats swabs are also received in low numbers, often associated by a non-invasive clinical presentation.

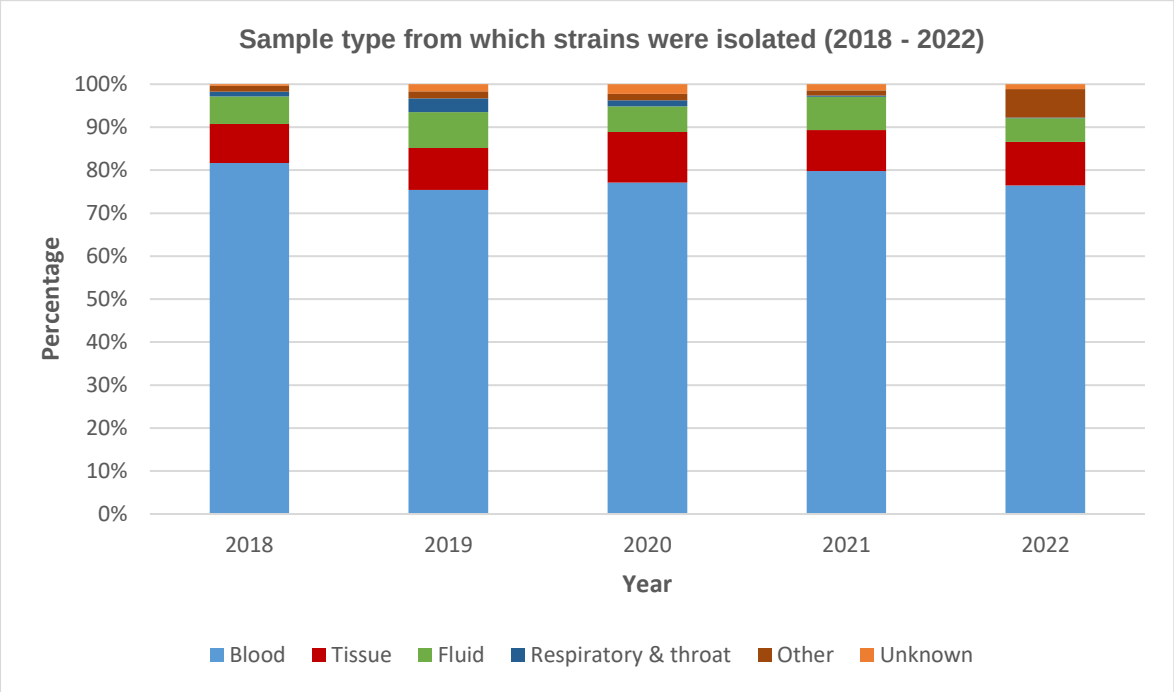

Figure 5: Sample type from which invasive β-hemolytic Streptococci non group B were isolated per year (2018 - 2022)

The most common clinical presentation of infection with invasive β-hemolytic Streptococci non group B is septicemia, followed by numerous other infection sites or syndromes (Tables 1a and 1b). Some examples of 'other' are abscesses, mastitis and adenitis. Sometimes multiple clinical presentations are provided for a single strain, most common is a combination of septicemia with any other clinical presentation. In such case, the most severe/invasive syndrome was counted for.

*Table 1a: Clinical presentation of patients with invasive **S. pyogenes** infection (2018 – 2022)*

Year	Septicemia n (%)	Cellulitis n (%)	Wound infection n (%)	Pneumonia n (%)	Monoarthritis n (%)	Osteomyelitis n (%)	Fasciitis n (%)	STSS n (%)	Puerperal sepsis n (%)	Other n (%)	Unknown n (%)	Total n
2018	174 (49)	20 (6)	27 (8)	32 (9)	6 (2)	6 (2)	11 (3)	2 (0.6)	6 (2)	52 (15)	20 (6)	356
2019	224 (53)	28 (7)	34 (8)	18 (4)	7 (2)	6 (1)	12 (3)	6 (1)	9 (2)	58 (14)	24 (6)	426
2020	92 (50)	16 (9)	5 (3)	13 (7)	4 (2)	3 (2)	13 (7)	4 (2)	3 (2)	21 (11)	11 (6)	185
2021	75 (49)	18 (12)	11 (7)	6 (4)	2 (1)	2 (1)	7 (5)	3 (2)	2 (1)	21 (14)	7 (5)	154
2022	276 (49)	28 (5)	42 (7)	66 (12)	8 (1)	11 (2)	23 (4)	17 (3)	8 (1)	64 (11)	25 (4)	568

*Table 1b: Clinical presentation of patients with invasive **S. dysgalactiae** infection (2018 – 2022)*

Year	Septicemia n (%)	Cellulitis n (%)	Wound infection n (%)	Pneumonia n (%)	Monoarthritis n (%)	Osteomyelitis n (%)	Fasciitis n (%)	STSS n (%)	Puerperal sepsis n (%)	Other n (%)	Unknown n (%)	Total n
2018	84 (51)	18 (11)	12 (7)	6 (4)	5 (3)	3 (2)	0 (0)	0 (0)	0 (0)	24 (15)	13 (8)	165
2019	107 (63)	10 (6)	11 (7)	3 (2)	4 (2)	1 (0.6)	1 (0.6)	2 (1)	0 (0)	27 (16)	4 (2)	170
2020	100 (63)	14 (9)	15 (9)	5 (3)	4 (3)	2 (1)	3 (2)	1 (0.6)	0 (0)	10 (6)	5 (3)	159
2021	117 (63)	24 (13)	6 (3)	5 (3)	3 (2)	3 (2)	0 (0)	0 (0)	1 (0.5)	16 (9)	11 (6)	186
2022	160 (68)	10 (4)	17 (7)	10 (4)	6 (2)	1 (0.4)	1 (0.4)	0 (0)	0 (0)	22 (9)	7 (3)	234

Although there are similarities in clinical presentation between *S. pyogenes* and *S. dysgalactiae* infections, fasciitis, puerperal sepsis and septic toxic shock syndrome are more often linked to iGAS infection. Pneumonia is also more common for *S. pyogenes* infections. Furthermore a strong increase in pneumonia cases is observed in 2022 which is in line with observations in other countries [4].

2. STRAIN TYPING

Emm typing is performed on all *S. pyogenes* and *S. dysgalactiae* strains. *Emm*-types are classified in *emm*-clusters containing closely related M proteins [5]. Figure 6 shows the yearly distribution of the most prevalent *S. pyogenes emm* types from 2012 until 2022. There is a huge diversity of *emm* types, in total more than 40 different *emm* types were detected. *Emm*1 (cluster A-C3) and *emm*3 (cluster A-C5) are the most prevalent *emm* types in the early years with a combined proportion of 28% (2012) 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 [6].
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) [7]. These *emm* types are also frequently isolated in Belgium. In 2022, the proportion of *emm1* exceeded 30% for the first time and *emm*12 has become more common (16%).

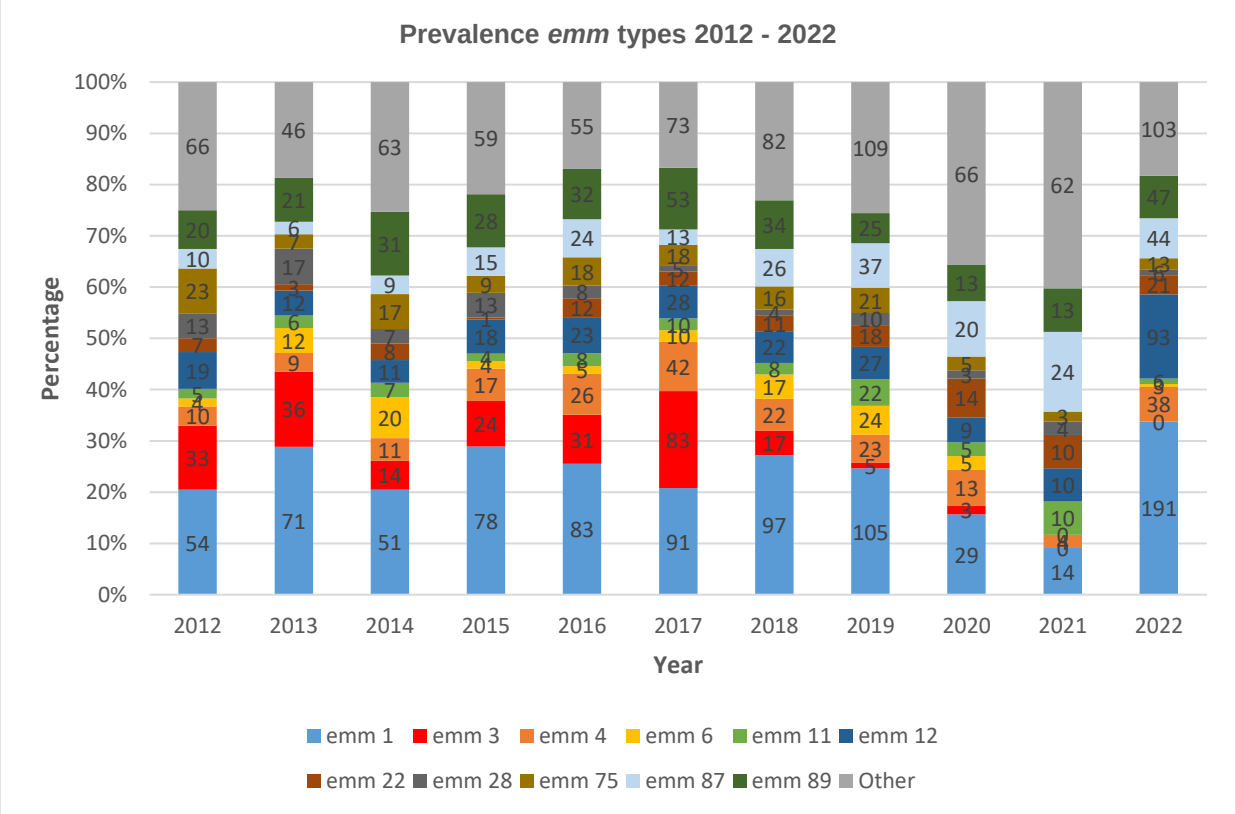

Figure 6: Prevalence of most common emm types from 2012 – 2022. Absolute numbers of isolates are presented in the bar of the graph

The *emm* types *emm*11 (cluster E6), *emm*22 (cluster E4), *emm*75 (cluster E6) and *emm*87 (cluster E3), that are not mentioned in the publication of Europe and North America, are also regularly detected in Belgium. Particularly in 2020 and 2021, beyond the scope of the publication, the prevalence of *emm*11, *emm*22, and *emm*87 increased in relative numbers. However, in 2022 the amount of *emm*11, *emm*22 and *emm*87 has decreased again.

A new subtype of *emm*1 was described in UK in 2019. This subtype is named the $M1_{UK}$ clone and is characterized by 27 SNPs in the *S. pyogenes* genome [4]. This clone shows an increased *speA* production explaining its more toxigenic behavior. This specific clone also emerged in Belgium. Figure 7 shows the sequenced *emm*1 types divided into $M1_{Global}$ and $M1_{UK}$. The first Belgian $M1_{UK}$ variants were detected in 2020. The observed increase in *emm*1 in 2022 is accompanied by a shift towards a higher proportion of $M1_{UK}$ variants (about 2/3 of the *emm*1 strains are $M1_{UK}$ variants).

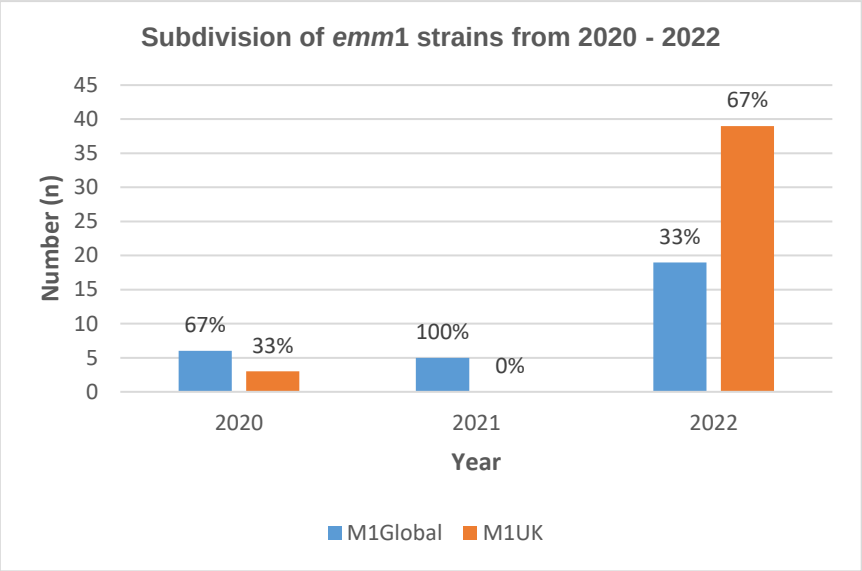

Figure 7: Subdivision of the sequenced emm*1 strains from 2020 – 2022 in M1$_{Global}$ and M1$_{UK}$ clones*

In 2021, the majority of infections in the age group 1 – 5 years were caused by *emm*87 (50%), while in 2022 the majority of infections was caused by *emm*1 (35%). The *emm* type distribution in the 80+ age group (Figure 8b) shows also some differences between 2021 and 2022. In 2021, most infections were caused by *emm*89 (29%) and other *emm* types (53%), while in 2022, most infections were caused by *emm*1 (21%) and *emm*12 (20%).

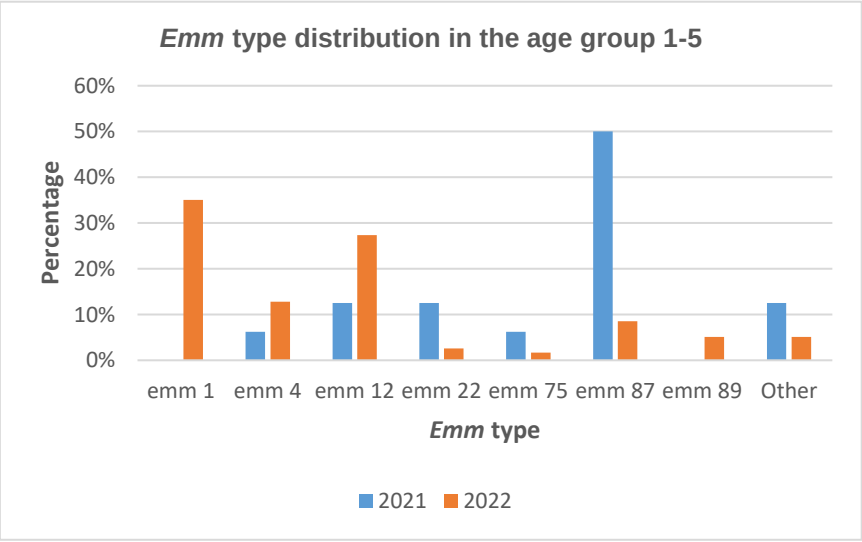

Figure 8a: Percentage of emm types found in the age group 1 – 5 years

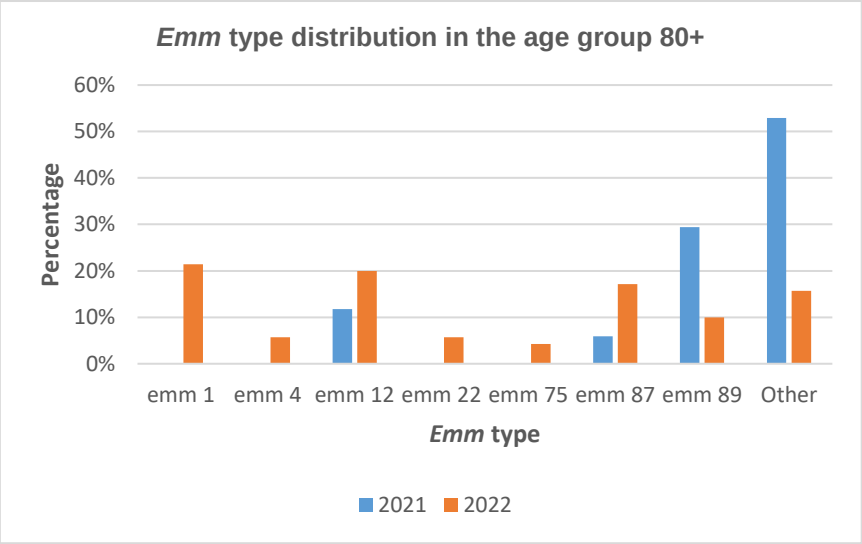

Figure 8b: Percentage of emm types found in the age group 80+ years

Figure 9 shows the prevalence of the 10 most common *S. dysgalactiae* types from 2016 until 2022. The biggest increase in absolute numbers was seen in the STG62647 type and its subtypes, the second most common type is STG485. In relative numbers there does not seem to be much variation over the years. Other countries have also observed an increase in *S. dysgalactiae* infections. In Western Norway, invasive infections by the *S. dysgalactiae* STG62647 type have been increasing since 2013 [8]. These infections are often associated with streptococcal toxic shock syndrome, necrotizing infections and endocarditis. However, in our data, the association of *S. dysgalactiae* infection with these clinical syndromes is not observed.

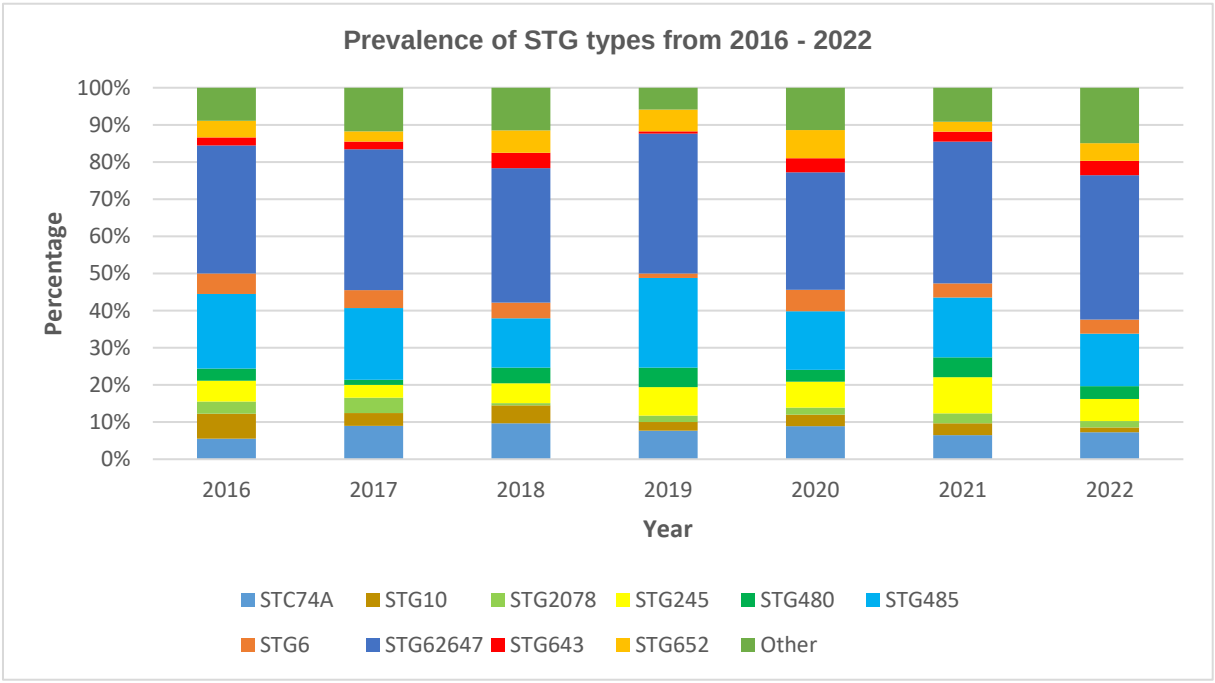

Figure 9: Prevalence of the 10 most common S. dysgalactiae STG types from 2016 – 2022

3. ANTIBIOTIC SUSCEPTIBILITY

From 2012 – 2018, the sensitivity to tetracycline, erythromycin and clindamycin was determined for all submitted *S. pyogenes* strains. From 2019 onwards, a yearly selection of 50 strains (with wide geographical distribution) was made for which susceptibility testing was performed. The percentage of resistant strains per antibiotic is shown in Figure 10. The resistance of *S. pyogenes* to tetracyclines has increased up to 38% in 2021. In 2022 the tetracycline resistance decreased drastically to 12%. It is uncertain if this drop in tetracycline resistance can be extrapolated to all strains as only a tenth is phenotypically tested but it is in line with genetic data (see table 2: tetracycline resistance genes detected in 14% of 2022 strains and 35% of 2021 strains). Resistance to erythromycin and clindamycin remains rather stable over the years.

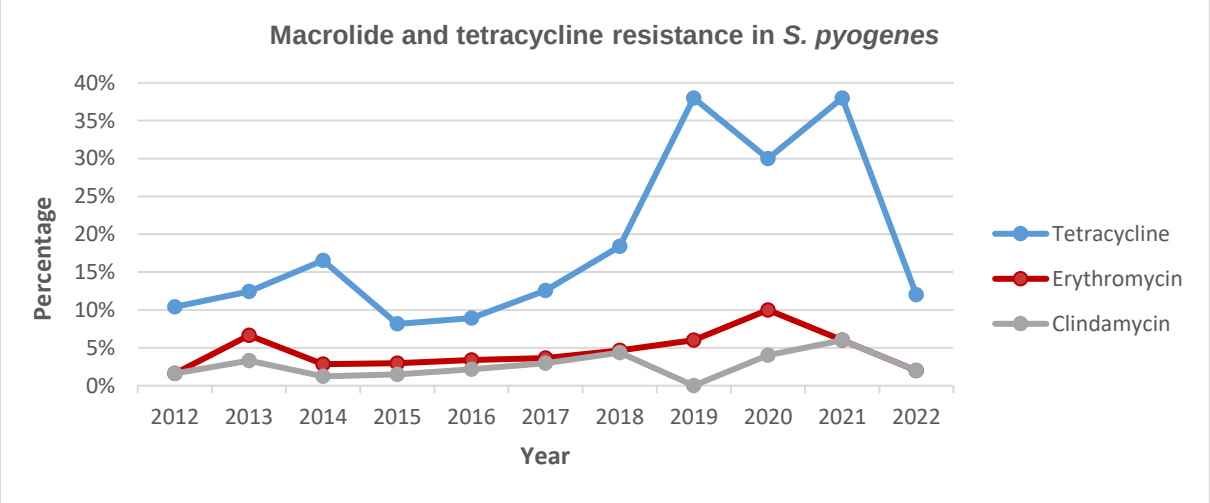

Figure 10: Percentage of macrolide and tetracycline resistance of S. pyogenes per year, results acquired by susceptibility testing

Upon specific request by the sending laboratories, multiplex PCR or WGS for the detection of tetracycline (Table 2) and macrolide resistance genes (Table 3) was performed. Since the number of tested strains per year varies significantly, detection of certain trends over time is difficult. However, it is clear that *tet*(M) is the most prevalent gene conferring tetracycline resistance while *erm*(A) used to be the most prevalent macrolide conferring resistance gene, but in the last years, both *erm*(A) and *erm*(B) are found equally.

*Table 2: Prevalence of **tetracycline resistance genes**: tet(K), tet(L), tet(M) and tet(O) in absolute and relative numbers in S. pyogenes isolates*

Year	Strains tested *n*	Resistance gene detected *n (%)*	***tet*(K)** *n (%)*	***tet*(L)** *n (%)*	***tet*(M)** *n (%)*	***tet*(O)** *n (%)*
2012	38	18 (47)	0 (0)	0 (0)	13 (72)	6 (33)
2013	45	21 (47)	0 (0)	1 (5)	15 (71)	6 (29)
2014	48	32 (67)	0 (0)	5 (16)	24 (75)	8 (25)
2015	48	22 (46)	0 (0)	0 (0)	16 (73)	6 (27)
2016	84	52 (62)	0 (0)	6 (12)	44 (85)	9 (17)
2017	98	70 (71)	1 (1)	1 (1)	61 (87)	10 (14)
2018	125	86 (69)	0 (0)	4 (5)	63 (73)	20 (23)
2019	14	5 (36)	1 (20)	0 (0)	4 (80)	0 (0)
2020	61	11 (18)	0 (0)	0 (0)	9 (82)	2 (18)
2021	72	25 (35)	0 (0)	1 (4)	23 (92)	1 (4)
2022	190	26 (14)	0 (0)	1 (4)	25 (96)	1 (4)

*Table 3: Prevalence of **macrolide resistance genes**: erm(A), erm(B) and mef in absolute and relative numbers in S. pyogenes isolates*

Year	Strains tested *n*	Resistance gene detected *n (%)*	***erm*(A)** *n (%)*	***erm*(B)** *n (%)*	***mef*** *n (%)*
2012	37	15 (41)	6 (40)	6 (40)	3 (20)
2013	45	16 (36)	9 (56)	5 (31)	2 (13)
2014	48	15 (31)	10 (67)	4 (27)	1 (7)
2015	48	15 (31)	8 (53)	5 (33)	3 (20)
2016	84	29 (35)	16 (55)	11 (38)	4 (14)
2017	98	32 (33)	15 (47)	12 (38)	5 (16)
2018	125	47 (38)	29 (62)	14 (30)	4 (9)
2019	14	3 (21)	2 (67)	1 (33)	0 (0)
2020	61	5 (8)	3 (60)	2 (40)	0 (0)
2021	72	5 (7)	1 (20)	3 (60)	1 (20)
2022	190	9 (5)	4 (44)	4 (44)	1 (11)

The presence of certain resistance genes seems to correlate with specific *emm* types (Table 4). Certain *emm* types (*emm*50, *emm*75 and *emm*82) lack resistance genes, in others (*emm1, emm4, emm5, emm12, emm87, emm89*) resistance genes are rarely encountered and certain types (*emm27, emm33, emm63, emm64, emm108, emm162*) always carry the *tet(M)* gene.

Table 4: The prevalence of resistance genes in different emm types of S. pyogenes isolates in 2014 - 2022

***Emm* type**	**Aantal (n)**	**Erm(A)**	**Erm(B)**	**Mef**	**Tet(K)**	**Tet(L)**	**Tet(M)**	**Tet(O)**
***Emm*1**	88	0%	1%	0%	0%	0%	1%	0%
***Emm*108**	24	0%	0%	0%	0%	0%	100%	0%
***Emm*11**	15	0%	54%	0%	0%	0%	17%	0%
***Emm*12**	24	0%	2%	0%	0%	0%	0%	0%
***Emm*162**	48	0%	0%	0%	0%	0%	100%	0%
***Emm*22**	42	2%	0%	2%	0%	0%	86%	0%
***Emm*25**	8	0%	0%	13%	0%	0%	88%	0%
***Emm*27**	6	0%	50%	0%	0%	0%	100%	0%
***Emm*28**	8	0%	38%	0%	0%	13%	25%	0%
***Emm*33**	6	0%	0%	0%	0%	0%	100%	0%
***Emm*4**	10	0%	0%	4%	0%	0%	4%	0%
***Emm*43**	5	0%	0%	0%	0%	0%	10%	0%
***Emm*5**	5	0%	0%	0%	0%	0%	7%	0%
***Emm*50**	16	0%	0%	0%	0%	0%	0%	0%
***Emm*63**	5	0%	0%	0%	20%	0%	100%	0%
***Emm*64**	37	0%	0%	0%	0%	0%	100%	0%
***Emm*75**	9	0%	0%	0%	0%	0%	0%	0%
***Emm*77**	10	49%	0%	0%	0%	0%	16%	59%
***Emm*82**	39	0%	0%	0%	0%	0%	0%	0%
***Emm*83**	26	0%	0%	0%	0%	10%	60%	0%
***Emm*87**	6	3%	0%	0%	0%	0%	3%	0%
***Emm*89**	7	8%	0%	0%	0%	0%	0%	0%
***Emm*90**	5	0%	0%	0%	0%	0%	50%	0%

4. VIRULENCE GENES

The virulence genes *speA*, *speC,* and *ssa* (Table 5) have been part of the routine virulence gene detection via PCR since 2012. The presence of *speC* has been stable (~45%) over the years, with a small increase during the COVID-19 pandemic to 56%. This is in contrast to the presence of *speA* where a remarkable decrease was observed in 2020-2021, but in 2022 *speA* expression shows an increase that reaches the same level as 2019. This phenomenon can be explained by the changes in prevalence of *emm*1 types over these years (see Figure 6), which is associated with *speA* expression [9].

Table 5: Prevalence of virulence genes: speA, speC and ssa genes by tested strains

Year	Number of tested strains *n*	***SpeA*** *n (%)*	***SpeC*** *n (%)*	***Ssa*** *n (%)*
2012	102	29 (28)	46 (45)	39 (38)
2013	121	67 (55)	51 (42)	19 (16)
2014	189	64 (34)	94 (50)	38 (20)
2015	175	100 (57)	68 (39)	40 (23)
2016	178	99 (56)	65 (37)	42 (24)
2017	116	51 (44)	47 (41)	40 (35)
2018	90	40 (44)	35 (39)	23 (26)
2019	122	42 (34)	57 (47)	30 (25)
2020	96	18 (19)	52 (54)	30 (31)
2021	99	8 (8)	56 (57)	29 (29)
2022	190	69 (36)	89 (47)	34 (18)

Since 2022, whole genome sequencing (WGS) is performed to detect a larger spectrum of virulence genes (Figure 11) instead of the previously used multiplex PCR. Virulence genes that are detected are multiple streptococcal pyrogenic exotoxins (*spe*), streptococcal superantigen (*ssa*), streptococcal mitogenic exotoxin Z (*smeZ*) and streptococcal C5a peptidase (*scpa*). Almost all strains contain *speG* (93% and *smeZ* (97%) next to the constitutively expressed *SpeB* and *SpeF*.

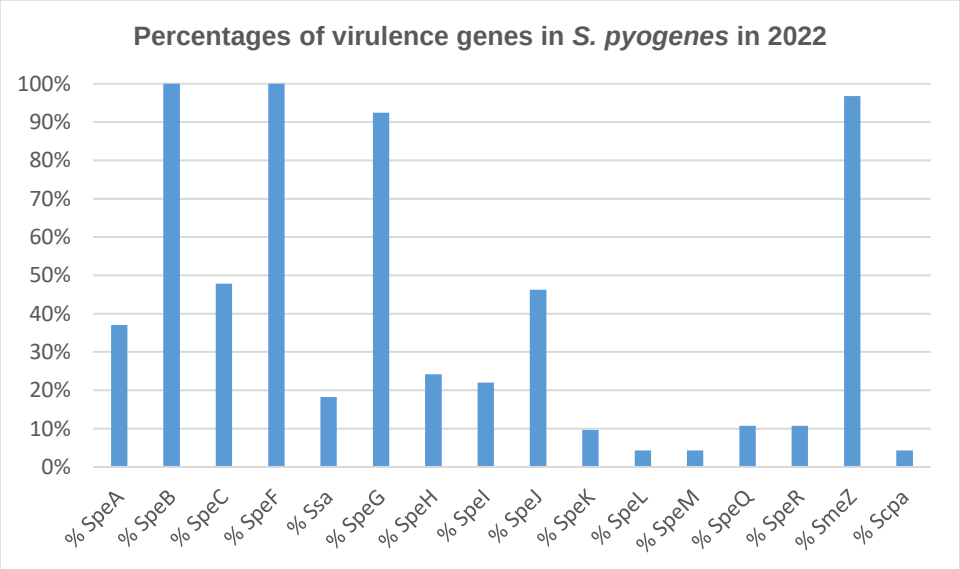

Figure 11: Percentages of virulence genes found in S. pyogenes strains in 2022

Table 6 shows the frequency of virulence genes in the 5 most prevalent *emm* types: *emm*1, *emm*4, *emm*12, *emm*87 and *emm*89. Certain virulence genes can be linked to specific *emm* types. All *emm*1 strains have virulence genes *speA*, *speG*, *speJ* and *SmeZ*. *SpeG* is a common virulence gene that can be found in almost every *emm* type, except for *emm*4 strains [9]. *SmeZ* is another virulence factor that can be found in almost all strains of every *emm* type. Other virulence factors are only detected in specific *emm* types: such as *ssa* in *emm*4 and *emm*87, *speH* and *speI* in *emm*12 or *speQ* and *speR* in *emm*87.

Table 6: The prevalence of virulence genes in the 5 most prevalent emm types: emm1, emm4, emm12, emm87 and emm89.

Emm type	SpeA	SpeC	Ssa	SpeG	SpeH	SpeI	SpeJ	SpeK	SpeQ	SpeR	SmeZ	Scpa
***Emm* 1**	100%	0%	0%	100%	0%	0%	100%	0%	0%	0%	100%	0%
***Emm* 4**	0%	88%	100%	0%	0%	0%	0%	0%	0%	0%	100%	13%
***Emm* 12**	3%	87%	0%	97%	100%	100%	0%	0%	0%	0%	97%	3%
***Emm* 87**	0%	83%	100%	100%	0%	0%	89%	17%	89%	89%	100%	11%
***Emm* 89**	0%	84%	0%	100%	0%	0%	0%	37%	0%	0%	100%	11%

5. 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 – 2022.

In 2022, the NRC received 571 ***S. pyogenes*** showing a steep incline 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) with a very steep increase from November 2022 onwards. Previous years did not show such pronounced seasonal variation. This increase is mainly caused by ***emm1*** *S. pyogenes* (30% of the isolates and 21% of the iGAS infections in 2022). A new *emm*1 subtype of *S. pyogenes*, the **$M1_{UK}$ clone**, emerged in Belgium in 2020. The increase in *emm1 S. pyogenes* in 2022 coincided with a shift towards a higher proportion of $M1_{UK}$ variants. Elevated Streptococcal pyrogenic exotoxin A (SpeA) production explains the more toxigenic behavior of this clone. Indeed, the virulence gene *speA* was present in all *emm*1 isolates. The highest incidence of invasive GAS strains was observed in children up to 5 years of age (n = 142, 17.5%) and in adults aged 50+. The incidence generally increases with age with the 80+ elderly representing almost a fifth of all strains (n = 145, 18%). Resistance to erythromycin and clindamycin remained stable and ≤10% between 2012 and 2022, while tetracycline resistance varied between 10 and 38% over the years. However, these variations need further investigation. *tet(M)* is the most prevalent tetracycline resistance gene, *erm(A)* and *erm(B)* are the most prevalent genes conferring resistance to macrolides

In 2022, the NRC received 234 ***S. dysgalactiae*** strains. The most common *S. dysgalactiae* types between 2016 and 2022 were STG6247 and STG485. The main infection sites of invasive β-hemolytic isolates are blood (> 75%) and tissue (> 9%) 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. A strong increase in pneumonia cases, mostly caused by *S. pyogenes* (4% of infections in 2021 to 12% in 2022), was observed in 2022.

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6. REFERENCES

[1] D. Shaw, R. Abad, Z. Amin-Chowdhury en A. Bautista, „Trends in invasive bacterial diseases during the first 2 years of the COVID-19 pandemic: analyses of prospective surveillance data from 30 countries and territories in the IRIS Consortium," *The Lancet Digital Health,* 27 07 2023.

[2] Centers for Disease Control and Prevention, „Increase in Pediatric Invasive Group A Streptococcal Infections," 10 03 2022. [Online]. Available: https://www.cdc.gov/mmwr/volumes/72/wr/mm7210a4.htm. [Geopend 18 09 2023].

[3] R. Guy, K. L. Henderson, J. Coelho, H. Hughes, E. L. Mason, S. M. Gerver, A. Demirjian, C. Watson , A. Sharp, C. S. Brown en T. Lamagni , „Increase in invasive group A streptococcal infection notifications, England, 2022," *Eurosurveillance,* vol. 28, nr. 1, 2023.

[4] V. Holdstock, J. Twynam-Perkins, T. Bradnock, E. M. Dickson, K. Harvey-Wood, P. Kalima, J. King, W. J. Olver, M. Osman, A. Sabharwal, A. Smith, S. Unger, L. Pollock, R. Langley, P. Davies en T. C. Williams, „National case series of group A streptococcus pleural empyema in children: clinical and microbiological features," *The Lancet Infectious Diseases,* vol. 23, nr. 2, pp. 154-156, 2023.

[5] M. Sanderson-Smith et al., „A Systematic and Functional Classification of Streptococcus pyogenes That Serves as a New Tool for Molecular Typing and Vaccine Development," *The Journal of Infectious Diseases,* vol 210, nr 8, pp. 1325 - 1338, 15 10 2014.

[6] P. Villalón, J. A. Sáez-Nieto, V. Rubio-López, M. J. Medina-Pascual, N. Garrido, G. Carrasco, S. Pino-Rosa en S. Valdezate , „Invasive Streptococcus pyogenes disease in Spain: a microbiological and epidemiological study covering the period 2007-2019," *European Journal of Clinical Microbiology & Infectious Diseases,* pp. 2295-2303, 2021.

[7] G. Gherardi, L. A. Vitali en R. Creti, „Prevalent emm Types among Invasive GAS in Europe and North America since Year 2000," *Frontiers in Public Health,* 2018.

[8] O. Oppegaard, M. Glambek, D. G. Skutlaberg, S. Skrede, A. Sivertsen en B. R. Kittang, „Streptococcus dysgalactiae Bloodstream Infections, Norway, 1999-2021," *Emerging Infectious Diseases,* pp. 260-267, 2 2023.

[9] B. Luca-Harari, J. Darenberg, S. Neal, T. Siljander, L. Strakova, A. Tanna, R. Creti, K. Ekelund, M. Koliou, P. T. Tassios, M. van der Linden, M. Straut, J. Vuopio-Varkila, A. Bouvet, A. Efstratiou, C. Schalén, B. Henriques-Normark en A. Jasir, „Clinical and Microbiological Characteristics of Severe Streptococcus pyogenes Disease in Europe," *Journal of Clinical Microbiology,* vol. 4, nr. 47, pp. 1155-1165, 2009.

7. RECENT NRC PUBLICATIONS

1. Kerstens J, Durmus B, Lambrecht S, Baar I, Ieven MM, Van Der Zijden T, et al. Meningoencephalitis with Streptococcus equi Subspecies equi Leading to a Dural Arteriovenous Fistula. Case Rep Neurol Med. 2021 Apr 15;2021:1–6.

2. Coppens J, Xavier BB, Loens K, Lammens C, Ieven M, Matheeussen V, et al. Remarkable genome stability among emm1 group a streptococcus in Belgium over 19 years. Genome Biol Evol. 2019;11(5).

3. Tuerlinckx D, Gueulette E, Loens K, Goossens H, Smeesters PR. Group A streptococcal meningitis: emm type distribution and theoretical vaccine coverage in children. Acta Clin Belg. 2016 May 3;71(3):138–41.

4. Saegeman V, Cossey V, Loens K, Schuermans A, Glaser P. Streptococcus Gallolyticus Subsp. Pasteurianus Infection In A Neonatal Intensive Care Unit. Pediatr Infect Dis J. 2016 Nov;35(11):1272–5.

5. Sanderson-Smith M, De Oliveira DMP, Guglielmini J, McMillan DJ, Vu T, Holien JK, et al. A Systematic and Functional Classification of Streptococcus pyogenes That Serves as a New Tool for Molecular Typing and Vaccine Development. J Infect Dis. 2014 Oct 15;210(8):1325–38.