REPORT 2021

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

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This report describes the activities performed by the National Reference Centre (NRC) for invasive β-hemolytic Streptococci non group B up until 2021, 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 and emm typing using Sanger sequencing or Whole Genome Sequencing (WGS).

1. STRAIN IDENTIFICATION AND DEMOGRAPHICS

The strains received by the National Reference Centre 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*).

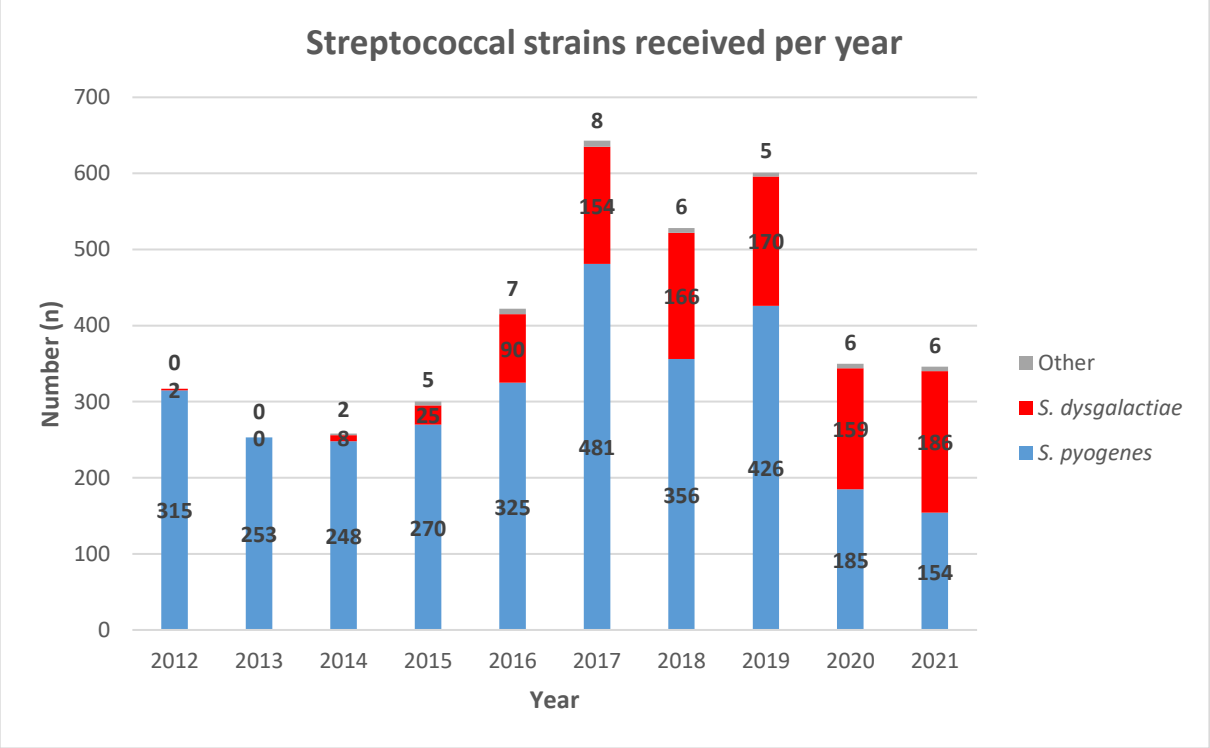

Figure 1: Strains received per year with differentiation between S. pyogenes, S. dysgalactiae and other species (S.equi, S.canis, S. uberis)

Since the start of the NRC, the number of strains increased every year with a peak in 2017. Thereafter, a small decline was observed with a remarkable drop in 2020 due to the COVID-19 pandemic. As this drop might be explained by both lower incidence and reduced ability to send samples to the NRC due to high workload in the laboratories, comparison of results to previous years should be done with caution. 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.

Figure 2 shows the strains received in 2021 by age group. The age distribution is comparable to the previous years. The highest incidence of invasive GAS strains was observed in children up to 5 years of age (n=23) and in adults aged 56+. The incidence increases with age with the +80 elderly representing a quarter of all strains (77/ 346 strains, 22.3%).

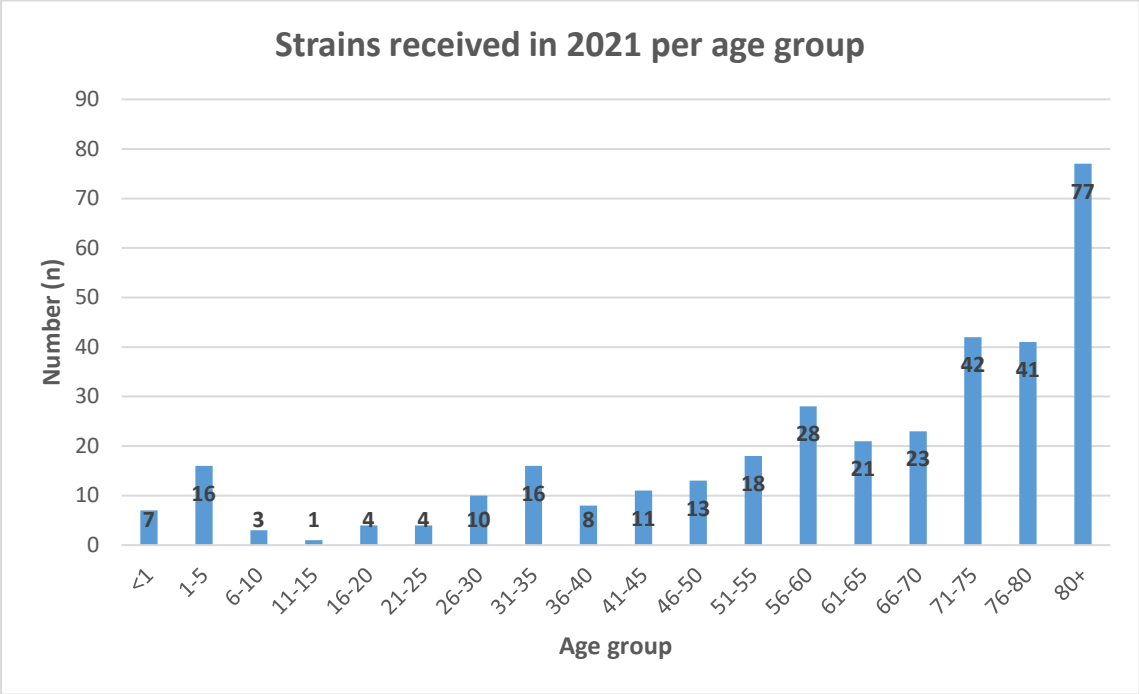

Figure 2: Number of strains received in 2021 per age group

Figure 3 shows the geographical distribution of strains received in 2021 based on the patient's postal code. The strains originate from all over Belgium (and also the southern part of the Netherlands) with the highest prevalence in urban areas, which correlates with the population density in these regions. The southern part of Belgium is underrepresented.

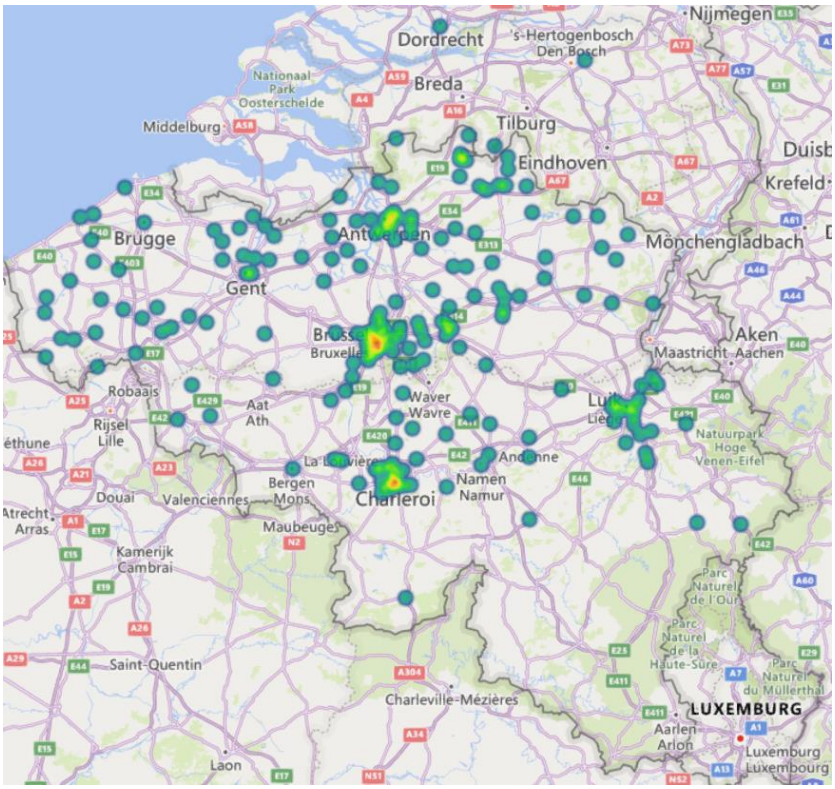

Figure 3: Geographical distribution of strains received in 2021 based on patient's postal code

The sample types from which the strains originate are shown in Figure 4. Strains have been isolated from both sterile and non-sterile samples. The main source of invasive β-hemolytic isolates is blood (> 75%), followed by (non-) surgical wounds (> 6%). The category "Fluid" consists of pleural, synovial, cerebrospinal and ascites fluid. Thereafter, the category "Biopsy" consists of tissue of glands, lower legs and toes. '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.

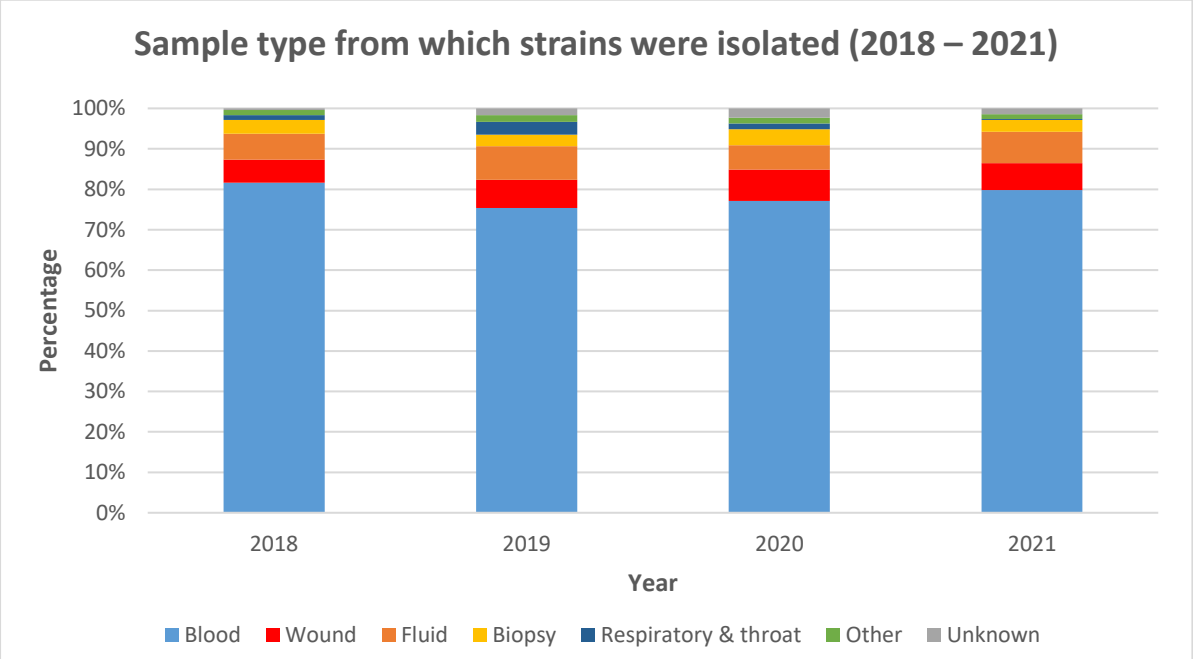

Figure 4: Sample type from which invasive β-hemolytic Streptococci non group B were isolated per year (2018 - 2021)

The most common clinical presentation of infection with invasive β-hemolytic Streptococci non group B is septicemia (> 50%), followed by numerous other infection sites or syndromes (Table 1). Some examples of 'other' are abscesses, mastitis and adenitis.

Table 1: Clinical presentation of patients with invasive β-hemolytic Streptococci non group B infection (2018 – 2021)

Year	Septicemia *n (%)*	Cellulitis *n (%)*	Wound infection *n (%)*	Pneumonia *n (%)*	Monoarthritis *n (%)*	Fasciitis *n (%)*	Osteomyelitis *n (%)*	Puerperal sepsis *n (%)*	STSS *n (%)*	Other *n (%)*
2018	264 (50.0)	49 (9.3)	40 (7.6)	29 (5.5)	11 (2.1)	7 (1.3)	6 (1.1)	5 (0.9)	2 (0.4)	115 (21.8)
2019	344 (57.2)	42 (7.0)	46 (7.7)	13 (2.2)	13 (2.2)	8 (1.3)	4 (0.7)	7 (1.2)	7 (1.2)	117 (19.5)
2020	219 (62.6)	27 (7.7)	24 (6.9)	3 (0.9)	8 (2.3)	8 (2.3)	4 (1.1)	3 (0.9)	5 (1.4)	49 (14.0)
2021	203 (58.7)	35 (10.1)	24 (6.9)	6 (1.7)	6 (1.7)	4 (1.2)	4 (1.2)	3 (0.9)	3 (0.9)	58 (16.8)

2. 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 5. The resistance of *S. pyogenes* to tetracyclines is increasing and up to 40% in 2021. In contrast, resistance to macrolides and clindamycin is lower and declining after a peak in 2019.

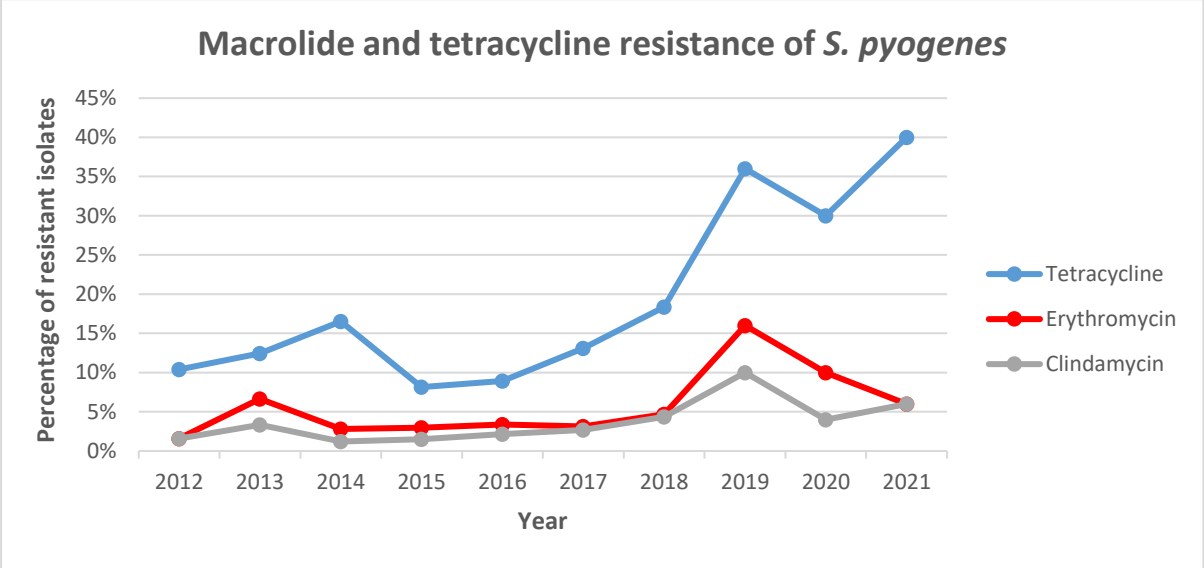

Figure 5: Percentage of macrolide and tetracycline resistance of S. pyogenes per year

Upon specific request by the sending laboratories, multiplex PCR or WGS for the detection of macrolide and tetracycline resistance genes was performed (table 2 and table 3). Since the number of tested strains per year varies significantly, detection of certain trends over time is difficult. However, it is clear that between 2012 and 2021 Tet(M) is the most prevalent gene conferring tetracyclin resistance and erm(A) is the most prevalent macrolide conferring resistance gene.

Table 2: Prevalence of tetracycline resistance genes

Year	Strains tested *(n)*	Resistance gene detected *(n)*	Tet(K) *n (%)*	Tet(L) *n (%)*	Tet(M) *n (%)*	Tet(O) *n (%)*
2012	37	19	0 (0.0)	0 (0.0)	13 (68.4)	6 (31.6)
2013	45	22	0 (0.0)	1 (4.5)	15 (68.2)	6 (27.3)
2014	48	37	0 (0.0)	5 (13.5)	24 (64.9)	8 (21.6)
2015	48	22	0 (0.0)	0 (0.0)	16 (72.7)	6 (27.3)
2016	84	59	0 (0.0)	6 (10.2)	44 (74.6)	9 (15.3)
2017	101	76	1 (1.3)	1 (1.3)	64 (84.2)	10 (13.2)
2018	125	87	0 (0.0)	4 (4.6)	63 (72.4)	20 (23.0)
2019	14	5	1 (20.0)	0 (0.0)	4 (80.0)	0 (0.0)
2020	67	13	0 (0.0)	1 (7.7)	10 (76.9)	2 (15.4)
2021	82	29	0 (0.0)	1 (3.4)	27 (93.1)	1 (3.4)

Table 3: Prevalence of macrolide resistance genes

Year	Strains tested *(n)*	Resistance gene detected *(n)*	Erm(A) *n (%)*	Erm(B) *n (%)*	Mef *n (%)*
2012	37	15	6 (40.0)	6 (40.0)	3 (20.0)
2013	45	16	9 (56.3)	5 (31.3)	2 (12.5)
2014	48	15	10 (66.7)	4 (26.7)	1 (6.7)
2015	48	16	8 (50.0)	5 (31.3)	3 (18.7)
2016	84	31	16 (51.6)	11 (35.5)	4 (12.9)
2017	101	32	15 (46.9)	12 (37.5)	5 (14.3)
2018	125	47	29 (61.7)	14 (29.8)	4(8.5)
2019	14	3	2 (66.6)	1 (33.3)	0 (0.0)
2020	67	7	2 (28.6)	4 (57.1)	1 (14.3)
2021	82	5	1 (20.0)	2 (40.0)	2 (40.0)

3. VIRULENCE

The results for detection of a small selection of virulence genes (SpeA, SpeC and Ssa) is shown in table 4. The presence of SpeC is stable (~40%) over the years also during the COVID-19 pandemic, in contrast to the presence of SpeA where a remarkable decrease can be observed. Isolates with speA are associated with the most severe infections [1]. The presence of virulence genes is detected with a multiplex PCR. Whole genome sequencing will be performed from 2022 onwards to detect a larger spectrum of virulence genes. Also, because in the recent years the number of strains in which none of the 3 above mentioned virulence genes could be detected, increased (30% in 2020 and 33% in 2021 compared to around 10-15% in the years before).

Table 4: Prevalence of superantigenes SpeA, SpeC and Ssa genes by tested strains

Year	Number of tested strains *(n)*	Number of strains without SpeA, SpeC or Ssa *(n)*	SpeA *n (%)*	SpeC *n (%)*	Ssa *n (%)*
2012	102	24	29 (28.4)	46 (45.1)	39 (38.2)
2013	121	11	67 (55.4)	51 (42.1)	19 (15.7)
2014	189	29	64 (33.9)	94 (49.7)	38 (20.1)
2015	175	10	100 (57.1)	68 (38.9)	40 (22.9)
2016	178	19	99 (55.6)	65 (36.5)	42 (23.6)
2017	126	17	57 (45.2)	52 (41.3)	44 (34.9)
2018	90	12	40 (44.4)	35 (38.9)	23 (25.6)
2019	122	16	42 (34.4)	57 (46.7)	30 (24.6)
2020	57	17	10 (17.5)	26 (45.6)	13 (22.8)
2021	54	18	2 (3.7)	26 (48.1)	6 (11.1)

4. STRAIN TYPING

Emm typing was performed on all submitted *S. pyogenes* strains. Figure 6 shows the yearly distribution of the most prevalent emm types. There is a huge diversity of different emm types. In total more than 40 different emm types were detected. Emm 1 and emm 3 are the most prevalent emm types in the early years with a combined proportion of 25% (2012) up to 43% (2013) but their prevalence decreased from 2018 onwards to 10% in 2021. High prevalence of emm 1 and emm 3 is also detected in Spain between 2007 -2019 [2]. The seven most prevalent emm types identified between 2000 - 2017 in Europe and North America are emm 1, emm 28, emm 89, emm 3, emm 12, emm 4, and emm 6 [3]. These emm-types are also frequently isolated in Belgium. The emm types emm 11, emm 22, emm75 and emm 87, 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.

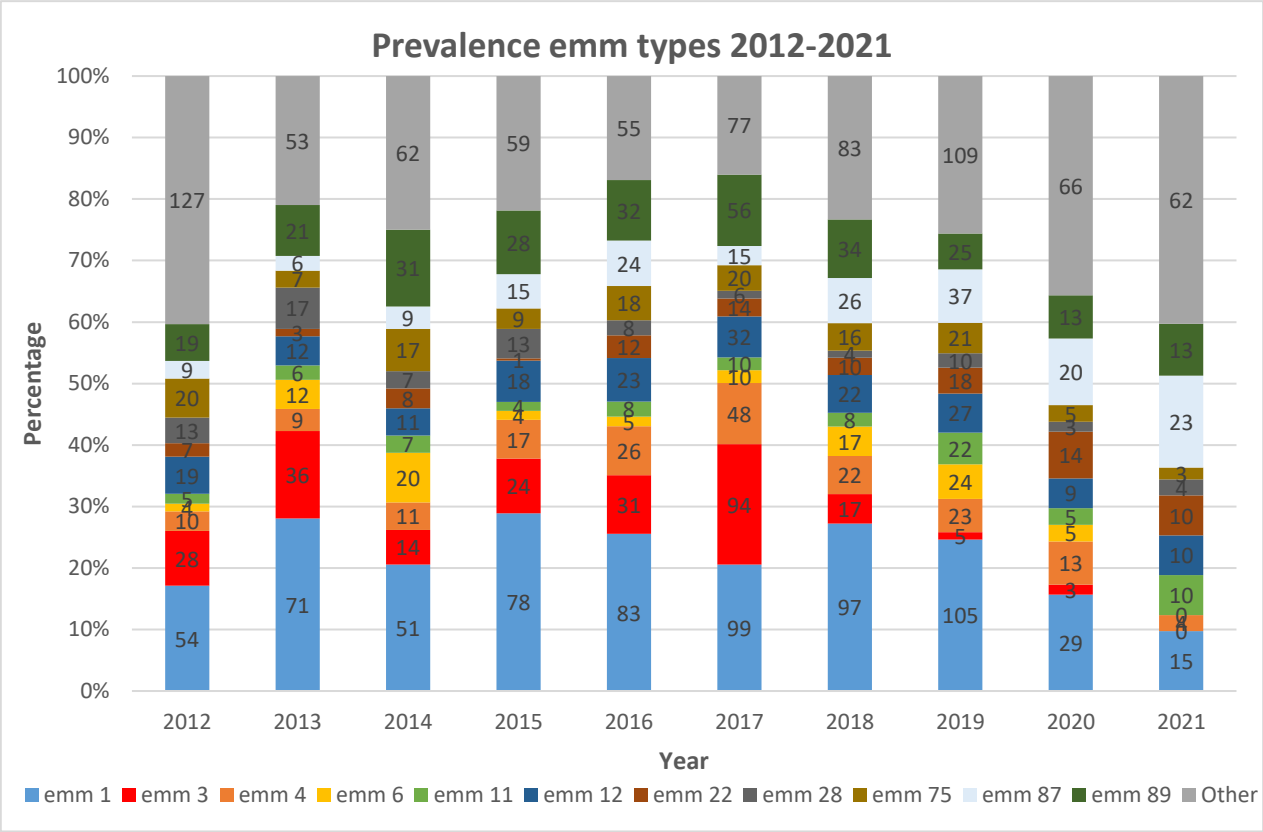

Figure 6: Prevalence of most common emm types from 2012 – 2021. Absolute numbers of isolates are presented in the bar of the graph

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