NETHERLANDS REFERENCE LABORATORY FOR BACTERIAL MENINGITIS

BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2021

Amsterdam UMC
University Medical Centers

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1 INTRODUCTION

This is the **50th** Annual Report of the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) of the Academic Medical Center (AMC) and the National Institute of Public Health and the Environment (RIVM). The NRLBM is located within the Department of Medical Microbiology and Infection Prevention of the Amsterdam UMC, location AMC in Amsterdam, The Netherlands. Nearly all Dutch clinical microbiology laboratories collaborate with the NRLBM by submitting bacterial isolates and/or cerebrospinal fluid samples from patients with meningitis as well as other invasive diseases and we are most grateful to our colleagues for their collaboration.

The NRLBM started collecting isolates of *Neisseria meningitidis* in 1959 and of other meningitis-causing bacteria in 1975. In the archives of the NRLBM approximately 91,750 isolates are now available for studies on the epidemiology of invasive bacterial infections, particularly bacterial meningitis, and on the pathogenicity and antibiotic susceptibility of isolates.

The objectives of the NRLBM are:
- to perform surveillance of bacterial meningitis and other invasive bacterial infections;
- to describe the (molecular) epidemiology of bacterial meningitis and select invasive bacterial infections in the Netherlands;
- to provide insights and leads for the development of potential vaccine components;
- to provide data about antibiotic susceptibility of isolates.

The information is presented in tables and figures and shortly discussed in the text.

We welcome your opinion and suggestions on this report.

Amsterdam, June, 2022

N.M. van Sorge, PhD, Professor | head of the NRLBM
Dr. W. Freudenburg, medical microbiologist

2 ISOLATES, CSF SPECIMENS AND SERA RECEIVED

The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) receives and collects isolates from cerebrospinal fluid (CSF) and blood from patients with proven meningitis (CSF and/or blood culture positive), bacteraemia and suspected meningitis (blood culture positive only), and patients with invasive disease with an isolate obtained from an otherwise sterile site (Figure 2.1). Unless otherwise indicated, every isolate from CSF represents a patient with meningitis, from CSF and blood a patient with meningitis and bacteraemia and from blood a patient with bacteraemia. When CSF is noted as the isolation source, this could indicate an isolate or positive PCR from CSF or CSF and blood. Incidences have been calculated by dividing the number of annually-received isolates (in a particular patient age group) by the number of inhabitants (within that same age group) multiplied by 100,000. Population statistics were obtained from Statistics Netherlands[1] using StatLine using 1 January as the reference date. By estimation, the NRLBM receives about 90% of the isolates from bacterial meningitis patients in the Netherlands[2].

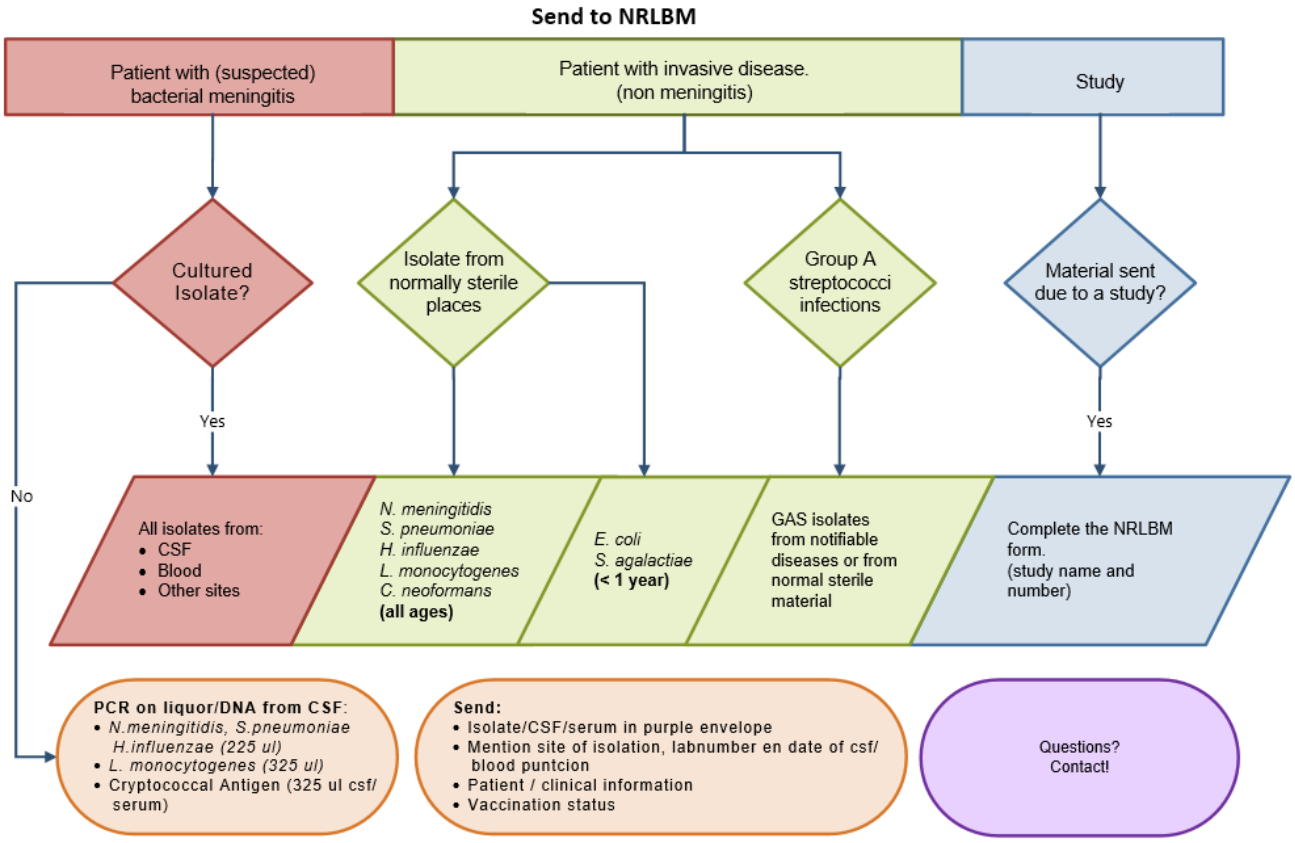


Figure 2.1. *Isolates and materials received by the NRLBM.*

[1] CBS - Statline Statistics Netherland www.cbs.nl

[2] Evaluation of the surveillance system for invasive meningococcal disease (IMD) in the Netherlands, 2004–2016; Brandwagt 2019

In 2021, the NRLBM received solates from CSF and/or blood and CSF and/or serum samples of 1,840 patients of which 1,766 were cultured or positive in antigen or PCR tests (**table 2.1/table 11.1**). Of all patients, 214 were culture- or PCR-confirmed cases of bacterial meningitis.

Table 2.1

	Number of specimens
Isolates (CSF and/or blood)	*1,735*
PCR- or antigen-positive samples of CSF, sera and other fluids	*31*
Total positive isolates and PCR- or antigen-positive samples	**1,766**
PCR- or antigen-negative samples of CSF, sera and other fluids	**74**
Total	**1,840**

In 2021, 49 clinical microbiology laboratories submitted isolates or samples to the NRLBM. Table 2.2 shows the received isolates or positive PCR samples from 1,766 patients according to the species and laboratory where cases were diagnosed. From 2003 onwards, the NRLBM requested nine sentinel laboratories, evenly distributed across the country and covering 28% of the Dutch population, to submit pneumococcal isolates from CSF and/or blood from patients of all ages.The nine sentinel laboratories are highlighted in orange in table 2.2.

Table 2.2 Number of isolates or PCR-positive samples from CSF and/or blood received in 2021, according to laboratory and bacterial species.

Location	Laboratory	Bacterial species[#]												
		Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	Nv	Total
Alkmaar	MCA lab. Med. Microbiologie	2	6	27	2	4	1	1	-	-	-	-	-	**43**
Amersfoort	Meander Medisch Centrum	-	5	19	1	3	6	-	-	-	-	-	-	**34**
Amsterdam	Amsterdam UMC	4	5	38	11	8	2	5	1	5	2	13	-	**94**
	Onze Lieve Vrouwe Gasthuis	2	2	36	-	8	2	10	-	-	-	-	-	**60**
	Slotervaart / ATAL	-	-	3	1	-	-	-	-	-	-	-	-	**4**
Apeldoorn	Gelre Ziekenhuizen	-	6	24	-	2	1	2	-	-	-	-	-	**35**
Arnhem	Rijnstate	1	4	22	-	1	-	4	-	-	-	-	-	**32**
Breda	Amphia Ziekenhuis	1	6	24	3	3	1	-	-	-	-	-	-	**38**
Capelle ad IJssel	IJsselland Ziekenhuis	-	1	4	-	1	2	-	-	-	-	-	-	**8**
Delft	Reinier Haga MDC	-	3	20	-	4	2	1	-	-	-	-	-	**30**
Den Bosch	Regionaal laboratorium Den Bosch	-	5	46	2	6	2	1	-	-	1	1	-	**64**
Den Haag	Haga Ziekenhuis, loc. Leyenburg	1	1	18	2	4	2	-	-	-	-	1	-	**29**
	MA Haaglanden, loc Westeinde	-	4	16	2	2	1	-	-	2	-	2	-	**29**
Deventer	Deventer Ziekenhuis	1	2	18	1	-	1	-	-	-	-	-	-	**23**
Doetinchem	Slingeland Ziekenhuis	-	5	12	-	2	3	1	-	-	-	-	-	**23**
Dordrecht	RLM Dordrecht / Gorinchem	2	9	26	1	8	1	2	-	-	-	-	-	**49**
Ede	Gelderse Vallei	-	1	35	3	4	1	-	-	-	-	1	-	**45**
Goes	Lab. v. Med.Microb. & Imm., ADRZ	-	2	14	-	2	-	-	-	-	-	-	-	**18**
Gouda	Groene Hart Ziekenhuis	-	2	14	3	1	-	-	-	-	-	-	-	**20**

Location	Laboratory	Bacterial species[#]												
		Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	Nv	Total
Groningen	Certe, Lab. v. Infectieziekten	-	12	49	2	3	3	2	-	-	1	-	-	**72**
	UMCG	1	2	8	2	1	1	-	-	-	-	-	-	**15**
Haarlem	Streeklab voor de Volksgezondheid	2	3	40	2	1	5	8	-	-	-	-	-	**61**
Harderwijk	St. Jansdal Ziekenhuis	1	5	12	1	3	-	-	-	-	-	-	-	**22**
Hengelo	LabMicTa	5	10	45	4	4	2	5	1	-	-	1	-	**77**
Hilversum	Centraal Bact. Ser. Lab.	1	2	12	2	1	1	-	-	-	-	-	-	**19**
Hoorn	Westfries gasthuis	1	4	39	2	5	3	-	-	-	-	-	-	**54**
Leeuwarden	Izore, centrum infectieziekten Friesland	-	4	54	5	7	-	7	-	-	1	1	-	**79**
Leiden	Alrijne ziekenhuis	-	4	22	3	2	-	-	1	-	-	-	-	**32**
	LUMC, KML, Lab.voor Bacteriologie	-	2	22	2	3	5	1	-	-	1	1	-	**37**
Maastricht	Acad. Ziekenhuis Maastricht	-	2	11	-	-	-	-	-	-	-	-	-	**13**
Nieuwegein	St. Antonius Ziekenhuis	-	7	31	1	4	1	6	-	-	-	2	-	**52**
Nijmegen	Canisius Wilhelmina Zknhs	1	3	19	-	-	1	-	-	-	-	-	-	**24**
	UMC St. Radboud	-	-	26	10	1	1	-	-	-	-	-	-	**38**
Neth Antilles	Medical Microbology, Curacao/St.Maarten	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Roermond	St. Laurentius Ziekenhuis	1	1	1	-	-	-	-	-	-	-	-	-	**3**
Roosendaal	Bravis Ziekenhuis	1	-	8	3	2	2	-	-	-	-	-	-	**16**
Rotterdam	Erasmus MC Med. Microbiologie	1	2	29	10	8	3	-	-	-	-	-	-	**53**
	Ikazia Ziekenhuis	-	1	7	-	4	1	-	-	-	-	-	-	**13**
	Maasstad Ziekenhuis	-	5	22	1	1	4	-	-	-	-	1	-	**34**
	St.Franciscus Gasthuis	2	3	19	-	2	2	1	-	-	-	-	-	**29**
Sittard	Zuyderland Medisch Centrum	-	1	22	-	4	2	8	-	-	1	1	-	**39**
Terneuzen	MICROVIDA, location Terneuzen	-	1	6	-	-	-	-	-	-	-	-	-	**7**
Tilburg	Streeklab. Tilburg	-	3	41	5	3	4	-	-	-	2	-	-	**58**
Utrecht	Diakonessenhuis	1	1	9	-	4	1	-	-	-	-	-	-	**16**
	St. Antonius	-	-	-	-	-	-	-	-	-	-	-	-	**-**
	UMC Med. Microbiologie	3	2	24	4	9	1	-	-	-	-	2	-	**45**
Veldhoven	PAMM, Lab. Med. Microbiologie	-	13	67	1	5	3	2	-	-	-	-	-	**91**
Venlo	Vie Curie medisch centrum	1	1	10	-	1	1	-	-	-	-	-	-	**14**
Zwolle	Isala Klinieken LMMI	1	4	45	8	6	5	4	-	-	-	1	-	**74**
Total		**37**	**167**	**1117**	**100**	**147**	**80**	**71**	**3**	**7**	**9**	**28**	**-**	**1766**

Nm: *N. meningitidis*; **Hi:** *H. influenzae*; **Sp:** *S. pneumoniae*; **Ec:** *E. coli*; **Sag:** *S. agalactiae*; **Lm:** *L. monocytogenes*; **Spy:** *S. pyogenes*; **Sau:** *S. aureus*; **Cns:** Coagulase-negative staphylococci; **Cn:** *C. neoformans*; **ot:** other bacteria; nv: non viable.

The distribution of the received isolates over the 5-year period 2017 - 2021 is presented in table 2.3. The total number of isolates increased from 2,118 in 2017 to 2,685 in 2019 (mainly due to changed submission criteria), which decreased to 1,766 isolates (~35%) in 2021. This decrease is likely attributable to the introduction of containment policies that were implemented in response to the COVID-19 pandemic. The decrease was particularly impressive for *N. meningitidis*, with a 76% decrease from 2019 to 2021 (157 isolates versus 37 isolates) and *S. pneumoniae* with 676 (38%) fewer isolates in 2021 versus 2019 . In contrast, the number of *H. influenzae* isolates only showed a 26% decrease, despite the fact that this pathogen is also transmitted via respiratory droplets. For neonatal pathogens, *E. coli* and *S. agalactiae*, no effect of the COVID-19 containment measures was observed compared to the previous years.

Table 2.3 Number of isolates from CSF and/or blood received in the years 2017 – 2021

Species	2017			2018			2019			2020			2021		
	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total
***N. meningitidis*[1]**	67	134	201	70	135	205	53	104	157	27	39	66	19	18	37
H. influenzae	30	194	224	23	216	239	23	203	226	21	181	202	24	143	167
S. pneumoniae	148	1255	1403	152	1757	1909	165	1628[2]	1793	108	1007	1115	87	1030	1117
E. coli	8	41	49	12	50	62	18	78	96	19	75	94	20	80	100
S. agalactiae	24	63	87	27	79	106	23	97	120	22	107	129	19	128	147
L. monocytogenes	20	71	91	9	56	65	26	81	107	11	70	81	12	68	80
S. pyogenes	7	11	18	3	9	12	14	122	136	5	112	117	2	69	71
S. aureus	5	0	5	7	0	7	11	0	11	10	0	10	3	0	3
Coag.neg.Staph.	6	0	6	4	0	4	2	0	2	4	1	5	7	0	7
C. neoformans	7	2	9	8	5	13	8	2	10	6	5	11	8	1	9
Others	9	13	22	18	7	25	16	11	27	16	22	38	13	15	28
non viable	0	3	3	0	7	7	0	0	0	0	1	1	0	0	0
Total	**331**	**1787**	**2118**	**333**	**2321**	**2654**	**359**	**2326**	**2685**	**249**	**1620**	**1869**	**214**	**1552**	**1766**

[1]Including PCR-positive patients
[2] 319 (2021) blood isolates from 9 sentinel labs
CSF: CSF or CSF and blood
blood: blood only

The incidence of invasive bacterial infections of the different bacterial species over the years 2017 to 2021 is shown in table 2.4. Incidences follow the number of received isolates/samples, i.e. incidence of *N. meningitidis* and *S. pneumoniae* decreased substantially from 2019 to 2021, whereas the incidence for *H. influenzae* decreased to a lesser extend. Incidences for *E. coli* and *S. agalactiae* invasive disease remained similar or increased, respectively.

Table 2.4 Incidence of invasive bacterial infections per species per 100,000 inhabitants, 2017 - 2021

Species	2017	2018	2019	2020	2021
N. meningitidis	1.18	1.19	0.91	0.38	0.21
H. influenzae	1.31	1.39	1.31	1.16	0.96
S. pneumoniae	8.21	11.11	10.37	6.41	6.39
E. coli	0.29	0.36	0.56	0.54	0.57
S. agalactiae	0.51	0.62	0.69	0.74	0.84
L. monocytogenes	0.53	0.38	0.62	0.47	0.46
S. pyogenes	0.11	0.07	0.79	0.67	0.41
S. aureus	0.03	0.04	0.06	0.06	0.02
Coag. neg. Staph.	0.04	0.02	0.01	0.03	0.04
C. neoformans	0.05	0.08	0.06	0.06	0.05
others	0.13	0.15	0.16	0.22	0.16
non viable	0.02	0.04	-	0.01	-
Total	**12.40**	**15.45**	**15.54**	**10.74**	**10.11**

Table 2.5 shows the distribution of isolates according to the source from which they were cultured. The top five species are comprised by *S. pneumoniae*, *H. influenzae, S. agalactiae, S. pyogenes, and E. coli*.

Table 2.5 Total number and proportion of isolates from CSF and/or blood received in 2021, according to bacterial species and source.

Species		CSF or CSF and blood, n	Blood only, n	Total, n	%
Neisseria meningitidis		19	18	37	**2.1**
Haemophilus influenzae [1, 2]		24	143	167	**9.5**
Streptococcus pneumoniae		87	1030	1117	**63.2**
Escherichia coli [3]		20	80	100	**5.7**
Streptococcus agalactiae		19	128	147	**8.3**
Listeria monocytogenes		12	68	80	**4.5**
Streptococcus pyogenes		2	69	71	**4.0**
Staphylococcus aureus		3	0	3	**0.2**
Coagulase-negative staphylococcus [4]		7	0	7	**0.4**
Cryptococcus neoformans[5]		8	1	9	**0.5**
Others total		13	15	28	**1.6**
Others	*Klebsiella aerogenes*	*1*	*0*	*1*	
	Pseudomonas aeruginosa	*2*	*0*	*2*	
	Salmonella species	*1*	*0*	*1*	
	Corynebacterium simulans	*1*	*0*	*1*	
	Enterobacter aerogenes	*1*	*0*	*1*	
	Serratia marcescens	*1*	*0*	*1*	
	Neisseria gonorrhoeae	*0*	*1*	*1*	
	Moraxella osloensis	*1*	*0*	*1*	
	Morganella morganii	*1*	*0*	*1*	
	Streptococcus dysgalactiae ssp equisimilis	*1*	*2*	*3*	
	Streptococcus gallolyticus	*0*	*1*	*1*	
	Streptococcus gallolyticus ssp gallolyticus	*0*	*3*	*3*	
	Streptococcus gallolyticus ssp pasteurianus	*0*	*2*	*2*	
	Streptococcus gordonii	*0*	*1*	*1*	
	Streptococcus infantis	*0*	*1*	*1*	
	Streptococcus intermedius	*1*	*0*	*1*	
	Streptococcus lutetiensis	*0*	*1*	*1*	
	Streptococcus mitis[6]	*0*	*1*	*1*	
	Streptococcus sanguinis	*0*	*1*	*1*	
	Cutibacterium acnes	*1*	*0*	*1*	
	Eggerthella lenta	*1*	*0*	*1*	
Non viable		0	0	0	**0**
Total		214	1552	1766	**100.0**

1 **In four patients, both *Haemophilus influenzae* and *Streptococcus pneumoniae* were isolated from blood.**

2 **In one patient, both *Haemophilus influenz*ae and *Streptococcus pyogenes* were isolated from blood.**

3 **In one patient, *Escherichia coli, Staphylococcus epidermidis* and *Streptococcus agalactiae* were isolated from blood together (0 months)**

4 **Coagulase-negative staphylococci, 5 *Staphylococcus epidermidis* were isolated from CSF; one*Staphylococcus capitis* and one *Staphylococcus caprae* were isolated from CSF**

5 **From the 9 Cryptococcus isolates, 8 *C. neoformans were isolated from CSF*, 1 *C. deneoformans* wasisolated from blood**

6 **In one patient, both *Streptococcus mitis* and *Streptococcus parasanguis* were isolated from blood**

3 BACTERIAL MENINGITIS – general overview

In 2021, the NRLBM received CSF isolates or PCR-positive CSF samples from 214 patients (Table 2.3 and 11.1). The proportion of meningococcal, pneumococcal, and haemophilus cases among meningitis patients was 9%, 41%, and 8%, respectively (Figure 3.1). The neonatal pathogens *S. agalactiae* and *E. coli* represented 8.9% and 9.4% of the meningitis cases, respectively (Figure 3.1)

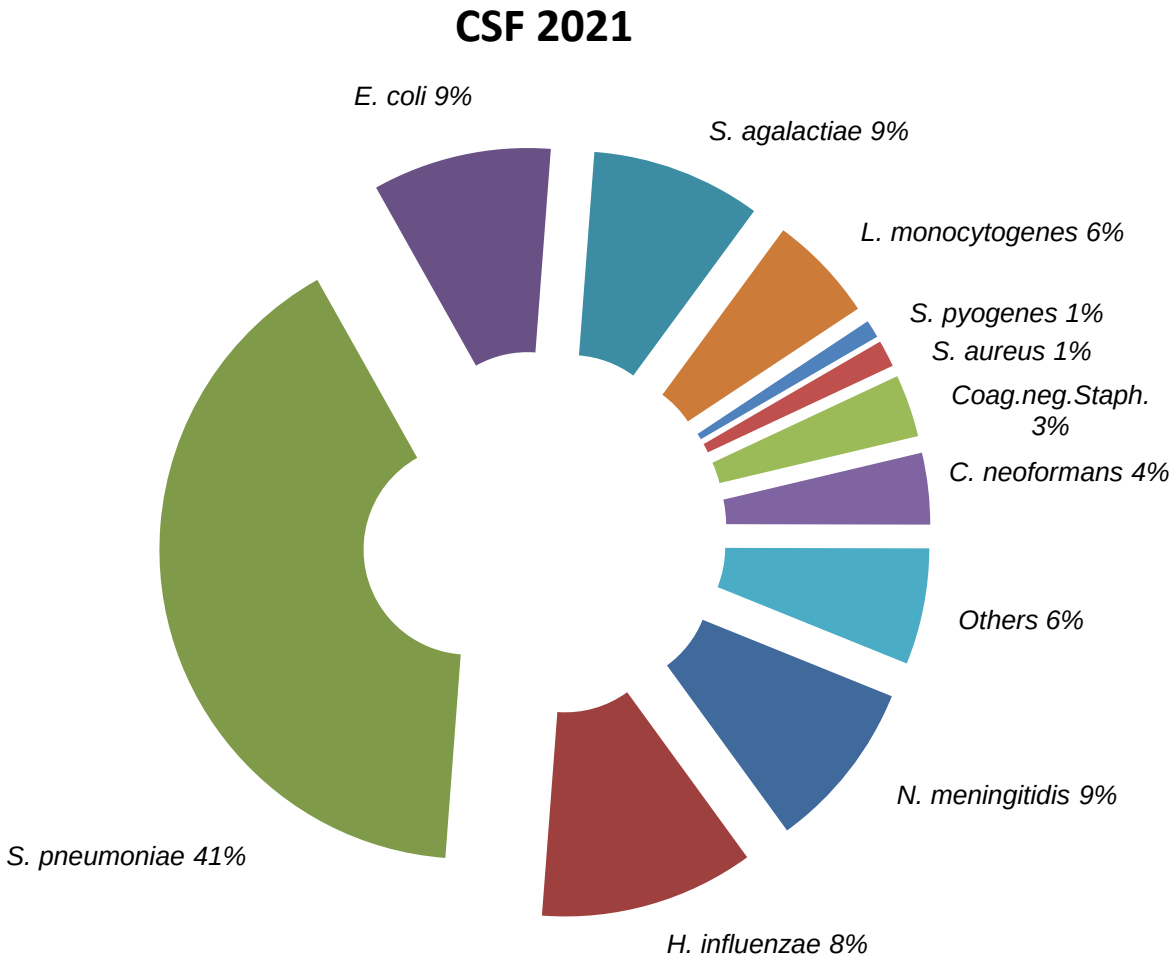


Figure 3.1 *Proportional distribution of CSF isolates and CSF-positive samples according to bacterial species, 2021*

Figure 3.2 shows the total annual number of bacterial isolates from CSF during the period 1991-2021. The trend line shows a decrease over the last three decades. The incidence per 100,000 inhabitants has stabilized around 2.0 from approximately 2010 until 2019, and further decreased to 1.2 in 2021 likely as a result of COVID-19 containment measures (Figure 3.2). Bacterial meningitis cases over the same 30-year period according to specific species, i.e. *N. meningitidis, H. influenzae* and *S. pneumoniae*, are presented in figure 3.3. Comparing meningitis incidence pre- and post-vaccination, the incidence of *Haemophilus* meningitis decreased from 1.6 per 100,000 in 1992 to 0.14 per 100,000 in 2021 and has remained at this low level. For meningococcal meningitis, the incidence decreased from 3.1/100,000 in 1993 to 0.10/100,000 in 2021, as a result of a decline in the number of cases caused by serogroups

B, C and W meningococci. The rapid decline in meningococcal meningitis around 2002 is largely attributed to nationwide vaccination against serogroup C, which started in 2002 and immediate showed an effect in 2003. After an increase in meningococcal meningitis between 2016 and 2018 as a result of MenW, the number of of meningococcal meningitis cases decreased again to 19 in 2021. This is likely the result of two events; the introduction of the MenACWY vaccine in the National Immunisation Programme as of 1 May 2018 and the COVID-19 containment measures in 2020. Pneumococcal meningitis showed a slight increase in annual incidence between 1991 and 2004 from 1.0 to 1.6 per 100,000 inhabitants. The introduction of the 7-valent conjugated polysaccharide vaccine (PCV-7) against pneumococci for children in the National Immunisation Programme in June 2006, and the switch to 10-valent (PCV-10) in 2011, decreased the incidence of pneumococcal meningitis to 0.95 per 100,000 in 2019. In 2021, the incidence of pneumococcal meningitis further decreased to 0.50 per 100,000 inhabitants, likely as a result of COVID-19 containment measures.

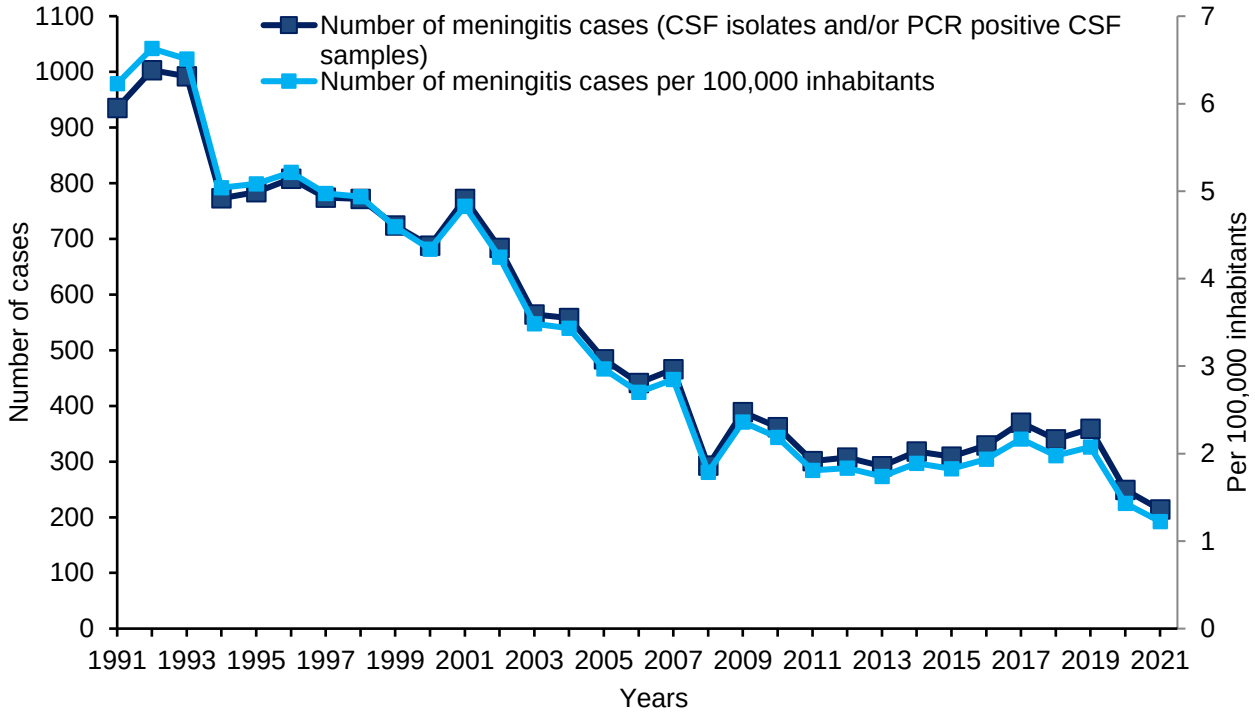


Figure 3.2 *All cause meningitis cases and incidence, 1991-2021*

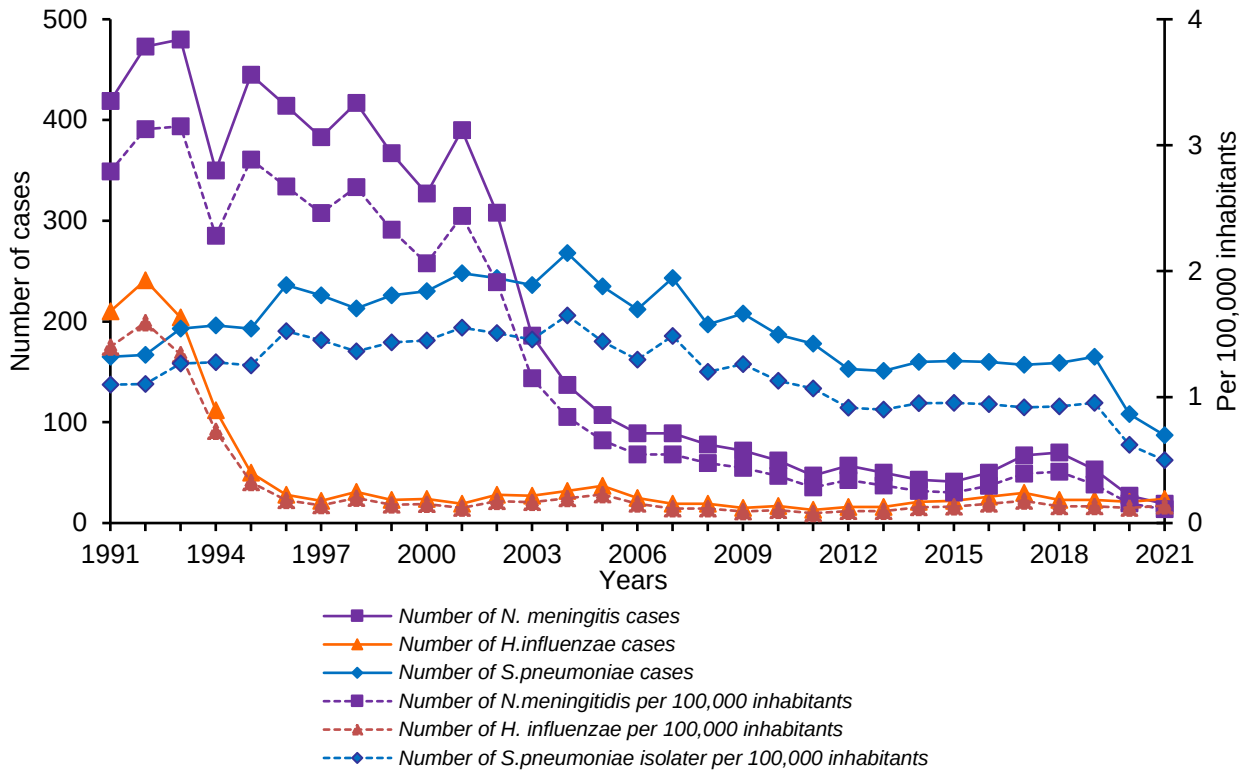


Figure 3.3 Number of cases and incidence of *meningococcal, haemophilus and pneumococcal meningitis (isolates and/or positive PCR from CSF), 1991-2021*

Table 3.1 shows the number of CSF isolates by annual quarter grouped by bacterial species. In contrast to previous years, most isolates were received during the second and fourth quarter of the year instead of the first quarter of the year. Again this is likely a result of COVID-19 containment measures, which varied in stringency at different times of the year.

Table 3.1 Isolates and PCR-positive samples from CSF by annual quarter according to bacterial species, 2021

	ANNUAL QUARTER					
SPECIES	**First**	**Second**	**Third**	**Fourth**	**Total**	**%**
N. meningitidis	4	3	7	5	**19**	***8.9***
H. influenzae	4	10	3	7	**24**	***11.2***
S. pneumoniae	10	24	15	38	**87**	***40.7***
E. coli	5	3	2	10	**20**	***9.4***
S. agalactiae	5	5	2	7	**19**	***8.9***
L. monocytogenes	5	3	4	0	**12**	***5.6***
S. pyogenes	1	0	1	0	**2**	***0.9***
S. aureus	1	1	0	1	**3**	***1.4***
Coag.neg.Staph.	2	2	0	3	**7**	***3.3***
C. neoformans	2	3	3	0	**8**	***3.7***
Others	6	2	2	3	**13**	***6.1***
non viable	0	0	0	0	**0**	***0***
Total	**45**	**56**	**39**	**74**	**214**	***100.0***
%	***21***	***26***	***18***	***35***	***100.0***	

Tables 3.2 and 3.3 show the distribution of bacterial species isolated from CSF according to patient age and the age-specific incidence per 100,000 individuals, respectively. *S. agalactiae* and *E. coli* are still the predominant species isolated from neonates (i.e. younger than 1 month), and together represented 83% of all isolates in this age group. In contrast, in infants 1-11 months of age, the predominant species were *H. influenzae* and *S. pneumoniae* (together 63%). Since the introduction of the *H. influenzae* b vaccine in 1993, the number of *H. influenzae* b meningitis cases in the age group 0-4 year has strongly decreased, from 231 in 1992 to 10 in 2021. Overall, for children ages 0-4 years, *S. pneumoniae* was the predominant cause of bacterial meningitis, representing 24.4% of all cases in this age group

Table 3.2 Isolates and PCR-positive samples from CSF grouped according to patients' age, 2021

	AGE (MONTHS)			AGE (YEARS)									TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-64**	**65-79**	**≥80**	**Total, n**	**%**
N. meningitidis	0	3	4	7	0	2	7	1	0	0	0	2	0	**19**	***8.9***
H. influenzae	2	13	0	15	1	0	0	0	2	0	5	1	0	**24**	***11.2***
S. pneumoniae	0	13	7	20	2	2	1	4	13	9	19	15	2	**87**	***40.7***
E. coli	12	4	0	16	0	0	0	0	0	1	2	1	0	**20**	***9.4***
S. agalactiae	13	5	0	18	0	0	0	0	1	0	0	0	0	**19**	***8.9***
L. monocytogenes	0	0	0	0	0	0	0	0	0	0	7	4	1	**12**	***5.6***
S. pyogenes	0	1	0	1	1	0	0	0	0	0	0	0	0	**2**	***0.9***
S. aureus	0	0	0	0	0	0	0	0	2	0	1	0	0	**3**	***1.4***
Coag.neg.Staph.	1	1	0	2	0	0	0	1	0	1	3	0	0	**7**	***3.3***
C. neoformans	0	0	0	0	0	0	0	0	1	0	5	2	0	**8**	***3.7***
Others	2	1	0	3	1	0	0	0	1	2	3	2	1	**13**	***6.1***
non viable	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0***
Total, n	*30*	*41*	*11*	*82*	*5*	*4*	*8*	*6*	*20*	*13*	*45*	*27*	*4*	**214**	***100***
%	***14.5***	***9.2***	***7.6***	***38.3***	***2.3***	***1.9***	***3.7***	***2.8***	***9.4***	***6.1***	***21.0***	***12.6***	***1.9***	***100***	

As anticipated from table 3.2, the incidence of all-cause bacterial meningitis was highest in the 0-11 month age group (table 3.3) with 42.19 cases per 100,000. The overall incidence of bacterial meningitis decreased from 2.08 in 2019 to 1.22 per 100,000 in 2021.

Table 3.3 Age-specific incidence of bacterial meningitis per 100,000 inhabitants according to bacterial species, 2021

	AGE (YEARS)											
SPECIES	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-64**	**65-79**	**≥80**	Total
N. meningitidis	1.78	0.58	-	0.21	0.68	0.04	-	-	-	0.08	-	0.11
H. influenzae	8.91	-	0.11	-	-	-	0.09	-	0.14	0.04	-	0.14
S. pneumoniae	7.73	1.02	0.22	0.21	0.10	0.18	0.60	0.42	0.52	0.57	0.24	0.50
E. coli	9.51	-	-	-	-	-	-	0.05	0.05	0.04	-	0.11
S. agalactiae	10.70	-	-	-	-	-	0.05	-	-	-	-	0.11
L. monocytogenes	-	-	-	-	-	-	-	-	0.19	0.15	0.12	0.07
S. pyogenes	0.59	-	0.11	-	-	-	-	-	-	-	-	0.01
S. aureus	-	-	-	-	-	-	0.09	-	0.03	-	-	0.02
Coag.neg.Staph.	1.19	-	-	-	-	0.04	-	0.05	0.08	-	-	0.04
C. neoformans	-	-	-	-	-	-	0.05	-	0.14	0.08	-	0.05
Others	1.78	-	0.11	-	-	-	0.05	0.09	0.08	0.08	0.12	0.07
non viable	-	-	-	-	-	-	-	-	-	-	-	-
Total	**42.19**	**1.60**	**0.56**	**0.42**	**0.78**	**0.27**	**0.92**	**0.60**	**1.22**	**1.03**	**0.48**	**1.22**

Table 3.4 shows the number of CSF isolates per species according to patient gender. For most species the Male/Female ratio varied between 1 and 2, except for *E. coli, S. agalactiae, L. monocytogenes, C. neoformans* and "other", which affected males more than twice as often as females and for *H. infleunzae* nearly twice as many females than males were affected. The overall M/F ratio was 1.5.

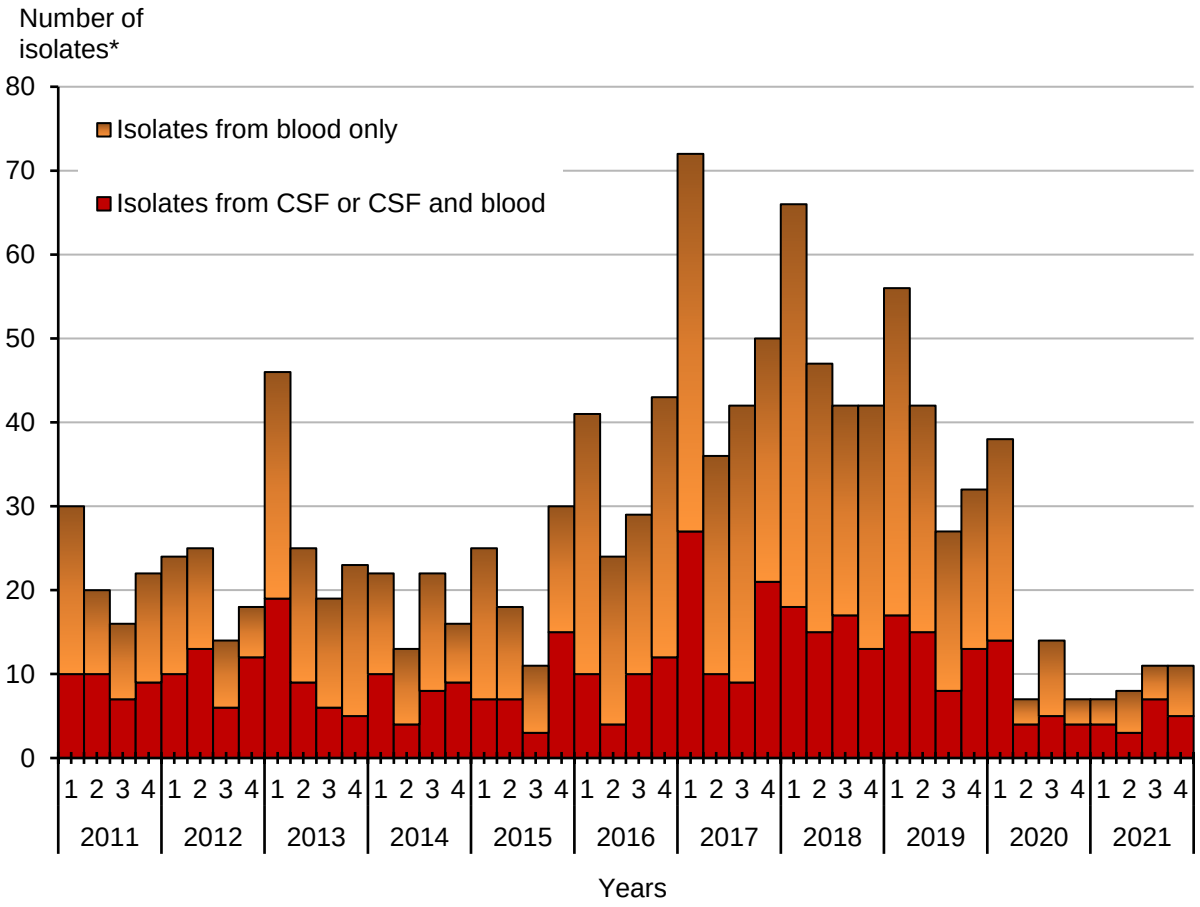
Table 3.4 Isolates and PCR positive samples from CSF according to patients' gender, 2021

SPECIES	M	F	M/F – ratio	Sex not known	Total
N. meningitidis	11	6	**1.8**	2	**19**
H. influenzae	9	15	**0.6**	-	**24**
S. pneumoniae	45	40	**1.1**	2	**87**
E. coli	15	5	**3.0**	-	**20**
S. agalactiae	13	5	**2.6**	1	**19**
L. monocytogenes	11	1	**11.0**	-	**12**
S. pyogenes	1	1	**1.0**	-	**2**
S. aureus	2	0	**-**	1	**3**
Coag.neg.Staph.	3	4	**0.8**	-	**7**
C. neoformans	6	2	**3.0**	-	**8**
Others	10	3	**3.3**	-	**13**
non viable	0	0	**-**	-	**0**
Total	**126**	**82**	**1.5**	**6**	**214**
%	***58.9***	***38.3***		***2.8***	***100***

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2021, the NRLBM received 25 *Neisseria meningitidis* isolates of which 7 were isolated from CSF (or CSF and blood; 15 in 2020) and 18 from blood only (39 in 2020). In addition, 12 culture-negative CSF samples tested positive for meningococci by PCR bringing the total number of received meningococcal isolates or PCR-positive CSF or blood to 37. The distribution of isolates received throughout the year was different from previous years in that the number of isolates decreased greatly in the second quarter of 2020 and remained low throughout 2021 (figure 4.1).



* Number of isolates; culture or PCR-positive CSF or blood samples.

Figure 4.1 *Seasonal distribution of meningococcal disease, 2011-2021*

4.2 Antibiotic susceptibility

All isolates (25/25) were susceptible to penicillin according to the new EUCAST break point (MIC ≤ 0.25 µg/ml; Table 4.1). Resistance to penicillin is therefore even less prevalent than in previous years (Tables 4.2, 4.3) The proportion of penicillin susceptible and penicillin resistant isolates was similar for isolates from blood and CSF (Tables 4.2, 4.3). In general, mutations in *penA,* encoding a penicillin binding protein, confer meningococcl with reduced penicillin susceptibility. All isolates were susceptible to Rifampicine.

Table 4.1 Penicillin susceptibility [3] of all received *N. meningitidis* isolates according to source of isolation (CSF and/or blood), 2021

Penicillin*				
	MIC ≤ 0.25 (S)	**MIC>0.25 (R)**	**Total**	**%**
CSF or CSF and blood	7	0	**7**	***28***
Blood only	18	0	**18**	***72***
Total	**25**	**0**	**25**	***100***
%	***100***	***0***	***100***	

* MIC values in µg/ml

Table 4.2 Penicillin susceptibility of *N. meningitidis* isolates from CSF, 2017-2021

Penicillin*					
	MIC ≤ 0.25 (S)		**MIC>0.25 (R)**		**Total**
	N	%	N	%	
2017	46	*100*	0	*0*	**46**
2018	53	*98*	1	*2*	**54**
2019	33	*100*	0	*0*	**33**
2020	14	*93*	1	*7*	**15**
2021	7	*100*	0	*0*	**7**

* MIC values in µg/ml

Table 4.3 Penicillin susceptibility of *N. meningitidis* isolates from blood only, 2017-2021

Penicillin*					
	MIC ≤ 0.25 (S)		**MIC>0.25 (R)**		**Total**
	N	%	N	%	
2017	128	*99*	1	*1*	**129**
2018	129	*98*	2	*2*	**131**
2019	102	*100*	0	*0*	**102**
2020	39	*100*	0	*0*	**39**
2021	18	*100*	0	*0*	**18**

* MIC values in µg/ml

[3] According to Eucast: https://eucast.org/clinical_breakpoints/

4.3 Serogroups

Serogroup B accounted for 84% of all received isolates / PCR-positive samples (Table 4.4), which is an increase compared to previous years (2020 61%; 2019 46%). However, observations across the entire collection period 1959 - 2021 (figure 4.2) show that the number of serogroup B isolates in 2021 was the lowest (31 cases) in 60 years. The proportion of serogroup W isolates decreased to 11% (table 4.4) compared to 18% in 2020, 38% in 2019, 50% in 2018, 41% in 2017, 34% in 2016 and 10% in 2015 (figure 4.2). Also in absolute numbers, the NRLBM received less serogroup W isolates (n = 4) compared to the previous 3 years (Figure 4.2). This reduction in meningococcal W cases is likely a result from the (catch-up) vaccination campaigs with MenACWY, the implementation of the MenACWY vaccine in the National Immunisation Programme as of 1 May 2018, as well as the COVID-19 containment measures in 2020 and 2021. Because meningococcal serogroup W also affects older children and because meningococcal carriage is highest in this age group, the vaccination has also been offered to teenagers in the year of their 14th birthday, as of 1 October 2018. The MenACWY vaccine was introduced to the vaccination program to counter an outbreak of meningococcal W between 2016-2018 and replaced the MenC vaccine.

Serogroup Y was responsible for 2.7% of all cases of invasive meningococcal disease in 2021 (Table 4.4). Similar to 2020, no serogroup C was isolated from CSF or blood in 2021, which had not been observed in the past 60 years of surveillance by the NRLBM. Both the proportion as well as absolute number of serogroup C isolates increased between 1991 and 2001 from approximately 10% in 1994 (66 cases) to 19% (105 cases) in 2000 and 40% (276 cases) in 2001 (figure 4.2). After implementation of the serogroup C vaccine in the National Immunisation Program in June 2002, a rapid decline and near eradication of MenC disease was observed. Overall, serogroups B and W have the highest incidence of invasive meningococcal disease (Table 4.5). Cases of invasive meningococcal disease are evenly distributed across the Netherlands (Figure 4.3).

Table 4.4 Number of meningococcal isolates according to serogroup and source of isolation, 2021

Source	Serogroup				Total, n
	B	W	Y	-*	
CSF	18	0	0	1	19
Blood	13	4	1	0	18
Total (%)	**31 (83.8)**	**4 (10.8)**	**1 (2.7)**	**1 (2.7)**	**37**

*Not enough DNA for group PCR

Table 4.5 Incidence of meningococcemia per 100,000 inhabitants according to serogroup and source of isolation, 2021

Source	Serogroup				Total, n
	B	W	Y	-*	
CSF	0.10	0.00	0.00	0.01	0.11
Blood	0.07	0.02	0.00	0.00	0.10
Total	**0.18**	**0.02**	**0.00**	**0.01**	**0.21**

*Not enough DNA for group PCR

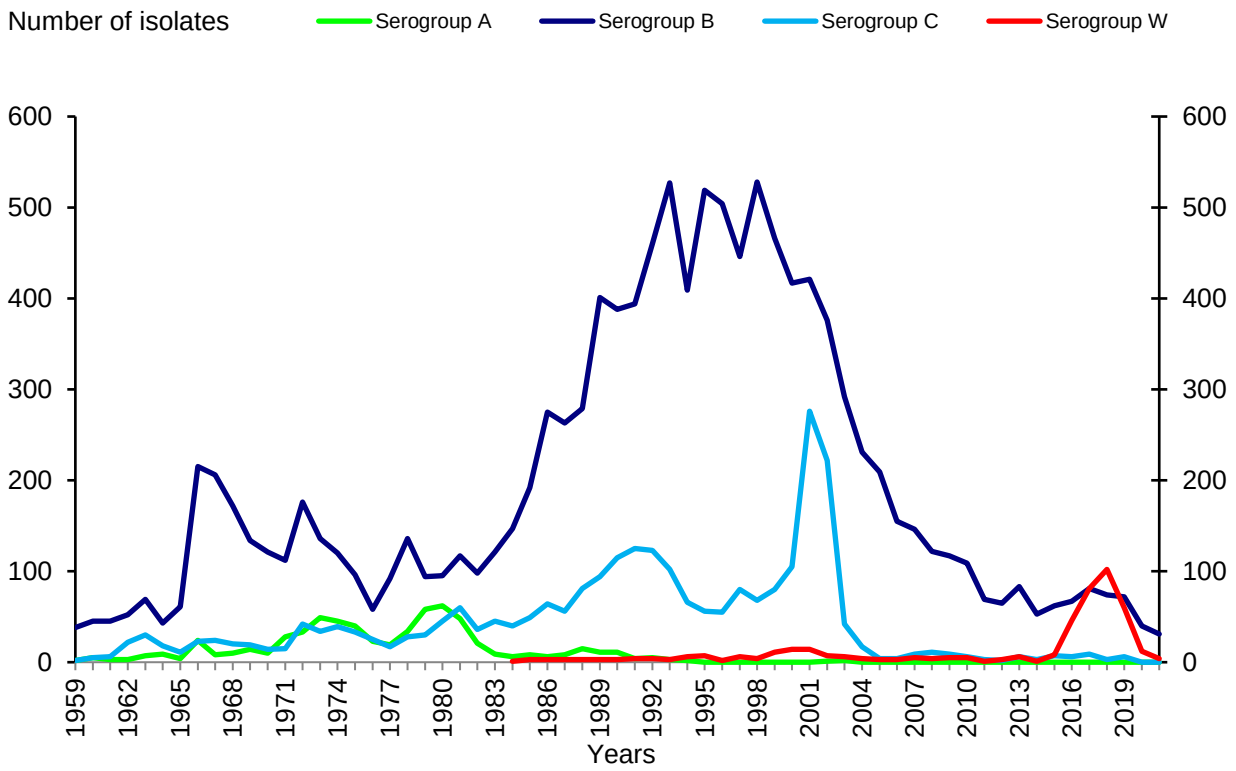


Figure 4.2. *Distribution of meningococcal serogroups A, B, C and W from 1959-2021.*

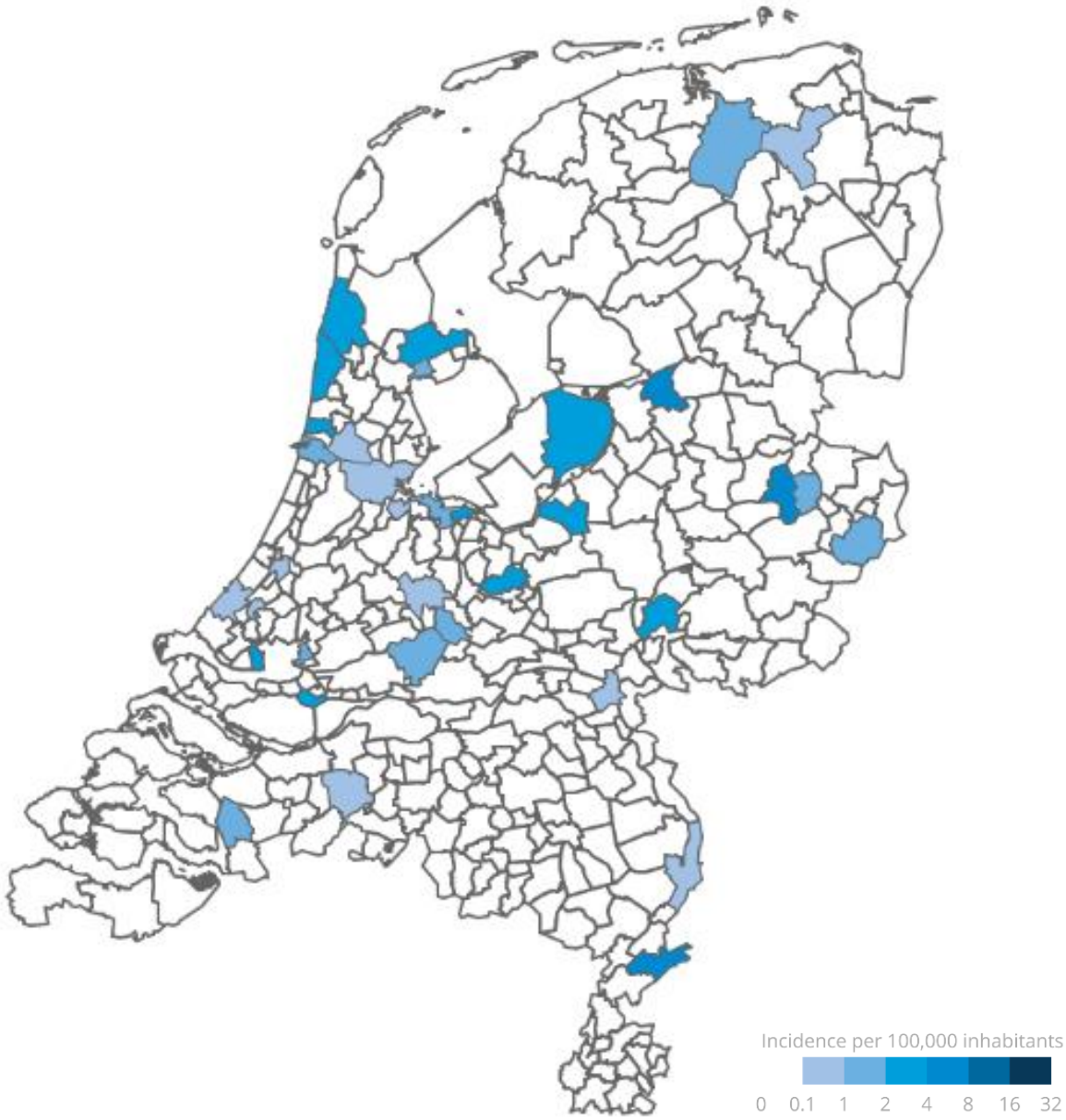


Figure 4.3 Geographical distribution of *N. meningitidis* cases based on incidence, *2021*. Incidence is calculated per municipality based on patient's place of residence.

4.4 Serogroup and age

Among serogroup B cases, 38% (14 of 37) of patients was below the age of 5 years and 84% (26/31) was between 0 and 25 years of age (table 4.6). In contrast, all cases caused by serogroup W were older than 25 years of age (table 4.6). In addition, 43% of serogroup B isolates (13/31) were isolated from CSF, compared to none of serogroup W isolates, suggesting that the clinical presentation and population at risk for infection may be different for serogroup B and W meningococci. Overall, the incidence of invasive meningococcal disease is highest in the age groups 0-4 and 15-19 with dominant contribution of serogroup B (table 4.7). Currently, the available MenB vaccines (Bexsero and Trumemba) are not included in the National Immunisation Programme.[4]

Table 4.6 Serogroups of *N. meningitidis* (isolates or PCR-positive samples from CSF and /or blood; absolute numbers) according to patient's age, 2021

	AGE (MONTHS)			AGE (YEARS)									TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	**n**	**%**
B	0	6	8	14	0	2	9	1	1	0	1	3	**31**	***83.8***
CSF	*0*	*3*	*4*	*7*	*0*	*2*	*7*	*1*	*0*	*0*	*0*	*1*	*18*	*48.6*
Blood	*0*	*3*	*4*	*7*	*0*	*0*	*2*	*0*	*1*	*0*	*1*	*2*	*13*	*35.2*
W	0	0	0	0	0	0	0	0	1	1	0	2	**4**	***10.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*0*	*2*	*4*	*10.8*
Y	0	0	0	0	0	0	1	0	0	0	0	0	**1**	***2.7***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*1*	*2.7*
-*	0	0	0	0	0	0	0	0	0	0	0	1	**1**	***2.7***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*2.7*
Total	0	6	8	**14**	**0**	**2**	**10**	**1**	**2**	**1**	**1**	**6**	**37**	***100.0***
CSF	*0*	*3*	*4*	***7***	***0***	***2***	***7***	***1***	***0***	***0***	***0***	***1***	***18***	***48.6***
Blood	*0*	*3*	*4*	***7***	***0***	***0***	***3***	***0***	***2***	***1***	***1***	***5***	***19***	***51.4***
%	***0***	***16.2***	***21.6***	***37.8***	***0***	***5.4***	***27.1***	***2.7***	***5.4***	***2.7***	***2.7***	***16.2***	***100.0***	

*Not enough DNA for serogroup PCR

[4] Gezondheidsraad. Vaccinatie tegen meningokokken. Den Haag: Gezondheidsraad, 2018; publicatienr. 2018/28
Werkagenda advisering Vaccinaties Gezondheidsraad 2022 (vierde kwartaal MenB)

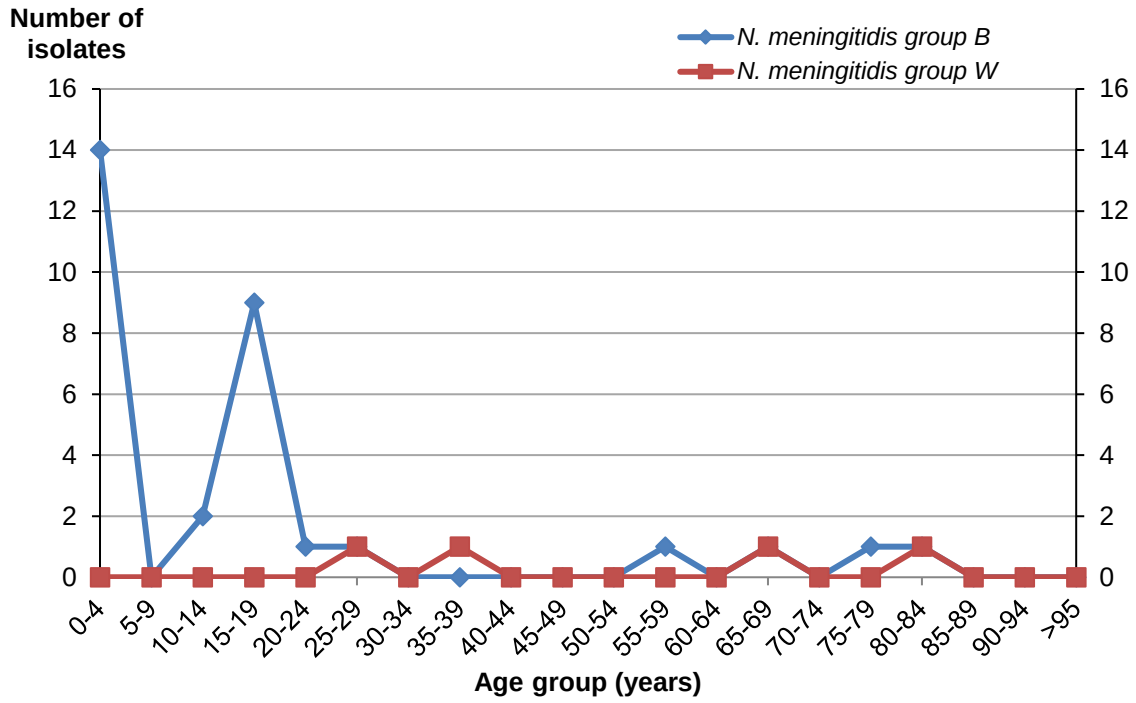
Table 4.7 Incidence of meningococcemia per 100,000 inhabitants according to different meningococcal serogroups and patient's age, 2021

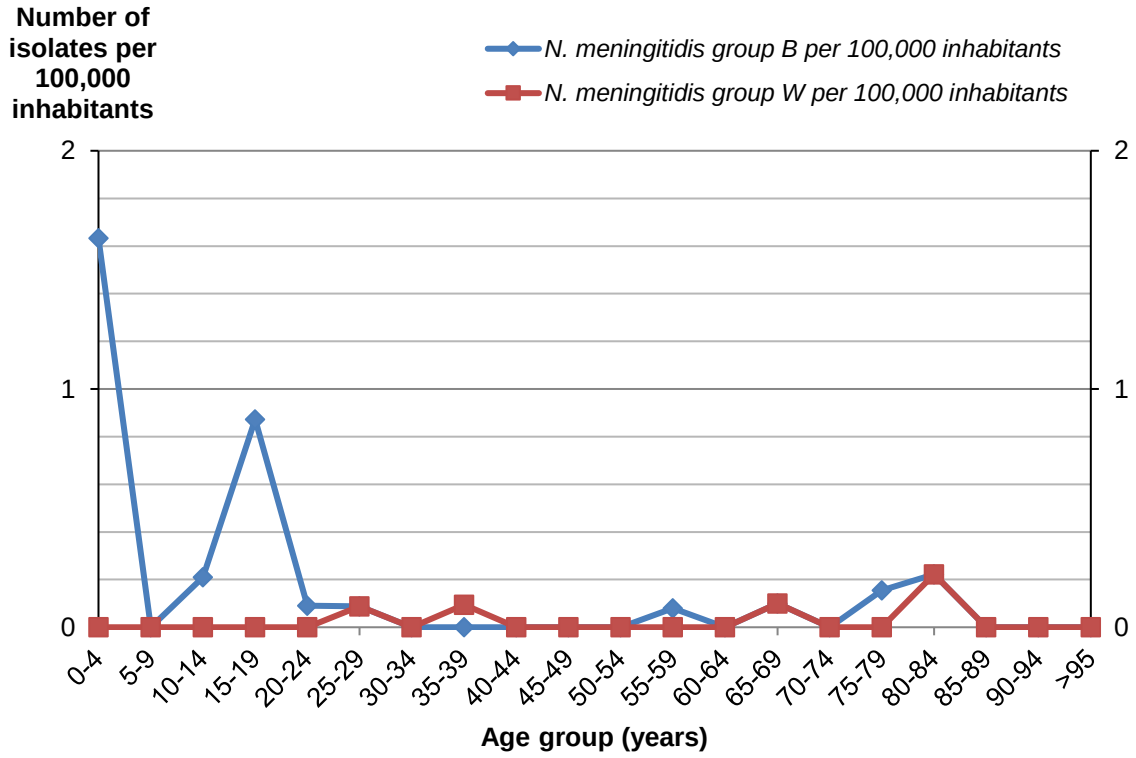
	AGE (YEARS)										TOTAL
Group	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	
B	3.57	1.16	0.00	0.21	0.87	0.09	0.09	0.00	0.03	0.09	**0.18**
CSF	*1.78*	*0.58*	*0.00*	*0.21*	*0.68*	*0.09*	*0.00*	*0.00*	*0.00*	*0.03*	***0.10***
Blood	*1.78*	*0.58*	*0.00*	*0.00*	*0.19*	*0.00*	*0.09*	*0.00*	*0.03*	*0.06*	***0.08***
W	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.02	0.00	0.06	**0.02**
CSF	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	***0.00***
Blood	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.09*	*0.02*	*0.00*	*0.06*	***0.02***
Y	0.00	0.00	0.00	0.00	0.10	0.00	0.0	0.00	0.00	0.00	**0.01**
CSF	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	***0.00***
Blood	*0.00*	*0.00*	*0.00*	*0.00*	*0.10*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	***0.00***
-*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	**0.01**
CSF	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	***0.00***
Blood	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.03*	***0.01***
Total	**3.57**	**1.16**	**0.00**	**0.21**	**0.97**	**0.09**	**0.18**	**0.02**	**0.03**	**0.17**	**0.21**
CSF	***1.78***	***0.58***	***0.00***	***0.21***	***0.68***	***0.09***	***0.00***	***0.00***	***0.0***	***0.03***	***0.10***
Blood	***1.78***	***0.58***	***0.00***	***0.00***	***0.29***	***0.00***	***0.18***	***0.02***	***0.03***	***0.14***	***0.11***

* Not enough DNA for serogroup PCR

Figure 4.5 shows the age distribution of patients with meningococcal disease caused by serogroups B and W. The age-specific incidence for serogroup B per 100,000 inhabitants in the age groups 0-4 years of age and 15 - 19 years years of age were 1.63 and 0.87, respectively (Figure 4.5B and Table 4.7). The age-specific incidence per 100,000 inhabitants for all age groups >19 years was below 0.25 (Table 4.7, Figure 4.5B). The age-specific incidence for serogroup W shows a different distribution compared to serogroup B (Figure 4.5B).

A





B

Figure 4.5 *A) Number of isolates and B) incidence of meningococcal disease* per 100,000 inhabitants *caused by serogroup B and W according to age groups, 2021*

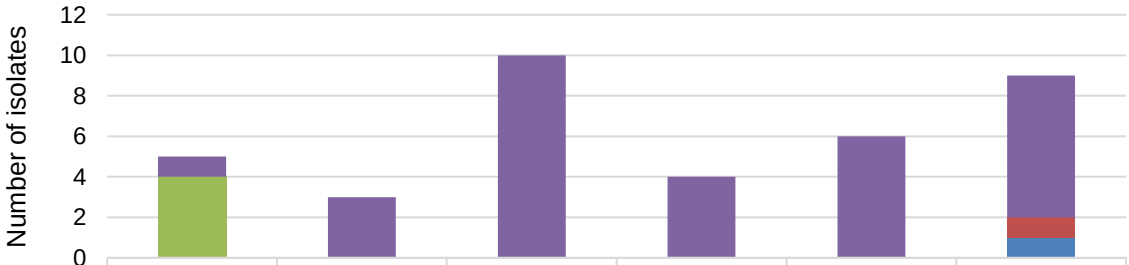
4.5 Distribution of PorA and FetA genosubtypes among meningococci

4.5.1 PorA

In addition to serogrouping, meningococci can be further subtyped based on the variation in PorA and FetA proteins. Previously, subtyping was performed using specific monoclonal antibodies for these proteins. However, from January 1, 2005, the NRLBM replaced antibody-based subtyping of PorA and FetA with molecular methods, i.e. DNA-sequencing of PorA and FetA DNA coding regions, due to discontinuation of the monoclonal antibodies.

The PorA epitopes that react with the monoclonal antibodies of the subtyping scheme are encoded by the *porA* variable regions VR1 and VR2. Since 2005, we routinely sequence the DNA regions which encode the VR1 and VR2 regions of *porA* of all meningococcal isolates. The DNA sequences are translated into putative amino acid sequences and compared with *porA* epitopes present in the PubMLST database https://pubmlst.org/neisseria/PorA/ (PubMLST – PorA typing[5])(PubMLST - PorA typing, sd). As an example for a PorA notation, (VR1,VR2): P1.7,4, in which VR1 is P1.7 indicates the VR1 region and the second P1.4 indicates the VR2 region, resulting in the combination P1.7,4.

In 2021, the NRLBM received 25 isolates and 12 PCR-positive samples. Of the culture-negative samples, nine could be completely subtyped. Overall, 16 different VR1/VR2 combinations were encountered among 31 serogroup B meningococci (2020: 25 different combinations; 2019: 30 different combinations; 2018: 37 different combinations). The proportion of dominant PorA genosubtypes has shifted tremendously in the last two decades: in 2000, genosubtype P1.7-2.4 represented 40% of all serogroup B isolates and gradually declined to only 10% in 2021 (table 4.8). In 2021, P1.22,14 was the most abundant genosubtype with 10 out of 31 isolates (32%; Figure 4.6). Approximately 84% (26/31 isolates) of the serogroup B isolates had at least one of the PorA epitopes present in the NonaMen vaccine currently in development (Table 4.8). All four serogroup W isolates, showed the same PorA VR1/VR2 combination P1.5.2 (100%; Figure 4.6)



	P1.5,2	P1.18-1,xx	P1.22,14	P1.22,xx	P1.7-2,xx	Other
B	1	3	10	4	6	7
W	4					
Y						1
NGD*						1

* No Group Detectable; Not enough DNA for serogroup PCR

Figure 4.6 *Distribution of PorA genosubtypes among all received meningococcal cases, 2021*

[5] *PubMLST - PorA typing*. Public databases for molecular typing: https://pubmlst.org/neisseria/PorA/

Table 4.8 PorA genosubtype distribution of *N. meningitidis* serogroup B isolates from 2017-2021 and hypothetical coverage by NonaMen vaccine.

	VR1.VR2 combination	YEAR									
		2017		2018		2019		2020		2021	
		No.	%	No.	%	No.	%	No.	%	No.	%
Vaccine types*	1.5-1, 2-2	1	**1.4**	0	**0.0**	0	**0.0**	1	**2.5**	0	**0.0**
	1.5-1, other	3	**4.2**	2	**2.7**	0	**0.0**	0	**0.0**	0	**0.0**
	1.5-2.10	2	**2.9**	0	**0.0**	1	**1.4**	0	**0.0**	0	**0.0**
	1.5-2, other	2	**2.9**	1	**1.3**	0	**0.0**	2	**5.0**	0	**0.0**
	1.7,16	1	**1.4**	1	**1.3**	1	**1.4**	1	**2.5**	0	**0.0**
	1.7, other	3	**4.2**	1	**1.3**	1	**1.4**	3	**7.5**	1	**3.2**
	1.7-1, 1	0	**0**	0	**0.0**	2	**2.8**	1	**2.5**	0	**0.0**
	1.7-1, other	1	**1.4**	0	**0.0**	1	**1.4**	0	**0.0**	0	**0.0**
	1.7-2,4	5	**7.0**	3	**4.1**	8	**11.1**	4	**10.0**	3	**9.7**
	1.7-2, other	2	**2.9**	6	**8.2**	11	**15.2**	5	**12.5**	3	**9.7**
	1.12-1,13	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.12-1, other	1	**1.4**	0	**0.0**	0	**0.0**	1	**2.5**	0	**0.0**
	1.18-1,3	0	**0.0**	1	**1.3**	0	**0.0**	0	**0.0**	0	**0.0**
	1.18-1, other	4	**5.6**	5	**6.8**	7	**9.7**	2	**5.0**	3	**9.7**
	1.19,15-1	1	**1.4**	1	**1.3**	1	**1.4**	1	**2.5**	0	**0.0**
	1.19, other	4	**5.6**	5	**6.8**	1	**1.4**	3	**7.5**	1	**3.2**
	1.22,14	21	**29.6**	20	**27.0**	19	**26.3**	2	**5.0**	10	**32.3**
	1.22,other	4	**5.6**	8	**10.8**	4	**5.6**	7	**17.5**	4	**12.9**
	Other, 1	0	**0.0**	0	**0.0**	1	**1.4**	1	**2.5**	0	**0.0**
	Other, 14	4	**5.6**	3	**4.1**	2	**2.8**	0	**0.0**	1	**3.2**
	Other, 16	2	**2.9**	2	**2.7**	3	**4.2**	1	**2.5**	0	**0.0**
	Subtotal vaccine types	61	**86.0**	59	**79.7**	63	**87.5**	35	**87.5**	26	**83.9**
NVT**	Other **Non Vaccine Type**	10	**14.0**	15	**20.3**	9	**12.5**	5	**12.5**	5	**16.1**
	Total	71	**100.0**	74	**100.0**	72	**100.0**	40	**100.0**	31	**100.0**

*based on a nonavalent PorA vaccine. NonaMen; serosubtypes P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1 and P1.18-1,3,6
**Non vaccine type

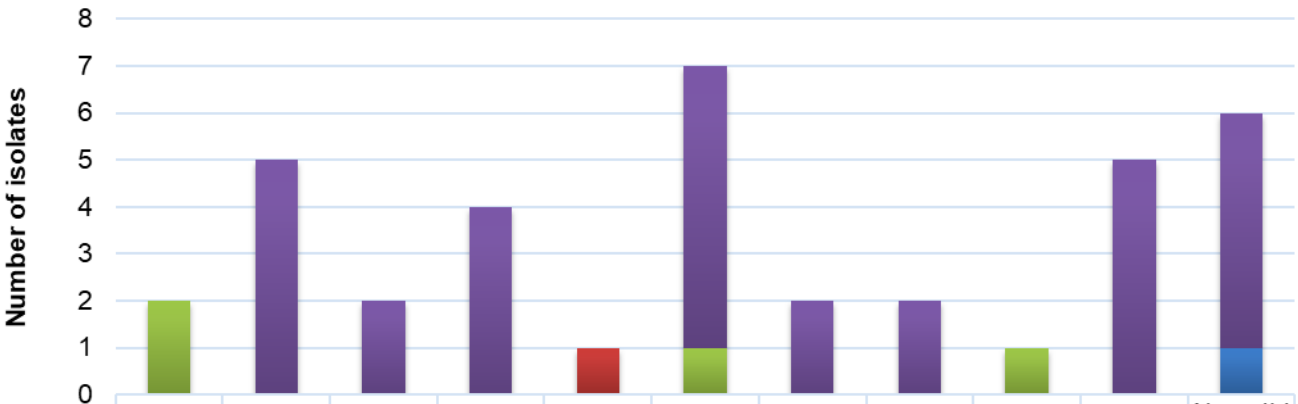
4.5.2 FetA

In addition to PorA epitope sequencing, meningococcal isolates are also characterized by FetA epitope sequencing. The outer membrane protein FetA is involved in iron uptake by meningococci and is considered as a potential vaccine component. Therefore, the variability of this protein has been investigated intensively. The most variable part of the protein, called VR, has been used to establish a typing scheme. Analogous to PorA typing, the VR part of *fetA* is sequenced and translated to a putative amino acid sequence. So far, approximately 270 VR sequences comprising 6 classes are identified, which are available at https://pubmlst.org/neisseria/FetA/. (PubMLST)[6]. As an example of a type designation: F5-2, in which the first digit indicates the class and the second digit the variant within this class.

In 2021, 11 different FetA variants were observed among 31 serogroup B meningococci, among which F3-3 (13%), F1-5 (16%) and F5-1 (19%) were the three dominant types (figure 4.7; table 4.9). In previous years, F1-5 constituted the dominant type within serogroup B meningococci (table 4.9), with strong linkage to PorA VR1/VR2 P1.7-2,4. Together, these types linked to the MLST clonal complex ST41/44. In 2021, 5 isolates were of FetA type F1-5, of which two were linked to P1.7-2,4 and 3 were linked to different PorA types. In total, 16 different PorA VR1/VR2 combinations and 11 different FetA variants were encountered among serogroup B meningococci. Although the numbers in 2021 are small, frequently found combinations were , P1.22,14:F5-1 (10%), P1.22,14:F5-5 (6%), P1.7-2,4:F1-5 (6%) and P1.7-2,16:F3-3 (6%).

In 2021, we received 4 serogroup W samples, all from blood. The 4 meningococcal serogroup W isolates displayed three different FetA types F1-1, F5-1 and one deletion (Figure 4.7, Table 4.9). All linked to PorA VR1/VR2 P1.5,2 and MLST clonal complex 11.

Figure 4.7 *Distribution of meningococcal* fetA *genosubtypes, 2021*



	F1-1	F1-5	F1-7	F3-3	F3-4	F5-1	F5-2	F5-5	Deletion	Other	No valid reaction
B		5	2	4		6	2	2		5	5
W	2					1			1		
Y					1						
NGD*											1

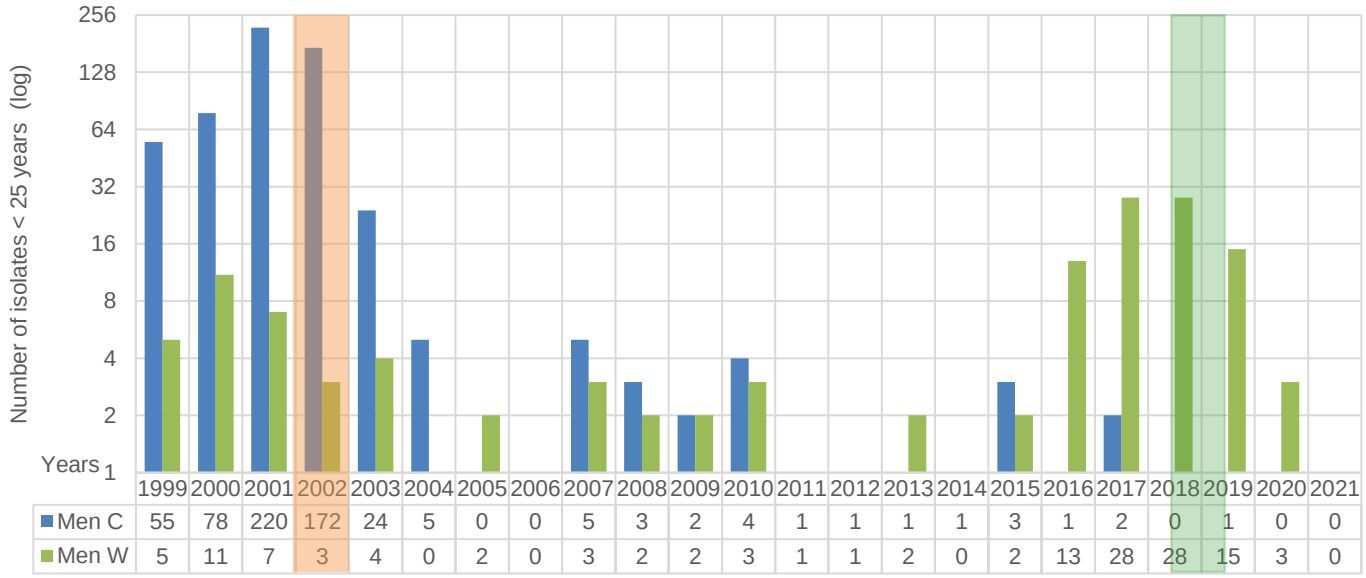
[6] *PubMLST - FetA variable region typing*. Public databases for molecular typing: https://pubmlst.org/neisseria/FetA/

Table 4.9 Temporal distribution in *fetA* genosubtype among *N. meningitidis* serogroups B, C and W isolates, 2017-2021

	Men B					Men C					Men W				
Years / FetA	2017	2018	2019	2020	2021	2017	2018	2019	2020	2021	2017	2018	2019	2020	2021
F1-1	0	1	2	0	0	0	0	0	0	0	72	90	53	12	2
F1-5	12	7	12	5	5	1	0	0	0	0	0	1	2	0	0
F1-7	9	4	6	1	2	0	0	1	0	0	0	0	0	0	0
F3-3	7	9	14	7	4	3	1	2	0	0	0	1	0	0	0
F3-4	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
F3-6	0	1	0	0	0	2	1	1	0	0	0	0	0	0	0
F3-7	0	0	0	0	0	0	0	0	0	0	2	3	1	0	0
F3-9	0	2	1	1	0	2	1	1	0	0	0	0	0	0	0
F4-1	3	2	0	1	0	0	0	0	0	0	1	0	1	0	0
F5-1	17	12	12	2	6	0	0	0	0	0	0	0	0	0	1
F5-2	1	2	3	2	2	0	0	0	0	0	0	0	1	0	0
F5-5	8	11	6	3	2	0	0	0	0	0	0	1	0	0	0
F5-8	0	1	1	3	0	0	0	0	0	0	0	1	0	0	0
F5-12	1	1	3	4	0	0	0	0	0	0	0	0	0	0	0
F5-36	2	1	1	3	0	0	0	0	0	0	0	2	0	0	0
Deletion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Other	22	20	11	8	10	1	0	1	0	0	6	2	1	0	0
Total	**82**	**74**	**72**	**40**	**31**	**9**	**3**	**6**	**0**	**0**	**81**	**102**	**60**	**12**	**4**

4.6 Vaccination prospects N. meningitidis

In the Netherlands, vaccination against serogroup C meningococcal disease was introduced in June 2002. All children born on or after June 1st, 2001 are vaccinated at the age of 14 months as part of the regular National Immunisation Programme. In addition, between June 2002 and October 2002, children and adolescents from 12 months to 19 years were vaccinated. In 2016-2018, the number of cases of meningococcal W disease showed a dramatic increased in the Netherlands. In response, the MenC vaccine was replaced by one that protects against meningococcal sergroups A, C, W and Y as of 1 May 2018. Because meningococcal type W is also hazardous for older children and because carriage is highest in this age group, the vaccination has also been offered to teenagers in the year they turn 14, as of 1 October 2018, including a catch-up campaign for 14-18 year olds between October 2018-June 2019. In 2021, no cases due to serogroup C or W meningococcal disease were reported in patients < 25 years of age (Figure 4.8).



Years	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Men C	55	78	220	172	24	5	0	0	5	3	2	4	1	1	1	1	3	1	2	0	1	0	0
Men W	5	11	7	3	4	0	2	0	3	2	2	3	1	1	2	0	2	13	28	28	15	3	0

Figure 4.8 *Number of N.meningitidis serogroup C and W isolates in patients < 25 years of age, 1999-2021. Start of vaccination with MenC and MenACWY vaccine is indicated in orange and green color, respectively.*

Two meningococcal group B vaccines are registered in the Netherlands but not included in the National Immunization programme (RIVM, meningococcal B vaccination)[7]

[7] RIVM. *Meningokokken B vaccinatie*. (Dutch) https://lci.rivm.nl/richtlijnen/meningokokken-b-vaccinatie

5 HAEMOPHILUS INFLUENZAE

5.1 General features

In total, 167 *Haemophilus influenzae* isolates or PCR-positive CSF samples were submitted to the NRLBM in 2021, which is a decrease compared to the 202 isolates received in 2020 (table 2.3, figure 3.3, figure 5.1). Twenty-four isolates were from CSF (or CSF and blood; 14.4%) and 143 from blood only (85.6%). Sixty-eight (41%) of the isolates were *H. influenzae* type b (table 5.1), which is the highest number since the introduction of vaccination in 1993 (figure 5.3). The increase is visible among different age groups (Figure 5.5).

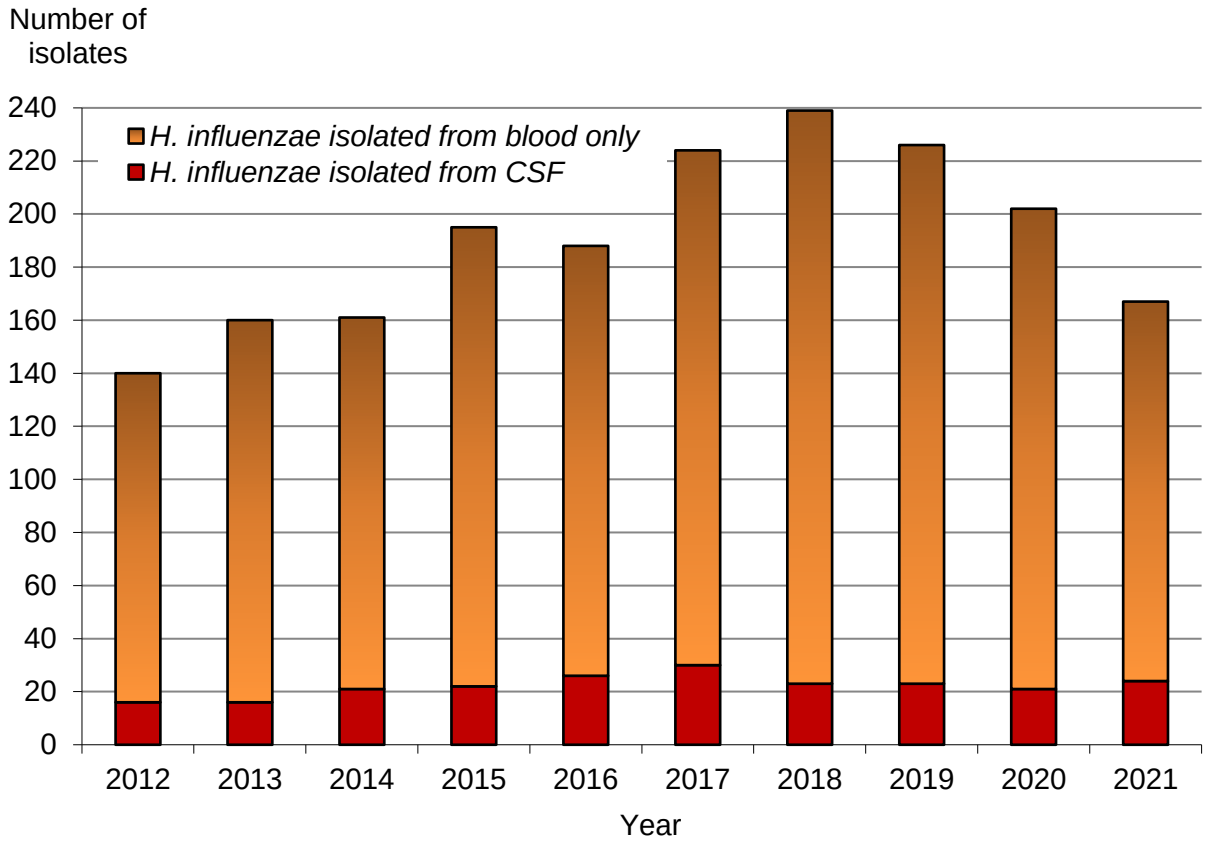


Figure 5.1 *Number of received H. influenzae isolates according to isolation source, 2012-2021*

5.2 Antibiotic susceptibility

The proportion of ß-lactamase-producing invasive *H. influenzae* isolates (CSF and/or blood) was 6.0% in 2021 (Figure 5.2). During the history of the NRLBM, the proportion of ß-lactamase-producing invasive *H. influenzae* isolates has always fluctuated for unknown reasons.

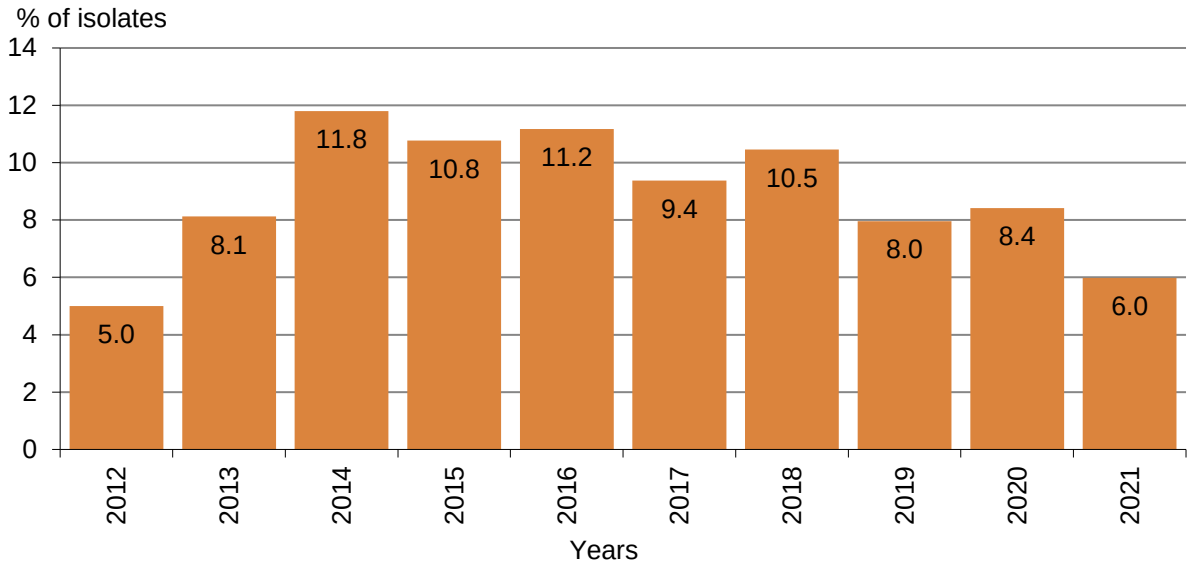


Figure 5.2 *Percentage β-lactamase-producing H. influenzae strains among received isolates, 2012-2021*

5.3 Serotype and age

In 2021, the number of *H. influenzae* type b isolates increased compared to the previous year, representing 41% of all received *H. influenzae* isolates compared to approximately 30% in 2020 and 16% in 2019. This is the highest number and proportion of *H. influenzae* b isolates in the last 15 years (Figure 5.3). Currently, it is unclear what the underlying causes for this increase in *H. influenzae* b is, especially when most other respiratory-transmitted pathogens decreased as a result of Covid-19 containment measures. We observed 26 cases of invasive *H. influenzae* type b disease among children younger than 2 years of age (Table 5.1 and figure 5.4; 24 in 2020; 11 in 2019; 15 in 2018; 7 in 2017). In contrast, the number of non-typeable *H. influenzae* isolates decreased further, for the first time in 20 years, representing 50% of all received isolates (84 of 167 isolates), compared to 58% in 2020 and 73% of all isolates in 2019 (Figure 5.3). Five non-typeable isolates were isolated from CSF (or CSF and blood) and 79 were isolated from blood only (table 5.1). Since 2000, the number of non-typeable *H. influenzae* isolates has steadily increased, which also explains the rise in total *H. influenzae* invasive infections over the same period (Figure 5.3). In addition, since 2008, the number of cases due to serotype f has been slowly increasing, but has been lower in 2020 and 2021, likely as a result of containment measures in response to the COVID-19 pandemic (Figure 5.3).

Table 5.1 Serotype distribution of all received *H. influenzae isolates* according to serotype patient's age, 2021

	AGE (MONTHS)				AGE (YEARS)					TOTAL	
Type	**0**	**1-11**	**12-23**	**24-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Hi - a	0	1	0	0	1	0	0	0	1	**2**	***1.2***
CSF	*0*	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	***1***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	***1***	
Hi - b	0	10	7	9	26	2	1	6	33	**68**	***40.7***
CSF	*0*	*7*	*2*	*1*	*10*	*1*	*0*	*1*	*2*	***14***	
Blood	*0*	*3*	*5*	*8*	*16*	*1*	*1*	*5*	*31*	***54***	
Hi - e	0	1	0	0	1	0	0	0	2	**3**	***1.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*0*	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*2*	***3***	
Hi - f	0	1	0	0	1	0	0	0	8	**9**	**5.4**
CSF	*0*	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*2*	***3***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*6*	***6***	
n.t.*	2	2	3	1	8	4	3	12	57	**84**	***50.3***
CSF	*1*	*0*	*1*	*0*	*2*	*0*	*0*	*1*	*2*	***5***	
Blood	*1*	*2*	*2*	*1*	*6*	*4*	*3*	*11*	*55*	***79***	
**** ND**	1	0	0	0	1	0	0	0	0	**1**	***0.6***
Total	**3**	**15**	**10**	**10**	**38**	**6**	**4**	**18**	**101**	**167**	***100.0***
CSF	***2***	***9***	***3***	***1***	***15***	***1***	***0***	***2***	***6***	***24***	***14.4***
Blood	***1***	***6***	***7***	***9***	***23***	***5***	***4***	***16***	***95***	***143***	***85.6***
%	***1.8***	***9.0***	***6.0***	***6.0***	***22.8***	***3.6***	***2.4***	***10.8***	***60.4***	***100.0***	

* non-typeable
** not enough DNA in CSF voor typing PCR.

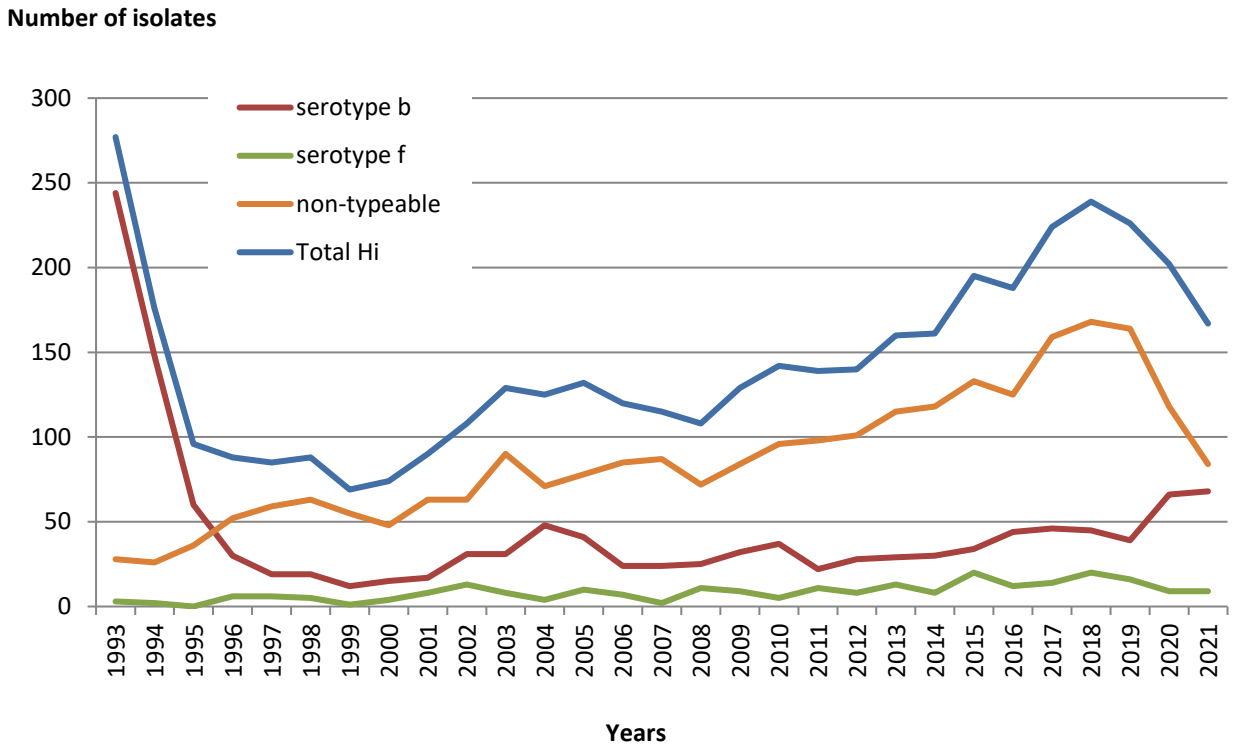


Figure 5.3 *Number of cases due to H. influenzae serotypes b, f and non-typeable H. influenzae, 1992-2021*

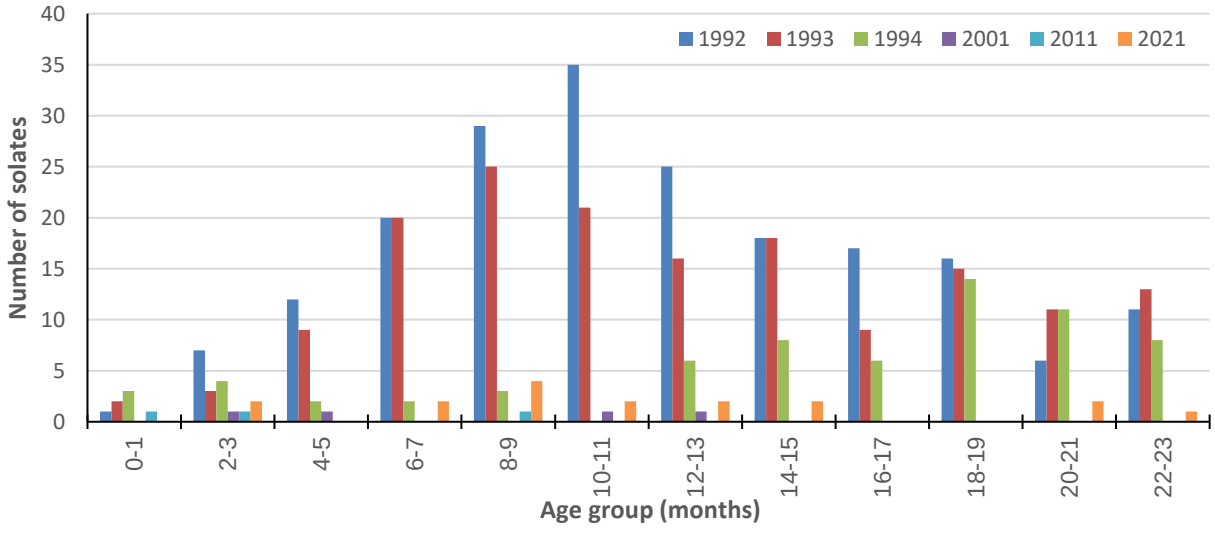


Figure 5.4 *Age distribution among children < 2 years of age of H. influenzae type b invasive disease for indicated years between 1992 – 2021. The H. influenzae type b vaccine was introduced in 1993.*

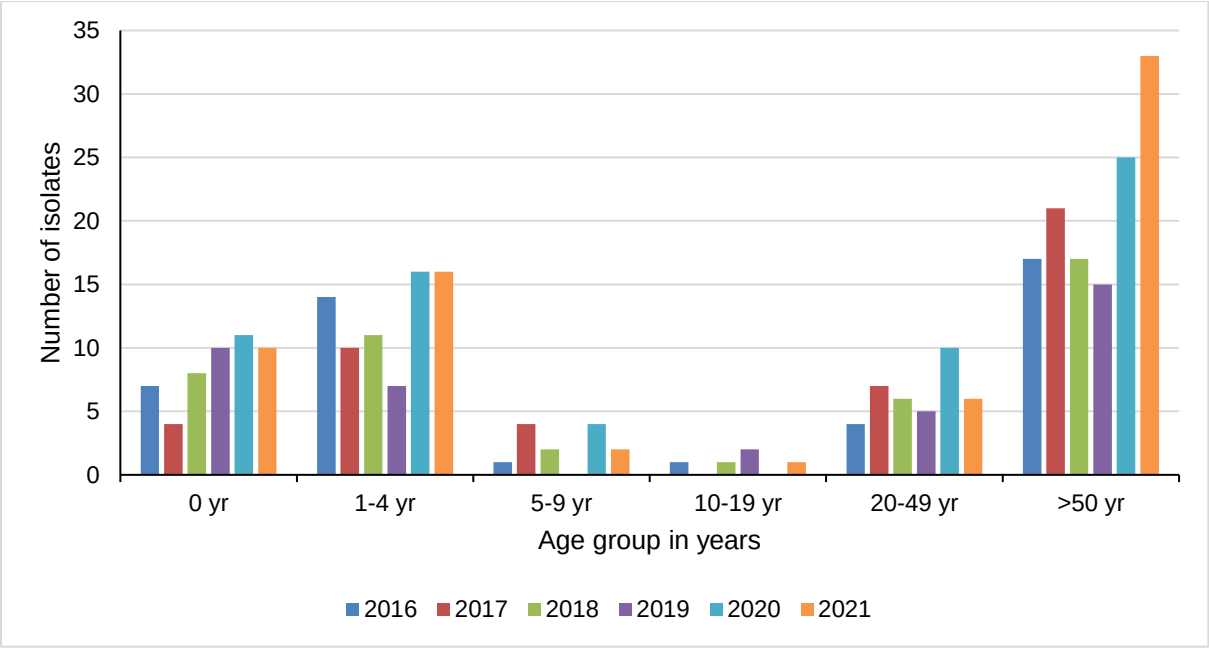


Figure 5.5 *Distribution of H. influenzae type b (CSF and/or blood) per age group. 2017-2021.*

5.4 Distribution of non-typeable H. influenzae

The proportion of non-typeable *H. influenzae* isolates increased from 6% in 1992 to about 73% in 2019 and decreased to 50% in 2021 (table 5.2), likely as a result of Covid-19 containment measures. The vast majority of non-typeable *H. influenzae* isolates are from blood (95%) in accordance to previous years (Table 5.2 and figure 5.6). Sixty-eight percent of invasive infections with non-typeable *H. influenzae* occurred mainly in individuals of 50 years or older (Tables 5.1 and 5.2). Among non-typeable *H. influenzae* isolates, biotype II was the predominant biotype during the last ten years (Figure 5.7).

Table 5.2 Number and proportion of non-typeable *H.influenzae isolates* from CSF and/or blood according to age, 2012- 2021

n.t.*	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	≥80	T	Csf / Blood	% **
2012	16	0	2	1	13	6	10	18	21	14	**101**	12/89	72.1
2013	8	1	2	1	7	4	5	27	29	31	**115**	6/109	71.9
2014	11	2	0	5	6	5	11	31	27	19	**117**	15/102	72.7
2015	12	3	1	5	6	9	19	34	19	24	**132**	14/118	67.7
2016	10	1	0	3	6	6	9	39	25	24	**123**	10/113	65.4
2017	18	1	3	4	8	11	16	33	37	28	**159**	21/138	71.0
2018	16	2	7	5	8	9	14	30	32	45	**168**	9/159	70.3
2019	12	0	2	8	14	8	17	29	39	35	**164**	12/152	72.6
2020	9	2	4	5	2	7	13	24	30	21	**118**	5/113	58.4
2021	8	4	3	2	6	4	9	22	22	4	**84**	5/79	50.3

* non-typeable

** % non-typeable / total *H. influenza* isolates

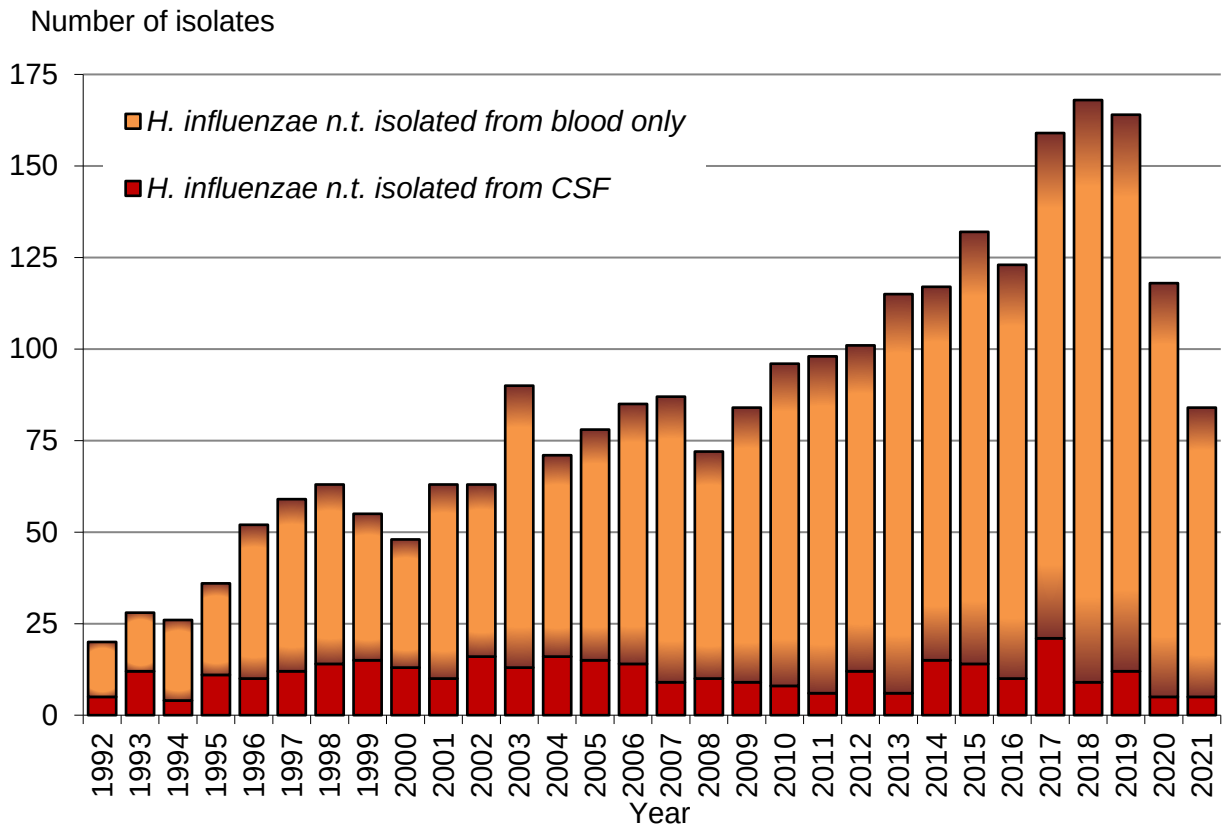


Figure 5.6 *Non-typeable H. influenzae isolates from CSF or blood received between 1992 - 2021*

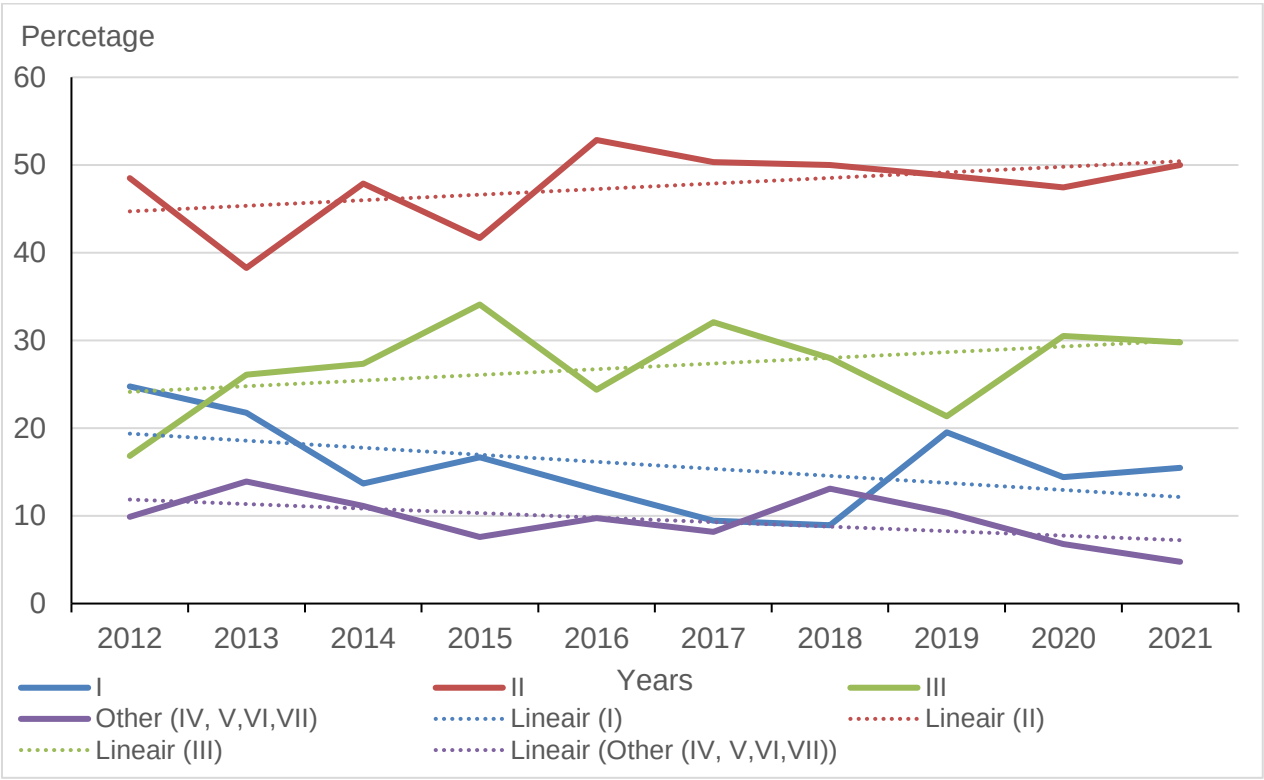


Figure 5.7 *Biotype distributions of non-typeable H. influenzae isolates from CSF and/or blood from 2012 – 2021.*

5.5 Geographical distribution of H. influenza

We also plotted the geographical distribution of all *H. influenzae* cases (Fig. 5.8A) and *H. influenzae* b cases (Fig. 5.8B) per 100,000 inhabitants based on the patient's residence to identify whether there was indication for clustering. No apparent pattern emerged from this visualization.

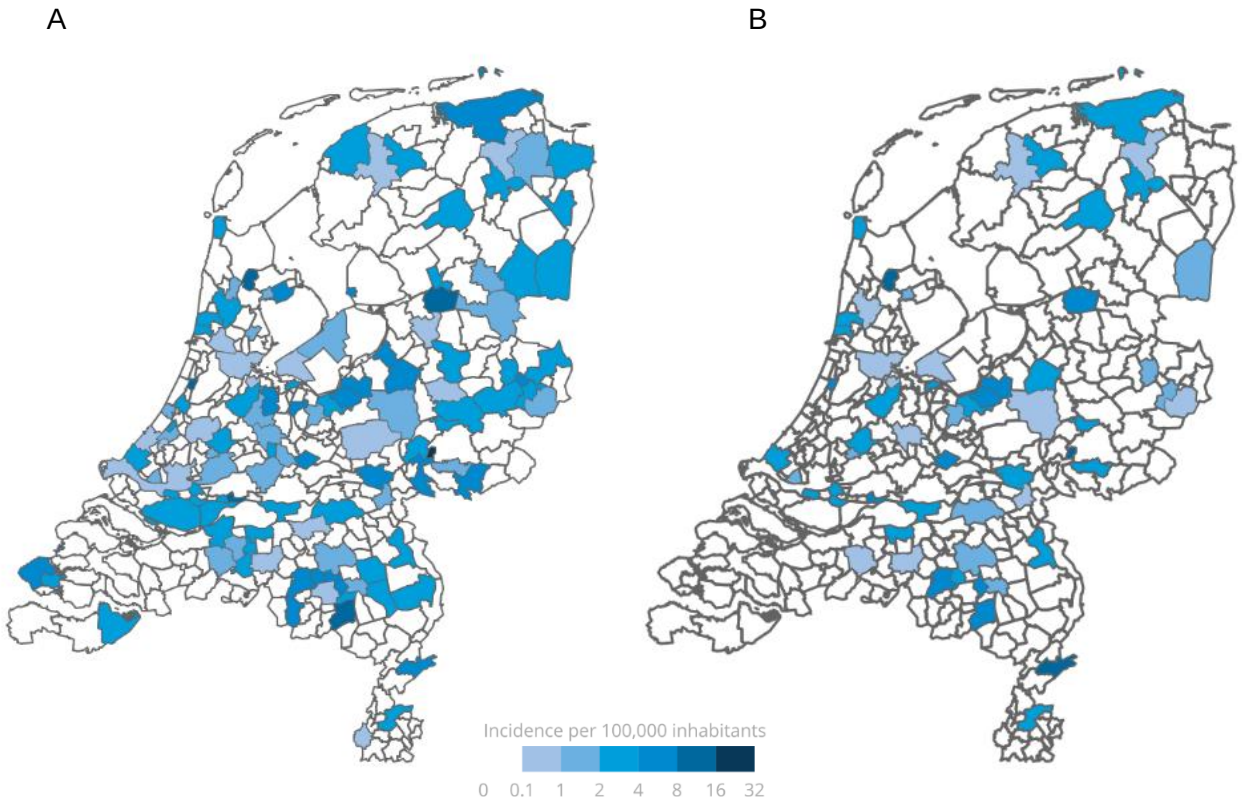


Figure 5.8. Geographical visualization of H. influenzae incidence for (A) all *H. influenzae* types and (B) *H. influenzae* b cases. Incidence is calculated per 100,000 inhabitants and place of residence of patient.

5.6 Vaccination prospects H. influenzae

The first implemented *H. influenzae* b vaccine consisted of the type b capsular polysaccharide conjugated to the tetanus toxoid protein (PRP-T). Since July 1993, children born after the 1st of April 1993 were vaccinated with the PRP-T vaccine, at the ages of 3, 4, 5, and 11 months. In 1999, the vaccine was administrated at the age of 2, 3, 4 and 11 months. In 2002, the Hib vaccine was given in combination with a pentavalent combination consisting of DTwP-IPV/Hib, with the whole cell pertussis (wP) component being changed to the acellular pertussis vaccine in 2004 (DTaP-IPV/Hib). In 2011, the Hepatitis B vaccine was added to this pentavalent combination vaccine (DTP3a-HBV-IPV/Hib). From Dec 2018, a different hexavalent vaccine product was used in which the composition of the administrated Hib conjugate vaccine changed from a conjugate with tetanus toxoid to a conjugate with *N. meningitidis* outer membrane protein complex (DTP5a-HBV-IPV-Hib). Finally, the vaccination schedule for this hexavalent vaccine that includes the Hib component has changed from a 3+1 to a 2+1 schedule (administrated at 3, 5, and 11 months of age) from January 2020.

The effect of vaccination on the frequency of *H. influenzae* meningitis cases is shown in figure 5.9. The number of *H. influenzae* meningitis cases caused by *H. influenzae* type b showed a steep decline since the introduction of the vaccine, while the number of cases caused by *H. influenzae* non-type b remained similar. In 2021, we received 30 *H. influenzae* type b isolates from patients that were vaccine-eligible (<28 years of age); 11 patients were CSF culture positive and 19 from blood. (CSF cases 2020: 9; 2019: 7) (figures 5.9 and 5.10). Of the 11 meningitis patients, 2 patients were not (completely) vaccinated at all and from nine patients, vaccination status was unknown.

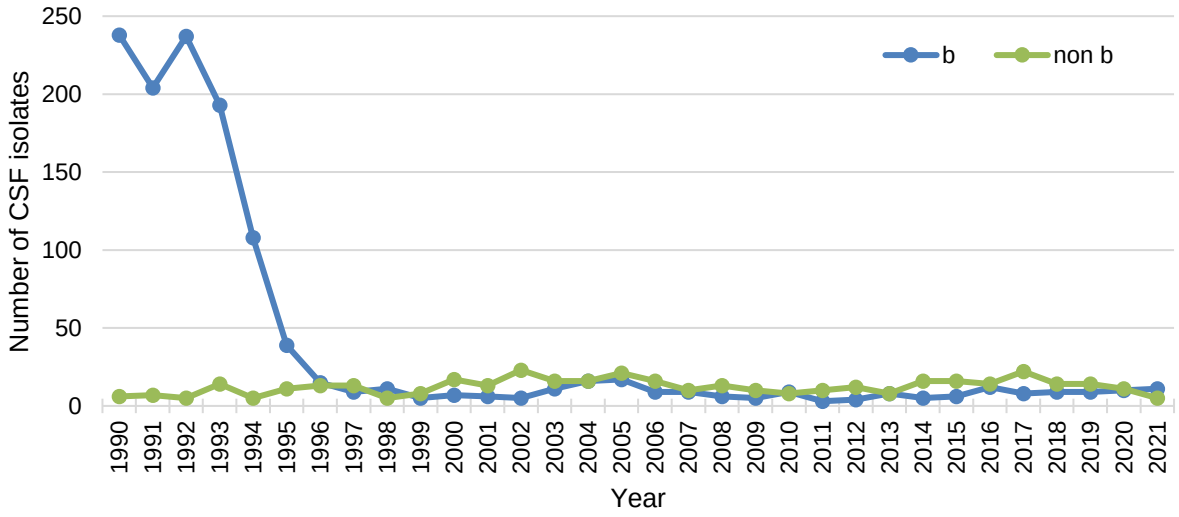


Figure 5.9 *The number of H. influenzae type b and non-type b cases, 1990 - 2021*

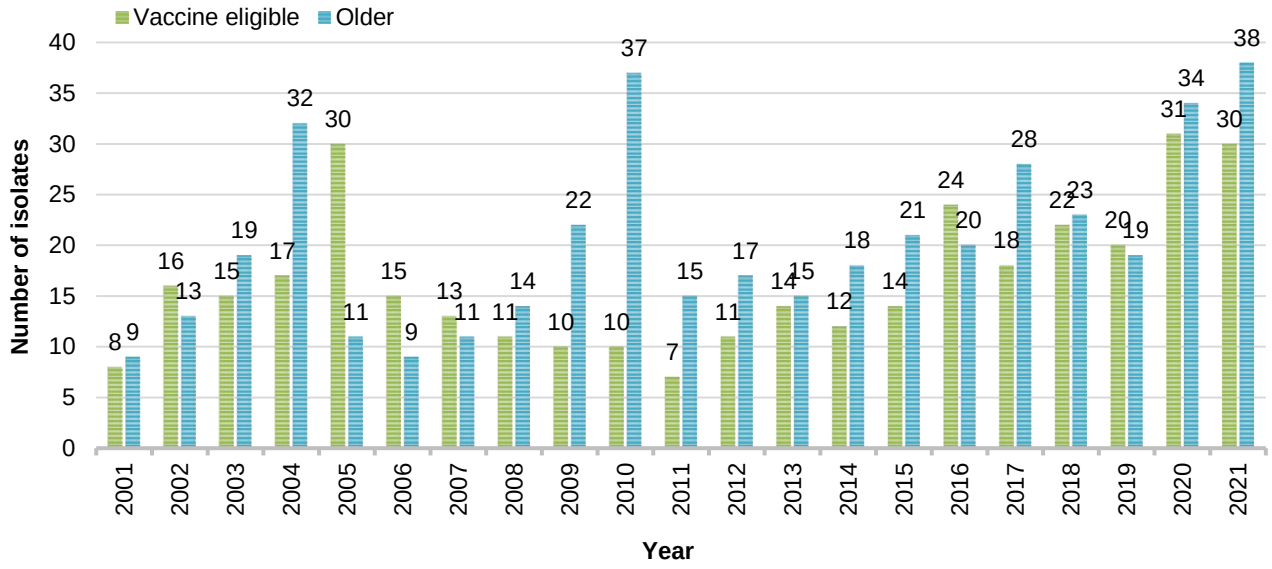


Figure 5.10 *The number of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 2001 –2021*

6 STREPTOCOCCUS PNEUMONIAE

6.1 General features

From 2003 onwards, the NRLBM requested nine sentinel laboratories, evenly distributed across the country and covering 28% of the Dutch population, to submit pneumococcal isolates from CSF and/or blood from patients of all ages. All medical microbiology laboratories were requested to submit pneumococcal isolates from CSF (or CSF and blood), with confirmed or suspected meningitis. From 2006, the 7-valent pneumococcal polysaccharide conjugate vaccine (PCV7) was introduced in the National Immunisation Programme and all medical microbiology laboratories were requested to submit all invasive pneumococcal isolates from patients in the age group 0-4 years. PCV7 was replaced by the 10-valent pneumococcal polysaccharide conjugate vaccine (PCV10) from March 1, 2011 onwards. Criteria for isolate submission remained similar until 2017. From 2017 onwards, all medical microbiology laboratories were requested to submit all invasive pneumococcal isolates without restriction to age of the patient. In 2021, the NRLBM received 1,117 isolates (or PCR positive samples) nationwide of which 345 (31%) pneumococcal isolates (CSF and/or blood) were received from the 9 sentinel laboratories. Of the 1,117 nationwide submitted isolates, 80 isolates were from CSF (or CSF and blood). The NRLBM also received 7 PCR-positive, culture-negative (CSF or blood) samples. The incidence of pneumococcal meningitis gradually increased from 1.0 per 100,000 individuals in 1990 to 1.6 per 100,000 individuals in 2004. The introduction of the PCV7/PCV10 vaccin decreased pneumococcal meningitis incidence to 0.5 per 100,000 individuals in 2021 (Figure 6.1).

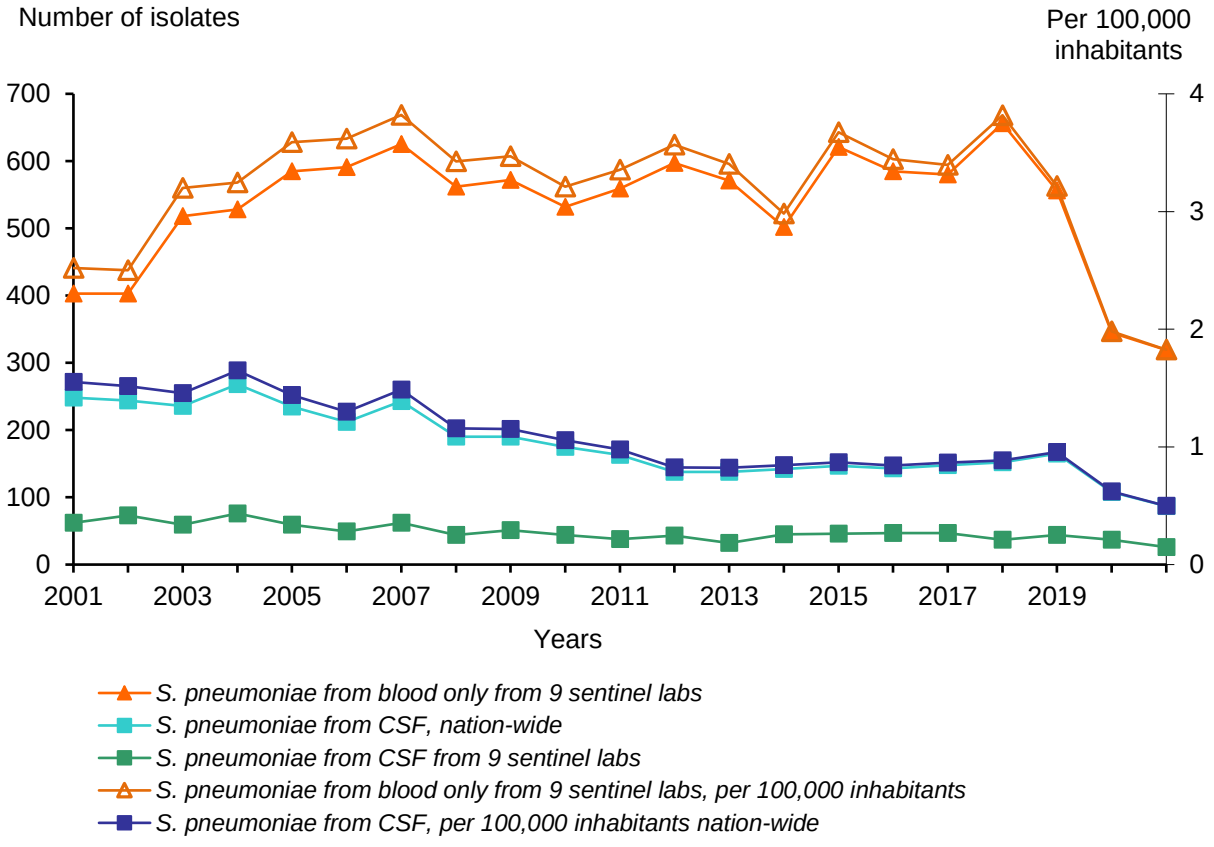


Figure 6.1 *Number of submitted S. pneumoniae isolates and invasive pneumococcal disease incidence based in isolates from blood or CSF, 2001-2021*

Figure 6.2 shows the number of *S. pneumoniae* isolates and incidence according to the patients' age. The incidence of pneumococcal meningitis is highest among patients in the age groups 0-4 and 60-69 years (Figure 6.2; top graph), whereas the incidence of pneumococcal bacteremia is highest in patients 65+ years of age, with about 3-fold higher incidence in the 95+ years of age (Figure 6.2; bottom graph). The absolute number of isolates from patients with bacteremia is highest in the age group 60-74 years (Figure 6.2; bottom graph). Figure 6.3 shows the geographical distribution of invasive pneumococcal disease per township based on patient's place of residence and per 100,000 inhabitants. There is no apparent pattern of clustering of cases.

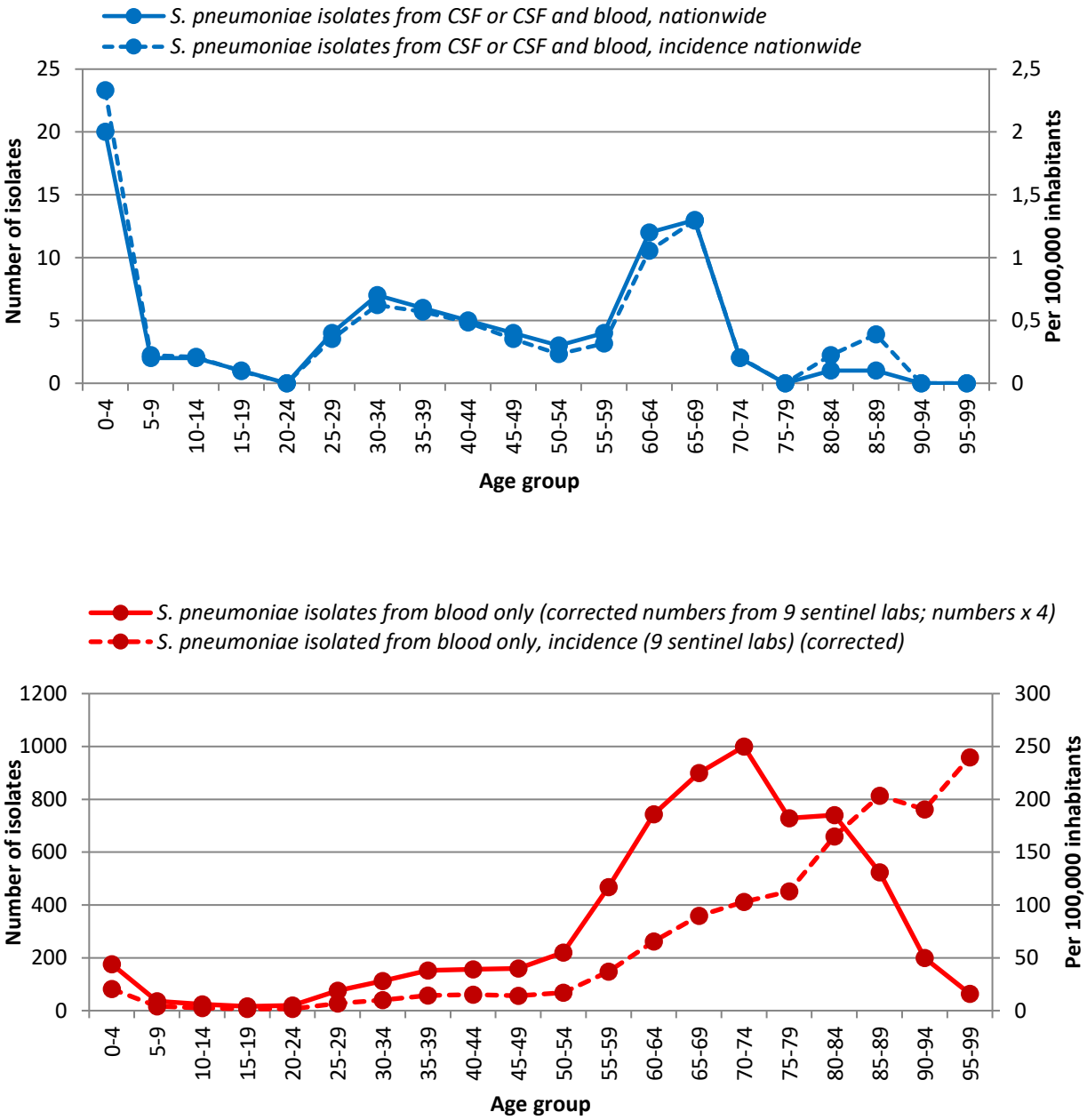


Figure 6.2 *S. pneumoniae isolates received per age group and incidence per 100,000 inhabitants according to isolation source in 2021. Top graph: isolates from CSF/CSF and blood. Bottom graph: isolates from blood only (9 sentinel labs) actual numbers x 4*

S. pneumonia meningitis

S. pneumonia bacteremia

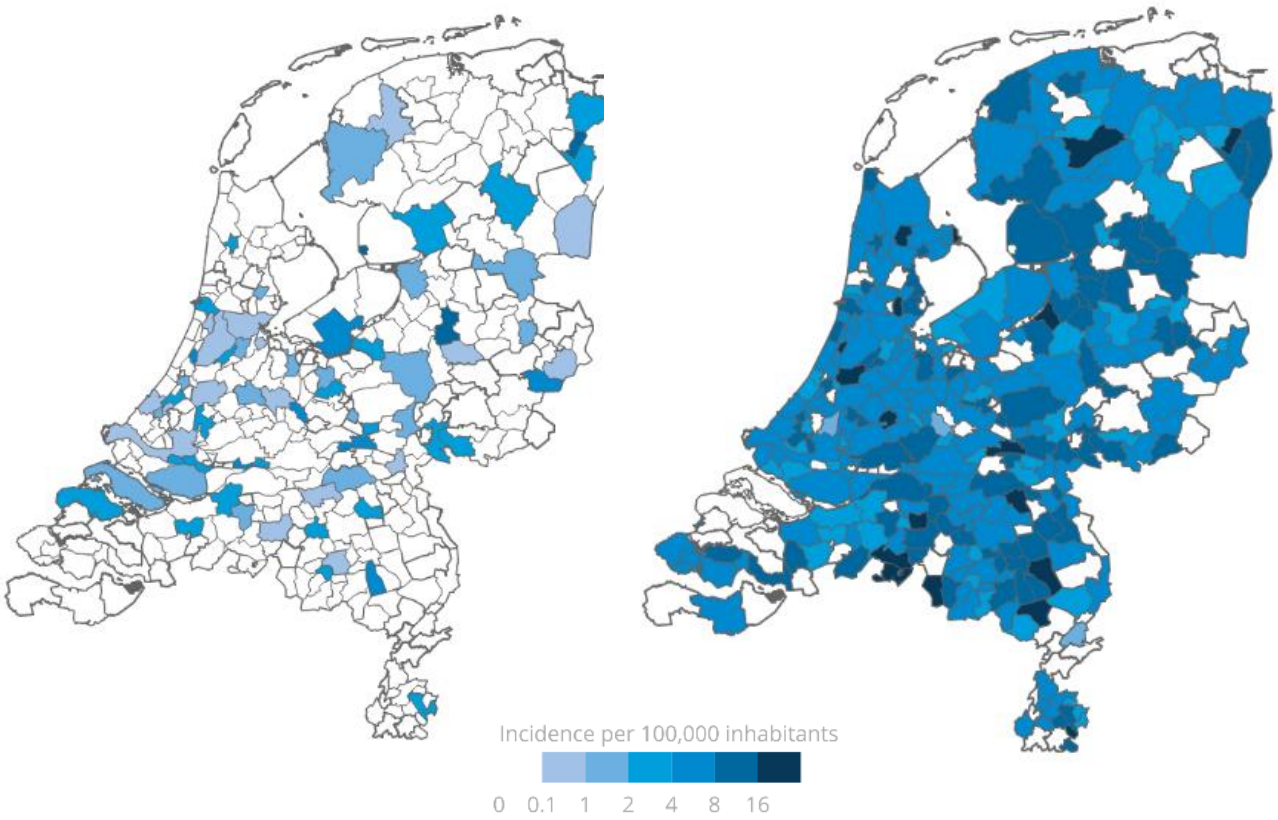


Figure 6.3 *Geographical distribution of S. pneumoniae invasive disease incidence per 100,000 inhabitants, left; meningitis based on isolates from CSF or CSF and blood, right; bacteremia based on isolates from blood only (nationwide). Data plotted based on patient's place of residence.*

6.2 Antibiotic susceptibility

Among 345 isolates from 9 sentinel labs, 26 were from CSF and 319 from blood only, 31 (9%) isolates were intermediately susceptible to penicillin (0.06 < MIC ≤ 2.0 mg/L; table 6.1). Among the blood isolates received from labs nationwide, one isolate (0.1%) was resistant to penicillin (Table 6.1). Among 87 nationwide patients with *S. pneumoniae* from CSF (or CSF and blood), 8 (9.2%) strains were resistant to penicillin (MIC > 0.06 mg/L). From 7 patients no MIC values were obtained as no *S. pneumoniae* isolate was available.

Table 6.1 Penicillin* susceptibility of *S. pneumoniae* isolates, 2021

MIC for CSF isolates (Nationwide)	S MIC ≤ 0.06	I	R MIC > 0.06	ND** (PCR)	Total
CSF/CSF and blood	72 (82.8%)	n.a.	8 (9.2%)	7 (8%)	87
MIC for blood isolates	**MIC ≤ 0.06**	**0.06< MIC ≤ 2.0**	**MIC >2.0**		
Blood only (9 sentinel labs)	287 (90.0%)	31 (9.7%)	0	1 (0.3%)	319
Blood only (nationwide)	953 (92.5%)	75 (7.3%)	1 (0.1%)	1 (0.1%)	1030

* MIC values in mg/L according to EUCAST guidelines
** No MIC value known because no isolate was available (PCR-positive patient)
n.a. not applicable for meningitis

6.3 Distribution according to serotype

The distribution of serotypes, grouped by vaccine type (VT) and by age of the patient, for isolates from CSF (or CSF and blood) or blood only (submitted by the 9 sentinel labs) is presented in tables 6.2 and 6.4, respectively. Disease caused by PCV10-covered serotypes is less than 3% for meningitis (table 6.2) and less than 2% for bacteremia (table 6.4). Serotypes that would be additionally covered by the PCV13 vaccine (serotypes 3, 6A and 19A) account for approximately 26% and 33% of all isolates from meningitis and bacteremia patients, respectively (Tables 6.2, 6.4). The incidence of pneumococcal meningitis per 100,000 inhabitants per vaccine type and age of the patient is shown in table 6.3. Incidence of meningitis caused by PCV10 vaccine types is nearly eliminated in all age groups. Nonetheless, meningitis incidence is still highest in the age group 0-11 months, followed by non-vaccinated age groups 30-39, 50-64 and 65-79 years as a result of disease caused by non-PCV10 serotypes (Table 6.3). Effect of PCV10 introduction on serotype distribution among meningitis and bacteremia patients can be seen in tables 6.5 and 6.6, respectively. There is an overall reduction in the number of PCV10-covered serotypes for the period 2011-2021 of >90%. However, the overall number of invasive pneumococcal disease isolates has remained fairly consistent up to 2019 due to an increase in the number of isolates of non-vaccine serotypes. Especially serotypes 3, 8 and 19A have been showing in increase over these years. Serotypes 3 and 19A would be covered by PCV13 and serotype 8 by PPV23. Since 2020 though, a decrease of ~48% in the total number of submitted pneumococcal isolates from invasive disease was observed, which is likely the result of the COVID-19 containment measures that also impact pneumococcal transmission.

Table 6.2 Serotype and age distribution of *S. pneumoniae* isolates from CSF (or CSF and blood; nationwide isolation collection), 2021. Serotypes are grouped by vaccine type.

			AGE (MONTHS)			AGE (YEARS)											
		TYPE	0	1-11	12-59	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total	%
23-valent vaccine (all above types except 6A)	10-valent vaccine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	-	1	-	-	1	
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		7F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		18C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19F	-	-	-	-	1	-	-	-	-	-	-	-	-	1	
		23F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		Subtotal PCV10	-	-	-	-	*1*	-	-	-	-	-	*1*	-	-	*2*	***2.3***
	13-valent vaccine	3	-	-	-	-	1	1	-	2	5	1	4	2	-	16	
		6A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19A	-	1	2	3	-	-	-	-	-	-	1	2	1	7	
		Subtotal PCV13	-	*1*	*2*	*3*	*2*	*1*	-	*2*	*5*	*1*	*6*	*4*	*1*	*25*	***28.7***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		8	-	3	-	3	-	-	-	-	2	3	2	3	1	14	
		9N	-	-	-	-	-	-	-	-	-	1	1	2	-	4	
		10A	-	1	-	1	-	-	-	-	2	-	-	-	-	3	
		11A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		12F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		15B	-	-	1	1	-	-	-	-	-	-	-	1	-	2	
		17F	-	-	-	-	-	-	-	1	-	-	1	-	-	2	
		20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		22F	-	-	-	-	-	-	-	-	-	-	3	-	-	3	
		33F	-	2	-	2	-	-	-	-	1	-	-	-	-	3	
Subtotal PPV23			-	*7*	*3*	*10*	*2*	*1*	-	*3*	*10*	*5*	*13*	*10*	*2*	*56*	***64.4***
		Other	-	5	3	8	-	1	1	1	2	3	4	4	-	24	
Type unknown			-	1	1	2	-	-	-	-	1	1	2	1	-	7	
		Total	**-**	**13**	**7**	**20**	**2**	**2**	**1**	**4**	**13**	**9**	**19**	**15**	**2**	**87**	***100.0***

*Total 23 valent vaccine= sum of all above types – 6A
* From 3 patients with a pneumococcus detected in CSF there is no serotype known

Table 6.3 Age-specific incidence of pneumococcal meningitis nationwide (isolates from CSF or CSF and blood) per 100,000 inhabitants according to vaccine serotype, 2021

	AGE (YEARS)											
TYPE	0	1-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total
10-valent	-	-	0.11	-	-	-	-	-	0.03	-	-	0.01
13-valent	0.59	0.29	0.22	0.11	-	0.09	0.23	0.05	0.16	0.15	0.12	0.14
23-valent	4.16	0.44	0.22	0.11	-	0.13	0.46	0.23	0.35	0.38	0.24	0.32
Other	3.57	0.58	-	0.11	0.10	0.05	0.14	0.19	0.16	0.19	-	0.18
Total	**7.73**	**1.12**	**0.22**	**0.21**	**0.10**	**0.18**	**0.60**	**0.42**	**0.52**	**0.57**	**0.24**	**0.50**

Table 6.4 Serotype and age-dependent distribution of *S. pneumoniae* isolates from blood submitted by the 9 sentinel laboratories, 2021.

			AGE (MONTHS)			AGE (YEARS)											
		TYPE	0	1-11	12-59	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total	%
23-valent vaccine (all above types except 6A)	13-valent vaccine	10-valent vaccine 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	1	1	-	-	2	
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		6B	-	-	-	-	-	-	-	-	-	-	-	-	1	1	
		7F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		14	-	-	-	-	-	-	-	-	-	-	1	-	-	1	
		18C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19F	-	-	-	-	-	-	-	-	-	-	-	1	-	1	
		23F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		Subtotal PCV10	-	-	-	-	-	-	-	-	-	*1*	*2*	*1*	*1*	*5*	***1.6***
		3	-	-	-	-	-	-	-	-	1	2	2	14	10	29	
		6A	-	-	-	-	-	-	-	-	-	-	1	-	-	1	
		19A	-	4	1	5	-	-	-	1	4	4	18	28	14	74	
		Subtotal PCV13	-	*4*	*1*	*5*	-	-	-	*1*	*5*	*7*	*23*	*43*	*25*	*109*	***34.2***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		8	-	-	-	-	-	-	2	-	2	6	17	27	9	63	
		9N	-	-	-	-	-	-	-	-	1	2	6	6	3	18	
		10A	-	-	-	-	-	-	-	-	-	-	-	3	1	4	
		11A	-	-	-	-	-	-	-	-	-	-	4	4	-	8	
		12F	-	-	-	-	-	-	-	-	-	-	2	1	-	3	
		15B	-	-	-	-	-	1	-	-	-	-	-	2	4	7	
		17F	-	-	-	-	-	-	-	-	-	-	-	1	2	3	
		20	-	-	-	-	-	-	-	1	-	-	-	3	1	5	
		22F	-	-	-	-	-	-	-	-	-	1	7	6	3	17	
		33F	-	-	-	-	-	-	-	-	-	-	2	4	2	8	
Subtotal PPV23			-	*4*	*1*	*5*	-	*1*	*2*	*2*	*8*	*16*	*61*	*100*	*50**	*245*	***76.8***
		Other	-	2	4	6	-	-	-	-	3	3	15	37	9	73	***23.0***
Type unknown			-	1	-	1	-	-	-	-	-	-	-	-	-	1	
		Total	**-**	**7**	**5**	**12**	**-**	**1**	**2**	**2**	**11**	**19**	**76**	**137**	**59**	**319**	***100.0***

*Total 23 valent vaccine= sum of all above types – 6A

Table 6.5 Changes in serotype distribution of pneumococcal CSF isolates (nationwide isolate collection). Introduction of PCV10 in Immunisation Programme is shaded in gray, 2011-2021

			TYPE	2011*	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
23-valent vaccine (all above types except 6A)	13-valent vaccine	10-valent vaccine	1	1	1	3	4	1	2	1	-	-	-	-
			4	2	4	2	2	-	1	1	2	1	-	1
			5	-	3	-	-	-	-	-	-	-	-	-
			6B	2	-	-	-	1	-	-	-	-	2	-
			7F	28	16	15	8	7	4	2	2	1	-	-
			9V	-	3	1	1	-	2	-	-	-	-	-
			14	2	1	-	-	1	-	-	2	-	-	-
			18C	5	2	2	-	1	-	1	1	1	-	-
			19F	6	4	2	4	2	5	6	1	3	1	1
			23F	2	1	-	-	1	-	1	-	-	-	-
			Subtotal PCV10	*48*	*35*	*25*	*19*	*14*	*14*	*12*	*8*	*6*	*3*	*2*
			3	7	13	16	13	16	25	20	20	21	20	16
			6A (not in 23 valent)	1	1	1	3	-	1	-	-	-	-	-
			19A	16	6	9	7	10	8	16	13	20	15	7
			Subtotal PCV13	*72*	*55*	*51*	*42*	*40*	*48*	*48*	*41*	*47*	*38*	*25*
			2	-	-	-	-	-	-	-	-	-	-	-
			8	17	9	16	23	24	18	21	23	26	8	14
			9N	7	4	2	6	6	3	6	4	4	4	4
			10A	7	9	7	12	5	7	7	7	11	2	3
			11A	5	1	1	3	2	3	2	8	-	4	-
			12F	7	10	9	8	9	12	8	11	5	3	-
			15B	3	1	-	-	-	5	7	2	7	1	2
			17F	3	1	1	1	-	-	1	2	-	1	2
			20	-	-	1	1	1	-	-	-	-	-	-
			22F	16	11	8	8	11	11	8	8	10	6	3
			33F	5	6	3	2	4	4	6	6	3	1	3
			Subtotal PPV23*	*141*	*106*	*98*	*103*	*102*	*110*	*114*	*112*	*113*	*68*	*56*
			6C	4	2	6	3	6	5	3	3	11	12	3
			7B	-	-	1	-	-	-	-	-	-	2	-
			7C	-	-	-	-	-	-	-	-	1	-	1
			10F	-	-	-	-	-	-	-	-	-	-	-
			10B	-	1	-	1	1	-	1	-	1	1	1
			12A	-	-	-	-	-	-	-	-	-	-	-
			13	-	-	-	-	-	-	-	-	-	-	1
			15A	1	1	4	6	7	2	4	3	1	-	2
			15C	-	3	-	-	1	-	3	1	1	1	1
			16F	4	-	5	2	1	3	1	5	-	-	1
			17A	-	-	-	-	-	-	-	-	-	-	-
			18F	-	-	-	-	-	-	-	-	-	-	-
			18A	-	-	-	-	-	-	-	-	-	-	-
			18B	-	1	-	-	-	-	-	-	-	-	-
			21	1	-	-	-	-	-	-	2	-	1	1
			22A	-	-	-	-	1	1	-	-	-	-	-
			23A	2	4	4	4	5	5	5	8	6	5	1
			23B	2	5	7	8	11	6	11	8	10	9	10
			24F	1	4	4	7	7	1	2	1	5	-	-
			24B	-	2	-	-	-	-	-	-	-	-	-
			25	-	-	-	-	-	-	-	-	-	-	-
			27	-	1	-	2	1	1	-	1	1	1	-
			28F	-	-	1	-	-	-	-	-	-	-	-
			28A	1	-	-	-	-	-	-	-	-	-	-
			29	-	1	-	-	-	-	-	-	-	-	-
			31	-	1	-	1	-	1	1	-	-	-	-
			33A	-	-	-	-	-	-	-	-	-	-	-
			34	1	-	-	-	1	1	1	2	2	1	-
			35F	1	-	2	1	2	5	1	3	3	4	2
			35B	-	1	3	1	1	1	-	2	-	-	-
			35D	-	-	-	-	-	-	-	1	1	-	-
			37	1	2	1	-	-	-	-	-	-	-	-
			38	-	2	1	-	-	-	-	-	2	-	-
			Rough (n.t.)	-	-	-	-	-	-	1	-	-	-	-
			Type unknown	-	-	-	-	-	-	-	-	10	3	7
			Total	163	138	138	142	147	143	148	152	165	108	87

Table 6.6 Changes in serotype distribution of *S. pneumoniae* from blood submitted by the 9 sentinel laboratories, 2011-2021. Serotypes are grouped by vaccine type.

			TYPE	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
23-valent vaccine (all above types except 6A)	13-valent vaccine	10-valent vaccine	1	40	50	40	41	41	22	8	8	-	-	-
			4	27	11	13	6	6	6	6	6	1	6	2
			5	11	8	9	2	1	-	1	-	-	-	-
			6B	3	3	3	3	4	1	-	2	3	1	1
			7F	91	92	75	53	56	36	27	23	4	1	-
			9V	5	2	4	1	5	-	2	3	-	-	-
			14	19	12	8	2	7	8	4	2	7	8	1
			18C	8	4	8	2	2	2	1	1	-	-	-
			19F	9	3	5	7	8	6	9	7	4	1	1
			23F	5	3	1	2	1	1	1	-	1	-	-
			Subtotal PCV10	*218*	*188*	*166*	*119*	*131*	*82*	*59*	*52*	*20*	*17*	*5*
			3	36	45	40	31	35	45	51	71	45	30	29
			6A (not in 23 valent)	2	6	2	-	2	-	4	4	1	1	1
			19A	63	78	61	44	78	75	82	101	84	54	74
			Subtotal PCV13	*319*	*317*	*269*	*194*	*246*	*202*	*196*	*228*	*150*	*102*	*109*
			2	-	-	-	-	-	-	-	-	-	-	-
			8	59	88	108	93	136	151	143	159	146	89	63
			9N	17	20	19	21	26	32	29	31	31	20	18
			10A	14	8	6	16	15	11	11	8	10	13	4
			11A	9	14	16	8	6	6	9	8	5	7	8
			12F	19	25	22	28	30	18	28	22	26	12	3
			15B	4	1	7	7	2	8	6	8	10	3	7
			17F	8	7	4	8	6	6	5	4	2	4	3
			20	4	-	1	4	2	3	5	6	10	3	5
			22F	37	41	45	34	43	28	39	45	47	24	17
			33F	15	22	12	12	19	18	12	28	13	6	8
			Subtotal PPV23*	*503*	*537*	*507*	*425*	529	483	479	*543*	449	282	*245*
			6C	7	10	10	7	21	20	15	24	22	18	19
			7B	-	-	-	-	-	-	-	1	1	3	-
			7C	-	-	-	-	-	-	-	1	4	5	2
			9A	-	1	-	1	-	1	-	-	-	-	-
			10F	-	-	-	1	-	-	-	-	-	-	-
			10B	-	-	1	-	-	-	1	2	2	1	1
			11B	-	-	-	-	-	-	-	-	2	-	-
			11D	-	-	-	-	-	-	-	-	3	2	1
			12A	-	-	-	-	-	-	-	1	-	-	-
			13	1	-	-	-	-	1	-	-	-	-	-
			15F	-	-	1	-	-	1	-	-	-	-	-
			15A	2	7	13	14	18	21	16	14	12	7	6
			15C	2	1	4	4	3	2	1	1	3	1	2
			16F	7	6	7	5	2	9	9	5	4	5	6
			17A	2	-	-	-	-	-	-	-	-	-	-
			18F	-	-	-	-	2	-	-	-	-	-	-
			18A	1	-	-	-	-	-	-	-	-	-	-
			18B	-	1	1	-	-	-	-	-	-	-	-
			21	-	-	2	1	-	-	1	1	-	2	-
			22A	1	-	1	-	1	-	-	1	-	-	-
			23A	2	6	6	7	7	12	15	14	11	6	9
			23B	9	3	6	15	5	11	17	11	17	4	15
			24F	3	2	4	4	7	1	6	3	7	-	1
			25F	-	-	-	-	1	-	1	-	-	-	-
			27	1	-	1	-	1	1	-	-	-	-	1
			28	-	-	-	-	-	-	-	-	1	-	-
			29	-	1	-	-	-	-	-	-	-	-	-
			31	2	6	2	2	4	4	3	6	1	-	-
			33A	-	1	-	-	-	1	-	1	-	-	-
			34	-	1	2	1	-	1	1	3	4	1	1
			35F	6	5	6	7	7	6	3	6	3	3	3
			35A	-	1	-	-	-	-	-	-	-	-	-
			35B	3	1	7	6	8	8	2	8	3	3	3
			35D	-	-	-	-	-	-	-	-	2	-	1
			37	-	-	-	1	1	-	-	-	-	-	-
			38	3	-	1	2	2	1	5	4	1	1	1
			40	-	-	1	-	-	-	-	-	-	-	-
			Rough (n.t.)	2	-	-	-	-	-	1	1	1	-	1
			Type unknown	-	-	-	-	-	-	-	-	-	-	1
			Total	559	596	585	503	621	584	580	655	556	345	319

6.4 Vaccination

The first pneumococcal polysaccharide conjugated vaccine contained 7 serotype-specific polysaccharides linked to inactive diphtheria toxin (PCV7). Since July 2006, children born after the 1st April 2006 were vaccinated with PCV7 at the ages of 2, 3, 4 and 11 months. In April 2011, the 10-valent vaccine (PCV10) was introduced for all newborns from March 1st 2011. In 2021, 2.3% of the CSF isolates were of a serotype covered by the PCV10 vaccine (table 6.2). There were only 2 patients with pneumococcal meningitis due to pneumococci with a PCV-10 vaccine serotype (4 and 19F; Table 6.5). One patient was not vaccinated because of age (60 years of age). The other patient (7 year) was immunocompromised. The beneficial effect of vaccination is partly countered by an increase in the number of cases due to non-vaccine types (figure 6.4).

The pneumococcal polysaccharide vaccine covers 23 serotypes (PPV23). Sixty-four percent of the CSF isolates were of a serotype that would be covered by this vaccine (table 6.5). (2020: 63%; 2005: 90% pre-vaccination). In 2021, 69-79 year-olds are offered a vaccination with PPV23 through the National Immunisation Programme.

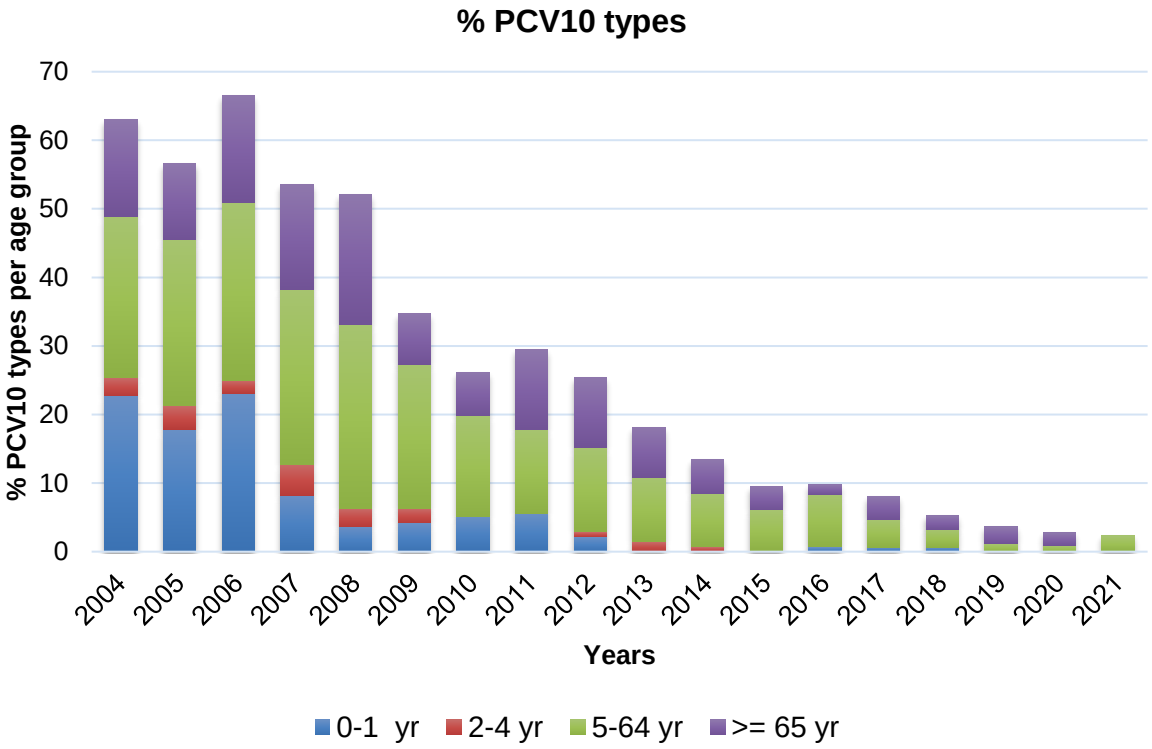


Figure 6.4 *The age distribution of S. pneumoniae invasive disease due to pneumococci of serotypes included in PCV-10, 2004-2021.*

7 ESCHERICHIA COLI

The NRLBM received 100 *Escherichia coli* isolates, 20 isolated from CSF (or CSF and blood) and 80 from blood only (Figure 7.1, Table 7.1). Sixty percent of the *E. coli* meningitis cases occurred in the first month of life (Table 7.1). Overall, 96% of invasive diseases cases caused by *E. coli* are in the age group 0-12 months (Table 7.1), matching with isolate submission criteria. Before 2016 the number of received isolates was rather stable with 15-30 isolates per year. From 2017, there is a marked increase, especially in received blood isolates, which is likely explained by increased submission as result of an ongoing study on neonatal meningitis (NOGBS study)[8]

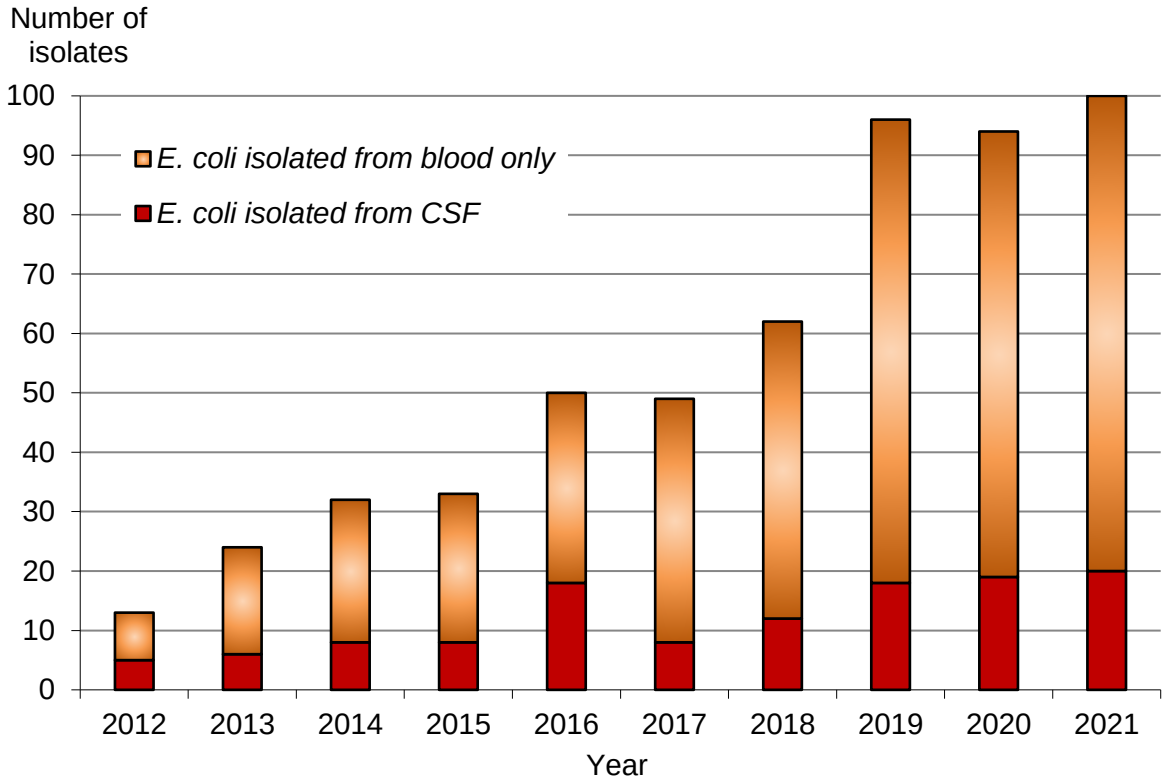


Figure 7.1 *Number of E. coli isolates received according to isolation source, 2012-2021*

Since 2016, K1 expression is determined by phage typing. In 2021, approximately 53% of the received *E. coli* isolates carried the K1 antigen (Table 7.1).

[8] *NOGBS study* Neuroinfecties Amsterdam: https://meningitisamc.nl/professionals/wetenschappelijk-onderzoek-professionals/nogbs-studie

Table 7.1 Number of *E. coli* isolates grouped according to serotype, patient's age, and source of isolation, i.e. CSF and/or blood, 2021

	AGE (MONTHS)			AGE (YEARS)					TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Non K1	35	8	0	43	0	0	1	3	**47**	***47***
CSF	*5*	*1*	*0*	*6*	*0*	*0*	*1*	*3*	***10***	
Blood	*30*	*7*	*0*	*37*	*0*	*0*	*0*	*0*	***37***	
K1	39	14	0	53	0	0	0	0	**53**	***53***
CSF	*7*	*3*	*0*	*10*	*0*	*0*	*0*	*0*	***10***	
Blood	*32*	*11*	*0*	*43*	*0*	*0*	*0*	*0*	***43***	
Total	**74**	**22**	**0**	*96*	*0*	*0*	*1*	*3*	**100**	***100***
CSF	*12*	*4*	*0*	*16*	*0*	*0*	*1*	*3*	*20*	*20*
Blood	*62*	*18*	*0*	80	*0*	*0*	*0*	*0*	*80*	*80*
%	***74***	***22***	***0***	***96***	***0***	***0***	***1***	***3***	***100***	

Since 2012, *E. coli* isolates received by the NRLBM are additionally characterized by O- and H-typing using Whole Genome Sequencing. O-typing refers to the O-group-specific genes within the O-antigen gene clusters, whereas H-typing determines the H-antigen genes that encode for the different flagellar types. Within the K1 isolates, 55% were of H-type H7 and 21% of type H4. H-type H4 was also dominant among the non-K1 isolates (26%), with H18 and H1 accounting together for more than one-third of the non-K1 isolates (table 7.2)

Table 7.2 H-type distribution among K1 and non-K1 *E. coli* isolates from CSF or blood, 2017-2021

TYPE	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1
	2017	**2018**	**2019**	**2020**	**2021**
H1	0 / 7	1 / 6	1 / 9	0 / 5	0 / 9
H4	4 / 1	3 / 7	7 / 12	8 / 8	11 / 12
H5	1 / 4	3 / 3	5 / 9	3 / 8	1 / 5
H6	3 / 1	2 / 2	1 / 1	6 / 0	4 / 2
H7	9 / 2	17 / 1	22 / 3	27 / 1	29 / 2
H9	0 / 4	0 / 1	-	0 / 1	0 / 2
H18	0 / 3	0 / 6	3 / 10	0 / 8	0 / 7
H31	0 / 2	-	0 / 3	0 / 4	5 / 1
Other	2 / 7	4 / 6	3 / 7	2 / 13	3 / 7
Total	**19 / 30**	**30 / 32**	**42 / 54**	**46 / 48**	**53 / 47**

The types O6 (13%) and O15 (13%) are most prevalent among non-K1 isolates, while the types O1 (30%), O18/O18ac (24%) and O25 (15%) are most frequent among K1 isolates. The 12 isolates showed in the group 'Other' were all different O-types (Figure 7.2)

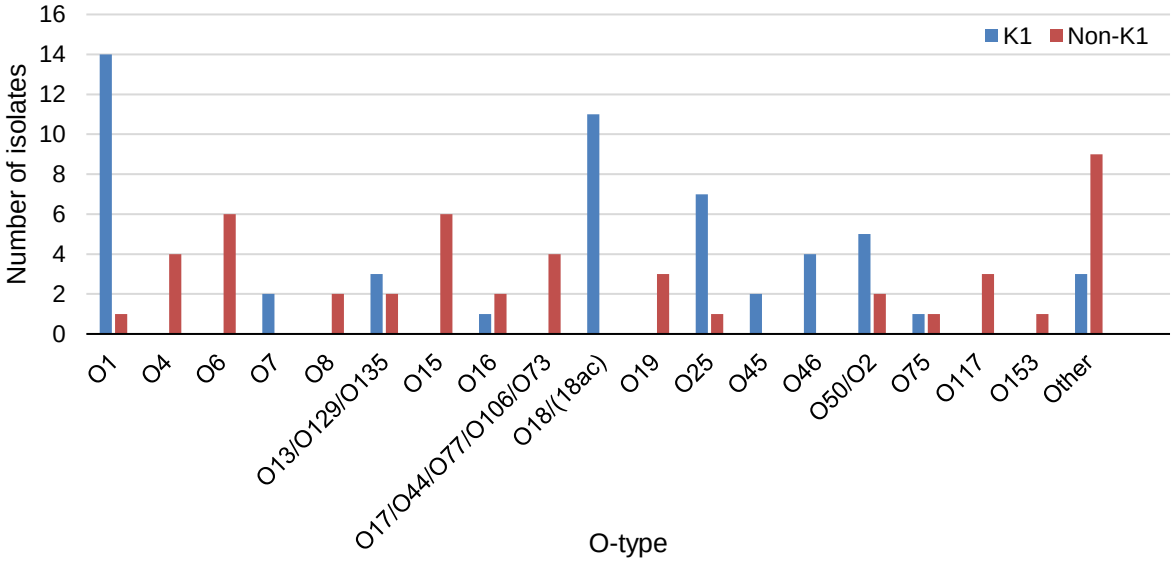


Figure 7.2 Distribution of O-types among K1 and non-K1 *E. coli* isolates from CSF and/or blood, 2021

Among K1 isolates, the O/H combination O1:H7 (26%) was most prevalent while among non-K1 isolates, O6:H1 was dominant (11%)(Figure 7.3).

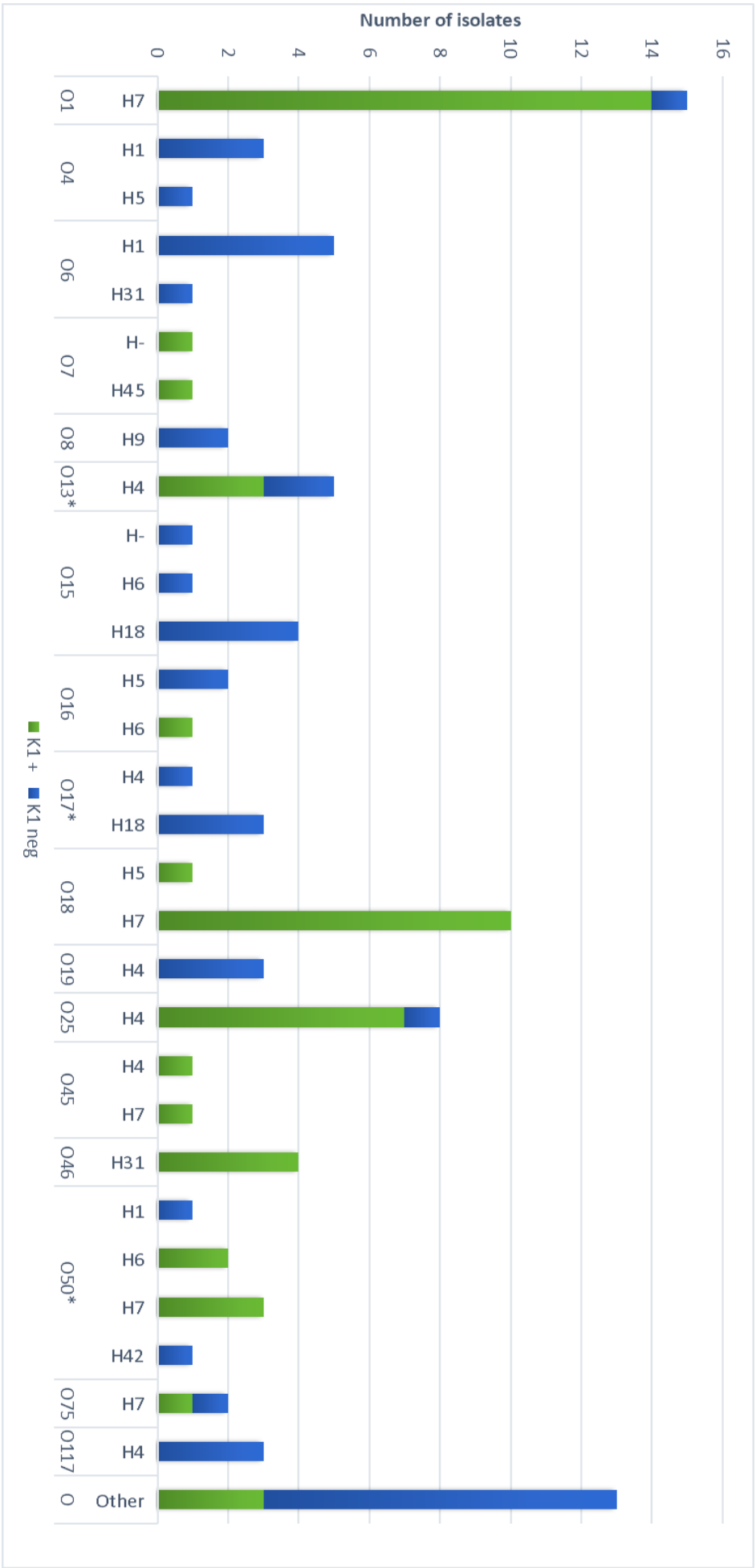


Figure 7.3 Distribution of O- and H-type combinations among K1 and non-K1 *E. coli* isolates from CSF and/or blood, 2021

*O13 = O13/O129/O135

O17 = O17/O44/O77/O106/O73

O50 = O50/O2O

8 STREPTOCOCCUS AGALACTIAE – (group B)

In 2021, the NRLBM received 147 *Streptococcus agalactiae* isolates, which is an increase compared to the 129 isolates in 2020 and 120 isolates in 2019 (figure 8.1). Nineteen (13%) *S. agalactiae* isolates were from CSF (or CSF and blood) and 128 (87%) from blood only (table 8.1, figure 8.1). Seventy-six percent of all the cases occurred in the first month of life, with 68% of the isolates recovered from CSF and 77% from blood (Table 8.1). Overall, 98% of invasive *S. agalactiae* disease cases occurred within the age group 0-4 years, with 96.6% occurring in the first year of life (table 8.1). As in previous years, Serotype III was most prevalent, accounting 54.4% of the cases (table 8.1, figure 8.2). Serotypes Ia and and V accounted for 17.7 and 11.5% of all cases (Table 8.1). From 2017, there is a marked increase in the number of received isolates, especially those recovered from blood. This is likely partially explained by increased submission as result of an ongoing study on neonatal meningitis (NOGBS study)[9].

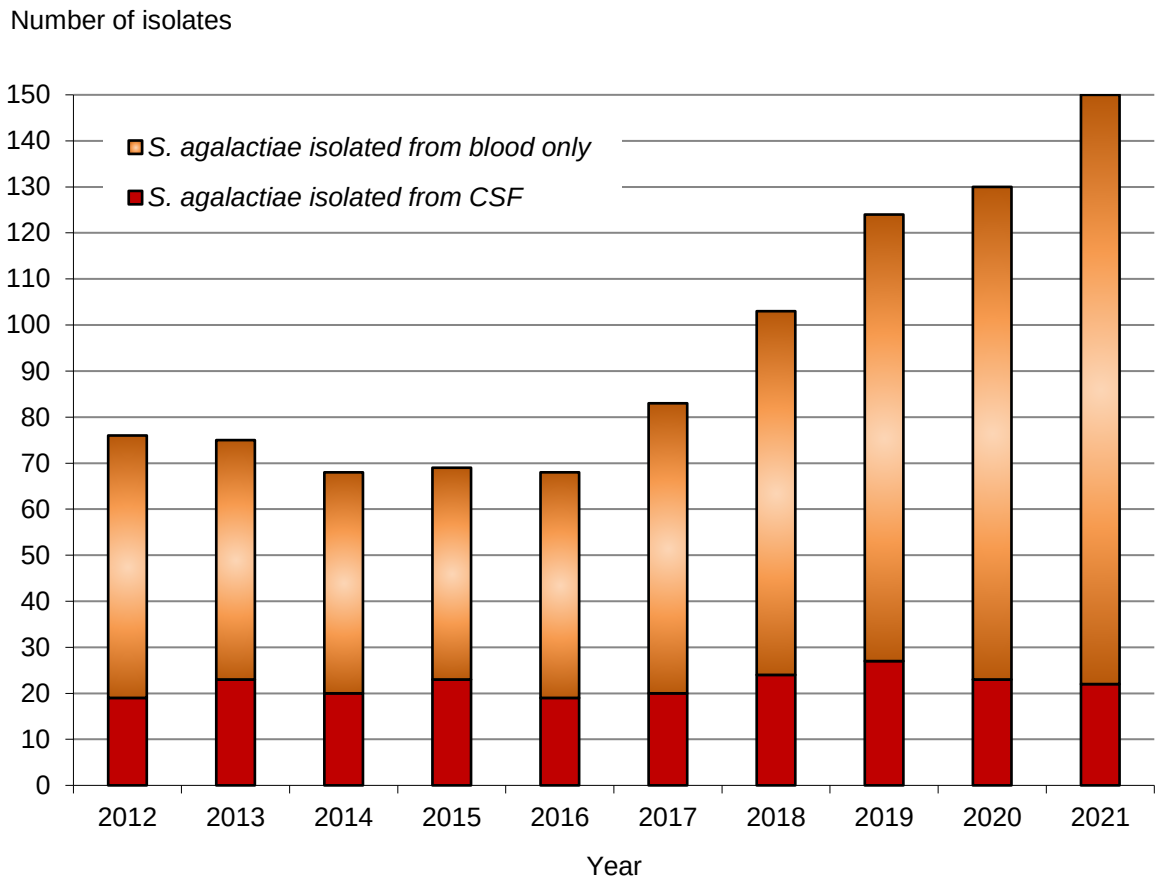


Figure 8.1 *Distribution of S. agalactiae isolates, 2012 - 2021*

[9] *NOGBS study* Neuroinfecties Amsterdam: https://meningitisamc.nl/professionals/wetenschappelijk-onderzoek-professionals/nogbs-studie

Table 8.1 Serotype distribution of *S. agalactiae* isolates from CSF and/or blood by age of patients, 2021

	AGE (MONTHS)			AGE (YEARS)					TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Ia	22	4	0	26	0	0	0	0	**26**	***17.7***
CSF	*1*	*1*	*0*	*2*	*0*	*0*	*0*	*0*	***2***	
Blood	*21*	*3*	*0*	*24*	*0*	*0*	*0*	*0*	***24***	
Ib	2	1	0	3	0	0	0	0	**3**	***2.0***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*2*	*1*	*0*	*3*	*0*	*0*	*0*	*0*	***3***	
II	14	0	0	14	0	0	1	0	**15**	***10.2***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*14*	*0*	*0*	*14*	*0*	*0*	*1*	*0*	***15***	
III	58	19	2	79	0	0	1	0	**80**	***54.4***
CSF	*11*	*4*	*0*	*15*	*0*	*0*	*1*	*0*	***16***	
Blood	*47*	*15*	*2*	*64*	*0*	*0*	*0*	*0*	***64***	
IV	2	0	0	2	0	0	0	0	**2**	***1.4***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*2*	*0*	*0*	*2*	*0*	*0*	*0*	*0*	***2***	
V	11	5	0	16	0	0	0	1	**17**	***11.5***
CSF	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	***1***	
Blood	*10*	*5*	*0*	*15*	*0*	*0*	*0*	*1*	***16***	
VI	1	1	0	2	0	0	0	0	**2**	***1.4***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*1*	*1*	*0*	*2*	*0*	*0*	*0*	*0*	***2***	
IX	2	0	0	2	0	0	0	0	**2**	***1.4***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*2*	*0*	*0*	*2*	*0*	*0*	*0*	*0*	***2***	
Total	**112**	**30**	**2**	**144**	**0**	**0**	**2**	**1**	**147**	***100.0***
CSF	***13***	***5***	***0***	***18***	***0***	***0***	***1***	***0***	***19***	***12.9***
Blood	***99***	***25***	***2***	***126***	***0***	***0***	***1***	***1***	***128***	***87.1***
%	***76.2***	***20.4***	***1.4***	***98.0***	***0***	***0***	***1.4***	***0.6***	***100.0***	

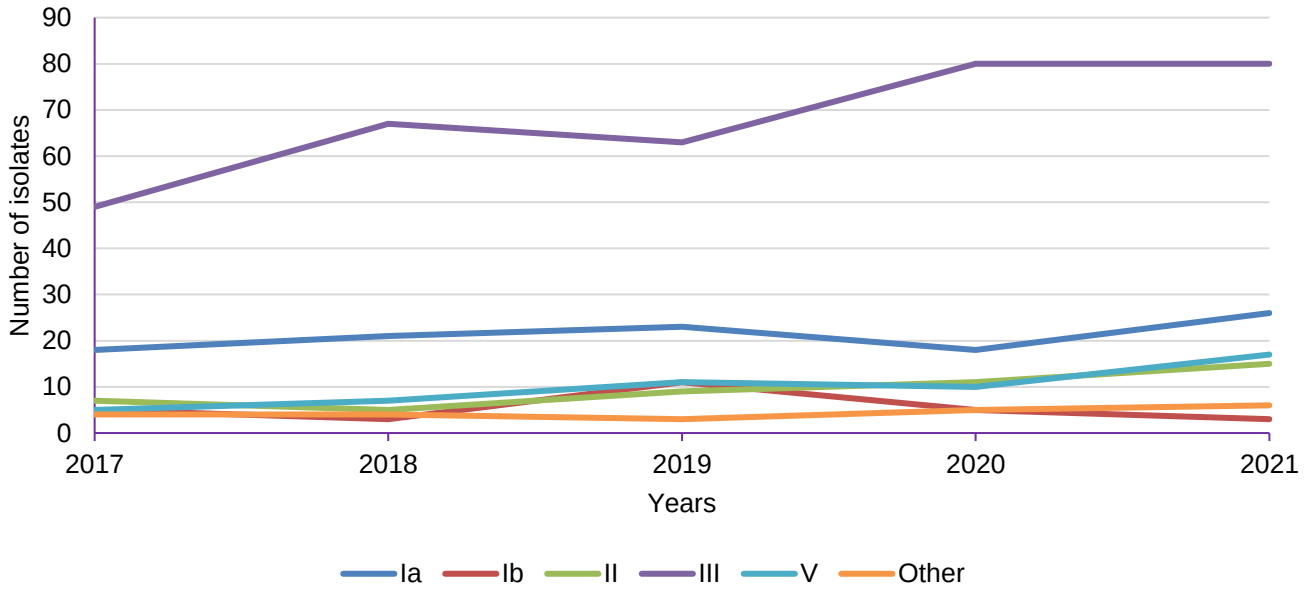


Figure 8.2 *Distribution of S. agalactiae serotypes, 2017 - 2021*

9 LISTERIA MONOCYTOGENES

Eighty *Listeria monocytogenes*[10] isolates were submitted to the NRLBM. Of these, 12 (15%) were from CSF (or CSF and blood) and 68 (85%) from blood only (Figure 9.1). The large majority (85%) occurred among individuals over 50 years of age (Table 9.1). Similar to previous years, serotypes 1/2a and 4b were most prevalent in 2021 (Table 9.1), accounting for 32.5% and 58.8% of the cases, respectively. The distribution of serotypes is similar to previous years (Figure 9.2)

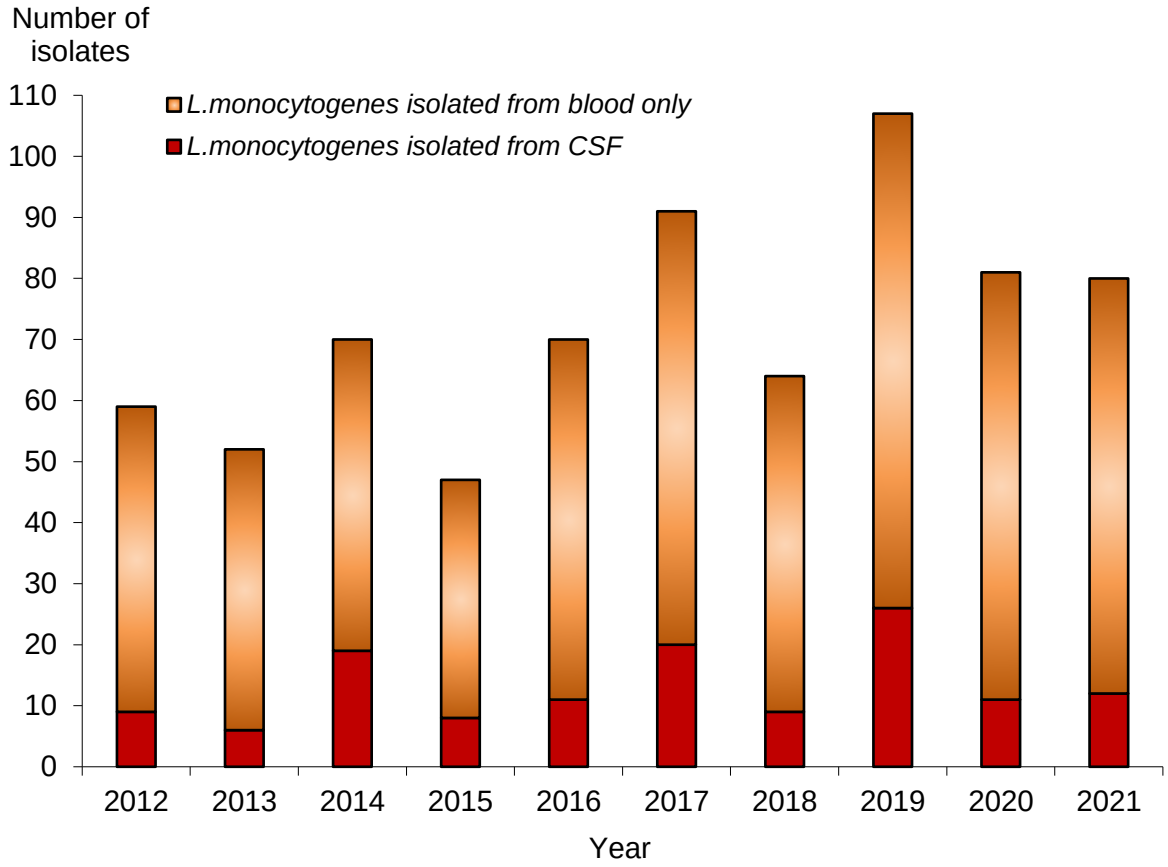


Figure 9.1 *Number of L. monocytogenes isolates grouped by isolation source, 2012-2021*

[10] RIVM. (Dutch article) *Vleeswaren waarschijnlijk bron 20 patiënten met Listeria*. RIVM: https://www.rivm.nl/nieuws/vleeswaren-waarschijnlijk-bron-20-patienten-met-listeria

Table 9.1 Total number of *L. monocytogenes* isolates from CSF and/or blood grouped according to age of patient and serotype, 2021

	AGE (YEARS)					TOTAL	
Group	**0-4**	**5-19**	**20-49**	**50-79**	**≥80**	**T**	**%**
1/2a	4	0	0	20	2	**26**	***32.5***
CSF	*0*	*0*	*0*	*4*	*0*	***4***	
Blood	*4*	*0*	*0*	*16*	*2*	***22***	
1/2b	1	0	0	4	1	**6**	***7.5***
CSF	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*1*	*0*	*0*	*4*	*1*	***6***	
4b	5	0	2	24	16	**47**	***58.8***
CSF	*0*	*0*	*0*	*6*	*1*	***7***	
Blood	*5*	*0*	*2*	*18*	*15*	***40***	
4e	0	0	0	1	0	**1**	***1.2***
CSF	*0*	*0*	*0*	*1*	*0*	***1***	
Blood	*0*	*0*	*0*	*0*	*0*	***0***	
Total	**10**	**0**	**2**	**49**	**19**	**80**	***100.0***
CSF	***0***	***0***	***0***	***11***	***1***	***12***	***15.0***
Blood	***10***	***0***	***2***	***38***	***18***	***68***	***85.0***
%	***12.5***	***0***	***2.5***	***61.2***	***23.8***	***100.0***	

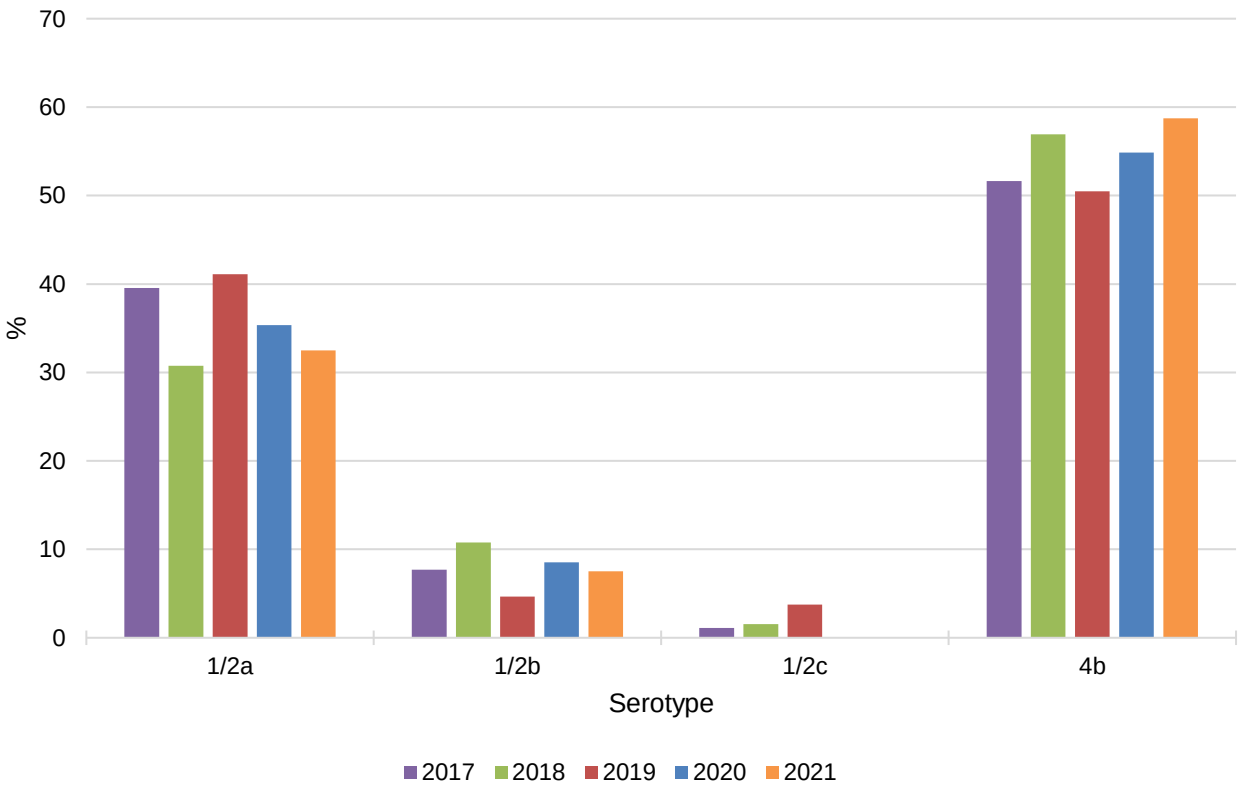


Figure 9.2 *Percentage of L. monocytogenes isolates grouped by serotype, 2017-2021*

10 STREPTOCOCCUS PYOGENES – (group A)

Until 2019, the NRLBM received *Streptococcus pyogenes* isolates associated with meningitis only. From April 2019, the NRLBM also receives *S. pyogenes* isolates from other invasive infections (iGAS) that are submitted through 9 sentinel laboratories that cover approximately 25% of the Dutch population. In addition, the NRLBM participated in a 2-year pilot study to gain insight into puerperal sepsis/fever caused by *S. pyogenes* at the national level (pGAS) starting in 2019 and ending July 2021.

Since 2015, all received *S. pyogenes* isolates are *emm*-typed by sequencing the hypervariable part of the *emm* gene (CDC – Streptococcus Laboratory)[11], which encodes the surface-expressed M protein. Currently, over 220 different *emm* genotypes are recognized. In 2014, an *emm*-cluster based system was proposed, clustering related M proteins based on shared binding and functional properties (Sandersom-Smith, 2014)[12].

In 2021, 137 *S. pyogenes* isolates were submitted to the NRLBM, 2 isolated from CSF (or CSF and blood; 1.5%), 69 from blood only (50.4%) and 66 from other sites (48.1%; Table 10.1). Almost 86% of *S. pyogenes* infections occurred in patients above 20 years of age (Table 10.1). The *emm* typing and *emm*-cluster based data of all isolates are displayed in Table 10.2. In total 25 different *emm* types were identified (Table 10.2). Over 65% of isolates belonged to *emm*-clusters E4 (49.6%) and E1 (15.3%; table 10.2), with predominant *emm* types within these clusters being *emm*89, *emm*22.0, *emm*28.0 and *emm*4.0. Remarkably, only 7.3% of the isolates belonged to *emm* 1.0, which is globally most associated with invasive GAS infections (Table 10.2). Seven isolates were submitted as Group A *Streptococcus* but turned out to be *S. dysgalactiae subsp. equisimilis* expressing the Lancefield group A antigen, a phenomenon that has been described previously.

Table 10.1 *S. pyogenes* isolates from CSF and/or blood according to patient's age, 2021.

TYPE	AGE (YEARS)					TOTAL	
	0-4	5-9	10-19	20-49	≥50	T	%
CSF	1	1	0	0	0	**2**	***1.5***
No studie	1	1	0	0	0	**2**	
Blood	8	4	2	27	28	**69**	***50.4***
No studie	1	3	2	6	9	**21**	
pGAS	0	0	0	11	0	**11**	
iGAS	7	1	0	10	19	**37**	
Other	1	1	2	58	4	**66**	***48.1***
No studie	0	0	0	2	0	**2**	
pGAS	0	0	1	45	0	**46**	
iGAS	1	1	1	11	4	**18**	
Total	**10**	**6**	**4**	**85**	**32**	**137**	***100***
%	***7.3***	***4.4***	***2.9***	***62.0***	***23.4***	***100***	

[11]CDC - Streptococcus Laboratory. Centers for Disease Control and Infections: https://www.cdc.gov/streplab/groupa-strep/resources.html

[12]Sanderson-Smith, M. D. (2014, feb 10). A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. *J Infect Dis 210, 1325-1338*.

Table 10.2 *Emm*-type and *emm*-cluster distribution of *S. pyogenes* isolates, 2021

Cluster	*emm* type	CSF	Blood	Other*	*1*	*2*	*3*	*4*	Total	%T
E1		**0**	**8**	**12**	***4***	***2***	***5***	***2***	**21**	**15.3**
	4.0	0	8	12	*4*	*1*	*5*	*2*	20	
	4.19	0	0	1	*0*	*1*	*0*	*0*	1	
E2		**0**	**3**	**0**	***0***	***0***	***0***	***0***	**3**	**2.2**
	76.17	0	1	0	*0*	*0*	*0*	*0*	1	
	90.2	0	1	0	*0*	*0*	*0*	*0*	1	
	92.0	0	1	0	*0*	*0*	*0*	*0*	1	
E3		**0**	**3**	**11**	***3***	***0***	***8***	***0***	**14**	**10.2**
	9.4	0	0	1	*1*	*0*	*0*	*0*	1	
	44.0	0	0	1	*0*	*0*	*1*	*0*	1	
	82.0	0	0	2	*1*	*0*	*1*	*0*	2	
	87.0	0	3	7	*1*	*0*	*6*	*0*	10	
E4		**1**	**34**	**33**	***1***	***1***	***28***	***3***	**68**	**49.6**
	2.0	0	1	0	*0*	*0*	*0*	*0*	1	
	8.0	0	1	0	*0*	*0*	*0*	*0*	1	
	22.0	0	8	8	*0*	*0*	*8*	*0*	16	
	28.0	0	7	9	*0*	*0*	*8*	*1*	16	
	73.0	0	1	0	*0*	*0*	*0*	*0*	1	
	77.0	0	2	4	*0*	*0*	*4*	*0*	6	
	89.0	1	14	12	*1*	*1*	*8*	*2*	27	
E6		**0**	**2**	**6**	***0***	***0***	***6***	***0***	**8**	**5.8**
	11.0	0	1	1	*0*	*0*	*1*	*0*	2	
	75.0	0	0	5	*0*	*0*	*5*	*0*	5	
	94.0	0	1	0	*0*	*0*	*0*	*0*	1	
A-C3		**1**	**9**	**0**	***0***	***0***	***0***	***0***	**10**	**7.3**
	1.0	1	9	0	*0*	*0*	*0*	*0*	10	
A-C4		**0**	**6**	**3**	***0***	***0***	***3***	***0***	**9**	**6.7**
	12.0	0	3	1	*0*	*0*	*1*	*0*	4	
	12.37	0	3	1	*0*	*0*	*1*	*0*	4	
	12.68	0	0	1	*0*	*0*	*1*	*0*	1	
D4		**0**	**3**	**0**	***0***	***0***	***0***	***0***	**3**	**2.2**
	53.12	0	1	0	*0*	*0*	*0*	*0*	1	
	80.7	0	1	0	*0*	*0*	*0*	*0*	1	
	83.1	0	1	0	*0*	*0*	*0*	*0*	1	
Deletie		**0**	**1**	**0**	***0***	***0***	***0***	***0***	**1**	**0.7**
	Deletie	0	1	0	*0*	*0*	*0*	*0*	1	
Total		**2**	**69**	**66**	*8*	*3*	*50*	*5*	**137**	**100**

*1: abscess; 2: Throat, nose, ear, BAL, sputum; 3: Cervix, fluor, vagina, lochia, urine; 4: Synovial fluid, wound

11 DNA or ANTIGEN DETECTION

The NRLBM received 105 culture-negative specimens of CSF, serum or other bodily fluids for antigen or DNA detection (Table 2.1). Lateral Flow Assay for cryptoccal antigen (LFA assay) was used to detect *C. neoformans*. PCR was performed with primers and probes specific for *N. meningitidis* (targeting *ctrA*) for *S. pneumoniae* (targeting *pia*) and for *H. influenzae* (*siaT* gene). When CSF was positive in the meningococcal PCR, the same sample was subjected to serogroup-specific PCR.

Of 105 culture-negative samples, 31 (30%) were positive for one of the target species by PCR or LFA test. Of these, 13 (42%) were positive for *N. meningitidis* (of which 12 were identified as serogroup B), 14 (45%) were positive for *S. pneumoniae* and 4 (13%) were positive for *H. influenzae*.

Table 11.1 CSF and serum samples tested for antigens or DNA, 2021

	CSF * (or DNA from CSF)	SERA or other fluids	TOTAL
***C. neoformans* (LFA)**	0	0	**0**
DNA of			
N. meningitidis group B	12	0	**12**
N. meningitidis	1	0	**1**
S. pneumoniae	13	1	14
H. influenzae	4	0	4
Sub Total	**30**	**1**	**31**
Antigen or PCR negative	57	3	**60**
LFA negative	9	5	**14**
Total	**97**	**9**	**105**

*From 8 patients with *S. pneumoniae* isolated from blood, the CSF was culture-negative but PCR-positive for pneumococcal DNA. Those were counted as CSF patients.
From 1 patient with *N. meningitidis* isolated from blood, the CSF was culture-negative but PCR-positive for meningococcal DNA. This one was counted as CSF.
Of 1 patient with positive Hi PCR a blood isolate was received. Those patient was counted as CSF.
The 9 fluids other than CSF were blood, sera, ascites and punctate.

12 PUBLICATIONS

1. A. Steens, K.R. Stanoeva, M.J. Knol, R. Mariman, H.E. de Melker, N.M. van Sorge. Increase in invasive disease caused by *Haemophilus influenzae* b, the Netherlands, 2020 to 2021. ***Eurosurveillance*** 2021 Oct; 26 (42): 2100956.

2. Angela B. Brueggemann, Melissa Jansen van Rensburg, David Shaw, Noel McCarthy, Keith A Jolley, Martin CJ Maiden, Mark PG van der Linden, Samantha CG Almeida, Alba Redin Alonso, Zahin Amin-Chowdhury, Amaresh Perez Argüello, Désirée E Bennett , Ray Borrow, Maria-Cristina C Brandileone, Karen Broughton, Bin Cao, Eun Hwa Choi, YW Chu, Stephen A Clark, Heike Claus, Juliana Coelho, Mary Corcoran, Simon Cottrell, Robert J Cunney, Tine Dalby, Heather Davies, Linda de Gouveia, Ala-Eddine Deghmane, Walter Demczuk, Stefanie Desmet, Richard J Drew, Mignon du Plessis, Helga Erlendsdottir, Norman K Fry, Kurt Fuursted, Steve J Gray, Birgitta Henriques-Normark, Thomas Hale, Markus Hilty, Steen Hoffmann, Hilary Humphreys, Margaret Ip, Susanne Jacobsson, Jana Kozakova, Karl G Kristinsson, Pavla Krizova, Alicja Kuch, Shamez N Ladhani, Thiên-Trí Lâm, Vera Lebedova, Ana Paula S Lemos, Laura Lindholm, David Litt, Irene Martin, Delphine Martiny, Wesley Mattheus, Martha McElligott , Mary Meehan, Susan Meiring, Paula Mölling, Eva Morfeldt, Julie Morgan, Robert M Mulhall, Carmen Muñoz-Almagro, David Murdoch, Martin Musilek, Monique Perrin, Malorie Perry, Richard Roberts, Maria Roberts, Assaf Rokney, Merav Ron, Kevin Scott, Carmen L Sheppard, Lotta Siira, Anna Skoczyńska, Hans-Christian Slotved, Andrew J Smith, Joon Young Song, Muhamed-Kheir Taha, Maija Toropainen, Dominic Tsang, Anni Vainio, Nina M van Sorge, Emmanuelle Varon, Jiri Vlach, Ulrich Vogel, Sandra Vohrnova, Anne von Gottberg, Rosemeire C Zanella, Fei Zhou. The Invasive Respiratory Infection Surveillance (IRIS) Initiative reveals significant reductions in invasive bacterial infections during the COVID-19 pandemic. 2021 ***Lancet Digital Health*** 2021 Jun; 3(6): e360-e370. doi: 10.1016/S2589-7500(21)00077-7

3. M. Middeldorp, A. van Lier, N. van der Maas, I. Veldhuijzen, W. Freudenburg, N.M. van Sorge, E.A.M. Sanders, M.J. Knol, H.E. de Melker. Short-term impact of COVID-19 pandemic on incidence of vaccine preventable diseases and participation in routine infant vaccinations in the Netherlands in the period March-September 2020. ***Vaccine*** 2021 Feb 12; 39 (7): 1039-1043

4. M. Ohm, S.J.M. Hahné, A. van der Ende, E.A.M. Sanders, W.L.M. Ruijs, N.M. van Sorge, H.E. de Melker, M.J. Knol. Vaccine impact and effectiveness of meningococcal serogroup ACWY conjugate vaccine implementation in the Netherlands: a nationwide surveillance study. ***Clin Infect Dis*** 2021 Sep 15; ciab791

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