NETHERLANDS REFERENCE LABORATORY FOR BACTERIAL MENINGITIS

BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2022

Amsterdam UMC
University Medical Centers

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Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM)
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CONTENTS

1 INTRODUCTION

This is the **51st** Annual Report of the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) of the Amsterdam UMC 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. All Dutch clinical microbiology laboratories collaborate with the NRLBM by submitting bacterial isolates and/or biological samples (e.g. cerebrospinal fluid) 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 94,480 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 invasive bacterial infections with a longstanding focus on bacterial meningitis;
- to describe the (molecular) epidemiology of invasive bacterial infections;
- 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, July, 2023

Ilse de Beer - Schuurman, BSc
N.M. van Sorge, PhD, Professor | head of the NRLBM
W. Freudenburg - de Graaf, MD PhD, clinical microbiologist

2 ISOLATES, CSF SPECIMENS AND SERA RECEIVED

The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) receives, types and stores 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 only 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 2022 as the reference date. By estimation, the NRLBM receives about 90% of the isolates from bacterial meningitis patients in the Netherlands[2].

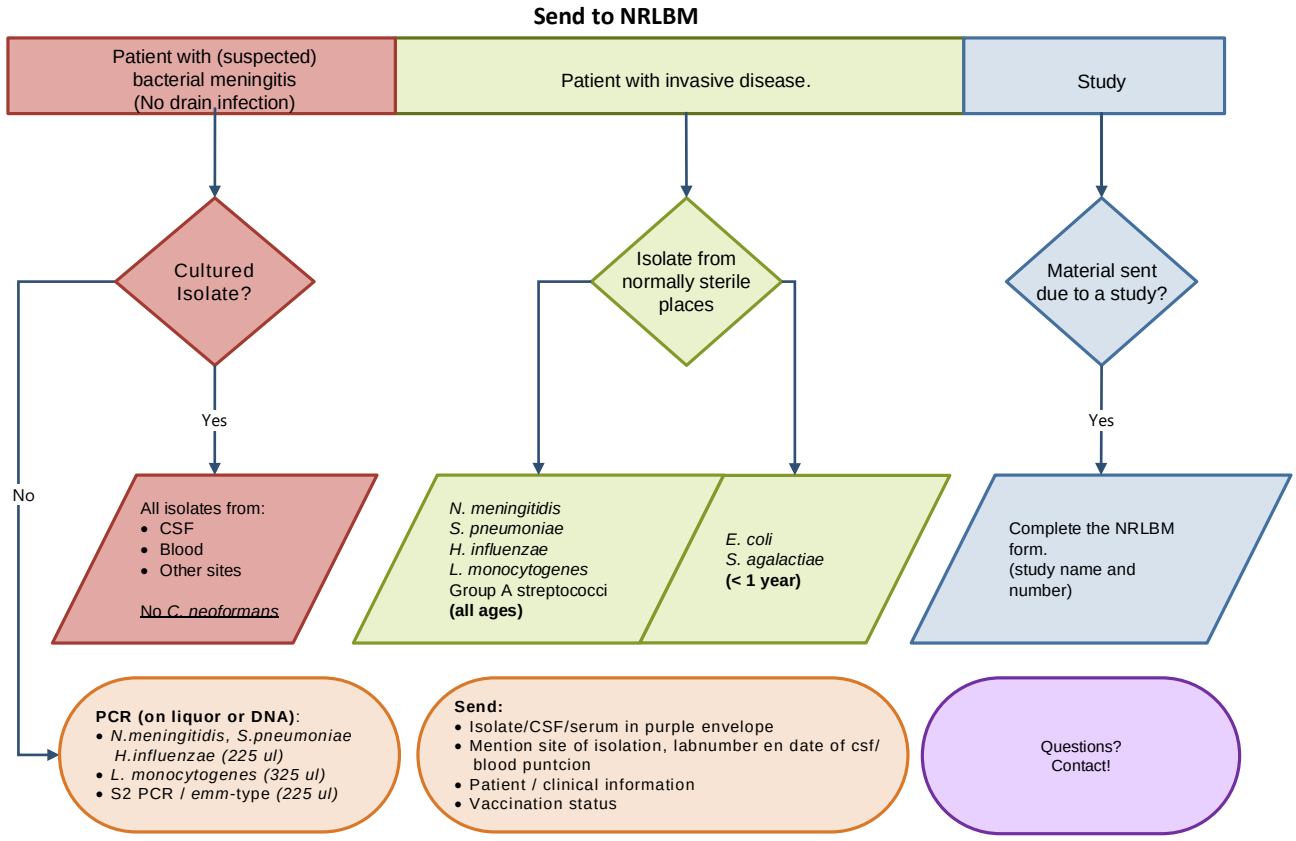


Figure 2.1. Overview of *isolates and materials received by the NRLBM.*

In 2022, the NRLBM received isolates from CSF and/or blood and samples of CSF, serum or other body fluids of 4,145 patients of which 2,747 were cultured or positive in antigen or PCR tests (**table 2.1/table 11.1**). Of all patients, 330 were culture- or PCR-confirmed cases of bacterial meningitis.

[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

Table 2.1 Overview of all samples received by the NRLBM in 2022

	Number of specimens
Isolates (CSF and/or blood)	*2,694*
PCR- or antigen-positive samples of CSF, sera and other fluids	*53*
Total positive isolates and PCR- or antigen-positive samples	**2,747**
PCR- or antigen-negative samples of CSF, sera and other fluids	**93**
Total	**2,840**

In 2022, 48 clinical microbiology laboratories submitted isolates or samples to the NRLBM. Table 2.2 shows the received isolates or positive PCR samples from 2,747 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 2022, according to laboratory and bacterial species.

Location	Laboratory MM; Medical Microbiology	Bacterial species[#]												
		Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	Nv	Total
Alkmaar	Northwest Clinics	4	7	53	-	7	4	1	-	-	-	1	1	**78**
Amersfoort	Meander Medical Center	1	6	26	-	2	2	1	-	-	-	-	-	**38**
Amsterdam	Amsterdam UMC	3	12	50	12	4	2	3	4	5	1	10	-	**106**
	OLVG	3	11	57	2	4	1	2	-	-	-	1	-	**81**
	ATAL medial (Slotervaart / Amstelland)	-	1	11	1	1	-	-	-	-	-	-	-	**14**
Apeldoorn	Eurofins, MMI Gelre Hospitals	1	7	45	3	1	5	-	-	-	1	-	-	**63**
Arnhem	Rijnstate Hospital	2	3	43	1	-	2	-	-	-	2	-	-	**53**
Breda	Microvida MMI Brabant & Zeeland	2	6	48	1	1	1	-	-	-	1	2	-	**62**
Capelle ad IJssel	IJsselland Hospital	-	1	26	1	3	2	-	-	-	-	-	-	**33**
Delft	Reinier Haga Medische Diagnostisch Centrum (RHMD)	1	5	44	-	5	2	-	-	-	-	1	-	**58**
Den Bosch	Jeroen Bosch Hospital, MM	1	12	86	2	4	1	-	-	-	-	1	-	**107**
Den Haag	Haga Hospital	-	5	23	-	2	1	-	-	-	-	-	-	**31**
	Haaglanden Medical Center	1	4	28	-	1	-	2	1	1	-	3	-	**41**
Deventer	Deventer Hospital, LMMI	2	2	24	-	1	4	-	1	-	-	-	-	**34**
Doetinchem	Slingeland Hospital, MM	2	5	43	1	1	1	-	-	-	-	-	1	**54**
Dordrecht	RLM Dordrecht / Gorinchem	3	5	37	1	4	1	1	-	-	-	-	-	**52**
Ede	Gelderse Vallei, MM	4	12	49	2	6	2	-	-	-	-	2	-	**77**
Goes	Microvida MMI Brabant & Zeeland	-	6	24	1	2	-	-	-	-	-	-	-	**33**
Gouda	Groene Hart Hospital	1	5	35	1	2	1	-	-	1	-	-	-	**46**
Groningen	Certe, MM	4	19	90	-	1	9	2	-	-	-	2	1	**128**
	UMCG, MMI	-	3	12	1	-	-	-	-	-	-	-	-	**16**
Haarlem	Streeklab voor de Volksgezondheid	1	12	64	1	3	3	-	-	-	-	1	-	**85**

Location	Laboratory MM; Medical Microbiology	Bacterial species#												
		Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	Nv	Total
Harderwijk	St. Jansdal	1	4	17	-	2	-	-	-	-	-	1	-	**25**
Hengelo	LabMicTa	-	8	79	1	7	2	1	-	-	-	-	-	**98**
Hilversum	CBS	1	6	18	1	-	1	2	-	-	2	-	-	**31**
Hoorn	Comicro, MML	3	7	44	4	3	3	-	-	-	1	-	-	**65**
Leeuwarden	MCL, Medische Microbiologie	5	11	75	2	7	3	-	-	-	-	1	-	**104**
Leiden	Eurofins Clinical Diagnostics, Alrijne Hospital	-	7	44	3	2	1	-	-	-	-	-	-	**57**
	LUMC,MM	2	9	23	4	2	3	1	-	-	-	3	-	**47**
Maastricht	Maastricht UMC+, MMI&I	1	3	25	-	-	1	-	-	-	-	-	-	**30**
Nieuwegein	Maatschap MMI, St. Antonius hospital	1	9	55	3	2	1	-	1	-	-	-	-	**72**
Nijmegen	Canisius Wilhelmina Hospital (CWZ)	1	4	25	2	2	1	-	-	-	-	-	-	**35**
	Radboud UMC	2	8	35	11	2	4	1	-	-	-	2	-	**65**
Neth Antilles	MM, Curacao/St.Maarten	-	-	3	-	-	-	-	-	-	-	-	-	**3**
Roermond	Laurentius hospital Roermond (LRZ)	-	4	12	-	-	-	-	-	-	-	-	-	**16**
Roosendaal	Microvida MMI Brabant & Zeeland, Bravis Hospital	1	2	37	1	-	1	-	-	-	-	1	-	**43**
Rotterdam	Erasmus MC, MMMI	2	10	30	15	4	1	3	-	-	3	2	1	**71**
	Ikazia Hospital, MMI	1	4	16	-	2	-	-	-	-	-	-	1	**24**
	Maasstad Laboratory, Maasstad Hospital	-	8	46	1	1	1	-	-	-	-	-	-	**57**
	Fransiscus Gasthuis & Vlietland	1	7	54	4	6	-	2	1	-	-	1	-	**76**
Sittard	Zuyderland Medisch Centrum	1	9	67	-	3	6	-	-	-	1	2	-	**89**
Terneuzen	Microvida MMI Brabant & Zeeland	1	2	13	-	-	-	-	-	-	-	-	-	**16**
Tilburg	Microvida MMI Brabant & Zeeland	-	7	62	1	2	1	-	-	-	2	-	-	**75**
Utrecht	Maatschap MMI, Diaconessenhuis Utrecht	2	3	21	-	2	1	-	-	-	-	-	-	**29**
	Maatschap MMI, St. Antonius hospital	-	-	1	-	-	-	-	-	-	-	-	-	**1**
	MMI, UMC Utrecht	2	8	28	4	2	4	1	-	-	-	5	-	**54**
Veldhoven	Eurofins, PAMM	11	24	101	1	6	5	2	-	-	1	1	-	**152**
Venlo	Vie Curie Medical Center	-	-	13	-	-	1	1	-	-	-	-	-	**15**
Zwolle	LMMI, Isala Hospital	4	9	79	3	4	4	1	-	-	1	2	-	**107**
Total		**79**	**322**	**1941**	**92**	**116**	**89**	**27**	**8**	**7**	**16**	**45**	**5**	**2747**

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 2018 - 2022 is presented in table 2.3. The total number of isolates was stable in 2018-2019 with approximately 2,650 samples. During the COVID-19 years 2020 and 2021, the number of samples decreased by 32% on average. This decrease is likely attributable to the introduction of containment policies that were implemented in response to the COVID-19 pandemic. For 2022, the number of samples (n=2,747) has steeply increased compared to 2021 and even exceeded the number of samples from 2019.

Table 2.3 Number of isolates from CSF and/or blood received in the years 2018 – 2022

Species[1]	2018			2019			2020			2021			2022		
	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total
N. meningitidis	70	135	205	53	104	157	27	39	66	19	18	37	41	38	79
H. influenzae	23	216	239	23	203	226	21	181	202	24	143	167	35	287	322
S. pneumoniae	152	1757	1909	165	1628[2]	1793	108	1007	1115	87	1030	1117	145	1796[2]	1941
E. coli	12	50	62	18	78	96	19	75	94	20	80	100	15	77	92
S. agalactiae	27	79	106	23	97	120	22	107	129	19	128	147	21	95	116
L. monocytogenes	9	56	65	26	81	107	11	70	81	12	68	80	10	79	89
S. pyogenes	3	9	12	14	122	136	5	112	117	2	69	71	19	8	27
S. aureus	7	0	7	11	0	11	10	0	10	3	0	3	8	0	8
Coag.neg.Staph.	4	0	4	2	0	2	4	1	5	7	0	7	7	0	7
C. neoformans	8	5	13	8	2	10	6	5	11	8	1	9	7	9	16
Others	18	7	25	16	11	27	16	22	38	13	15	28	22	23	45
non viable	0	7	7	0	0	0	0	1	1	0	0	0	0	5	5
Total	**333**	**2321**	**2654**	**359**	**2326**	**2685**	**249**	**1620**	**1869**	**214**	**1552**	**1766**	**330**	**2417**	**2747**

[1]Including PCR-positive samples
[2] 541 (2022) blood isolates from 9 sentinel labs
CSF: CSF or CSF and blood
blood: blood only
Non-viable; for 5 isolates from blood, 4 *S. pneumoniae* and 1 *H. influenzae* isolate

The incidence of invasive bacterial infections of the different bacterial species over the years 2018 to 2022 is shown in table 2.4. Incidences follow the number of received isolates/samples, with incidence of *S. pneumoniae* returning to pre-COVID19 levels. N. meningitis incidence is still at lower levels compared to 2019, which is likely a result of the COVID-19 restriction measures as well as the introduction of the MenACWY vaccine in 2018. Incidence for *H. influenzae* has been the highest of the past 5 years, wheres incidences for *E. coli* and *S. agalactiae* invasive disease in neonates remained similar or decreased, respectively.

Table 2.4 Incidence of invasive bacterial infections per species per 100,000 inhabitants, 2018 - 2022

Species	2018	2019	2020	2021	2022
N. meningitidis	1.19	0.91	0.38	0.21	0.45
H. influenzae	1.39	1.31	1.16	0.96	1.83
S. pneumoniae	11.11	10.37	6.41	6.39	11.03
E. coli	0.36	0.56	0.54	0.57	0.52
S. agalactiae	0.62	0.69	0.74	0.84	0.66
L. monocytogenes	0.38	0.62	0.47	0.46	0.51
S. pyogenes	0.07	0.79	0.67	0.41	0.15
S. aureus	0.04	0.06	0.06	0.02	0.05
Coag. neg. Staph.	0.02	0.01	0.03	0.04	0.04
C. neoformans	0.08	0.06	0.06	0.05	0.09
others	0.15	0.16	0.22	0.16	0.26
non viable	0.04	-	0.01	-	0.03
Total	**15.45**	**15.54**	**10.74**	**10.11**	**15.62**

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, E. coli and Listeria monocytogenes*.

Table 2.5 Total number and proportion of isolates from CSF and/or blood received in 2022, according to bacterial species and source.

Species		CSF or CSF and blood, n	Blood only, n	Total, n	%
Neisseria meningitidis		41	38	79	**2.9**
Haemophilus influenzae [1]		35	287	322	**11.7**
Streptococcus pneumoniae [2]		145	1796	1941	**70.6**
Escherichia coli [3]		15	77	92	**3.4**
Streptococcus agalactiae[4]		21	95	116	**4.2**
Listeria monocytogenes[5]		10	79	89	**3.2**
Streptococcus pyogenes		19	8	28	**1.0**
Staphylococcus aureus[6]		8	0	8	**0.3**
Coagulase-negative staphylococcus [7]		7	0	7	**0.3**
Cryptococcus neoformans		7	9	16	**0.6**
Others total		22	23	45	**1.6**
Others	*Klebsiella pneumonia*	*3*	*0*	*3*	
	Klebsiella variicola	*1*	*0*	*1*	
	Pseudomonas aeruginosa	*1*	*0*	*1*	
	Salmonella groep D	*1*	*0*	*1*	
	Acinetobacter lwoffi	*1*	*0*	*1*	
	Enterobacter cloacae	*2*	*0*	*2*	
	Enterobacter faecalis	*1*	*0*	*1*	
	Enterobacter faecium	*1*	*0*	*1*	
	Serratia marcescens	*1*	*0*	*1*	
	Moraxella osloensis	*1*	*0*	*1*	
	Streptococcus dysgalactiae	*0*	*1*	*1*	
	Streptococcus dysgalactiae ssp equisimilis	*0*	*12*	*12*	
	Streptococcus equi	*1*	*0*	*1*	
	Streptococcus gallolyticus ssp gallolyticus	*0*	*1*	*1*	
	Streptococcus gallolyticus ssp pasteurianus	*1*	*1*	*2*	
	Streptococcus infantis	*1*	*1*	*2*	
	Streptococcus mitis	*0*	*2*	*2*	
	Streptococcus oralis	*0*	*1*	*1*	
	Streptococcus oralis ssp oralis	*1*	*0*	*1*	
	Cutibacterium acnes	*1*	*0*	*1*	
	Candida albicans	*1*	*0*	*1*	
	Cryptococcus deneoformans	*0*	*1*	*1*	
	Granulicatella adiascens	*0*	*1*	*1*	
	Rothia mucilaginosa	*1*	*0*	*1*	
	Shewanella algae	*1*	*0*	*1*	
	Sphigomonas paucimobilis	*1*	*0*	*1*	
	Contamination	*0*	*2*	*2*	
Non viable		0	5	5	**0.2**
Total		330	2417	2747	**100.0**

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

2 **In two patients, both *Streptococcus pneumoniae* and *Streptococcus pyogenes* were isolated from blood.**

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

4 **In one patient, *Streptococcus agalactiae* and *Klebsiella pneumoniae* were isolated from CSF**

5 **In one patient, *Listeria monocytogenes* and *Streptococcus pyogenes* were isolated from blood**

6 **In one patient, *Staphylococcus aureus* and *Staphylococcus warneri* were isolated from CSF**

7 **Coagulase-negative staphylococci, 4 *Staphylococcus epidermidis* were isolated from CSF; two *Staphylococcus capitis* and one *Staphylococcus warneri was* isolated from CSF**

3 BACTERIAL MENINGITIS – general overview

In 2022, the NRLBM received CSF isolates or PCR-positive CSF samples from 330 patients (Table 2.3 and 11.1). The proportion of meningococcal, pneumococcal, and haemophilus cases among meningitis patients was 12%, 44%, and 11%, respectively (Figure 3.1). The neonatal pathogens *S. agalactiae* and *E. coli* represented 6% and 5% of the meningitis cases, respectively (Figure 3.1)

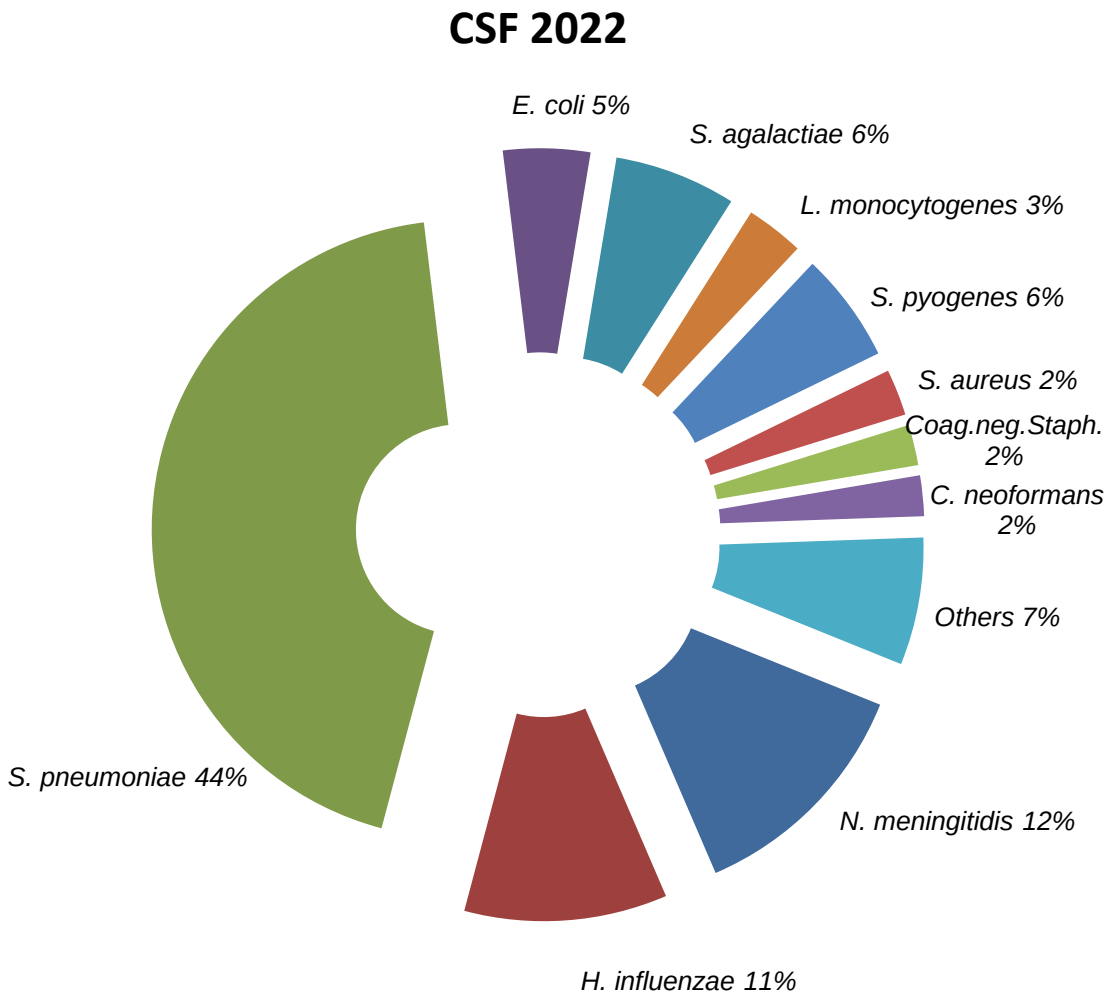


Figure 3.1 *Proportional distribution of CSF isolates and CSF-positive samples according to bacterial species, 2022*

Figure 3.2 shows the total annual number of bacterial isolates from CSF and CSF positive PCR's during the period 1992-2022. The trend line shows a decrease over the last three decades but incidence has stabilized around 2.0 from approximately 2010 until 2022, with a 2-year decreased incidence in 2020 and 2021 likely as a result of COVID-19 containment measures (Figure 3.2).

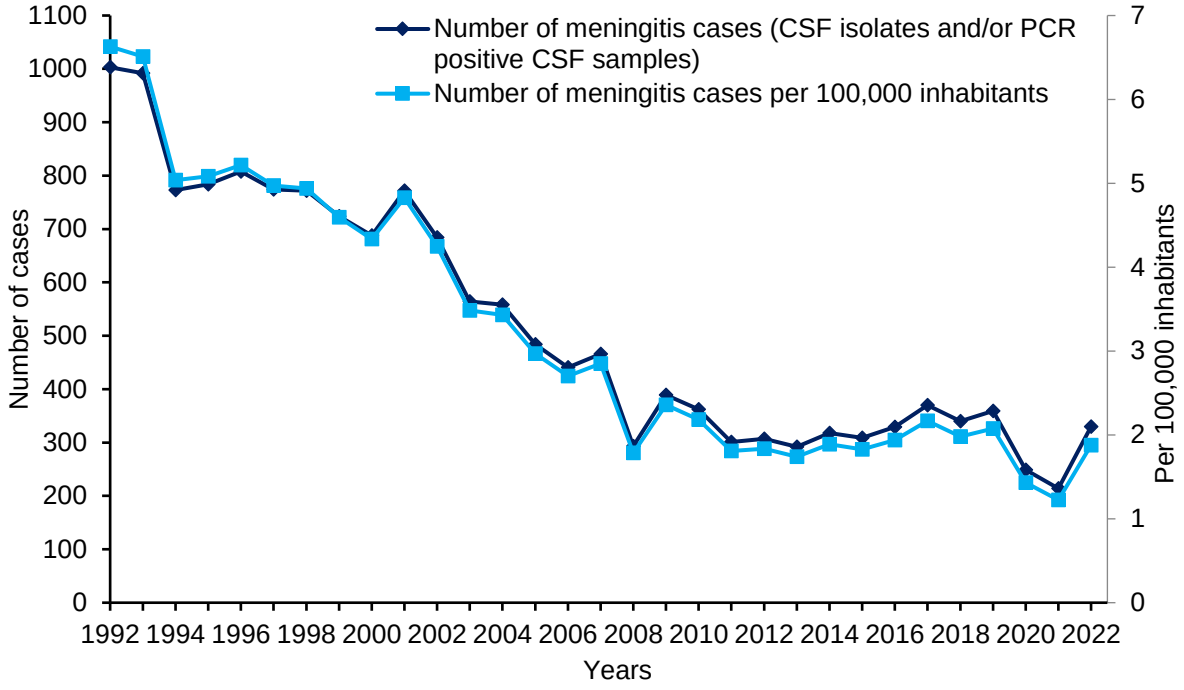


Figure 3.2 *All cause meningitis cases and incidence, 1992-2022*

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 less than 0.10 in 2009 but increasing to 0.20 per 100,000 in 2022. For meningococcal meningitis, the incidence decreased from 3.1/100,000 in 1993 to 0.23/100,000 in 2022. 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 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. Although the incidence of meningococcal meningitis is still low (0.23/100,000/year), the incidence is clearly increased compared to 2020/2021. 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) 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, but increased to almost pre-COVID19 levels in 2022 (0.82/100,000/year).

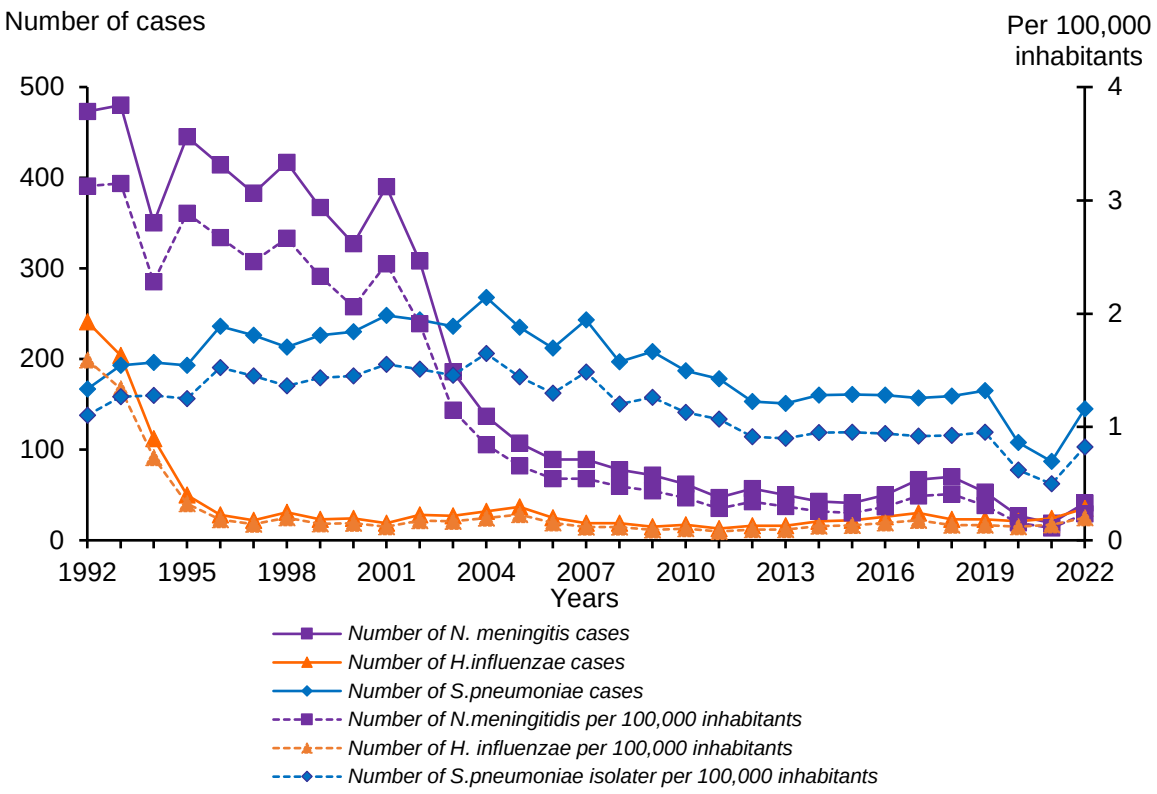


Figure 3.3 Number of cases and incidence of meningococcal, haemophilus and pneumococcal meningitis (isolates and/or positive PCR from CSF), 1992-2022

Table 3.1 shows the number of CSF isolates and/or CSF positive PCR's by annual quarter grouped by bacterial species. Most isolates were received during the 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 were fully lifted in March 2022.

Table 3.1 Isolates and PCR-positive samples from CSF by annual quarter according to bacterial species, 2022

	ANNUAL QUARTER					
SPECIES	**First**	**Second**	**Third**	**Fourth**	**Total**	**%**
N. meningitidis	4	12	9	16	**41**	***12.4***
H. influenzae	7	10	9	9	**35**	***10.6***
S. pneumoniae	37	40	20	48	**145**	***43.9***
E. coli	5	2	4	4	**15**	***4.6***
S. agalactiae	5	4	5	7	**21**	***6.4***
L. monocytogenes	2	2	3	3	**10**	***3.0***
S. pyogenes	5	4	4	6	**19**	***5.8***
S. aureus	4	1	1	2	**8**	***2.4***
Coag.neg.Staph.	1	1	2	3	**7**	***2.1***
C. neoformans	0	2	2	3	**7**	***2.1***
Others	8	1	6	7	**22**	***6.7***
non viable	0	0	0	0	**0**	***0.0***
Total	**78**	**79**	**65**	**108**	**330**	***100.0***
%	***23.6***	***24.0***	***19.7***	***32.7***	***100.0***	

Tables 3.2 shows the distribution of culture and non-culture positive CSF samples according to bacterial species and patient age. Table 3.3 shows the age-specific incidence per 100,000 individuals for the same samples. *S. agalactiae* and *E. coli* are still the predominant species isolated from neonates (i.e. younger than 1 month), and together represented 73% of all isolates in this age group. In contrast, in infants 1-11 months of age, the predominant species is *S. pneumoniae* (together with *H. influenzae*, *E. coli* and *S. agalactiae* 79%). 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 13 in 2022. Overall, for children ages 0-4 years, *S. pneumoniae* was the predominant cause of bacterial meningitis, representing 23.0% of all cases in this age group.

Table 3.2 Isolates/PCR-positive samples from CSF grouped according to species and age, 2022

	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	1	3	17	9	1	1	1	1	0	**41**	*12.4*
H. influenzae	0	4	10	14	1	0	0	1	4	0	4	11	0	**35**	*10.6*
S. pneumoniae	1	10	9	20	3	7	1	1	10	7	44	45	7	**145**	*43.9*
E. coli	10	4	0	14	0	0	0	1	0	0	0	0	0	**15**	*4.6*
S. agalactiae	12	5	0	17	0	0	0	2	0	0	0	2	0	**21**	*6.4*
L. monocytogenes	1	0	0	1	0	0	0	0	0	1	2	5	1	**10**	*3.0*
S. pyogenes	0	0	3	3	0	0	1	0	3	0	6	5	1	**19**	*5.8*
S. aureus	0	0	0	0	0	0	0	0	3	0	1	4	0	**8**	*2.4*
Coag.neg.Staph.	1	0	0	1	0	1	0	0	0	0	4	1	0	**7**	*2.1*
C. neoformans	0	0	0	0	0	0	0	1	1	1	2	2	0	**7**	*2.1*
Others	5	3	2	10	0	0	2	1	0	1	4	3	1	**22**	*6.7*
non viable	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	*0.0*
Total, n	*30*	*29*	*28*	*87*	*5*	*11*	*21*	*16*	*22*	*11*	*68*	*79*	*10*	**330**	*100*
%	***9.1***	***8.8***	***8.5***	***26.4***	***1.5***	***3.3***	***6.4***	***4.8***	***6.7***	***3.3***	***20.6***	***24.0***	***3.0***	***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 32.94 cases per 100,000. The overall incidence of bacterial meningitis increased from 1.22 in 2021 to 1.88 per 100,000 in 2022.

Table 3.3 Age-specific incidence of bacterial meningitis per 100,000 inhabitants, 2022

	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	1.67	0.58	0.11	0.31	1.66	0.40	0.05	0.05	0.03	0.04	-	0.23
H. influenzae	2.23	1.46	0.11	-	-	0.04	0.18	-	0.11	0.41	-	0.20
S. pneumoniae	6.14	1.31	0.34	0.73	0.10	0.04	0.45	0.33	1.19	1.68	0.82	0.82
E. coli	7.82	-	-	-	-	0.04	-	-	-	-	-	0.09
S. agalactiae	9.49	-	-	-	-	0.09	-	-	-	0.07	-	0.12
L. monocytogenes	0.56	-	-	-	-	-	-	0.05	0.05	0.19	0.12	0.06
S. pyogenes	0.59	-	0.11	-	-	-	-	-	-	-	-	0.01
S. aureus	-	0.44	-	-	0.10	-	0.14	-	0.16	0.19	0.12	0.11
Coag.neg.Staph.	0.56	-	-	0.10	-	-	-	-	0.11	0.04	-	0.04
C. neoformans	-	-	-	-	-	0.04	0.05	0.05	0.05	0.07	-	0.04
Others	4.47	0.29	-	-	0.20	0.04	-	0.05	0.11	0.11	0.12	0.13
non viable	-	-	-	-	-	-	-	-	-	-	-	-
Total	**32.94**	**4.08**	**0.56**	**1.15**	**2.05**	**0.71**	**0.99**	**0.52**	**1.83**	**2.96**	**1.17**	**1.88**

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*, which affected males six times more often as females.The overall M/F ratio was 1.5.

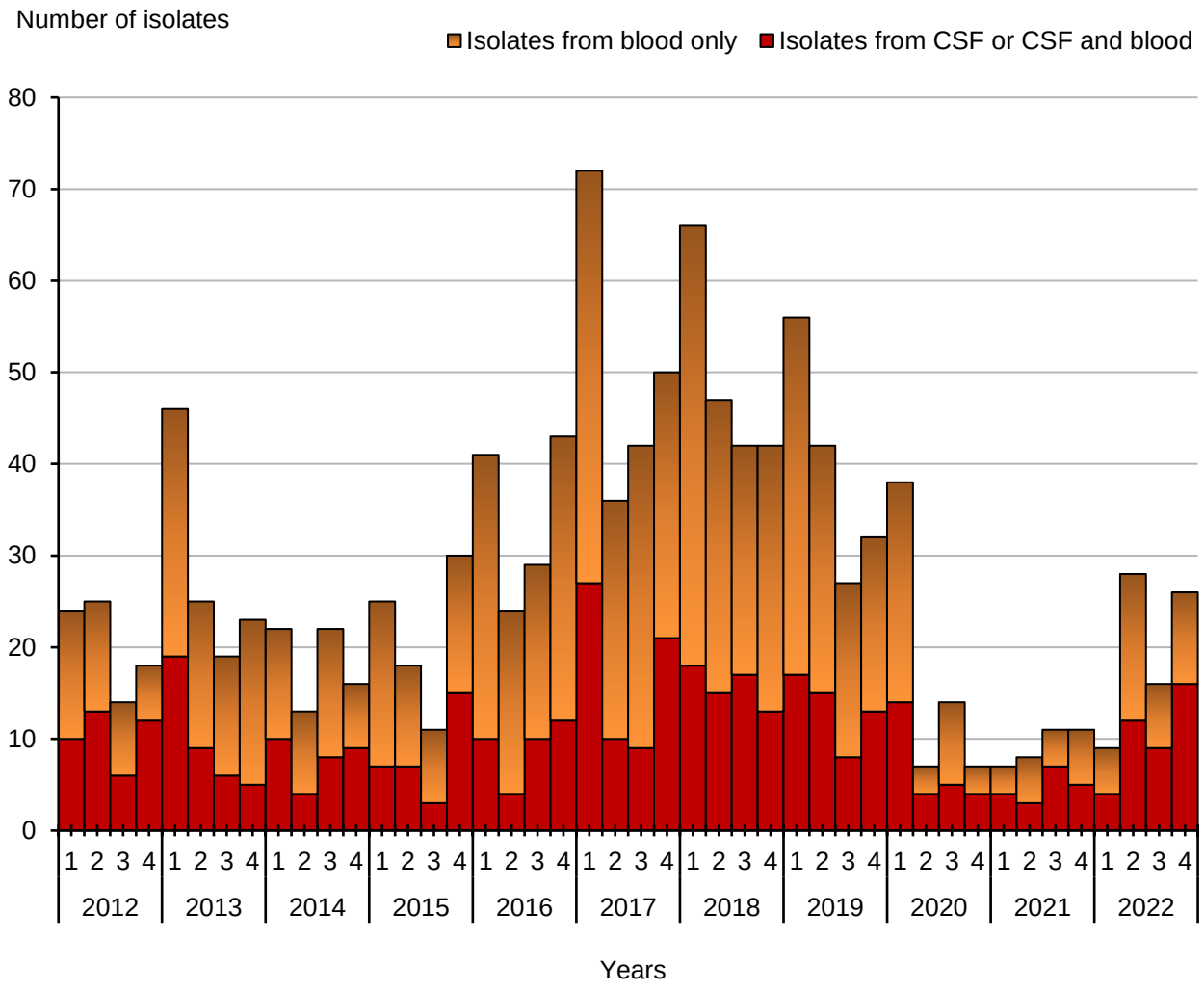
Table 3.4 Isolates and PCR-positive samples from CSF according to patients' gender, 2022

SPECIES	M	F	M/F – ratio	Sex not known	Total
N. meningitidis	26	14	**1.8**	1	**41**
H. influenzae	16	19	**0.8**	-	**35**
S. pneumoniae	76	67	**1.1**	2	**145**
E. coli	13	2	**6.5**	-	**15**
S. agalactiae	10	11	**0.9**	-	**21**
L. monocytogenes	6	4	**1.5**	-	**10**
S. pyogenes	7	12	**0.6**	-	**19**
S. aureus	4	4	**1.0**	-	**8**
Coag.neg.Staph.	5	2	**2.5**	-	**7**
C. neoformans	1	6	**0.2**	-	**7**
Others	14	8	**1.8**	-	**22**
non viable	0	0	**0**	-	**0**
Total	**178**	**149**	**1.2**	**3**	**330**
%	***53.9***	***45.1***		***0.9***	***100***

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2022, the NRLBM received 56 *Neisseria meningitidis* isolates of which 18 were isolated from CSF (or CSF and blood; 7 in 2021) and 38 from blood only (18 in 2021). In addition, 23 culture-negative CSF/blood samples tested positive for meningococci by PCR bringing the total number of received meningococcal isolates or PCR-positive CSF or blood to 79. The distribution of isolates received throughout the year was different from regular years in that the highest number of isolates was received in the second and fourth quarter of 2022 (figure 4.1).



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

Figure 4.1 *Seasonal distribution of meningococcal disease, 2012-2022*

4.2 Antibiotic susceptibility

All isolates (56/56) were susceptible to penicillin according to the new EUCAST break point (MIC ≤ 0.25 µg/ml; Table 4.1). In general, meningococcal resistance to penicillin is rare in the Netherlands (Tables 4.2, 4.3). 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), 2022

Penicillin*				
	MIC ≤ 0.25 (S)	**MIC>0.25 (R)**	**Total**	**%**
CSF or CSF and blood	18	0	**18**	***30***
Blood only	38	0	**38**	***70***
Total	**56**	**0**	**56**	***100***
%	***100***	***0***	***100***	

* MIC values in µg/ml

Table 4.2 Penicillin susceptibility of *N. meningitidis* isolates from CSF, 2018-2022

Penicillin*					
	MIC ≤ 0.25 (S)		**MIC>0.25 (R)**		**Total**
	N	%	N	%	
2018	53	*98*	1	*2*	**54**
2019	33	*100*	0	*0*	**33**
2020	14	*93*	1	*7*	**15**
2021	7	*100*	0	*0*	**7**
2022	18	*100*	0	*0*	**18**

* MIC values in µg/ml

Table 4.3 Penicillin susceptibility of *N. meningitidis* isolates from blood only, 2018-2022

Penicillin*					
	MIC ≤ 0.25 (S)		**MIC>0.25 (R)**		**Total**
	N	%	N	%	
2018	129	*98*	2	*2*	**131**
2019	102	*100*	0	*0*	**102**
2020	39	*100*	0	*0*	**39**
2021	18	*100*	0	*0*	**18**
2022	38	*100*	0	*0*	**38**

* MIC values in mg/L

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

4.3 Serogroups

Serogroup B accounted for 87% (n=69) of all received isolates / PCR-positive samples (Table 4.4), which is an increase in absolute numbers as well as proportion compared to previous years (2021 84%; 2020 61%; 2019 46%) and similar to number of MenB-positive samples pre-COVID-19. The proportion of serogroup W isolates decreased to 2.5% (table 4.4) compared to 11% in 2021, 18% in 2020, 38% in 2019, and 50% in 2018 (figure 4.2). Also in absolute numbers, the NRLBM received less serogroup W isolates (n = 2) 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. 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 and Z were both responsible for 2.5% (each n=2) of all cases of invasive meningococcal disease in 2022 (Table 4.4). In contrast to 2020 and 2021, 3 serogroup C were received, all isolated from blood. 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 has the highest incidence of invasive meningococcal disease, with the other serogroups represented nearly equally for the remaining cases (Table 4.5). Cases of invasive meningococcal disease are evenly distributed across the Netherlands (Figure 4.3).

Table 4.4 Number of meningococci according to serogroup and source of isolation, 2022

Source	Serogroup						Total, n
	B	C	W	Y	Z	NG*	
CSF	40	0	0	0	0	1	41
Blood	29	3	2	2	2	0	38
Total (%)	**69 (87.4)**	**3 (3.8)**	**2 (2.5)**	**2 (2.5)**	**2 (2.5)**	**1 (1.3)**	**79**

*Non groupable

Table 4.5 Incidence of meningococcemia per 100,000 inhabitants according to serogroup and source of isolation, 2022

Source	Serogroup						Total, n
	B	C	W	Y	Z	NG*	
CSF	0.23	0.00	0.00	0.00	0.00	0.01	0.23
Blood	0.16	0.02	0.01	0.01	0.01	0.01	0.22
Total	**0.39**	**0.02**	**0.01**	**0.01**	**0.01**	**0.01**	**0.45**

*Non groupable

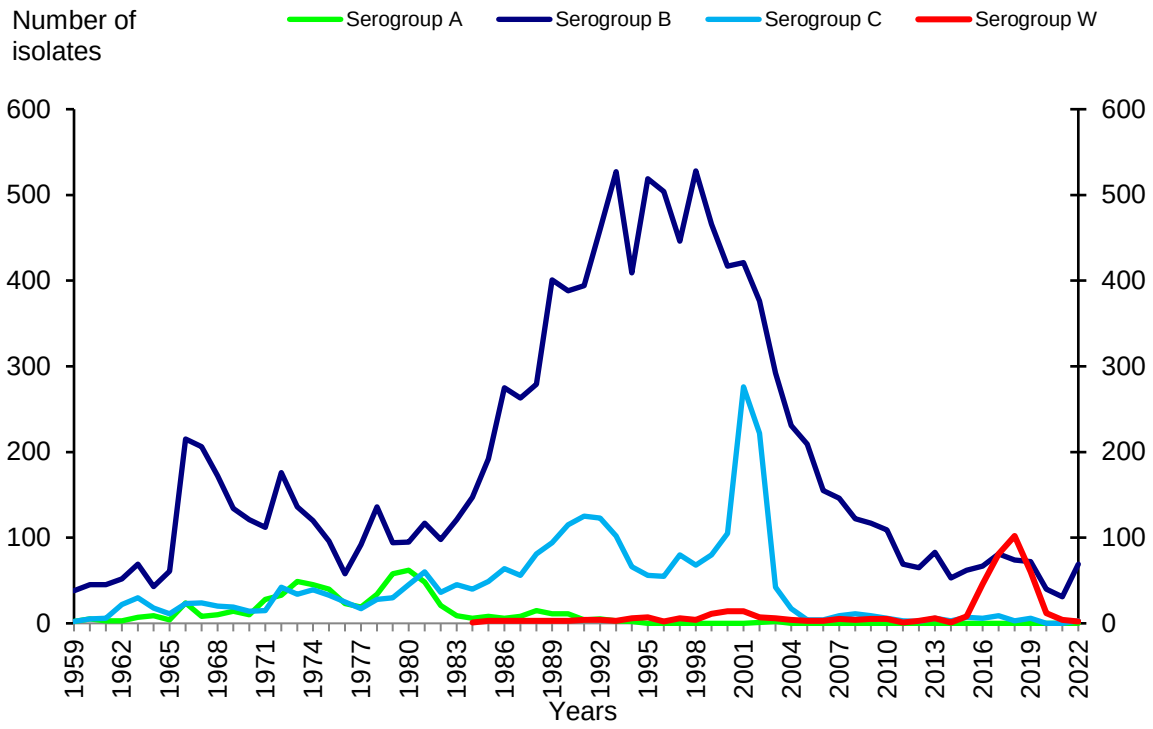
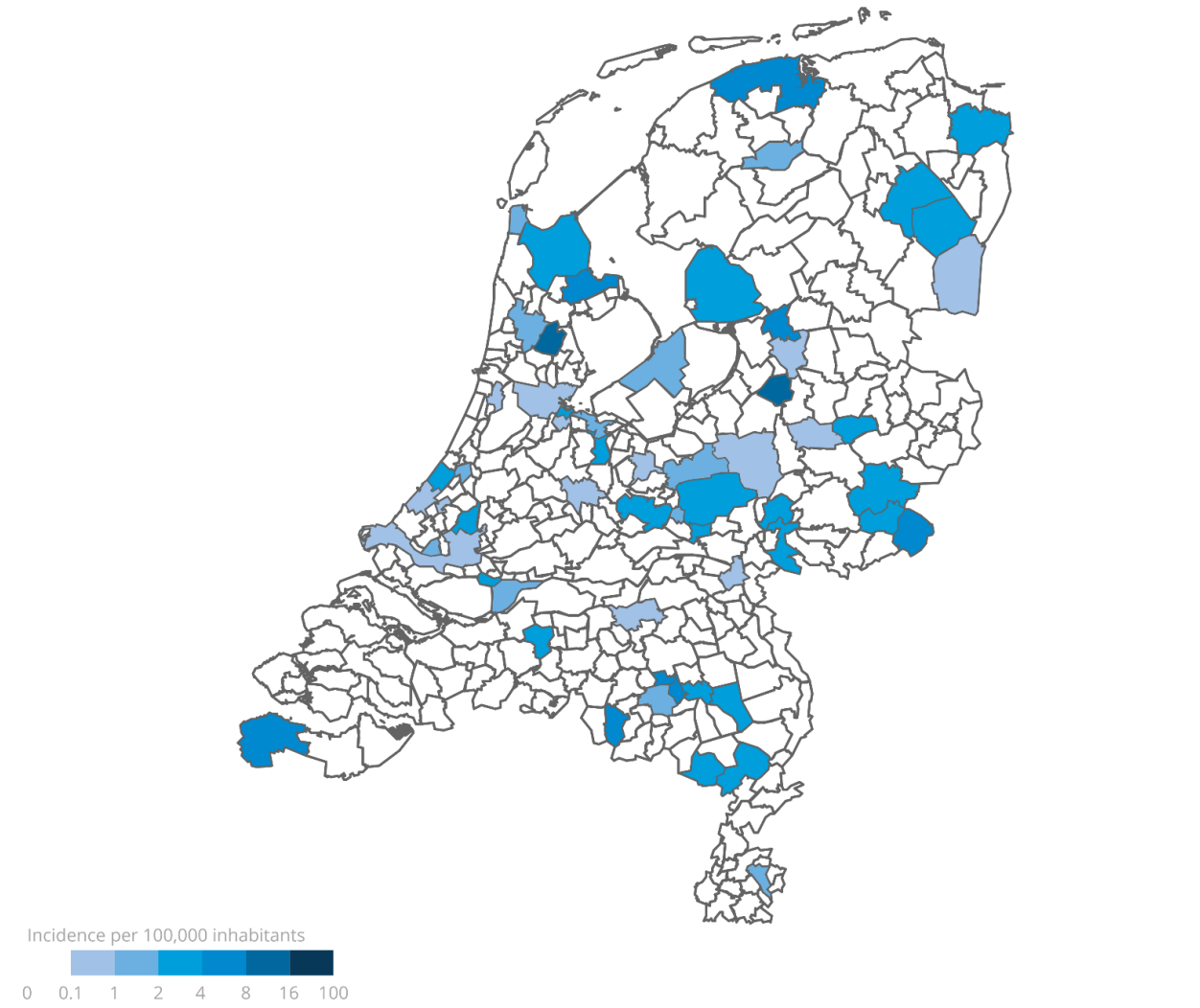


Figure 4.2. *Distribution of meningococcal serogroups A, B, C and W from 1959-2022.*

Figure 4.3 Geographical distribution of *N. meningitidis* (CSF and/or blood) cases based on incidence, *2022*. Incidence is calculated per municipality based on patient's place of residence.



4.4 Serogroup and age

Among serogroup B cases, 19% (13 of 69) of patients was below the age of 5 years and 80% (55/69) was between 0 and 25 years of age (table 4.6). In addition, 49% of serogroup B isolates (39/69) were isolated from CSF, compared to none of the serogroup C, W, Y and Z isolates, suggesting that the clinical presentation and population at risk for infection may be different for serogroup B and C, W, Y and Z meningococci. Overall, the incidence of invasive meningococcal disease is highest in the age groups < 1 year and 15-19 years of age 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 and isolation source, 2022

	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	5	8	13	1	4	28	9	1	4	4	5	**69**	***87.4***
CSF	*0*	*3*	*4*	*7*	*1*	*3*	*16*	*8*	*1*	*2*	*1*	*1*	*40*	*50.6*
Blood	*0*	*2*	*4*	*6*	*0*	*1*	*12*	*1*	*0*	*2*	*3*	*4*	*29*	*36.7*
C	0	0	0	0	1	0	0	0	0	0	2	0	**3**	***3.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Blood	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*2*	*0*	*3*	*3.8*
W	0	0	0	0	0	0	0	0	1	0	0	1	**2**	***2.5***
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*	*0*	*0*	*1*	*2*	*2.5*
Y	0	0	0	0	0	0	0	0	0	1	0	1	**2**	***2.5***
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*	*1*	*0*	*1*	*2*	*2.5*
Z	0	0	0	0	0	0	0	1	0	0	0	1	**2**	***2.5***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*1*	*2*	*2.5*
NG*	0	0	0	0	0	0	1	0	0	0	0	0	**1**	***1.3***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	*0*	*1*	*1.3*
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Total	0	5	8	**13**	**2**	**4**	**29**	**10**	**2**	**5**	**6**	**8**	**79**	***100.0***
CSF	*0*	*3*	*4*	***7***	***1***	***3***	***17***	***8***	***1***	***2***	***1***	***1***	***41***	***51.9***
Blood	*0*	*2*	*4*	***6***	***1***	***1***	***12***	***2***	***1***	***3***	***5***	***7***	***38***	***48.1***
%	***0***	***6.3***	***10.1***	***16.5***	***2.5***	***5.1***	***36.7***	***12.7***	***2.5***	***6.3***	***7.6***	***10.1***	***100.0***	

*Not enough DNA for serogroup PCR

[4] Gezondheidsraad. Vaccinatie tegen meningokokken. Den Haag: Gezondheidsraad, 2018; publicatienr. 2018/28 https://www.gezondheidsraad.nl/onderwerpen/vaccinaties/alle-adviezen-over-vaccinaties/vaccinatie-tegen-meningokokken-b-update
Werkagenda advisering Vaccinaties Gezondheidsraad 2022 (vierde kwartaal MenB)

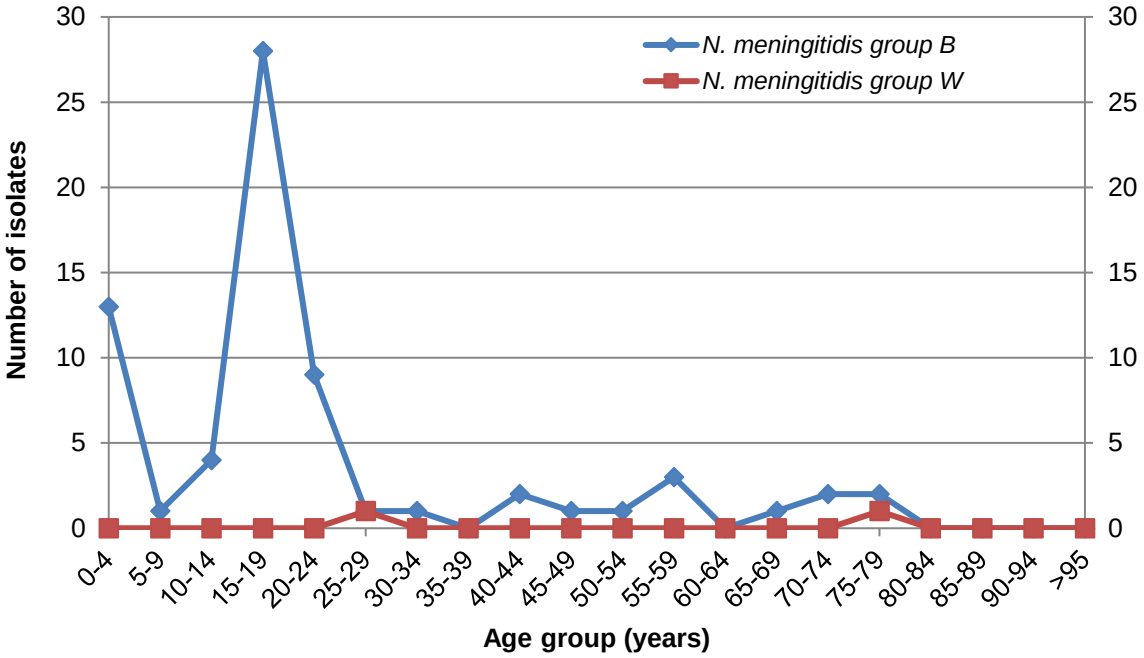
Table 4.7 Incidence of invasive meningococcal disease per 100,000 inhabitants according to meningococcal serogroup and patient's age, 2022

	AGE (YEARS)										TOTAL
Group	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	
B	2.79	1.17	0.11	0.42	2.73	0.79	0.09	0.09	0.11	0.14	**0.39**
CSF	*1.67*	*0.58*	*0.11*	*0.31*	*1.56*	*0.71*	*0.09*	*0.05*	*0.03*	*0.03*	***0.23***
Blood	*1.12*	*0.58*	*0.00*	*0.10*	*1.17*	*0.09*	*0.00*	*0.05*	*0.08*	*0.11*	***0.16***
C	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.05	0.00	**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*	*0.11*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.05*	*0.00*	***0.02***
W	0.00	0.00	0.00	0.00	0.00	0.00	0.09	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*	*0.09*	*0.00*	*0.00*	*0.03*	***0.01***
Y	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	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*	*0.02*	*0.00*	*0.03*	***0.01***
Z	0.00	0.00	0.00	0.00	0.00	0.09	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*	*0.09*	*0.00*	*0.00*	*0.00*	*0.03*	***0.01***
NG*	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
CSF	*0.00*	*0.00*	*0.00*	*0.10*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	***0.01***
Blood	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	***0.00***
Total	**2.79**	**1.17**	**0.22**	**0.42**	**2.83**	**0.88**	**0.18**	**0.11**	**0.16**	**0.23**	**0.45**
CSF	***1.67***	***0.58***	***0.11***	***0.31***	***1.66***	***0.71***	***0.09***	***0.05***	***0.03***	***0.03***	***0.23***
Blood	***1.12***	***0.58***	***0.11***	***0.10***	***1.17***	***0.18***	***0.09***	***0.07***	***0.13***	***0.20***	***0.22***

* Insufficient DNA for serogroup PCR

Figure 4.5 shows the age distribution of patients with invasive 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 1.50 and 2.73, respectively (Figure 4.5B and Table 4.7). The age-specific incidence per 100,000 inhabitants for all age groups >25 years was below 0.14 (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

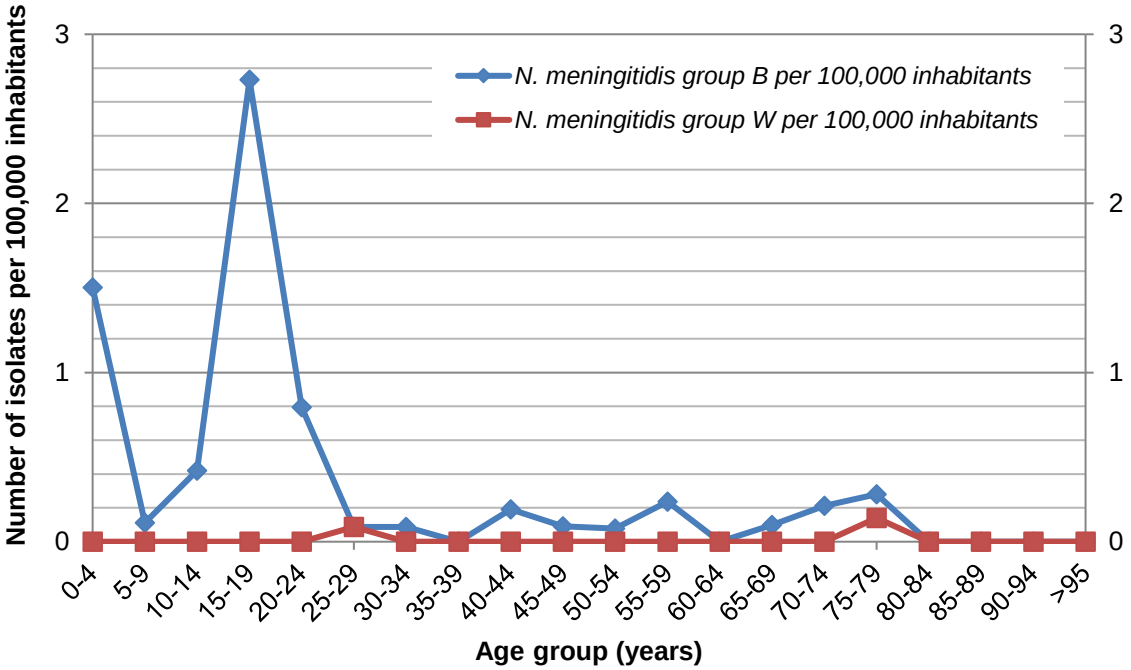


Figure 4.5 *A) Number of isolates and B) incidence of meningococcal disease* per 100,000 inhabitants *caused by serogroup B and W according to age groups, 2022*

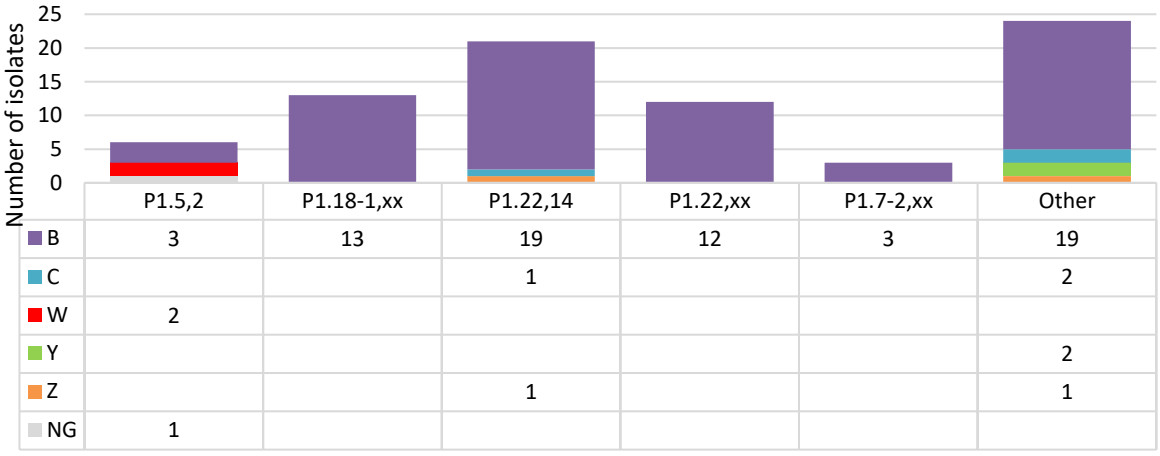
4.5 Distribution of PorA and FetA genosubtypes among meningococci

4.5.1 PorA

In addition to serogrouping, meningococci can be further subtyped based on the variation in PorA and FetA proteins. 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 monoclonal antibodies required for typing.

The PorA epitopes that react with the monoclonal antibodies of the subtyping scheme are encoded by the *porA* variable regions VR1 and VR2, which are now routinely determined by Sanger sequencing for 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 2022, the NRLBM received 56 isolates and 23 PCR-positive samples. Of the culture-negative samples, 16 could be completely subtyped and 7 not or not complete. Overall, 24 different VR1/VR2 combinations were encountered among 69 serogroup B meningococci (2021: 16 different combinations; 2020: 25 different combinations; 2019: 30 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 3% in 2022 (table 4.8). In 2021, P1.22,14 was the most abundant genosubtype with 19 out of 69 isolates (28%; Figure 4.6). Approximately 88% (61/69 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).



	P1.5,2	P1.18-1,xx	P1.22,14	P1.22,xx	P1.7-2,xx	Other
B	3	13	19	12	3	19
C			1			2
W	2					
Y						2
Z			1			1
NG	1					

Figure 4.6 *Distribution of PorA genosubtypes among all received meningococcal cases, 2022*

[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 2018-2022 and hypothetical coverage by NonaMen vaccine.

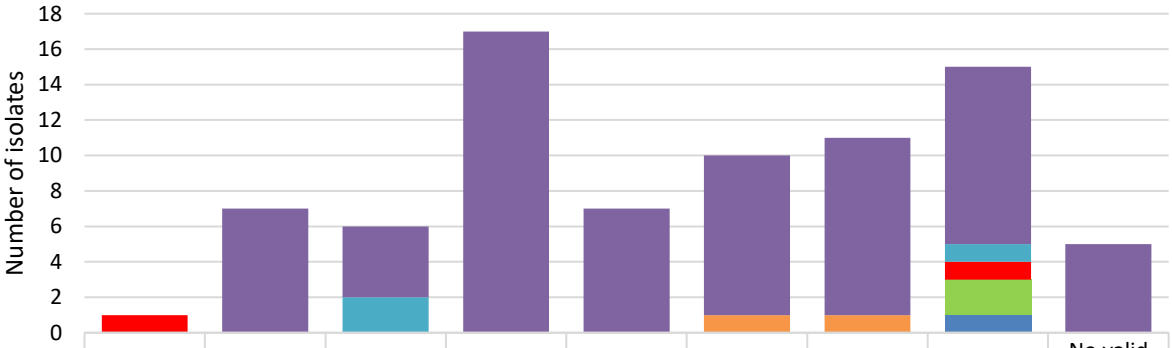
	VR1.VR2 combination	YEAR									
		2018		2019		2020		2021		2022	
		No.	%	No.	%	No.	%	No.	%	No.	%
Vaccine types*	1.5-1, 2-2	0	**0.0**	0	**0.0**	1	**2.5**	0	**0.0**	0	**0.0**
	1.5-1, other	2	**2.7**	0	**0.0**	0	**0.0**	0	**0.0**	2	**2.9**
	Other, 2-2	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	1	**1.5**
	1.5-2,10	0	**0.0**	1	**1.4**	0	**0.0**	0	**0.0**	0	**0.0**
	1.5-2, other	1	**1.3**	0	**0.0**	2	**5.0**	0	**0.0**	0	**0.0**
	Other,10	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.7,16	1	**1.3**	1	**1.4**	1	**2.5**	0	**0.0**	0	**0.0**
	1.7, other	1	**1.3**	1	**1.4**	3	**7.5**	1	**3.2**	0	**0.0**
	Other, 16	2	**2.7**	3	**4.2**	1	**2.5**	0	**0.0**	2	**2.9**
	1.7-1, 1	0	**0.0**	2	**2.8**	1	**2.5**	0	**0.0**	3	**4.3**
	1.7-1, other	0	**0.0**	1	**1.4**	0	**0.0**	0	**0.0**	0	**0.0**
	Other, 1	0	**0.0**	1	**1.4**	1	**2.5**	0	**0.0**	0	**0.0**
	1.7-2,4	3	**4.1**	8	**11.1**	4	**10.0**	3	**9.7**	1	**1.5**
	1.7-2, other	6	**8.2**	11	**15.2**	5	**12.5**	3	**9.7**	2	**2.9**
	Other 4	0	**0.0**	0	**0.0**	0	**0.0**	1	**3.2**	0	**0.0**
	1.12-1,13	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.12-1, other	0	**0.0**	0	**0.0**	1	**2.5**	0	**0.0**	3	**4.3**
	Other, 13	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.18-1,3	1	**1.3**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.18-1, other	5	**6.8**	7	**9.7**	2	**5.0**	3	**9.7**	13	**18.8**
	Other, 3	2	**2.7**	0	**0.0**	1	**0.0**	0	**0.0**	0	**0.0**
	1.19,15-1	1	**1.3**	1	**1.4**	1	**2.5**	0	**0.0**	0	**0.0**
	1.19, other	5	**6.8**	1	**1.4**	3	**7.5**	1	**3.2**	2	**2.9**
	Other, 15-1	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.22,14	20	**27.0**	19	**26.3**	2	**5.0**	10	**32.3**	19	**27.5**
	1.22,other	8	**10.8**	4	**5.6**	7	**17.5**	4	**12.9**	12	**17.4**
	Other, 14	3	**4.1**	2	**2.8**	0	**0.0**	1	**3.2**	1	**1.5**
	Subtotal vaccine types	61	**82.4**	63	**87.5**	36	**90.0**	27	**87.1**	61	**88.4**
NVT**	Other **Non Vaccine Type**	13	**17.6**	9	**12.5**	4	**10.0**	4	**12.9**	8	**11.6**
	Total	74	**100.0**	72	**100.0**	40	**100.0**	31	**100.0**	69	**100.0**

4.5.2 FetA

In addition to *porA* epitope sequencing, meningococcal isolates are also characterized by *fetA* epitope sequencing, which encodes the outer membrane protein FetA 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 Sanger 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 2022, 14 different *fetA* variants were observed among 69 serogroup B meningococci, among which F3-3 (25%), F5-5 (15%) and F5-12 (13%) 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 2022, 6 isolates were of *fetA* type F1-5, of which one was linked to P1.7-2,4 and 5 were linked to different *porA* types. In total, 27 different *porA* VR1/VR2 combinations and 14 different *fetA* variants were encountered among serogroup B meningococci. In 2022, frequently found combinations were, P1.22,14:F5-1 (7%), P1.22,14:F5-5 (13%) and P1.22,9:F5-12 (10%).

In 2022, we received 2 serogroup W samples, both from blood. The 2 meningococcal serogroup W isolates displayed two different *fetA* types F1-1 and F3-7 (Figure 4.7, Table 4.9). Both linked to *porA* VR1/VR2 P1.5,2 and MLST clonal complex 11.



	F1-1	F1-5	F1-7	F3-3	F5-1	F5-12	F5-5	Other	No valid reaction
B		7	4	17	7	9	10	10	5
C			2					1	
W	1							1	
Y								2	
Z						1	1		
NG								1	

Figure 4.7 *Distribution of meningococcal* fetA *genosubtypes, 2022*

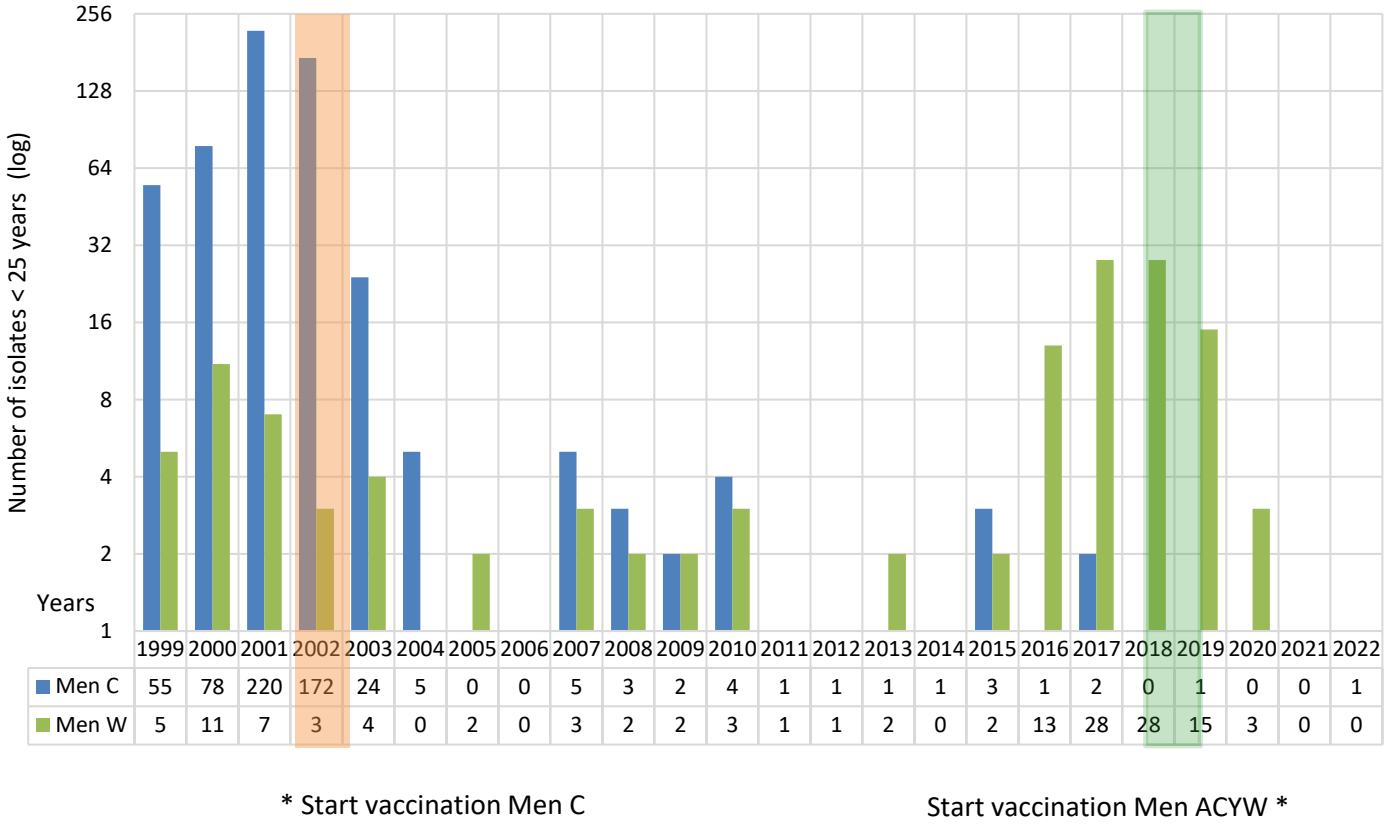
[6] *PubMLST - FetA variable region typing*. Public databases for molecular typing: https://pubmlst.org/neisseria/FetA/

Table 4.9 Temporal distribution in *fetA* genosubtype among *N. meningitidis* serogroups B, C and W isolates, 2018-2022

	Men B					Men C					Men W				
Years / FetA	2018	2019	2020	2021	2022	2018	2019	2020	2021	2022	2018	2019	2020	2021	2022
F1-1	1	2	0	0	0	0	0	0	0	0	90	53	12	2	1
F1-5	7	12	5	5	7	0	0	0	0	0	1	2	0	0	0
F1-7	4	6	1	2	4	0	1	0	0	2	0	0	0	0	0
F3-3	9	14	7	4	17	1	2	0	0	0	1	0	0	0	0
F3-4	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
F3-6	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0
F3-7	0	0	0	0	0	0	0	0	0	0	3	1	0	0	1
F3-9	2	1	1	0	0	1	1	0	0	0	0	0	0	0	0
F4-1	2	0	1	0	0	0	0	0	0	0	0	1	0	0	0
F5-1	12	12	2	6	7	0	0	0	0	0	0	0	0	1	0
F5-2	2	3	2	2	1	0	0	0	0	0	0	1	0	0	0
F5-5	11	6	3	2	10	0	0	0	0	0	1	0	0	0	0
F5-8	1	1	3	0	0	0	0	0	0	0	1	0	0	0	0
F5-12	1	3	4	0	9	0	0	0	0	0	0	0	0	0	0
F5-36	1	1	3	0	1	0	0	0	0	0	2	0	0	0	0
Deletion	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Other	20	11	8	10	13	0	1	0	0	0	2	1	0	0	0
Total	**74**	**72**	**40**	**31**	**69**	**3**	**6**	**0**	**0**	**3**	**102**	**60**	**12**	**4**	**2**

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 increase in the Netherlands. In response, the MenC vaccine was replaced by a vaccine that protects against meningococcal sergroups A, C, W and Y as of 1 May 2018. Because meningococcal type W also affected 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 2022, only one case due to serogroup C but no cases due to serogroup W meningococcal disease were reported in patients < 25 years of age (Figure 4.8).



	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
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	1
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	0

Figure 4.8 *Number of N.meningitidis serogroup C and W isolates in patients < 25 years of age, 1999-2022. 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*. https://lci.rivm.nl/richtlijnen/meningokokken-b-vaccinatie

5 HAEMOPHILUS INFLUENZAE

5.1 General features

In total, 322 *Haemophilus influenzae* isolates from CSF and/or blood or PCR-positive CSF samples were submitted to the NRLBM in 2022, which is a increase compared to the 167 isolates received in 2021 (table 2.3, figure 3.3, figure 5.1). Thirty-five isolates were from CSF (or CSF and blood; 10.8%) and 287 from blood only (89%). Sixty (20%) of the isolates were *H. influenzae* type b (table 5.1).

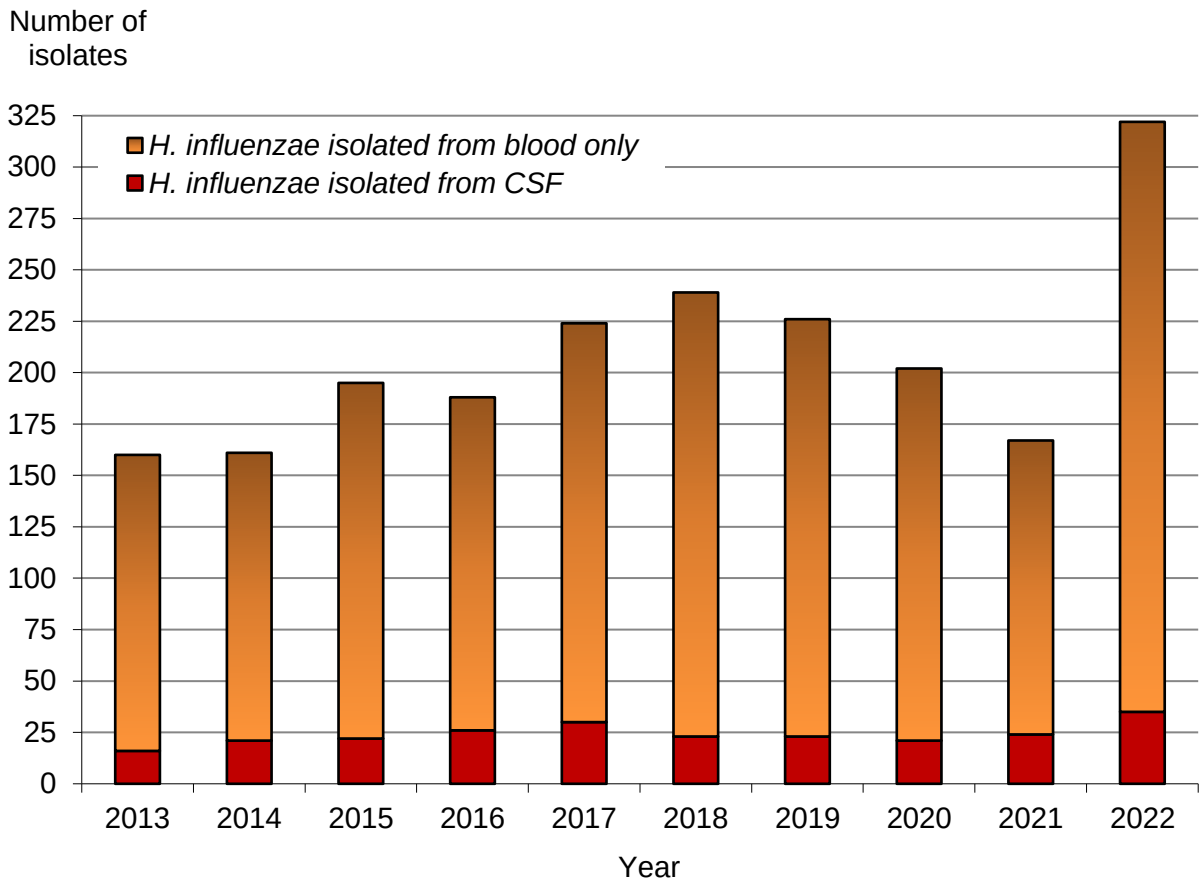


Figure 5.1 *Number of received H. influenzae isolates and CSF-positive samples according to isolation source, 2013-2022*

5.2 Antibiotic susceptibility

The proportion of ß-lactamase-producing invasive *H. influenzae* isolates (CSF and/or blood) was 8.0% in 2022 (Figure 5.2). Throughout the history of the NRLBM, the proportion of ß-lactamase-producing invasive *H. influenzae* isolates has always fluctuated for unknown reasons.

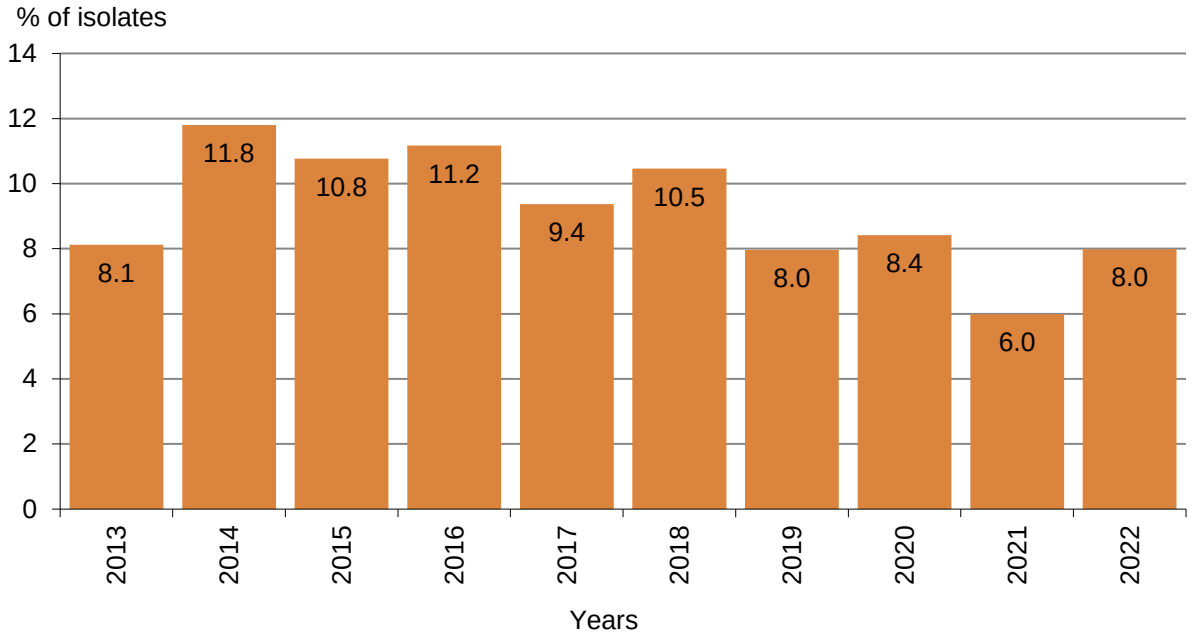


Figure 5.2 *Percentage β-lactamase-producing H. influenzae strains among received isolates, 2013-2022*

5.3 Serotype and age

In 2022, the number of *H. influenzae* type b isolates declined a little over 10% (60 versus 68) compared to 2021. Hib represented 19% of all received *H. influenzae* isolates compared to approximately 41% in 2021, 30% in 2020 and 16% in 2019. This is still among the highest number of Hib isolates in the last 15 years (Figure 5.3). It is unclear what the underlying causes for these high Hib numbers are. We observed 20 cases of invasive Hib disease among children younger than 2 years of age (Table 5.1; 26 in 2021, 24 in 2020; 11 in 2019; 15 in 2018; 7 in 2017). In contrast, the number of non-typeable *H. influenzae* isolates increased to 209 after lifting the corona-related restrictions in March 2022, the highest number in the last 30 years (Figure 5.3 and 5.6). Thirteen non-typeable isolates were isolated from CSF (or CSF and blood) and 190 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 *H. influenzae* serotype f has been steadily increasing, with a slight reduction in 2020 and 2021, likely associated to the 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, 2022

	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	0	0	0	0	0	0	2	4	**6**	***1.9***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*2*	*4*	***6***	
Hi - b	0	10	10	12	32	2	0	6	20	**60**	***18.6***
CSF	*0*	*4*	*7*	*2*	*13*	*0*	*0*	*0*	*0*	***13***	
Blood	*0*	*6*	*3*	*10*	*19*	*2*	*0*	*6*	*20*	***47***	
Hi - e	0	0	0	0	0	0	0	1	6	**7**	***2.2***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*6*	***7***	
Hi - f	0	0	3	0	3	2	0	3	32	**40**	**12.4**
CSF	*0*	*0*	*1*	*0*	*1*	*0*	*0*	*0*	*2*	***3***	
Blood	*0*	*0*	*2*	*0*	*2*	*2*	*0*	*3*	*30*	***37***	
n.t.*	5	5	4	6	20	2	2	36	149	**209**	***64.9***
CSF	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*5*	*13*	***19***	
Blood	*5*	*5*	*4*	*6*	*20*	*1*	*2*	*31*	*136*	***190***	
Total	**5**	**15**	**17**	**18**	**55**	**6**	**2**	**48**	**211**	**322**	***100.0***
CSF	***0***	***4***	***8***	***2***	***14***	***1***	***0***	***5***	***15***	***35***	***10.9***
Blood	***5***	***11***	***9***	***16***	***41***	***5***	***2***	***43***	***196***	***287***	***89.1***
%	***1.6***	***4.6***	***5.3***	***5.6***	***17.1***	***1.9***	***0.6***	***14.9***	***65.5***	***100.0***	

* non-typeable

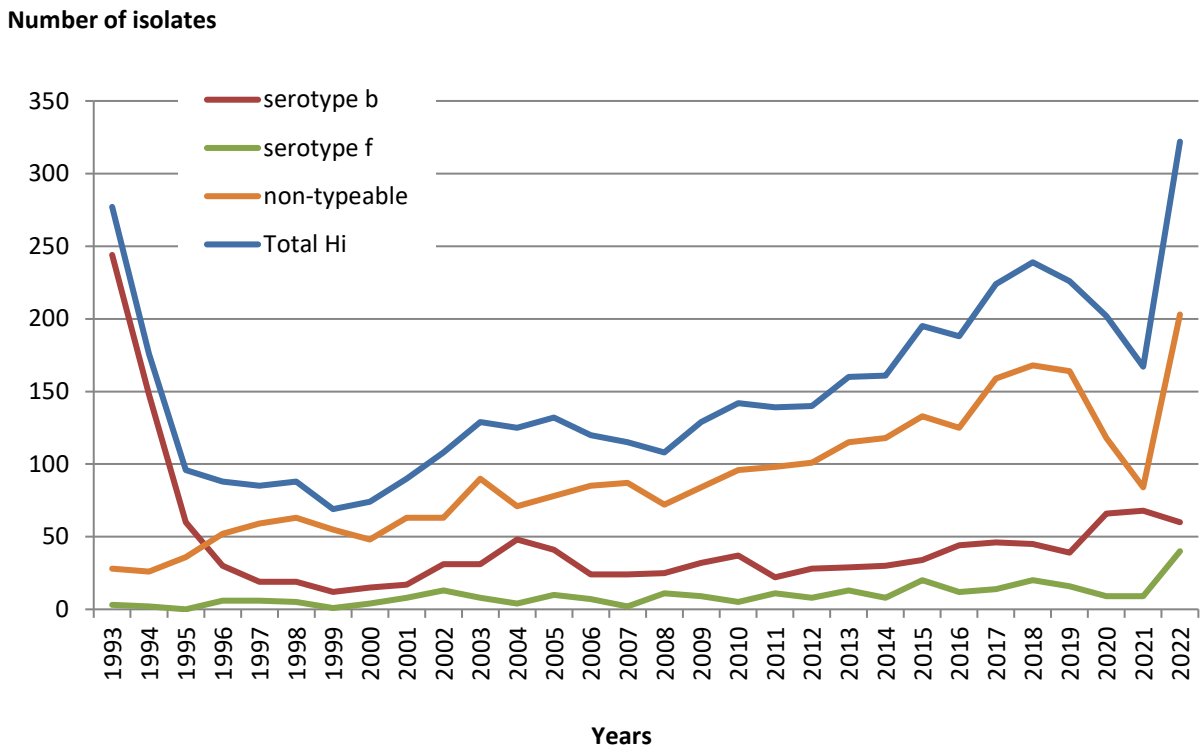


Figure 5.3 *Number of cases due to H. influenzae serotypes b, f and non-typeable H. influenzae, 1993-2022*

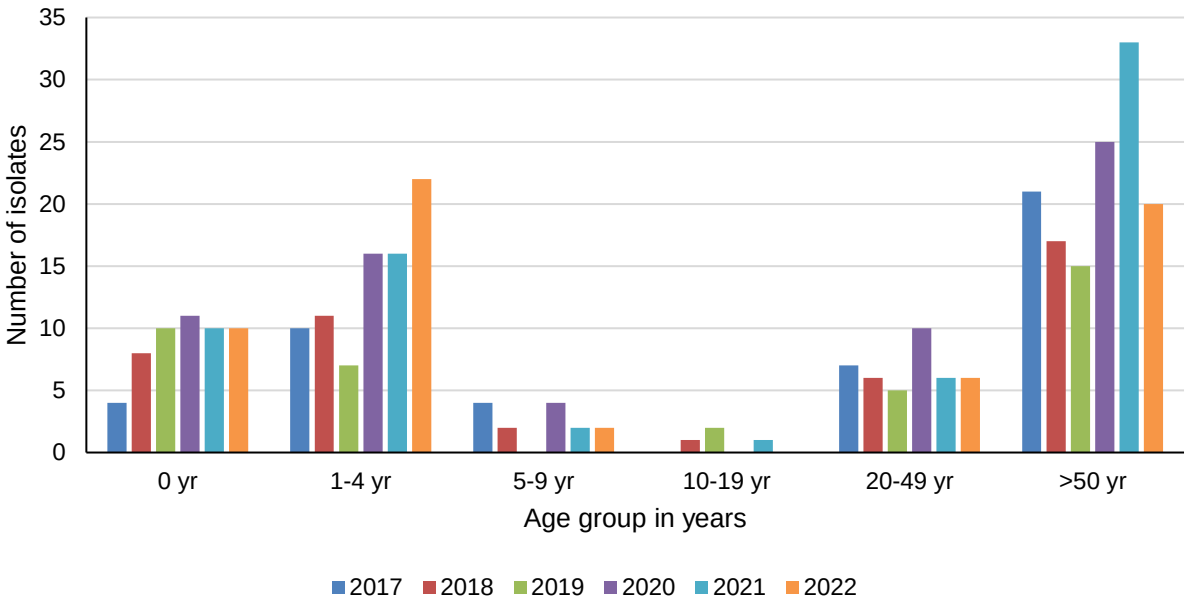


Figure 5.4 *Distribution of H. influenzae type b (CSF and/or blood) per age group. 2017-2021.*

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. After a decrease to 50% in 2021 (table 5.2), non-typeable *H. influenzae* isolates again represent 65% of *H. influenzae* isolates in 2022. The vast majority of non-typeable *H. influenzae* isolates are from blood (87%) in accordance to previous years (Table 5.2 and figure 5.5). Seventy-one 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 5.6).

Table 5.2 Number and proportion of non-typeable *H.influenzae isolates* from CSF and/or blood according to age, 2013- 2022

n.t.*	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	≥80	T	Csf / Blood	% **
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
2022	20	2	2	10	23	3	24	45	36	44	**209**	19/190	64.9

* non-typeable

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

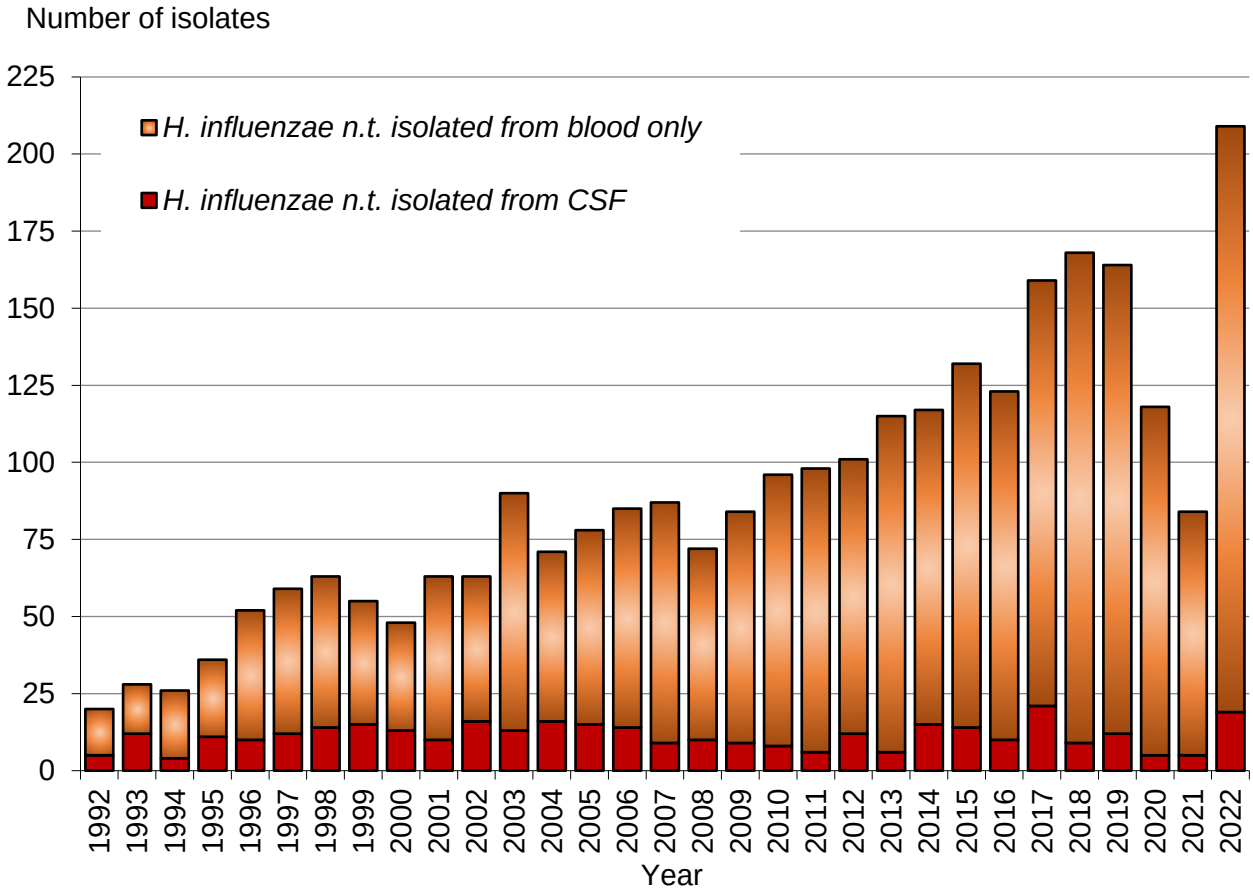


Figure 5.5 *Non-typeable H. influenzae isolates from CSF or blood received between 1992 - 2022*

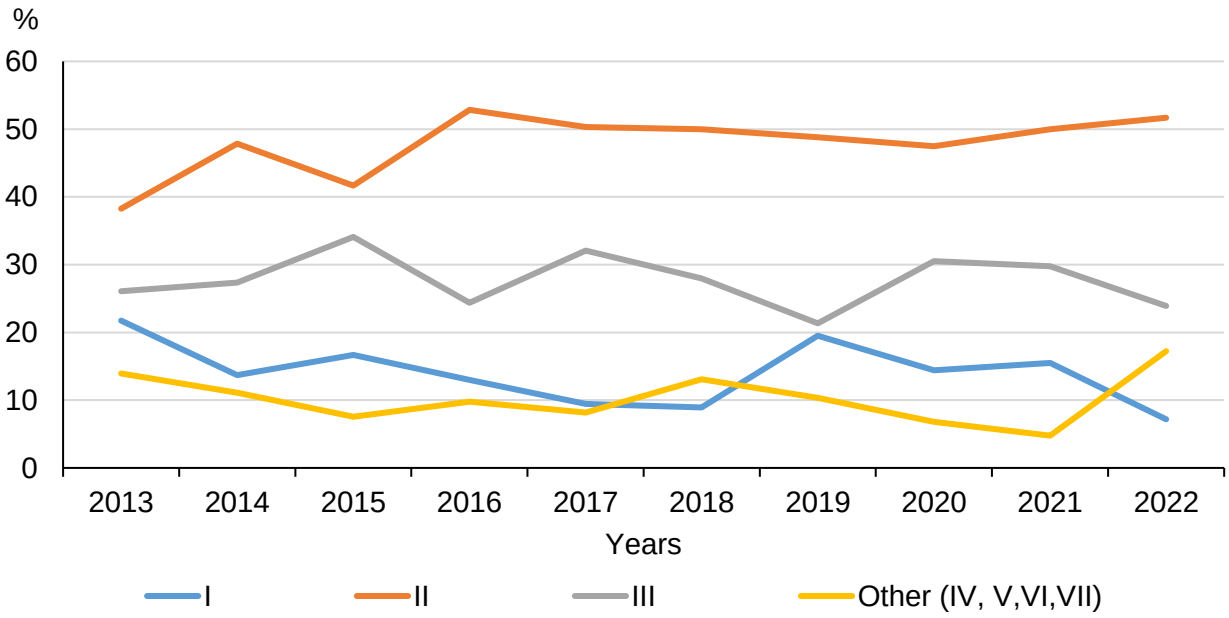


Figure 5.6 *Biotype distributions of non-typeable H. influenzae isolates from CSF and/or blood from 2013 – 2022.*

5.5 Geographical distribution of H. influenza

We plotted the geographical distribution of all *H. influenzae* cases (Fig. 5.7A) and *H. influenzae* b cases (Fig. 5.7B) 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.

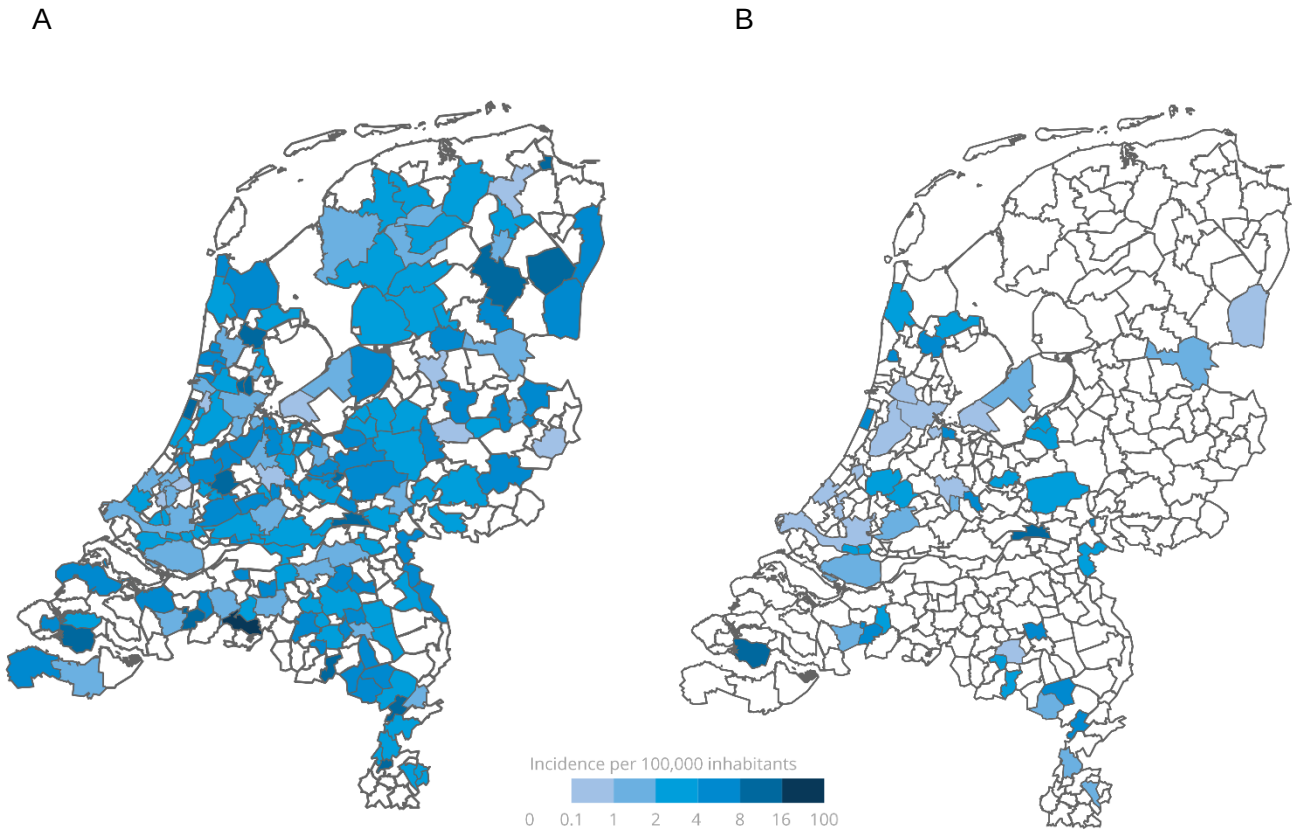


Figure 5.7. Geographical visualization of H. influenzae incidence for (A) all *H. influenzae* types and (B) *H. influenzae* b cases. Incidence is calculated per 100,000 inhabitants and place of residence of patient.

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 5.8. 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 2022, we received 34 *H. influenzae* type b isolates from patients that were vaccine-eligible (<29 years of age); 13 patients were CSF culture positive and 21 from blood. (CSF cases 2021: 11; 2020: 9; 2019: 7) (figures 5.8 and 5.9). Of the 13 meningitis patients, 6 were complety vaccinated, 6 patients were not (completely) vaccinated and from one patient, vaccination status was unknown.

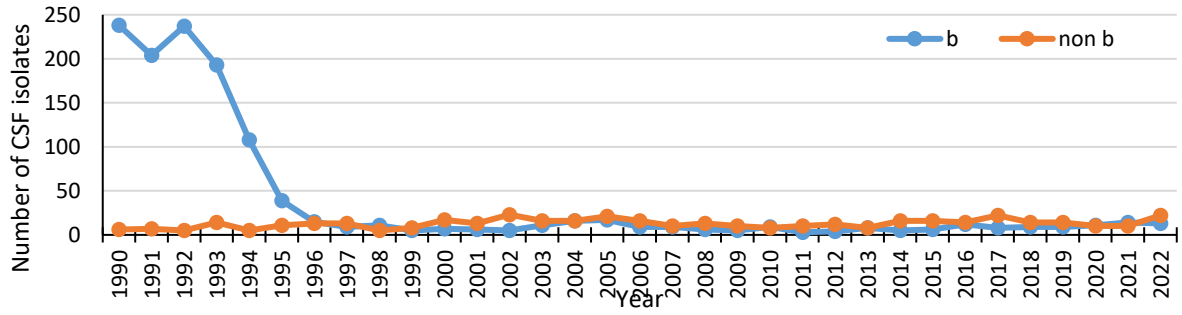


Figure 5.8 *The number of H. influenzae type b and non-type b cases, 1990 – 2022*

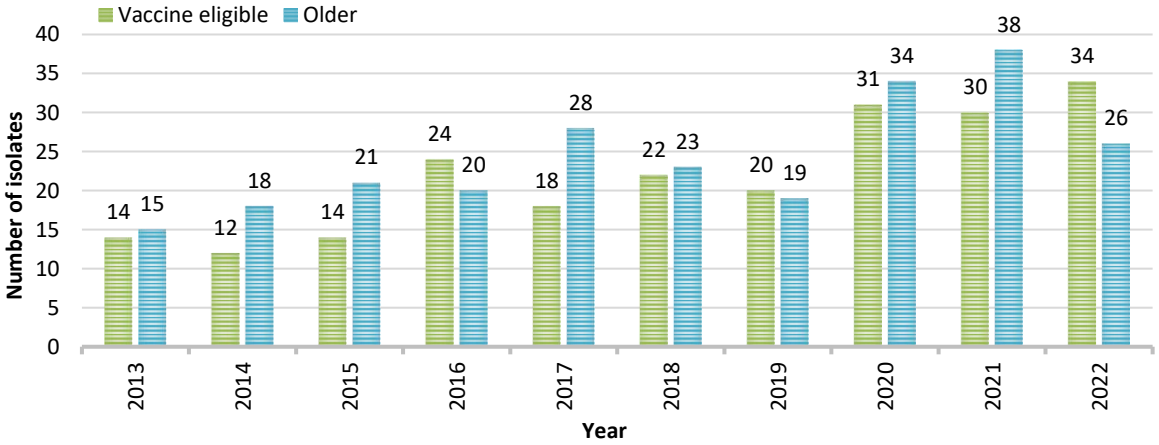


Figure 5.9 *The number of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 2013 –2022*

6 STREPTOCOCCUS PNEUMONIAE

6.1 General features

From 2003 onwards, the NRLBM requested nine sentinel laboratories, evenly distributed across the country and currently 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 2022, the NRLBM received 1,941 isolates (or PCR positive samples) nationwide of which 571 (30%) pneumococcal isolates (CSF and/or blood) were received from the 9 sentinel laboratories. Of the 1,941 nationwide submitted isolates, 145 isolates were from CSF (or CSF and blood). The NRLBM also received 18 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 0.8 per 100,000 individuals in 2022 (Figure 6.1).

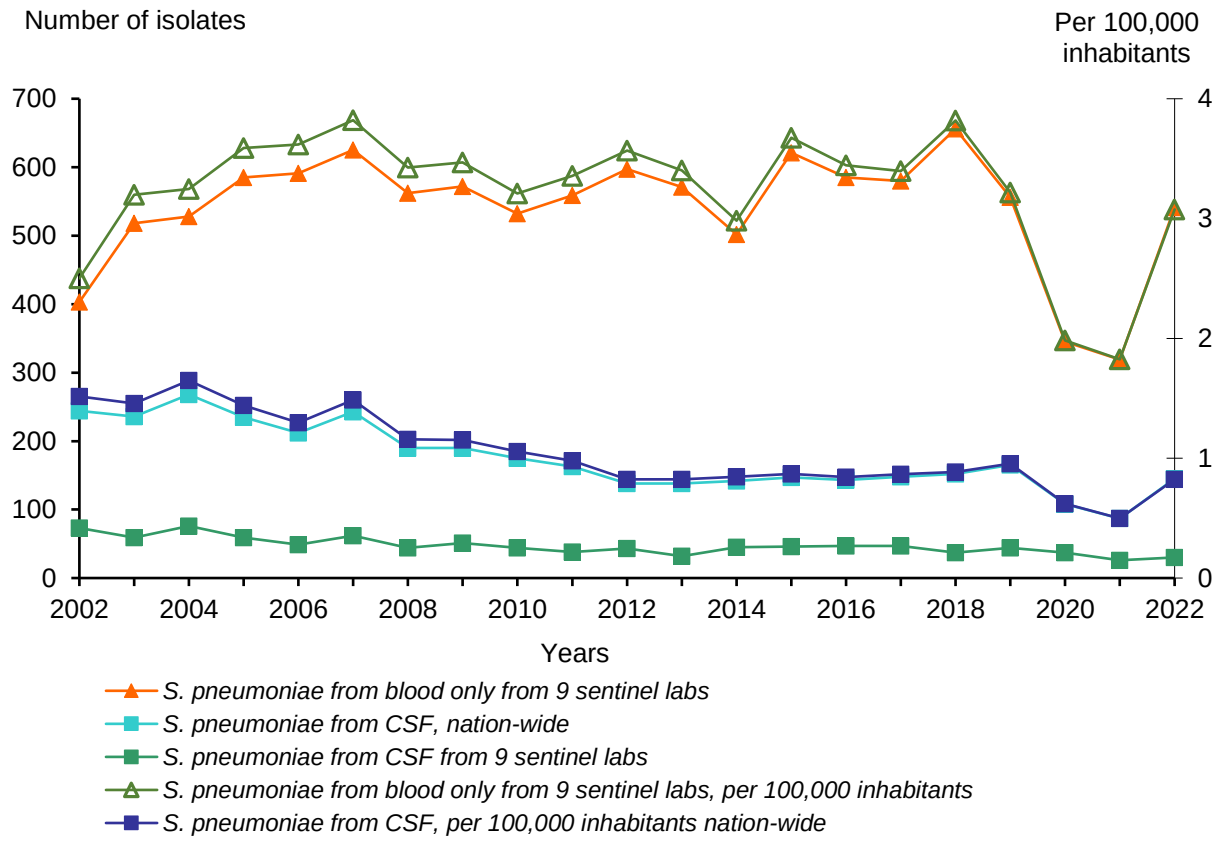


Figure 6.1 *Number of submitted S. pneumoniae isolates and invasive pneumococcal disease incidence based in isolates from blood or CSF, 2002-2022*

Figure 6.2 shows the number of *S. pneumoniae* isolates and incidence according to the patients' age group. The incidence of pneumococcal meningitis (CSF or CSF/blood) 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.

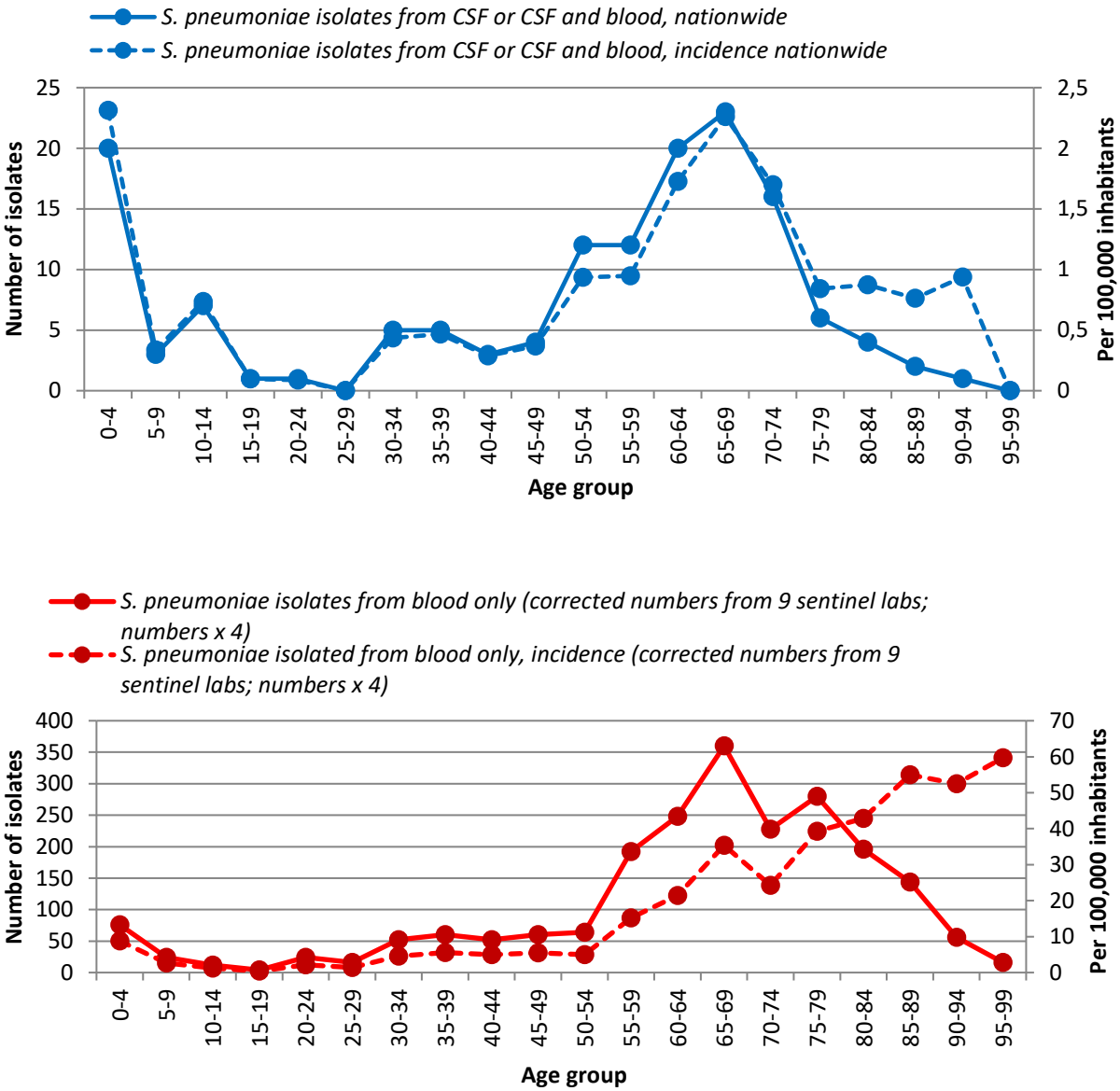


Figure 6.2 *S. pneumoniae isolates received per age group and incidence per 100,000 inhabitants according to isolation source in 2022. Top graph: isolates from CSF/CSF and blood. Bottom graph: isolates from blood only (9 sentinel labs) actual numbers x 4*

S. pneumonia meningitis

S. pneumonia bacteremia

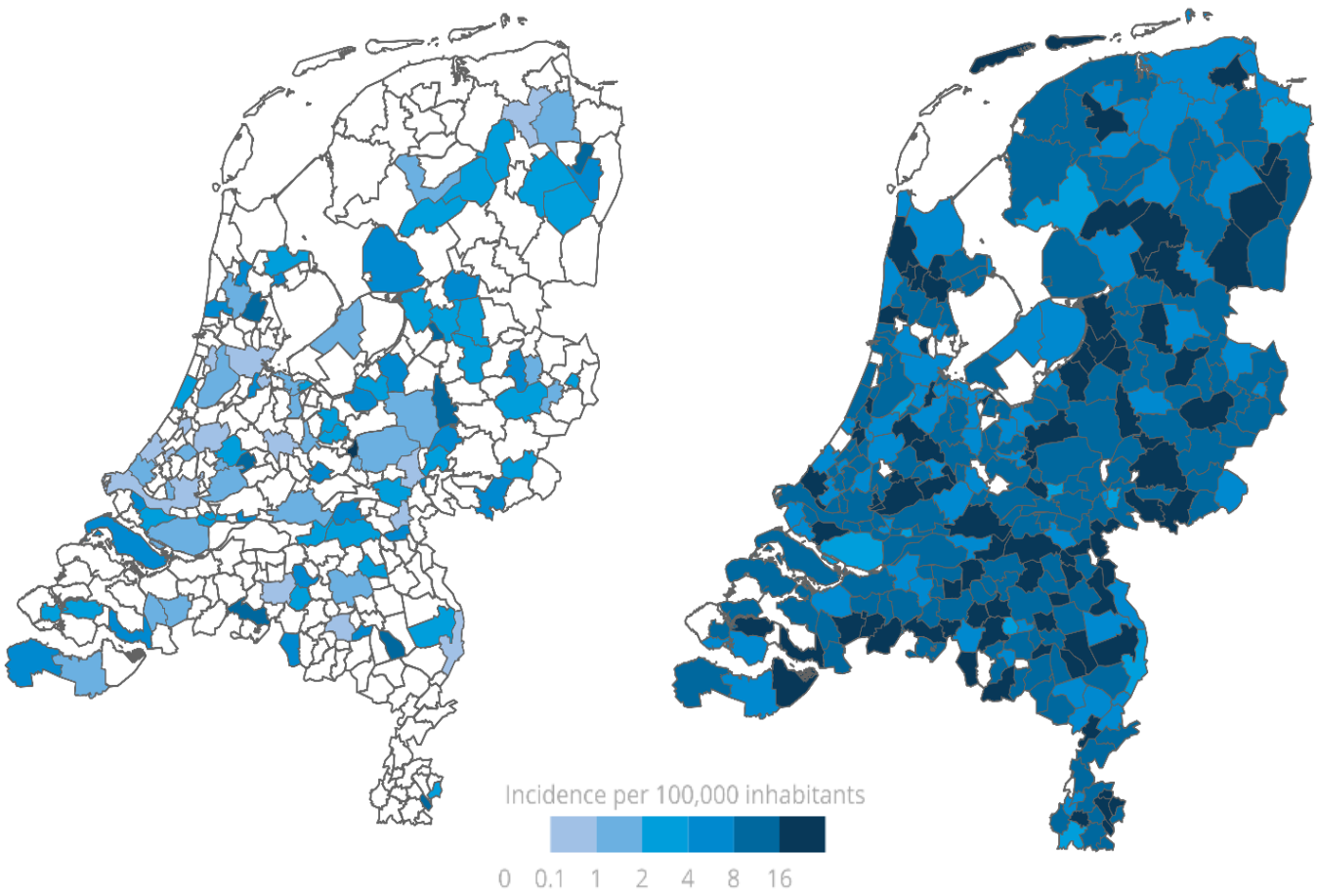


Figure 6.3 *Geographical distribution of S. pneumoniae invasive disease incidence per 100,000 inhabitants, left; meningitis based on isolates from CSF or CSF and blood, right; bacteremia based on isolates from blood only (nationwide). Data plotted based on patient's place of residence.*

6.2 Antibiotic susceptibility

Among 571 isolates from 9 sentinel labs, 28 were from CSF and 538 from blood only, 49 (8.6%) isolates were intermediately susceptible (0.06 < MIC ≤ 2.0 mg/L) and none were resistant to penicillin (table 6.1). Among the blood isolates received from labs nationwide, two isolates (0.1%) were resistant to penicillin (Table 6.1). Among 145 nationwide *S. pneumoniae* isolates from CSF (or CSF and blood), 12 (8.3%) strains were resistant to penicillin (MIC > 0.06 mg/L). From 11 patients (nationwide) no MIC values were obtained as no *S. pneumoniae* isolate was available.

Table 6.1 Penicillin* susceptibility of *S. pneumoniae* isolates, 2022

MIC for CSF isolates (Nationwide)	S MIC ≤ 0.06	I	R MIC > 0.06	ND** (PCR)	Total
CSF/CSF and blood	126 (86.9%)	n.a.	12 (8.3%)	7 (4.8%)	145
MIC for blood isolates	**MIC ≤ 0.06**	**0.06< MIC ≤ 2.0**	**MIC >2.0**		
Blood only (9 sentinel labs)	493 (91.1%)	45 (8.3%)	0	3 (0.6%)	541
Blood only (nationwide)	1672 (93.1%)	118 (6.6%)	2 (0.1%)	4 (0.2%)	1796

* MIC values in mg/L according to EUCAST guidelines
** No MIC value known because no isolate was available (PCR-positive culture-negative sample)
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 8.3% for meningitis (table 6.2) and 3.0% for bacteremia (table 6.4). Serotypes that would be additionally covered by the PCV13 vaccine (serotypes 3, 6A and 19A) account for approximately 22.7% and 36% 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 can be seen in tables 6.5 and 6.6, respectively. The overall reduction in the number of PCV10-covered serotypes for the period 2011-2021 is >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. Of the non-PCV13 serotypes, serotype 8 is most prevalent in meningitis (13.8%; Table 6.2) and invasive pneumococcal disease in blood (20.3%; Table 6.4).

Table 6.2 Serotype and age distribution of *S. pneumoniae* isolates from CSF (or CSF and blood; nationwide isolation collection), 2022. 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	4	-	-	5	**3.5**
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		7F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	-	-	-	-	-	1	-	-	-	1	**0.7**
		18C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		19F	-	-	-	-	-	2	1	-	1	1	-	-	-	5	**3.5**
		23F	-	-	-	-	-	-	-	-	-	-	1	-	-	1	**0.7**
		Subtotal PCV10	-	-	-	-	-	*2*	*1*	-	*1*	*3*	*5*	-	-	*12*	***8.3***
	13-valent vaccine	3	-	-	-	-	1	-	-	-	4	1	4	5	-	15	***10.3***
		6A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19A	-	3	2	5	-	-	-	-	1	1	7	4	-	18	***12.4***
		Subtotal PCV13	-	*3*	*2*	*5*	*1*	*2*	*1*	-	*6*	*5*	*16*	*9*	-	*45*	***31.0***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		8	1	2	-	3	-	-	-	-	-	1	5	11	-	20	***13.8***
		9N	-	-	-	-	-	-	-	-	-	1	-	1	-	2	***1.4***
		10A	-	1	2	3	-	-	-	-	-	-	4	4	-	11	***7.6***
		11A	-	-	-	-	-	-	-	-	-	-	-	1	-	1	***0.7***
		12F	-	-	-	-	-	-	-	-	-	-	1	1	-	2	***1.4***
		15B	-	1	1	2	-	-	-	-	1	-	-	-	-	3	***2.0***
		17F	-	-	-	-	-	-	-	1	-	-	1	-	-	2	***1.4***
		20	-	-	-	-	-	-	-	-	1	-	-	-	-	1	***0.7***
		22F	-	1	-	1	1	-	-	-	-	-	1	4	-	7	***4.8***
		33F	-	-	-	-	-	-	-	-	-	-	3	1	1	5	***3.5***
Subtotal PPV23			*1*	*8*	*5*	*14*	*2*	*2*	*1*	*1*	*8*	*7*	*31*	*32*	*1*	*99*	***68.3***
		Other	-	1	4	5	-	5	-	-	2	-	10	13	4	39	***26.9***
Type unknown			-	1	-	1	1	-	-	-	-	-	3	-	2	7	***4.8***
		Total	**1**	**10**	**9**	**20**	**3**	**7**	**1**	**1**	**10**	**7**	**44**	**45**	**7**	**145**	***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, 2022

	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	-	-	-	0.21	0.10	-	0.05	0.14	0.13	-	-	0.07
13-valent	1.67	0.29	0.11	0.21	0.10	-	0.27	0.23	0.43	0.34	-	0.26
23-valent	5.02	0.73	0.22	0.21	0.10	0.04	0.36	0.33	0.84	1.20	0.12	0.56
Other	1.12	0.58	0.11	0.52	-	-	0.09	-	0.35	0.49	0.70	0.26
Total	**7.82**	**1.02**	**0.34**	**0.73**	**0.10**	**0.04**	**0.45**	**0.33**	**1.19**	**1.68**	**0.82**	**0.82**

Table 6.4 Serotype and age-dependent distribution of *S. pneumoniae* isolates from blood submitted by the 9 sentinel laboratories, 2022.

			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	-	3	-	1	6	***1.1***
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		7F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		14	-	-	-	-	1	-	-	-	1	1	-	-	2	5	***0.9***
		18C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19F	1	-	-	1	-	-	-	-	-	-	-	2	-	3	***0.6***
		23F	-	-	1	1	-	-	-	-	-	-	-	-	1	2	***0.4***
		Subtotal PCV10	*1*	*-*	*1*	*2*	*1*	*-*	*-*	*1*	*2*	*1*	*3*	*2*	*4*	*16*	***3.0***
		3	-	1	-	1	1	1	-	1	2	2	14	33	13	68	***12.5***
		6A	-	-	-	-	-	-	-	-	-	1	-	-	-	1	***0.2***
		19A	-	-	6	6	-	-	-	1	7	4	31	51	26	126	***23.3***
		Subtotal PCV13	*1*	*1*	*7*	*9*	*2*	*1*	*-*	*3*	*11*	*8*	*48*	*86*	*43*	*211*	***39.0***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		8	1	2	-	3	-	-	-	3	10	9	28	42	15	110	***20.3***
		9N	-	-	-	-	-	-	-	-	-	1	1	6	3	11	***2.0***
		10A	-	-	1	1	-	-	-	1	-	2	3	2	5	14	***2.6***
		11A	-	-	-	-	-	-	-	-	-	-	1	2	2	5	***0.9***
		12F	-	-	-	-	-	-	-	1	1	-	11	3	1	17	***3.1***
		15B	-	-	1	1	-	-	-	-	-	-	-	1	1	3	***0.6***
		17F	-	-	-	-	1	-	-	-	-	-	-	1	2	4	***0.7***
		20	-	-	-	-	-	-	-	-	-	-	1	4	2	7	***1.3***
		22F	-	-	1	1	-	2	-	1	2	1	10	10	8	35	***6.5***
		33F	-	1	-	1	1	-	-	-	-	-	2	7	2	13	***2.4***
Subtotal PPV23			*2*	*4*	*10*	*16*	*4*	*3*	*-*	*9*	*24*	*21*	*105*	*164*	*84**	*430*	***79.4***
		Other	-	-	3	3	2	-	1	1	4	7	19	52	19	108	***20.0***
Type unknown			-	-	-	-	-	-	-	-	-	-	2	1	-	3	***0.6***
		Total	**2**	**4**	**13**	**19**	**6**	**3**	**1**	**10**	**28**	**28**	**126**	**217**	**103**	**541**	***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, 2011-2022

			TYPE	2011*	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
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
			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	-	-	-	1
			18C	5	2	2	-	1	-	1	1	1	-	-	-
			19F	6	4	2	4	2	5	6	1	3	1	1	5
			23F	2	1	-	-	1	-	1	-	-	-	-	1
			Subtotal PCV10	*48*	*35*	*25*	*19*	*14*	*14*	*12*	*8*	*6*	*3*	*2*	*12*
			3	7	13	16	13	16	25	20	20	21	20	16	15
			6A (not in 23 valent)	1	1	1	3	-	1	-	-	-	-	-	-
			19A	16	6	9	7	10	8	16	13	20	15	7	18
			Subtotal PCV13	*72*	*55*	*51*	*42*	*40*	*48*	*48*	*41*	*47*	*38*	*25*	*45*
			2	-	-	-	-	-	-	-	-	-	-	-	-
			8	17	9	16	23	24	18	21	23	26	8	14	20
			9N	7	4	2	6	6	3	6	4	4	4	4	2
			10A	7	9	7	12	5	7	7	7	11	2	3	11
			11A	5	1	1	3	2	3	2	8	-	4	-	1
			12F	7	10	9	8	9	12	8	11	5	3	-	2
			15B	3	1	-	-	-	5	7	2	7	1	2	3
			17F	3	1	1	1	-	-	1	2	-	1	2	2
			20	-	-	1	1	1	-	-	-	-	-	-	1
			22F	16	11	8	8	11	11	8	8	10	6	3	7
			33F	5	6	3	2	4	4	6	6	3	1	3	5
			Subtotal PPV23*	*141*	*106*	*98*	*103*	*102*	*110*	*114*	*112*	*113*	*68*	*56*	*99*
			6C	4	2	6	3	6	5	3	3	11	12	3	6
			7B	-	-	1	-	-	-	-	-	-	2	-	-
			7C	-	-	-	-	-	-	-	-	1	-	1	2
			10F	-	-	-	-	-	-	-	-	-	-	-	-
			10B	-	1	-	1	1	-	1	-	1	1	1	1
			11D	-	-	-	-	-	-	-	-	-	-	-	1
			12A	-	-	-	-	-	-	-	-	-	-	-	-
			13	-	-	-	-	-	-	-	-	-	-	1	-
			15A	1	1	4	6	7	2	4	3	1	-	2	-
			15C	-	3	-	-	1	-	3	1	1	1	1	1
			16F	4	-	5	2	1	3	1	5	-	-	1	1
			17A	-	-	-	-	-	-	-	-	-	-	-	-
			18F	-	-	-	-	-	-	-	-	-	-	-	-
			18A	-	-	-	-	-	-	-	-	-	-	-	-
			18B	-	1	-	-	-	-	-	-	-	-	-	-
			21	1	-	-	-	-	-	-	2	-	1	1	1
			22A	-	-	-	-	1	1	-	-	-	-	-	-
			23A	2	4	4	4	5	5	5	8	6	5	1	3
			23B	2	5	7	8	11	6	11	8	10	9	10	12
			24F	1	4	4	7	7	1	2	1	5	-	-	1
			24B	-	2	-	-	-	-	-	-	-	-	-	-
			27	-	1	-	2	1	1	-	1	1	1	-	2
			28F	-	-	1	-	-	-	-	-	-	-	-	-
			28A	1	-	-	-	-	-	-	-	-	-	-	-
			29	-	1	-	-	-	-	-	-	-	-	-	-
			31	-	1	-	1	-	1	1	-	-	-	-	-
			33A	-	-	-	-	-	-	-	-	-	-	-	-
			34	1	-	-	-	1	1	1	2	2	1	-	1
			35F	1	-	2	1	2	5	1	3	3	4	2	6
			35B	-	1	3	1	1	1	-	2	-	-	-	1
			35D	-	-	-	-	-	-	-	1	1	-	-	-
			37	1	2	1	-	-	-	-	-	-	-	-	-
			38	-	2	1	-	-	-	-	-	2	-	-	-
			Rough (n.t.)	-	-	-	-	-	-	1	-	-	-	-	-
			Type unknown	-	-	-	-	-	-	-	-	10	3	7	7
			Total	163	138	138	142	147	143	148	152	165	108	87	145

Table 6.6 Changes in serotype distribution of *S. pneumoniae* from blood submitted by the 9 sentinel laboratories, 2011-2022. Serotypes are grouped by vaccine type.

			TYPE	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
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	6
			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	5
			18C	8	4	8	2	2	2	1	1	-	-	-	-
			19F	9	3	5	7	8	6	9	7	4	1	1	3
			23F	5	3	1	2	1	1	1	-	1	-	-	2
			Subtotal PCV10	*218*	*188*	*166*	*119*	*131*	*82*	*59*	*52*	*20*	*17*	*5*	*16*
			3	36	45	40	31	35	45	51	71	45	30	29	68
			6A (not in 23 valent)	2	6	2	-	2	-	4	4	1	1	1	1
			19A	63	78	61	44	78	75	82	101	84	54	74	126
			Subtotal PCV13	*319*	*317*	*269*	*194*	*246*	*202*	*196*	*228*	*150*	*102*	*109*	*211*
			2	-	-	-	-	-	-	-	-	-	-	-	-
			8	59	88	108	93	136	151	143	159	146	89	63	110
			9N	17	20	19	21	26	32	29	31	31	20	18	11
			10A	14	8	6	16	15	11	11	8	10	13	4	14
			11A	9	14	16	8	6	6	9	8	5	7	8	5
			12F	19	25	22	28	30	18	28	22	26	12	3	17
			15B	4	1	7	7	2	8	6	8	10	3	7	3
			17F	8	7	4	8	6	6	5	4	2	4	3	4
			20	4	-	1	4	2	3	5	6	10	3	5	7
			22F	37	41	45	34	43	28	39	45	47	24	17	35
			33F	15	22	12	12	19	18	12	28	13	6	8	13
			Subtotal PPV23*	*503*	*537*	*507*	*425*	529	483	479	*543*	449	282	*245*	*430*
			6C	7	10	10	7	21	20	15	24	22	18	19	19
			7B	-	-	-	-	-	-	-	1	1	3	-	1
			7C	-	-	-	-	-	-	-	1	4	5	2	6
			9A	-	1	-	1	-	1	-	-	-	-	-	-
			10F	-	-	-	1	-	-	-	-	-	-	-	-
			10B	-	-	1	-	-	-	1	2	2	1	1	1
			11B	-	-	-	-	-	-	-	-	2	-	-	-
			11D	-	-	-	-	-	-	-	-	3	2	1	1
			12A	-	-	-	-	-	-	-	1	-	-	-	-
			13	1	-	-	-	-	1	-	-	-	-	-	-
			15F	-	-	1	-	-	1	-	-	-	-	-	-
			15A	2	7	13	14	18	21	16	14	12	7	6	11
			15C	2	1	4	4	3	2	1	1	3	1	2	4
			16F	7	6	7	5	2	9	9	5	4	5	6	13
			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	18
			23B	9	3	6	15	5	11	17	11	17	4	15	12
			24F	3	2	4	4	7	1	6	3	7	-	1	6
			24B	-	-	-	-	-	-	-	-	-	-	-	1
			25F	-	-	-	-	1	-	1	-	-	-	-	-
			27	1	-	1	-	1	1	-	-	-	-	1	1
			28A	-	-	-	-	-	-	-	-	1	-	-	1
			29	-	1	-	-	-	-	-	-	-	-	-	1
			31	2	6	2	2	4	4	3	6	1	-	-	1
			33A	-	1	-	-	-	1	-	1	-	-	-	-
			34	-	1	2	1	-	1	1	3	4	1	1	2
			35F	6	5	6	7	7	6	3	6	3	3	3	4
			35A	-	1	-	-	-	-	-	-	-	-	-	-
			35B	3	1	7	6	8	8	2	8	3	3	3	1
			35D	-	-	-	-	-	-	-	-	2	-	1	-
			37	-	-	-	1	1	-	-	-	-	-	-	-
			38	3	-	1	2	2	1	5	4	1	1	1	3
			40	-	-	1	-	-	-	-	-	-	-	-	-
			Rough (n.t.)	2	-	-	-	-	-	1	1	1	-	1	1
			Type unknown	-	-	-	-	-	-	-	-	-	-	1	3
			Total	559	596	585	503	621	584	580	655	556	345	319	541

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 2022, 8.3% of the CSF isolates were of a serotype covered by the PCV10 vaccine (table 6.2). There were 12 patients with pneumococcal meningitis due to pneumococci with a PCV-10 vaccine serotype (4, 19F and 23A; Table 6.5). Only 3 patients were born after April 2006 and were therefore eligible for PCV10 vaccination. 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-eight percent of the CSF isolates and 79% of blood isolates were of a serotype that would be covered by this vaccine (table 6.5). (2005: 90% pre-vaccination). From 2020, PPV23 is offered through the National Immunisation Programme to the elderly in yearly cohorts (2020: 1941-1947, 2021: 1948-1953, 2022:1953-1956), covering the ages 66-79 years.

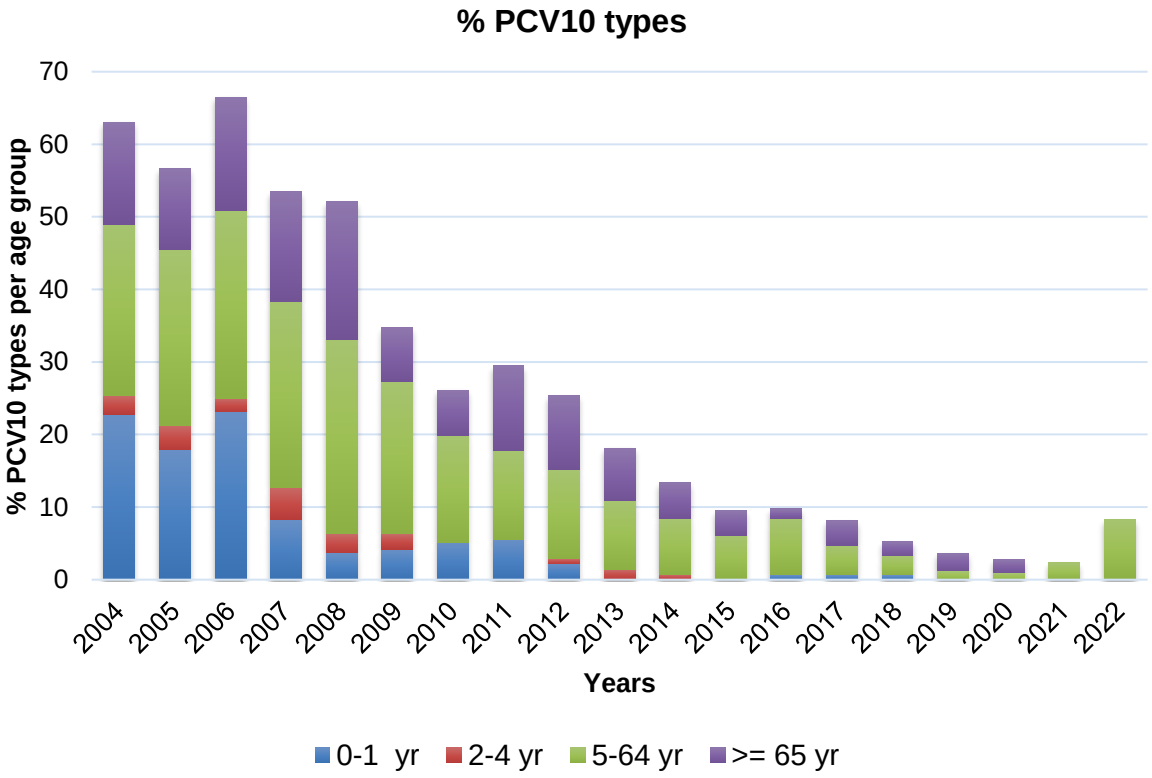


Figure 6.4 *Proportion of PCV-10 serotypes in patients with invasive pneumococcal disease per age category, 2004-2022.*

7 ESCHERICHIA COLI

The NRLBM received 92 *Escherichia coli* isolates (from children <1 year), 15 isolated from CSF (or CSF and blood) and 77 from blood only (Figure 7.1, Table 7.1). Sixty-five percent (n=60) of the *E. coli* meningitis and bacteremia cases occurred in the first month of life (Table 7.1). 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]. Since 2019, isolate submission has stabilized around 95 isolates per year (Figure 7.1).

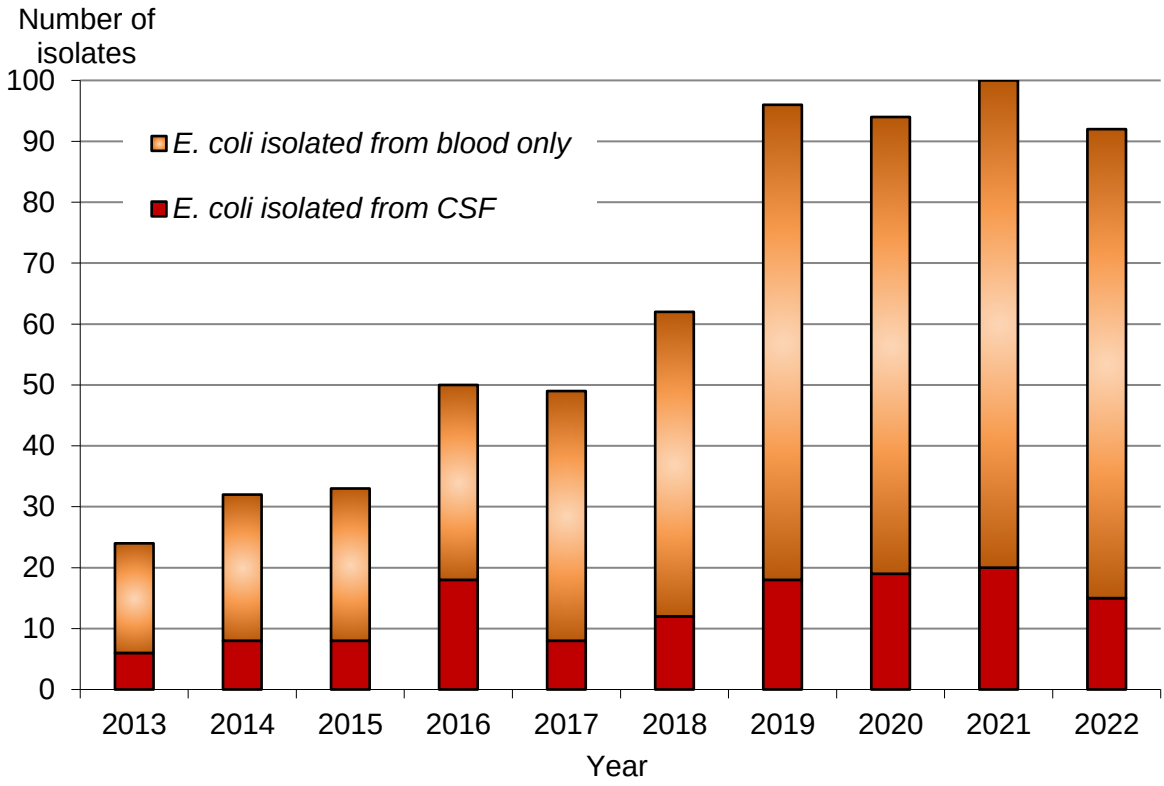


Figure 7.1 *Number of E. coli isolates received according to isolation source, 2013-2022*

Since 2016, K1 expression is determined by phage typing. In 2022, approximately 50% 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, 2022

	AGE (MONTHS)			AGE (YEARS)					TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Non K1	28	17	0	45	0	0	1	0	**46**	***50***
CSF	*2*	*1*	*0*	*3*	*0*	*0*	*1*	*0*	***4***	
*Blood**	*26*	*16*	*0*	*42*	*0*	*0*	*0*	*0*	***42***	
K1	32	14	0	46	0	0	0	0	**46**	***50***
CSF	*8*	*3*	*0*	*11*	*0*	*0*	*0*	*0*	***11***	
Blood	*24*	*11*	*0*	*35*	*0*	*0*	*0*	*0*	***35***	
Total	**60**	**31**	**0**	*91*	*0*	*0*	*1*	*0*	**92**	***100***
CSF	*10*	*4*	*0*	*14*	*0*	*0*	*1*	*0*	*15*	*20*
Blood	*50*	*27*	*0*	*77*	*0*	*0*	*0*	*0*	*77*	*80*
%	***65***	***34***	***0***	***99***	***0***	***0***	***1***	***0***	***100***	

* Note: Submission criteria, all *E. coli* isolates from patients with meningitis. For invasive disease (non meningitis), *E. coli* isolates only from children < 1 year.

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, 57% were of H-type H7 and 17% of type H4. H-type H4 was also dominant among the non-K1 isolates (20%), with H18 and H1 accounting together for more than fifty percent 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, 2018-2022

TYPE	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1
	2018	**2019**	**2020**	**2021**	**2022**
H1	1 / 6	1 / 9	0 / 5	0 / 9	0/11
H4	3 / 7	7 / 12	8 / 8	11 / 12	8/9
H5	3 / 3	5 / 9	3 / 8	1 / 5	5/5
H6	2 / 2	1 / 1	6 / 0	4 / 2	2/2
H7	17 / 1	22 / 3	27 / 1	29 / 2	26/3
H9	0 / 1	-	0 / 1	0 / 2	-
H18	0 / 6	3 / 10	0 / 8	0 / 7	0/5
H31	-	0 / 3	0 / 4	5 / 1	3/4
Other	4 / 6	3 / 7	2 / 13	3 / 7	2/7
Total	**30 / 32**	**42 / 54**	**46 / 48**	**53 / 47**	**46/46**

The types O6 (24%) and O25 (13%) are most prevalent among non-K1 isolates, while the types O1 (32%), O25 (11%) and O45 (11%) are most frequent among K1 isolates. The 5 isolates showed in the group 'Other' were all different O-types (Figure 7.2).

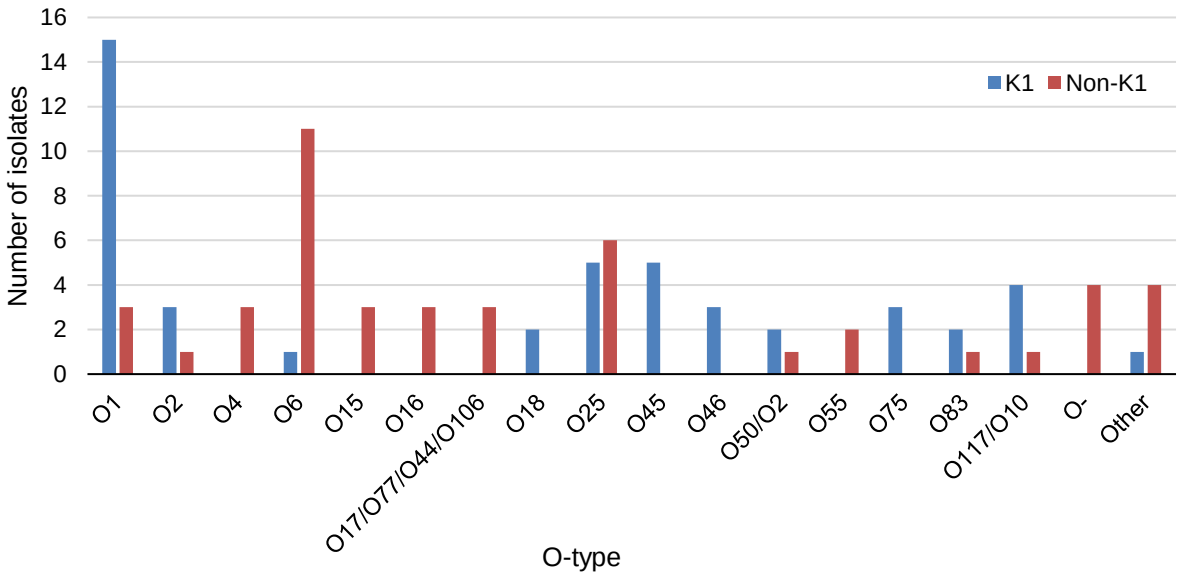


Figure 7.2 Distribution of O-types among K1 and non-K1 *E. coli* isolates from CSF and/or blood, 2022

Among K1 isolates, the O/H combination O1:H7 (32%) was most prevalent while among non-K1 isolates, O6:H1 and O25:H4 were dominant (13% each)(Figure 7.3).

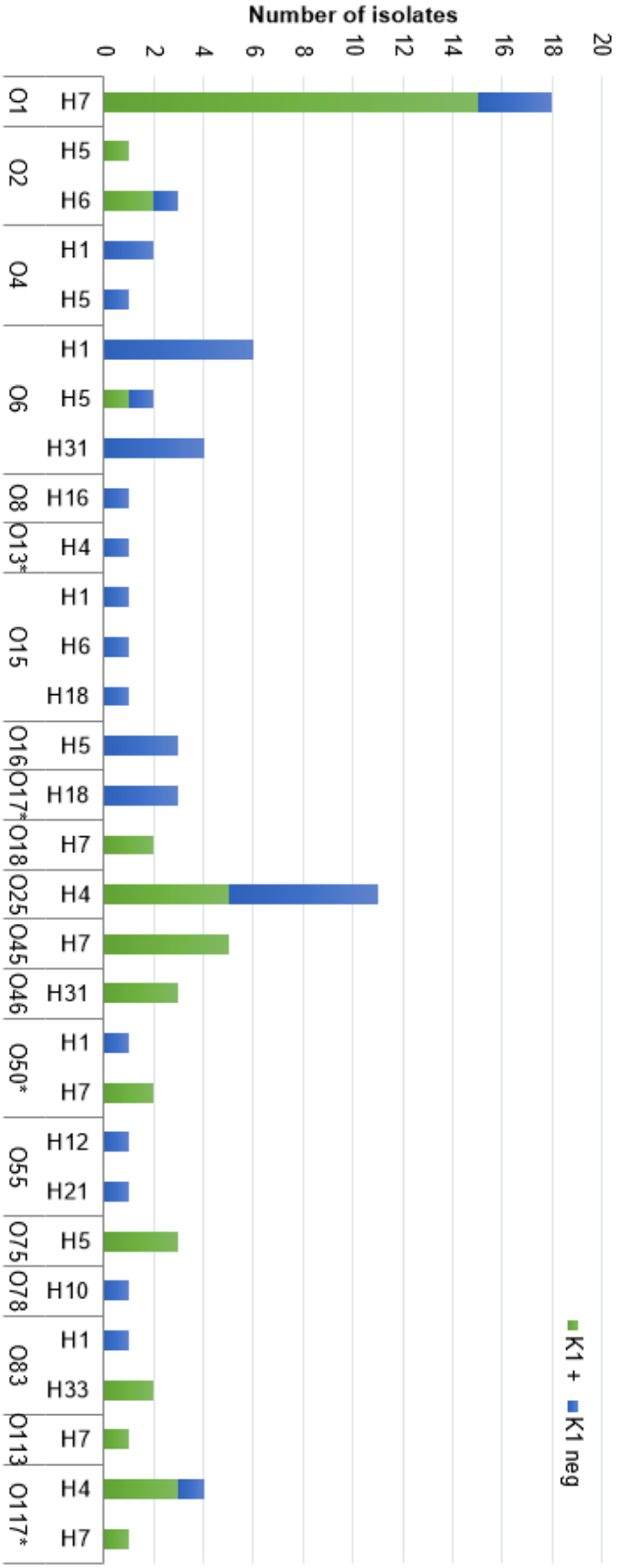


Figure 7.3 Distribution of O- and H-type combinations among K1 and non-K1 *E. coli* isolates from CSF and/or blood, 2022

*O13 = O13/O135

O17 = O77/O17/O44/O106

O50 = O50/O2O

O117 = O117/O107

8 STREPTOCOCCUS AGALACTIAE – (group B)

In 2022, the NRLBM received 116 *Streptococcus agalactiae* (Group B Streptococcus) isolates, which is a decrease compared to the 147 isolates in 2021 and 129 isolates in 2020 (figure 8.1). Twenty-one (18%) *S. agalactiae* isolates were from CSF (or CSF and blood) and 95 (82%) from blood only (table 8.1, figure 8.1). Seventy-six percent of all the cases (n=88) occurred in the first month of life, with18% of the isolates recovered from CSF and 82% from blood (Table 8.1). Overall, 96% of invasive *S. agalactiae* disease cases occurred within the first year of life (table 8.1). As in previous years, Serotype III was most prevalent, accounting for 60.3% of the cases (table 8.1, figure 8.2). Serotypes Ia and and V accounted for 13.8 and 7.8% 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].

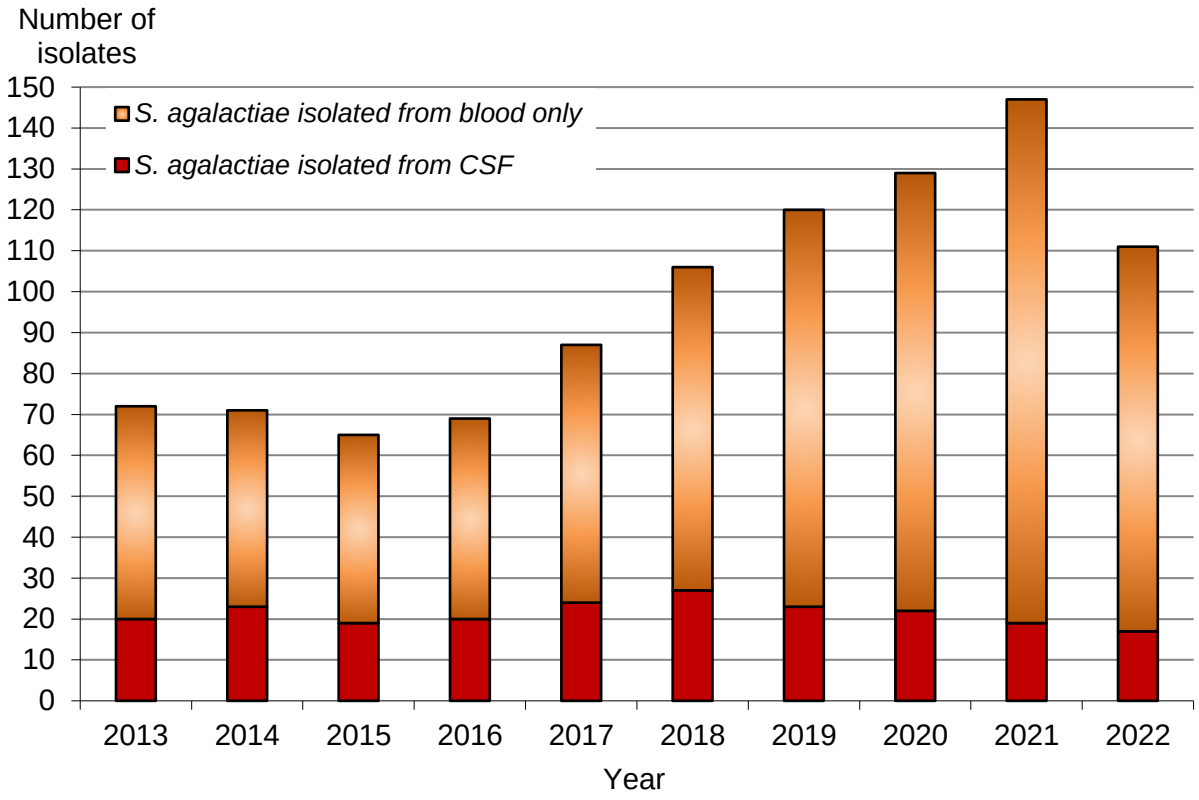


Figure 8.1 *Distribution of S. agalactiae isolates, 2013 - 2022*

[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, 2022.

	AGE (MONTHS)			AGE (YEARS)					TOTAL	
Group*	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Ia	14	2	0	16	0	0	0	0	**16**	***13.8***
CSF	*3*	*1*	*0*	*4*	*0*	*0*	*0*	*0*	***4***	
Blood	*11*	*1*	-	*12*	-	-	-	-	***12***	
Ib	3	1	0	4	0	0	0	0	**4**	***3.4***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*3*	*1*	-	*4*	-	-	-	-	***4***	
II	6	1	0	7	0	0	1	0	**8**	***7.0***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	***1***	
Blood	*6*	*1*	-	*7*	-	-	-	-	***7***	
III	53	13	0	66	0	0	1	3	**70**	***60.3***
CSF	*9*	*4*	*0*	*13*	*0*	*0*	*0*	*3*	***16***	
Blood	*44*	*9*	-	*53*	-	-	*1*	-	***54***	
IV	2	2	0	4	0	0	0	0	**4**	***3.4***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*2*	*2*	-	*4*	-	-	-	-	***4***	
V	7	2	0	9	0	0	0	0	**9**	***7.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*7*	*2*	-	*9*	-	-	-	-	***9***	
VI	1	1	0	2	0	0	0	0	**2**	***1.7***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*1*	*1*	-	*2*	-	-	-	-	***2***	
IX	2	1	0	3	0	0	0	0	**3**	***2.6***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*2*	*1*	-	*3*	-	-	-	-	***3***	
Total	**88**	**23**	**0**	**111**	**0**	**0**	**2**	**3**	**116**	***100.0***
CSF	***12***	***5***	***0***	***17***	***0***	***0***	***1***	***3***	***21***	***18.1***
Blood	***76***	***18***	***-***	***94***	***0***	***0***	***1***	***0***	***95***	***81.9***
%	***75.9***	***19.8***	***0***	***95.7***	***0***	***0***	***1.7***	***2.6***	***100.0***	

* Note: Submission criteria, all *S.agalactiae* isolates from patients with meningitis. For invasive disease (non meningitis), *S. agalactiae* isolates only from children < 1 year.

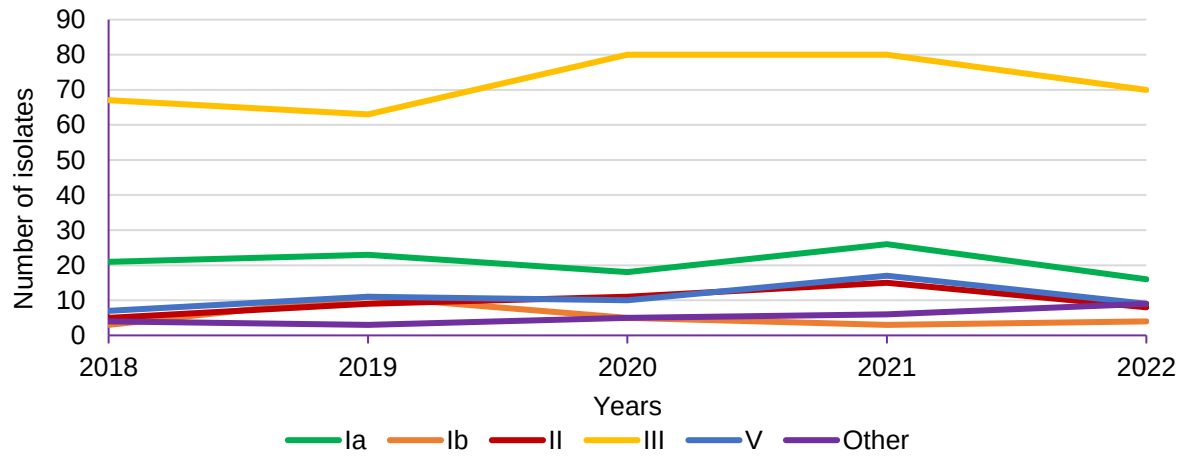


Figure 8.2 *Distribution of S. agalactiae serotypes, 2018 - 2022*

9 LISTERIA MONOCYTOGENES

Eighty-nine *Listeria monocytogenes*[10] isolates were submitted to the NRLBM, which is about average for the last five years (Figure 9.1). Of these, 10 (11%) were from CSF (or CSF and blood) and 79 (89%) from blood only (Figure 9.1). The large majority (78%) occurred among individuals over 50 years of age (Table 9.1). Similar to previous years, serotypes 1/2a and 4b were most prevalent in 2022 (Table 9.1), accounting for 47.2% and 45.0% of the cases, respectively. Compared to previous years, this is the first year that serotype 1/2a is the most dominant serotype, followed by serotype 4b (Figure 9.2)

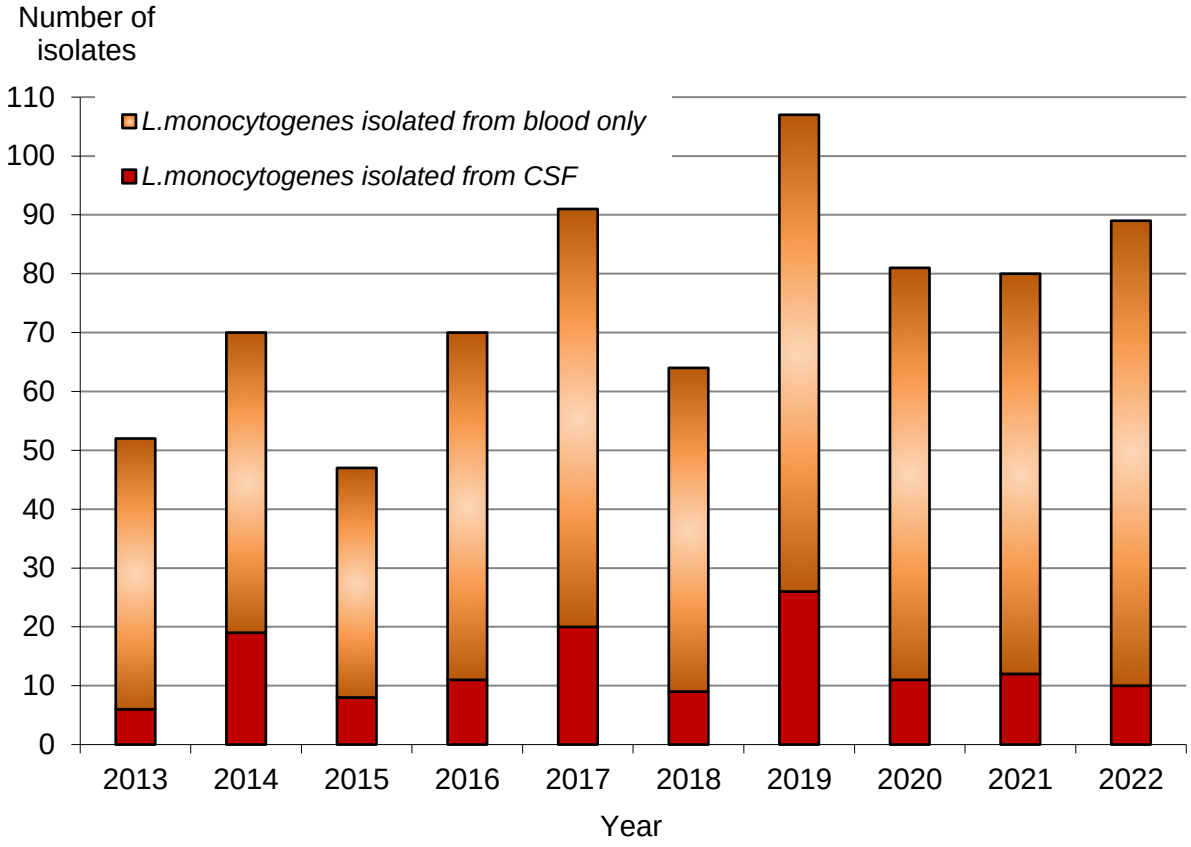


Figure 9.1 *Number of L. monocytogenes isolates grouped by isolation source, 2013-2022*

[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, 2022

	AGE (YEARS)					TOTAL	
Group	**0-4**	**5-19**	**20-49**	**50-79**	**≥80**	**T**	**%**
1/2a	4	0	2	24	12	**42**	***47.2***
CSF	*1*	*0*	*0*	*0*	*1*	***2***	
Blood	*3*	*0*	*2*	*24*	*11*	***40***	
1/2b	0	0	0	3	3	**6**	***6.7***
CSF	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*0*	*0*	*0*	*3*	*3*	***6***	
1/2c	0	0	0	1	0	**1**	***1.1***
CSF	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*0*	*0*	*0*	*1*	*0*	***1***	
4b	2	0	3	23	12	**40**	***45.0***
CSF	*0*	*0*	*1*	*7*	*0*	***8***	
Blood	*2*	*0*	*2*	*16*	*12*	***32***	
Total	**6**	**0**	**5**	**51**	**27**	**89**	***100.0***
CSF	***1***	***0***	***1***	***7***	***1***	***10***	***15.0***
Blood	***5***	***0***	***4***	***44***	***26***	***79***	***85.0***
%	***6.7***	***0***	***5.6***	***57.3***	***30.4***	***100.0***	

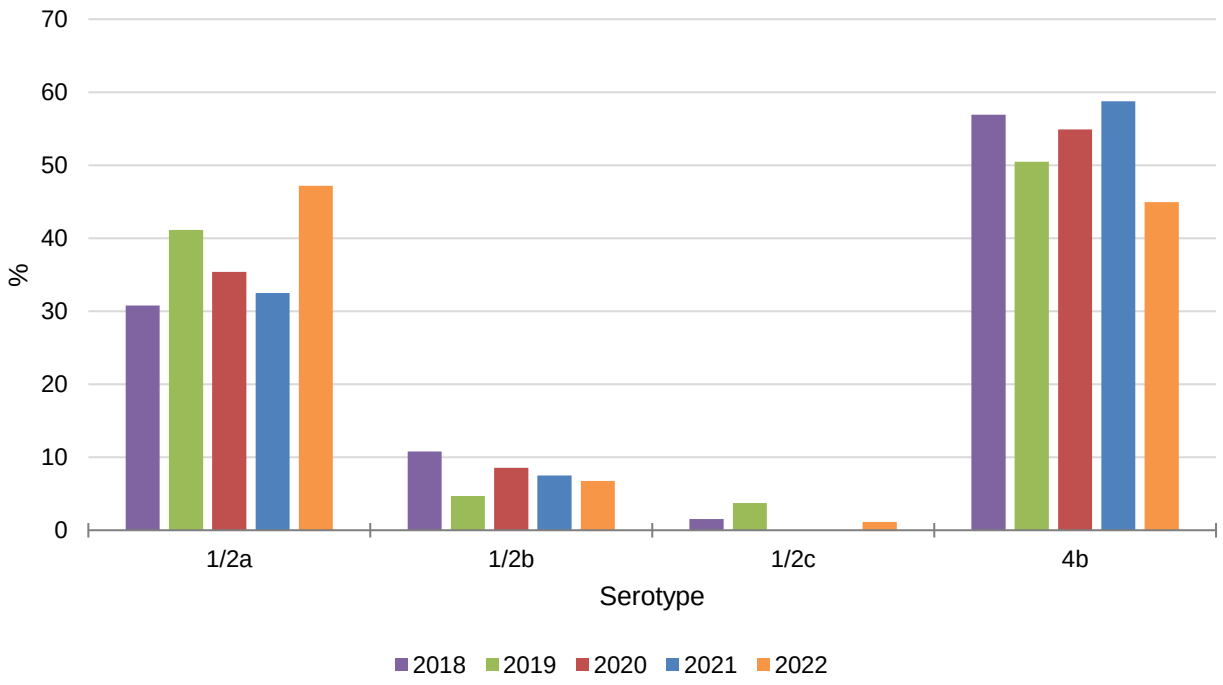


Figure 9.2 *Percentage of L. monocytogenes isolates grouped by serotype, 2018-2022*

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 28% 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. Finally, from April 2022, the NRLBM receives *S. pyogenes* isolates from all invasive infections (iGAS) from all medical microbiology laboratories. The results from iGAS surveillance will be published in a separate report.

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 2022, 1107 *S. pyogenes* isolates were submitted to the NRLBM, 19 were isolated from CSF and 8 were isolated from blood with clinical suspicion for meningitis (Table 10.1).

The *emm* typing and *emm*-cluster based data of all isolates are displayed in Table 10.2. There was a strong dominance (n=20; 74%) of *emm*1.0 cluster A-C3 among *S. pyogenes* isolates from meningitis patients (Table 10.2), followed by *emm*12.0 (n=3; 11%). Of the 14 emm1.0 CSF isolates that were analyzed by WGS, 11 (79%) belonged to the more virulent M1UK lineage[13,14].

Table 10.1 *S. pyogenes* isolates from CSF and/or blood according to patient's age, 2022.

TYPE	AGE (YEARS)					TOTAL	
	0-4	5-9	10-19	20-49	≥50	T	%
CSF	3	0	1	3	12	**19**	***70.4***
Blood	3	0	1	2	2	**8**	***29.6***
Total	**6**	**0**	**2**	**5**	**14**	**27**	***100***
%	***22.2***	***0***	***7.4***	***18.5***	***51.9***	***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 et al. A systematic and functional classification of *Streptococcus pyogenes* that serves as a new tool for molecular typing and vaccine development. *J Infect Dis* 2014.

[13] Lynskey N.N. et al. Emergence of dominant toxigenic M1T1 *Streptococcus pyogenes* clone during increased scarlet fever activity in England: a population-based molecular epidemiological study. *Lancet Infect Dis* 2019

[14] Van der Putten et al. Group A Streptococcal Meningitis With the M1UK Variant in the Netherlands. *JAMA* 2023

Table 10.2 *Emm*-type and *emm*-cluster distribution of meningitis related *S. pyogenes* isolates, 2022

Cluster	*emm* type	CSF	Blood	Total	%T
E1		**0**	**1**	**1**	**3.7**
	4.0	0	1	1	
E4		**1**	**1**	**2**	**7.4**
	22.0	0	1	1	
	28.0	1	0	1	
A-C3		**16**	**4**	**20**	**74.1**
	1.0	16	4	20	
A-C4		**2**	**2**	**4**	**14.8**
	12.0	2	1	3	
	12.37	0	1	1	
Total		**19**	**8**	**27**	**100**

11 DNA or ANTIGEN DETECTION

The NRLBM received 146 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*) for *H. influenzae* (*siaT* gene) and *L. monocytogenes*. When CSF was positive in the meningococcal or *H. influenzae* PCR, the same sample was subjected to serogroup and serotype-specific PCR.

Of 146 culture-negative samples, 53 (36%) were positive for one of the target species by PCR or LFA test. Of these, 25 (47%) were positive for *N. meningitidis*, 18 (34%) were positive for *S. pneumoniae* and 9 (17%) were positive for *H. influenzae*.

Table 11.1 CSF and serum samples tested for antigens or DNA, 2022

	CSF * (or DNA from CSF)	SERA or other fluids	TOTAL
***C. neoformans* (LFA)**	1	0	**1**
DNA of			
N. meningitidis group B	25	0	**25**
S. pneumoniae	14	4	18
H. influenzae	9	0	9
Sub Total	**49**	**4**	**53**
Antigen or PCR negative	71	4	**75**
LFA negative	17	1	**18**
Total	**137**	**9**	**146**

*From 7 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 2 patients with *N. meningitidis* isolated from blood, the CSF was culture-negative but PCR-positive for meningococcal DNA. Those were 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. I. Passaris, N. Mauder, M. Kostrzewa, I. Burckhardt, S. Zimmermann, **N.M. van Sorge**, H-C. Slotved, S. Desmet, P-J. Ceyssens. Validation of Fourier-Transform infrared Spectroscopy for serotyping of *Streptococcus pneumoniae*. ***J Clin Microbiol*** 2022 Jul 20: e0032522

2. A. Steens, M.J. Knol, W. Freudenburg-de Graaf, H.E. de Melker, A. van der Ende, **N.M. van Sorge**. Pathogen- and type-specific changes in invasive bacterial disease epidemiology during the first year of the COVID-19 pandemic in the Netherlands. ***Microorganisms*** 2022 May 5; 10(5): 972

3. 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*** 2022 Jul 6; 2173-2180. doi: 10.1093/cid/ciab791
4.
5. K. Korsten, W. Freudenburg-de Graaf, W.C.M. Bril-Keijzers, B.C.L. van der Putten, **N.M. van Sorge**. *Streptococcus dysgalactiae* subsp*. equisimilis* (SDSE) als veroorzaker van groep A streptokokken infecties (in Dutch). 2022 **Nederlands Tijdschrift voor Medische Microbiologie**

6. T.M. van Soest, N. Chekrouni, **N.M. van Sorge**, M.C. Brouwer, D. van de Beek. Bacterial meningitis presenting with a normal cerebrospinal fluid leukocyte count. ***J Infect***. 2022 May; 84 (5): 615-620

7. T.M. van Soest, N. Chekrouni, **N.M. van Sorge**, M.C. Brouwer, D. van de Beek. Community-acquired bacterial meningitis in patients of 80 years and older. ***J of American Geriatrics Society*** 2022 Jul; 70 (7): 2060-2069

8. L. Snoek, M.N. van Kassel, J.F. Krommenhoek, N.B. Achten, F.B. Plötz, **N.M. van Sorge**, M.C. Brouwer, D. van de Beek, M. W Bijlsma. Comparing the sensitivity of the neonatal early-onset sepsis calculator to the Dutch and the updated NICE guideline in an observational cohort of culture-positive cases. ***EClinicalMedicine*** 2022 Jan 10; 44:101270

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