NETHERLANDS REFERENCE LABORATORY FOR BACTERIAL MENINGITIS

BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2019

Reference Laboratory Bacterial Meningitis

AMC RIVM

Amsterdam
The Netherlands

Amsterdam UMC
University Medical Centers

BACTERIAL MENINGITIS IN THE NETHERLANDS
ANNUAL REPORT 2019

NETHERLANDS REFERENCE LABORATORY FOR BACTERIAL MENINGITIS

Academic Medical Center (AMC)
and
National Institute of Public Health and the Environment (RIVM),
Department of Medical Microbiology, AMC
PO Box 22660 , 1100 DD Amsterdam
The Netherlands
Telephone
+31 20 566 4874
+31 20 566 4864
+31 20 566 4861
E-mail: reflab@amc.uva.nl

Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM)
Bacterial meningitis in the Netherlands; annual report 2019
Amsterdam: University of Amsterdam, 2020

CONTENTS

CONTENTS....3

1 INTRODUCTION....6

2 ISOLATES, CSF SPECIMENS AND SERA RECEIVED....8

3 BACTERIAL MENINGITIS – general overview....13

4 NEISSERIA MENINGITIDIS....17
4.1 General features 17
4.2 Antibiotic susceptibility 18
4.3 Serogroups 19
4.4 Serogroup and age 21
4.5 Distribution of PorA and FetA genosubtypes among meningococci 25

5 HAEMOPHILUS INFLUENZAE....29
5.1 General features 29
5.2 Antibiotic susceptibility 30
5.3 Serotype and age 31
5.4 Distribution of non-typeable H. influenzae 33
5.5 Geographical distribution of H. influenza 35

6 STREPTOCOCCUS PNEUMONIAE....36
6.1 General features 36
6.2 Antibiotic susceptibility 38
6.3 Distribution according to serotype 39

7 ESCHERICHIA COLI....44

8 STREPTOCOCCUS AGALACTIAE – (group B)....48

9 LISTERIA MONOCYTOGENES....50

10 STREPTOCOCCUS PYOGENES – Group A....52

11 ANTIGEN AND DNA DETECTION....55

12 VACCINATION PROSPECTS....56
12.1 N. meningitidis 56
12.2 H. influenzae 57
12.3 S. pneumoniae 58

13 PUBLICATIONS....59

14 ACKNOWLEDGEMENTS....61

15 Bibliography....61

1 INTRODUCTION

This is the **48th** Annual Report of the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) of the Academic Medical Center (AMC) and the National Institute of Public Health and the Environment (RIVM). The NRLBM is located within the Department of Medical Microbiology of the Amsterdam UMC, location AMC in Amsterdam. Nearly all Dutch clinical microbiology collaborate with the NRLBM by submitting bacterial isolates and/or cerebrospinal fluid samples from patients with meningitis as well as other invasive diseases and we are most grateful to our colleagues for their collaboration.

The NRLBM started collecting isolates of *Neisseria meningitidis* in 1959 and of other meningitis-causing bacteria in 1975.

In the archives of the NRLBM approximately 78,300 isolates are now available for studies on the epidemiology of bacterial meningitis and on the pathogenicity and antibiotic susceptibility of isolates.

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

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

We welcome your opinion and suggestions on this report.

Amsterdam, November, 2020

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

2 ISOLATES, CSF SPECIMENS AND SERA RECEIVED

The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) collects isolates from cerebrospinal fluid (CSF) and blood from patients with proven meningitis (CSF and possibly blood culture positive) or with bacteraemia and suspected meningitis (blood culture positive only). Unless otherwise indicated, every isolate from CSF, from CSF and blood and from blood represents a patient with meningitis, a patient with meningitis and bacteraemia, or a patient with bacteraemia. When CSF is given as the isolation source this could indicate isolate from CSF or CSF and blood. Incidences have been calculated by dividing the number of annually-received isolates (in a certain patient age group) by the number of inhabitants (for a particular age group) in that multiplied by 100,000. Population statistics were obtained from Statistics Netherlands (CBS - Statline Statistics Netherland) using StatLine. By estimation, the NRLBM receives about 90% of the isolates from meningitis patients in the Netherlands. Hence, incidences presented in this report are likely to be a slight underestimation of true incidences.

In 2019, the NRLBM received isolates from CSF and/or blood from 2,685 patients. In addition, 57 CSF and/or serum samples were positive in antigen or PCR tests (**table 2.1/table 11.1**). Of all patients, 359 were clinically confirmed cases of bacterial meningitis.

Table 2.1

	Number of specimens
Isolates (CSF and/or blood)	2685
PCR or antigen positive samples of CSF, Sera and other fluids	57
PCR or antigen negative samples of CSF, Sera and other fluids	99
Total	**2841**

In 2019, 49 clinical microbiology laboratories submitted isolates to the NRLBM. Table 2.2 shows the 2,685 received isolates according to species and to laboratory where cases were diagnosed.

Table 2.2 Number of isolates from CSF and/or blood received in 2019, according to laboratory and bacterial species

Location	Laboratory	Bacterial species[#]											
		Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	Total
Alkmaar	MCA lab. Med. Microbiologie	1	6	32	3	2	2	1	-	-	-	-	**47**
Amersfoort	Meander Medisch Centrum	4	3	39	-	4	1	1	-	-	-	-	**52**
Amsterdam	Academisch Medisch Centrum	4	4	26	3	5	1	4	1	-	-	4	**52**
	Academisch ziekenhuis VU	1	2	31	6	3	2	1	2	1	-	2	**51**
	Onze Lieve Vrouwe Gasthuis	11	2	15	5	9	1	5	-	-	1	1	**50**
	Slotervaart / ATAL	-	1	15	1	3	4	-	-	-	-	-	**24**
Apeldoorn	Gelre Ziekenhuizen	2	6	64	-	3	3	4	-	-	-	3	**85**
Arnhem	Rijnstate	-	5	51	-	-	4	6	1	-	-	-	**67**
Breda	Amphia Ziekenhuis	5	6	42	4	6	2	2		-	-	-	**67**
Capelle ad IJssel	IJsselland Ziekenhuis	3	2	11	-	-	2	-		-	-	-	**18**
Delft	Reinier Haga MDC	5	6	3	1	6	4	4		-	-	-	**29**
Den Bosch	Regionaal laboratorium Den Bosch	2	8	61	-	-	2	1	1	-	-	-	**75**
Den Haag	Haga Ziekenhuis, loc. Leyenburg	7	2	33	2	8	-	-		-	-	-	**52**
	MA Haaglanden, loc Westeinde	1	6	27	-	2	1	1	1	-	-	-	**39**
Deventer	Deventer Ziekenhuis	2	1	19	-	3	1	-	1	-	-	-	**27**
Doetinchem	Slingeland Ziekenhuis	6	5	39	-	-	1	1	-	-	1	-	**53**
Dordrecht	RLM Dordrecht / Gorinchem	4	6	38	1	4	1	2	-	-	-	-	**56**
Ede	Gelderse Vallei	7	7	55	2	3	2	7	-	-	-	1	**84**
Goes	Lab. v. Med.Microb. & Imm., ADRZ	4	3	35	-	1	2	-	-	-	-	-	**45**
Gouda	Groene Hart Ziekenhuis	5	4	36	-	1	2	-	-	-	-	-	**48**
Groningen	Certe, Lab. v. Infectieziekten	8	16	89	-	-	6	2	-	-	-	1	**122**
	UMCG	2	4	4	1	-	3	-	-	-	-	-	**14**
Haarlem	Streeklab voor de Volksgezondheid	2	7	70	1	2	7	18	-	-	-	1	**108**
Harderwijk	St. Jansdal Ziekenhuis	3	2	13	1	6	-	1	-	-	-	-	**26**
Hengelo	LabMicTa	9	7	73	1	-	4	4	-	-	-	-	**98**
Hilversum	Centraal Bact. Ser. Lab.	2	2	6	-	2	1	-	-	-	-	1	**14**
Hoorn	Westfries gasthuis	2	6	44	2	2	3	-	-	-	2	1	**62**
Leeuwarden	Izore, centrum infectieziekten Friesland	3	9	96	-	8	2	26	-	-	-	-	**144**
Leiden	Alrijne ziekenhuis	2	8	52	-	-	-	1	1	-	-	-	**64**
	LUMC, KML, Lab.voor Bacteriologie	4	5	32	4	5	4	-	2	-	1	1	**58**
Maastricht	Acad. Ziekenhuis Maastricht	1	-	1	-	-	-	-	-	-	-	-	**2**
Nieuwegein	St. Antonius Ziekenhuis	4	8	56	-	2	4	33	-	-	-	1	**108**
Nijmegen	Canisius Wilhelmina Zknhs	2	2	32	1	-	2	1	-	-	-	-	**40**
	UMC St. Radboud	2	3	39	8	1	4	-	-	-	-	-	**57**
Roermond	St. Laurentius Ziekenhuis	-	1	-	-	-	-	-	-	-	-	-	**1**

Roosendaal	St. Fransiscus Ziekenhuis	-	1	33	2	4	-	1	-	-	-	-	**41**
Rotterdam	Erasmus MC Med. Microbiologie	5	7	33	15	2	3	3	-	-	-	-	**68**
	Ikazia Ziekenhuis	1	-	20	-	1	1	-	-	-	-	-	**23**
	Maasstad Ziekenhuis	5	4	15	2	3	1	-	-	-	-	1	**31**
	St.Franciscus Gasthuis	-	5	67	-	4	3	-	-	-	-	1	**80**
Sittard	Zuyderland Medisch Centrum	4	7	69	1	-	2	-	-	-	1	3	**87**
Terneuzen	Zorgsaam Zeeuws-Vlaanderen	2	1	5	-	-	-	-	-	-	-	-	**8**
Tilburg	Streeklab. Tilburg	3	6	30	3	1	2	-	-	-	-	-	**45**
Utrecht	Diakonessenhuis	2	3	2	-	2	2	-	-	-	-	-	**11**
	UMC Med. Microbiologie	6	5	37	18	7	4	1	-	1	-	2	**81**
Veldhoven	PAMM, Lab. Med. Microbiologie	4	11	121	3	3	9	4	-	-	1	-	**156**
Vredenburg	Medical Microbology, Curacao/St.Maarten	-	-	3	-	-	-	-	-	-	-	-	**3**
Venlo	Vie Curie medisch centrum	1	-	5	-	-	-	1	-	-	-	-	**7**
Zwolle	Isala Klinieken LMMI	4	11	74	5	2	2	-	1	-	3	3	**105**
Total		**157**	**226**	**1793**	**96**	**120**	**107**	**136**	**11**	**2**	**10**	**27**	**2685**

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 staphylococcus; **Cn:** *C. neoformans;* **ot:** other bacteria.

The distribution of the received isolates over the 5 year period 2015 through 2019 is presented in table 2.3. The total number of isolates increased from 1,404 in 2015 to 2,685 in 2019. The number of isolates from meningococcal disease increased from 84 in 2015 to 157 in 2019. Since June 2006, children born after the 1st April 2006 are vaccinated with a 10-valent conjugated polysaccharide vaccine (PCV10) against *Streptococcus pneumoniae*. The number of *S. pneumoniae* isolates from CSF decreased from more than 200 yearly before 2007 to 165 in 2019. The number of *Listeria monocytogenes* isolates increased by more than 50% from 47 in 2015 to 107 in 2019. The number of *Haemophilus influenzae* isolates increased from 195 in 2015 to 226 in 2019, mainly due to a higher number of *H. influenzae* isolates from blood.

Table 2.3 Number of isolates from CSF and/or blood received in the years 2015 – 2019

Species	2015			2016			2017			2018			2019		
	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total
***N. meningitidis*[1]**	33	51	84	36	100	136	67	134	201	70	135	205	53	104	157
H. influenzae	22	173	195	26	162	188	30	194	224	23	216	239	23	203	226
S. pneumoniae	147	754	901	143	762	905	148	1255	1403	152	1757	1909	165	1628[2]	1793
E. coli	8	20	28	18	32	50	8	41	49	12	50	62	18	78	96
S. agalactiae	19	46	65	20	49	69	24	63	87	27	79	106	23	97	120
L. monocytogenes	8	39	47	11	59	70	20	71	91	9	56	65	26	81	107
S. pyogenes	3	13	16	5	5	10	7	11	18	3	9	12	14	122	136
S. aureus	8	8	16	10	1	11	5	0	5	7	0	7	11	0	11
Coag.neg.Staph.	2	0	2	2	0	2	6	0	6	4	0	4	2	0	2
C. neoformans	7	2	9	7	3	10	7	2	9	8	5	13	8	2	10
others	30	10	40	19	18	37	9	13	22	18	7	25	16	11	27
non viable	0	1	1	0	2	2	0	3	3	0	7	7	0	0	0
Total	**287**	**1117**	**1404**	**297**	**1193**	**1490**	**331**	**1787**	**2118**	**333**	**2321**	**2654**	**359**	**2326**	**2685**

[1]Including PCR positive patients
[2] 556 (2019) blood isolates from 9 sentinel labs
CSF: CSF or CSF and blood
blood: blood only

The incidence of invasive bacterial infections of the different bacterial species from CSF and/or blood over the years 2015 to 2019 is shown in table 2.4. The incidence of *H. influenzae* infection was 38% lower compared to the pre-vaccination year (2.1 in 1992; 1.3 in 2019). The incidence *of H. influenzae* infection increased from 2010 until now, mainly due to an increase in the number of bacteraemia cases caused by unencapsulated (non-typeable) *H. influenzae*.

Table 2.4 Number of isolates from CSF and/or blood per 100,000 inhabitants, 2015 - 2019

Species	2015	2016	2017	2018	2019
N. meningitidis	0.50	0.80	1.18	1.19	0.91
H. influenzae	1.15	1.11	1.31	1.39	1.31
S. pneumoniae	5.33	5.33	8.21	11.11	10.37
E. coli	0.17	0.29	0.29	0.36	0.56
S. agalactiae	0.38	0.41	0.51	0.62	0.69
L. monocytogenes	0.28	0.41	0.53	0.38	0.62
S. pyogenes	0.09	0.06	0.11	0.07	0.79
S. aureus	0.09	0.07	0.03	0.04	0.06
Coag. neg. Staph.	0.01	0.01	0.04	0.02	0.01
C. neoformans	0.05	0.06	0.05	0.08	0.06
others	0.24	0.22	0.13	0.15	0.16
non viable	0.01	0.01	0.02	0.04	-
Total	**8.31**	**8.78**	**12.40**	**15.45**	**15.54**

Table 2.5 shows the distribution of isolates according to the compartment from which they were cultured. The predominant species were *N. meningitidis, H. influenzae* and *S. pneumoniae*.

Table 2.5 Total number of isolates from CSF and/or blood received in 2019, according to bacterial species and source.

Species		CSF or CSF and blood, n	Blood only, n	Total, n	%
Neisseria meningitidis		53	104	157	**5.8**
Haemophilus influenzae [1]		23	203	226	**8.4**
Streptococcus pneumoniae [2]		165	1628	1793	**66.7**
Escherichia coli		18	78	96	**3.6**
Streptococcus agalactiae		23	97	120	**4.5**
Listeria monocytogenes[3]		26	81	107	**4.0**
Streptococcus pyogenes[4]		14	122	136	**5.1**
Staphylococcus aureus[5]		11	0	11	**0.4**
Coagulase-negative staphylococcus [6]		2	0	2	**0.1**
Cryptococcus neoformans		8	2	10	**0.4**
Others total		16	11	27	**1.0**
Others	*Klebsiella pneumoniae*	*3*	*0*	*3*	
	Klebsiella aerogenes	*1*	*0*	*1*	
	Pseudomonas aeruginosa	*1*	*0*	*1*	
	Pasteurella multocida	*1*	*0*	*1*	
	Citrobacter koseri	*1*	*0*	*1*	
	Nocardia farcinica	*0*	*1*	*1*	
	Haemophilus parainfluenzae	*0*	*1*	*1*	
	Streptococcus australis	*1*	*0*	*1*	
	Streptococcus dysgalactiae ssp equisimilis	*2*	*2*	*4*	
	Streptococcus equi ssp ruminatorum	*0*	*1*	*1*	
	Streptococcus gallolyticus ssp gallolyticus	*0*	*1*	*1*	
	Streptococcus infantis	*0*	*1*	*1*	
	Streptococcus intermedius	*1*	*0*	*1*	
	Streptococcus mitis	*1*	*2*	*3*	
	Streptococcus oralis	*0*	*1*	*1*	
	Streptococcus oralis ssp oralis[7]	*1*	*0*	*1*	
	Streptococcus sanguinis	*1*	*0*	*1*	
	Indifferent streptococci	*0*	*1*	*1*	
	Enterococcus faecalis	*1*	*0*	*1*	
	Propionibacterium acnes	*1*	*0*	*1*	
Total		359	2326	2685	**100.0**

1 In six patients ***Haemophilus influenza*** and ***Streptococcus pneumoniae*** were isolated from blood.
2 In one patient ***Streptococcus pneumoniae*** and ***Streptococcus agalactiae*** were isolated from blood (2 months of age).
3 In one patient ***Listeria monocytogenes*** and ***Streptococcus pneumoniae*** were isolated from blood (57 years of age)
4 In one patient ***Streptococcus pyogenes*** was isolated from CSF and ***Streptococcus pneumoniae*** from blood
5 In one patient ***Staphylococcus aureus*** and ***Staphylococcus epidermidis*** was isolaten from CSF
6 Two coagulase-negative staphylococci were isolated from CSF; one ***Staphylococcus epidermidis*** and one ***Staphylococcus capitis***
7 In one patient ***Streptococcus oralis ssp oralis*** and ***Streptococcus sanguinis*** were isolated from CSF

3 BACTERIAL MENINGITIS – general overview

In 2019, the NRLBM received CSF isolates or PCR-positive CSF samples from 359 patients (Table 11.1). The proportion of meningococcal and pneumococcal cases among meningitis patients was 15% and 47%, respectively (Figure 3.1).

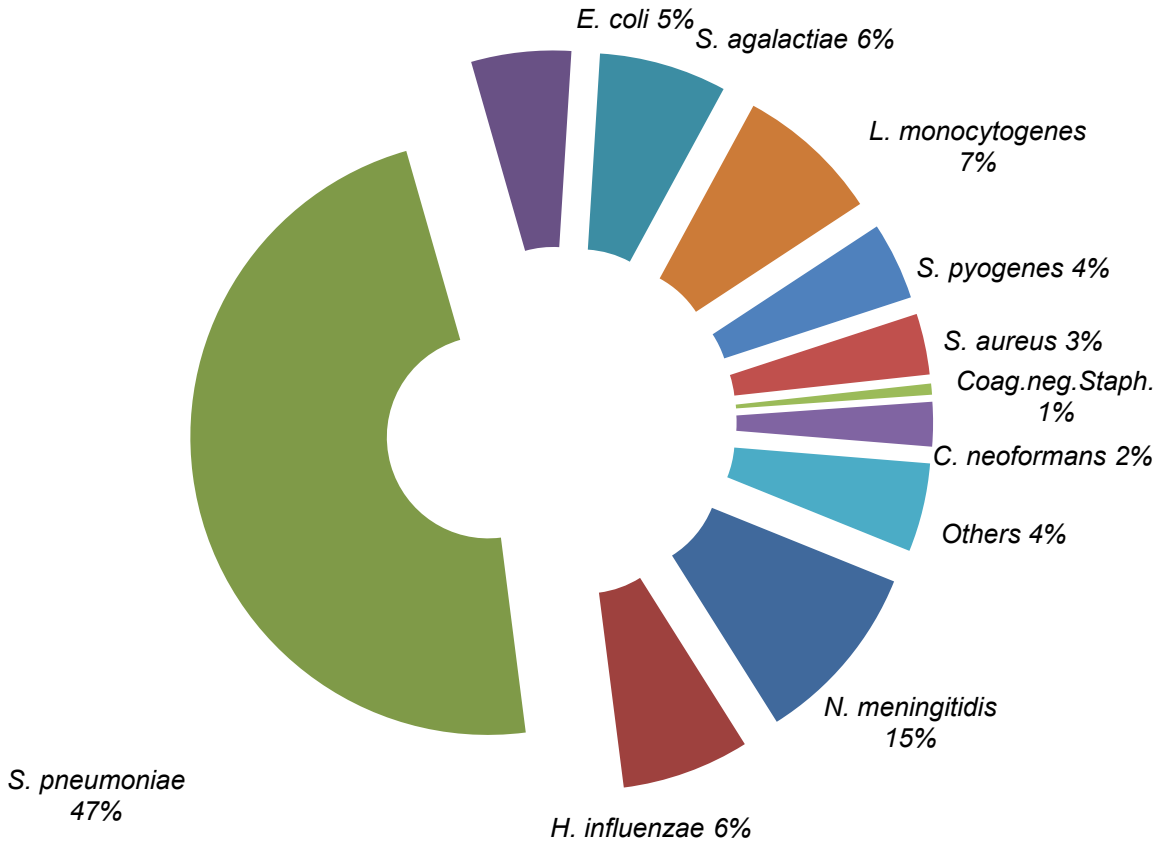


Figure 3.1 *Proportional distribution of CSF isolates and CSF positive samples according to bacterial species, 2019*

Figure 3.2 shows the annual total number of bacterial isolates from CSF during the period 1989-2019. The 30-year trend line shows a increase over the last three decades. The incidence per 100,000 inhabitants is quite stable around 2.0 (range 1.8 – 2.1) during the period 2010-2019 (Figure 3.2).
Bacterial meningitis cases over the same 30-year period according to specific species, i.e. *N. meningitidis, H. influenzae* and *S. pneumoniae*, are presented in figure 3.3. Comparing meningitis incidence pre- and post-vaccination, the incidence of *Haemophilus* meningitis decreased from 1.6 per 100,000 in 1992 to 0.13 per 100,000 in 2019 and has remained at this low level. For meningococcal meningitis (with by isolate or a positive PCR result) the incidence decrease from 3.1/100,000 in 1993 to 0.3/100,000 in 2019, mainly due to a decline in the number of cases caused by serogroups B and C meningococci. The decline in serogroup C meningococcal meningitis is largely attributed to nationwide vaccination, which started in 2002 and immediate showed a decrease in 2003, when *N. meningitidis* was not the main cause of bacterial meningitis anymore in the Netherlands. In 2019, the number of meningococcal meningitis cases decreased to 53, which is mainly explained by a decreased number of cases caused by serogroup W. Pneumococcal meningitis showed a slight increase in annual

incidence between 1991 and 2004 from 1.0 to 1.6 per 100,000 inhabitants. In 2019, the incidence of pneumococcal meningitis has decreased to 0.98 per 100,000 inhabitants due to inclusion of 10-valent conjugated polysaccharide vaccine against pneumococci for children in the National Immunisation Programme in June 2006.

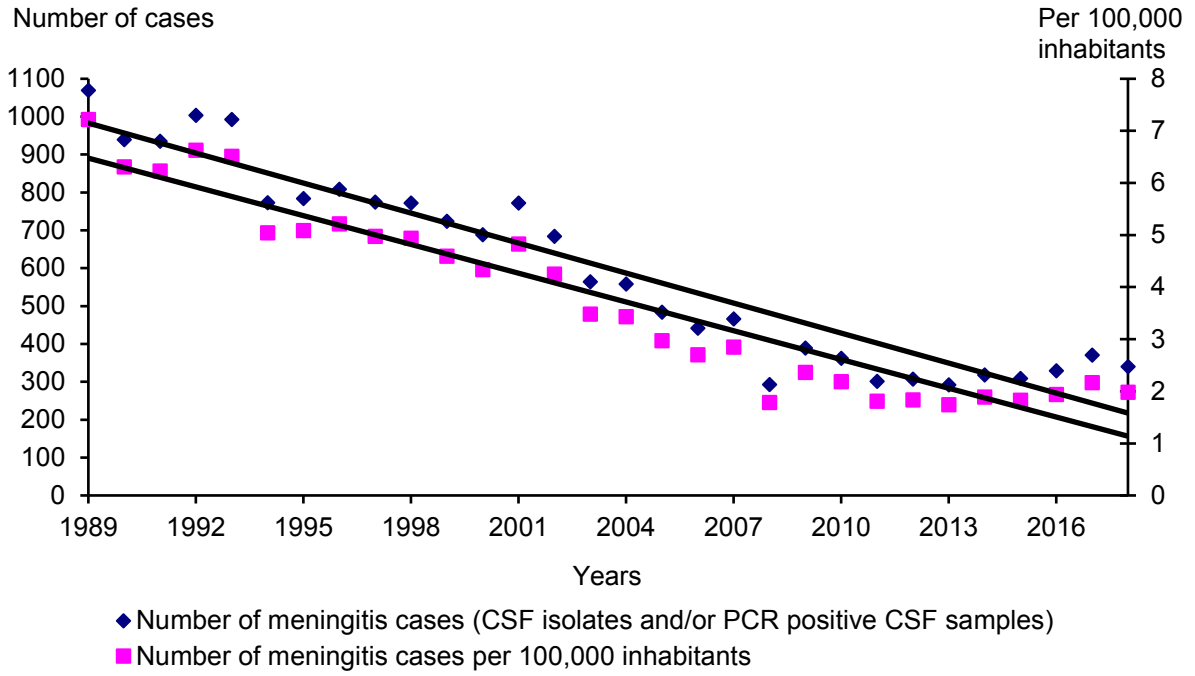


Figure 3.2 *All cause meningitis cases and incidence, 1989-2019*

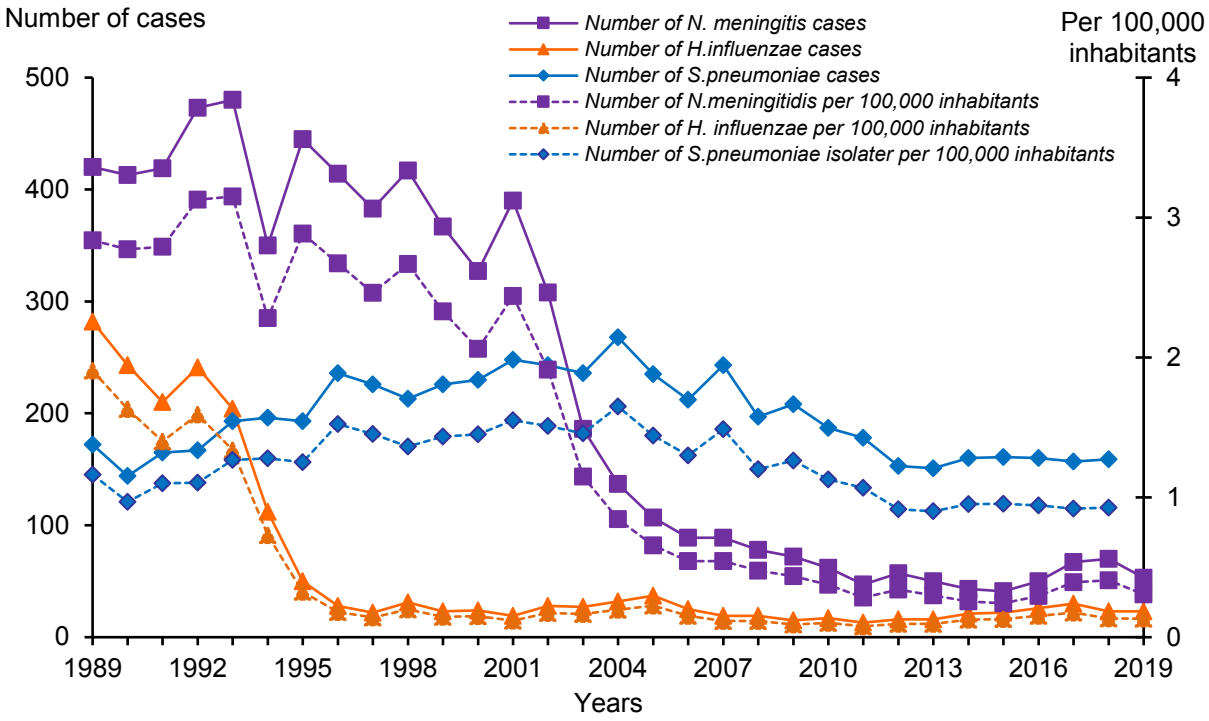


Figure 3.3 Number of cases and incidence of *meningococcal, haemophilus and pneumococcal meningitis (isolates and/or positive PCR from CSF), 1989-2019*

Table 3.1 shows the number of isolates from CSF by annual quarter grouped per bacterial species. As in previous years, most isolates were received during the first quarter of the year.

Table 3.1 Isolates and PCR-positive samples from CSF by annual quarter according to bacterial species, 2019

	ANNUAL QUARTER					
SPECIES	**First**	**Second**	**Third**	**Fourth**	**Total**	**%**
N. meningitidis	17	15	8	13	**53**	***14.8***
H. influenzae	7	6	6	4	**23**	***6.4***
S. pneumoniae	56	48	14	47	**165**	***46.0***
E. coli	3	7	4	4	**18**	***5.0***
S. agalactiae	4	6	6	7	**23**	***6.4***
L. monocytogenes	4	10	10	2	**26**	***7.2***
S. pyogenes	5	5	0	4	**14**	***3.9***
S. aureus	2	3	3	3	**11**	***3.1***
Coag.neg.Staph.	1	0	1	0	**2**	***0.6***
C. neoformans	2	1	3	2	**8**	***2.2***
Others	6	2	5	3	**16**	***4.4***
non viable	0	0	0	0	**0**	***0.0***
Total	**119**	**67**	**58**	**86**	**359**	***100***
%	***33.1***	***18.8***	***16.0***	***24.0***	***100.0***	

Tables 3.2 and 3.3 show the distribution of bacterial species isolated from CSF according to patient age and the age-specific incidence per 100,000 persons, respectively. *Streptococcus agalactiae* and *E. coli* are still the predominant species isolated from neonates (i.e. younger than 1 month), and together represented 86% of all isolates in this age group. In contrast, in infants 1-11 months of age, the predominant species was *S. pneumoniae* (56%). 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 9 in 2019.

Table 3.2 Isolates and PCR-positive samples from CSF grouped according to patients' age, 2019

	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	2	12	14	2	2	11	6	3	1	10	4	0	**53**	***14.8***
H. influenzae	0	3	6	9	0	0	0	0	2	1	6	5	0	**23**	***6.4***
S. pneumoniae	2	14	8	24	1	0	2	2	14	7	62	44	9	**165**	***46.0***
E. coli	12	3	0	15	0	0	0	1	1	0	1	0	0	**18**	***5.0***
S. agalactiae	20	2	0	22	0	0	0	0	0	0	0	1	0	**23**	***6.4***
L. monocytogenes	0	0	0	0	0	0	0	0	1	0	5	12	8	**26**	***7.2***
S. pyogenes	0	0	2	2	1	1	1	0	0	0	5	2	2	**14**	***3.9***
S. aureus	2	0	1	3	0	0	0	1	0	1	1	5	0	**11**	***3.1***
Coag.neg.Staph.	0	0	0	0	0	1	0	1	0	0	0	0	0	**2**	***0.6***
C. neoformans	0	0	0	0	0	0	1	0	0	1	2	4	0	**8**	***2.2***
Others	1	1	0	2	0	0	0	2	1	3	2	6	0	**16**	***4.4***
non viable	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
Total, n	*37*	*25*	*29*	*91*	*4*	*4*	*15*	*13*	*22*	*14*	*94*	*83*	*19*	**359**	***100***
%	***10.3***	***7.0***	***8.1***	***25.4***	***1.1***	***1.1***	***4.2***	***3.6***	***6.1***	***3.9***	***26.2***	***23.1***	***5.3***	***100***	

As anticipated from table 3.2, the incidence of bacterial meningitis was highest in the 0-11 month age group (table 3.3).

Table 3.3 Age-specific incidence of bacterial meningitis per 100,000 inhabitants according to bacterial species, 2019

	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	1.19	1.72	0.22	0.21	1.05	0.27	0.14	0.04	0.28	0.16	-	0.31
H. influenzae	1.78	0.86	-	-	-	-	0.09	0.04	0.17	0.20	-	0.13
S. pneumoniae	9.50	1.15	0.11	-	0.19	0.09	0.66	0.31	1.72	1.75	1.13	0.95
E. coli	8.91	-	-	-	-	0.05	0.05	-	0.03	-	-	0.10
S. agalactiae	13.06	-	-	-	-	-	-	-	-	0.04	-	0.13
L. monocytogenes	-	-	-	-	-	-	0.05	-	0.14	0.48	1.00	0.15
S. pyogenes	-	0.29	0.11	0.10	0.10	-	-	-	0.14	0.08	0.25	0.08
S. aureus	1.19	0.14	-	-	-	0.05	-	0.04	0.03	0.20	-	0.06
Coag.neg.Staph.	-	-	-	0.10	-	0.05	-	-	-	-	-	0.01
C. neoformans	-	-	-	-	0.10	-	-	0.04	0.06	0.16	-	0.05
Others	1.19	-	-	-	-	0.09	0.05	0.13	0.06	0.24	-	0.09
non viable	-	-	-	-	-	-	-	-	-	-	-	-
Total	**36.81**	**4.16**	**0.44**	**0.42**	**1.43**	**0.59**	**1.04**	**0.62**	**2.61**	**3.30**	**2.38**	**2.08**

Table 3.4 shows the number of CSF isolates per species according to patient gender. For most species the Male/Female ratio varied between 0.7 and 1.5. The overall M/F ratio was 0.9.

Table 3.4 Isolates and PCR positive samples from CSF according to patients' gender, 2019

SPECIES	M	F	M/F – ratio	Sex not known	Total	%
N. meningitidis	30	22	**1.4**	1	**53**	***14.8***
H. influenzae	10	13	**0.8**	0	**23**	***6.4***
S. pneumoniae	69	95	**0.7**	1	**165**	***46.0***
E. coli	12	5	**2.4**	1	**18**	***5.0***
S. agalactiae	5	17	**0.3**	1	**23**	***6.4***
L. monocytogenes	16	10	**1.6**	0	**26**	***7.2***
S. pyogenes	8	6	**1.3**	0	**14**	***3.9***
S. aureus	7	4	**1.8**	0	**11**	***3.1***
Coag.neg.Staph.	1	1	**1**	0	**2**	***0.6***
C. neoformans	5	3	**1.7**	0	**8**	***2.2***
Others	8	6	**1.3**	2	**16**	***4.4***
non viable	0	0	**-**	0	**0**	***0.0***
Total	**171**	**182**	**0.9**	**6**	**359**	***100***
%	***47.6***	***50.7***		***1.7***	*100*	

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2019, the NRLBM received 135 *Neisseria meningitidis* isolates of which 33 were isolated from CSF (or CSF and blood; 54 in 2018) and 102 from blood only (131 in 2018). In addition, 20 culture-negative CSF samples and 2 blood samples tested positive for meningococci by PCR. In total, we received meningococcal isolates or PCR-positive CSF or blood from 157 patients. The distribution of isolates received throughout the year showed the familiar seasonality with the highest number of isolates received in the first quarter of the year (figure 4.1).

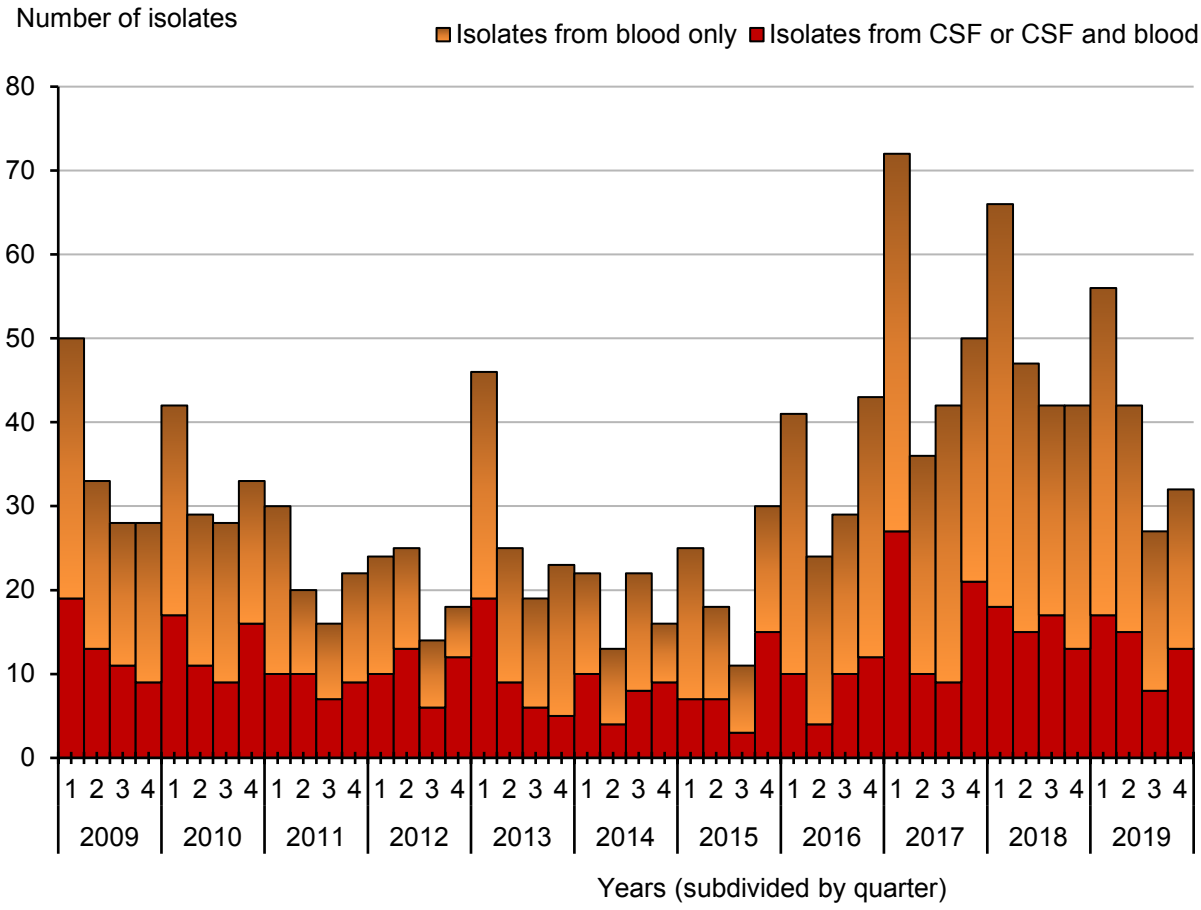


Figure 4.1 *Seasonal distribution of meningococcal disease, 2009-2019*

4.2 Antibiotic susceptibility

Ninety-five percent of all isolates (128/135) were susceptible to penicillin (MIC ≤ 0.064 µg/ml) and no isolate was resistant to penicillin (MIC > 0.25 µg/ml; Table 4.1). The proportion of penicillin susceptible and penicillin intermediate isolates was similar for isolates from blood and CSF (Tables 4.2, 4.3). In general, mutations in *penA,* encoding a penicillin binding protein, confer meningococcl with reduced penicillin susceptibility. All isolates were susceptible to Rifampicine.

Table 4.1 Penicillin susceptibility of all received *N. meningitidis* isolates according to source of isolation (CSF and/or blood), 2019

Penicillin*					
	MIC ≤ 0.064 (S)	**0.064< MIC≤0.25 (I)**	**MIC>0.25 (R)**	**Total**	**%**
CSF or CSF and blood	31	2	0	**33**	***24***
Blood only	97	5	0	**102**	***76***
Total	**128**	**7**	**0**	**135**	***100***
%	***95***	***5***	***0***	***100***	

* MIC values in µg/ml

Table 4.2 Penicillin susceptibility of *N. meningitidis* isolates from CSF, 2015-2019

Penicillin*							
	MIC ≤ 0.064 (S)		**0.064< MIC≤0.25 (I)**		**MIC>0.25 (R)**		**Total**
	N	%	N	%	N	%	
2015	32	*97.0*	1	*3.0*	0	*0.0*	**33**
2016	32	*88.0*	4	*12.0*	0	*0.0*	**36**
2017	37	*80.4*	9	*19.6*	0	*0.0*	**46**
2018	39	*72.2*	14	*25.9*	1	*1.9*	**54**
2019	31	*93.9*	2	*6.1*	0	*0.0*	**33**

* MIC values in µg/ml

Table 4.3 Penicillin susceptibility of *N. meningitidis* isolates from blood only, 2015-2019

Penicillin*							
	MIC ≤ 0.064 (S)		**0.064< MIC≤0.25 (I)**		**MIC>0.25 (R)**		**Total**
	N	%	N	%	N	%	
2015	48	*94.1*	3	*5.9*	0	*0.0*	**51**
2016	88	*88.0*	12	*12.0*	0	*0.0*	**100**
2017	104	*80.6*	24	*18.6*	1	*0.8*	**129**
2018	99	*75.6*	30	*22.9*	2	*1.5*	**131**
2019	97	*95.1*	5	*4.9*	0	*0.0*	**102**

* MIC values in µg/ml

4.3 Serogroups

Serogroup B accounted for 46% of all received isolates (Table 4.4), which is an increase compared to previous years (2018: 36%; 2017: 41%). However, the absolute number of group B isolates (n = 72; table 4.4) was approximately similar compared to 2018 (n = 74). Observations across the entire collection period 1959 - 2019 (figure 4.2) show that the number of serogroup B isolates was the lowest (53 cases) in 2014. The proportion of serogroup W isolates decreased to 38% (table 4.4) compared to 50% in 2018, 41% in 2017, 34% in 2016 and 10% in 2015 (figure 4.2). Also in absolute numbers, the NRLBM received less serogroup W isolates (n = 60) compared to the previous 2 years (Figure 4.2). This is likely due to the implementation of the MenACWY vaccine in the National Immunisation program as of 1 May 2018. Because meningococcal serogroup W also affects older children, the vaccination is also be offered to teenagers in the year they turn 14, as of 1 October 2018. The MenACWY vaccine was introduced to the vaccination program to counter an increase in the number of meningococcal W cases and replaced the MenC vaccine.

Serogroups C and Y were responsible for 3.8% and 10.8% of all cases of invasive meningococcal disease in 2019, respectively (Table 4.4). 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). In response, vaccination against serogroup C was included in the National Immunisation Programme in June 2002, resulting in a rapid decline and near eradication of the number of serogroup C isolates received by the NRLBM. Overall, serogroups B and W have the highest incidence of invasive meningococcal disease (Table 4.5). Cases of invasive meningococcal disease are evenly distributed across the Netherlands (Figure 4.3).

Table 4.4 Number of meningococcal isolates according to serogroup and source of isolation, 2019

Source	Serogroup					Total, n
	B	C	W	Y	NG	
CSF	*41*	*1*	*6*	*3*	*2*	*53*
Blood	*31*	*5*	*54*	*14*	*0*	*104*
Total (%)	**72 (45.9)**	**6 (3.8)**	**60 (38.2)**	**17 (10.8)**	**2 (1.3)**	**157**

Table 4.5 Incidence of meningococcemia per 100,000 inhabitants according to serogroup and source of isolation, 2019

Source	Serogroup					Total
	B	C	W	Y	NG	
CSF	*0.24*	*0.01*	*0.03*	*0.02*	*0.01*	*0.31*
Blood	*0.18*	*0.03*	*0.31*	*0.08*	*0*	*0.60*
Total	**0.42**	**0.04**	**0.35**	**0.10**	**0.01**	**0.91**

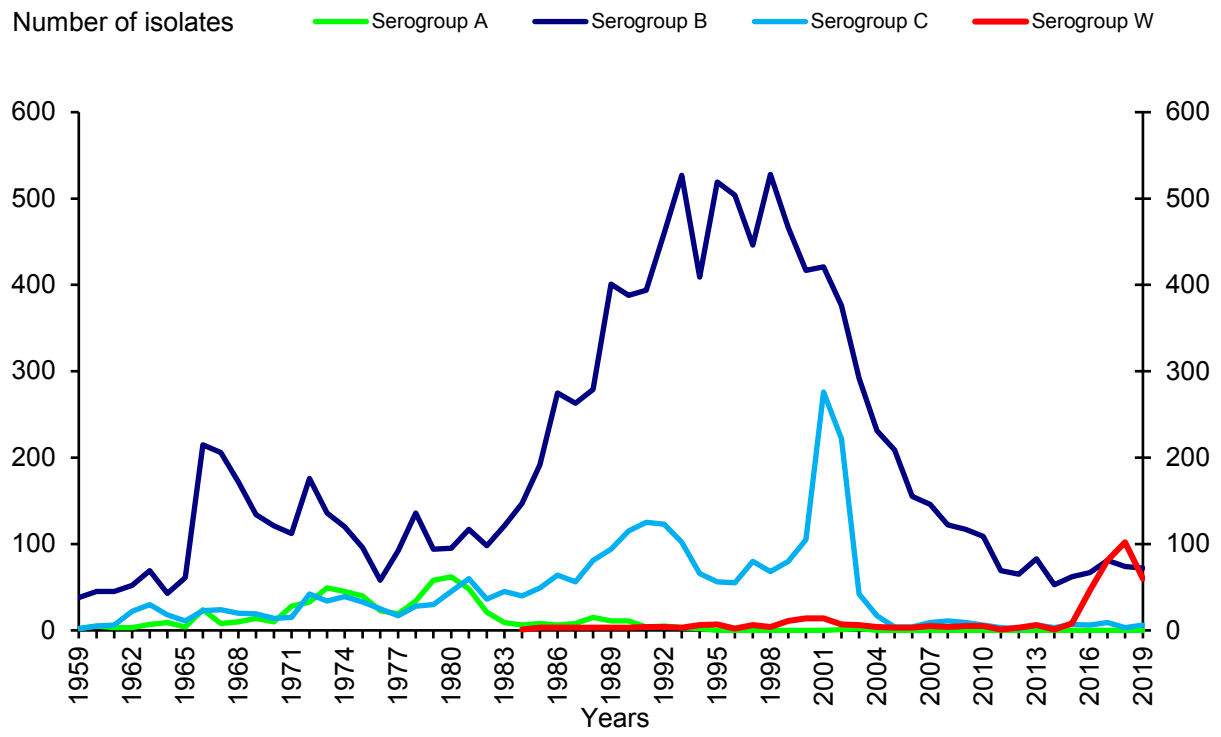


Figure 4.2. *Distribution of meningococcal serogroups A, B, C and W across the entire collection period from 1959-2019.*

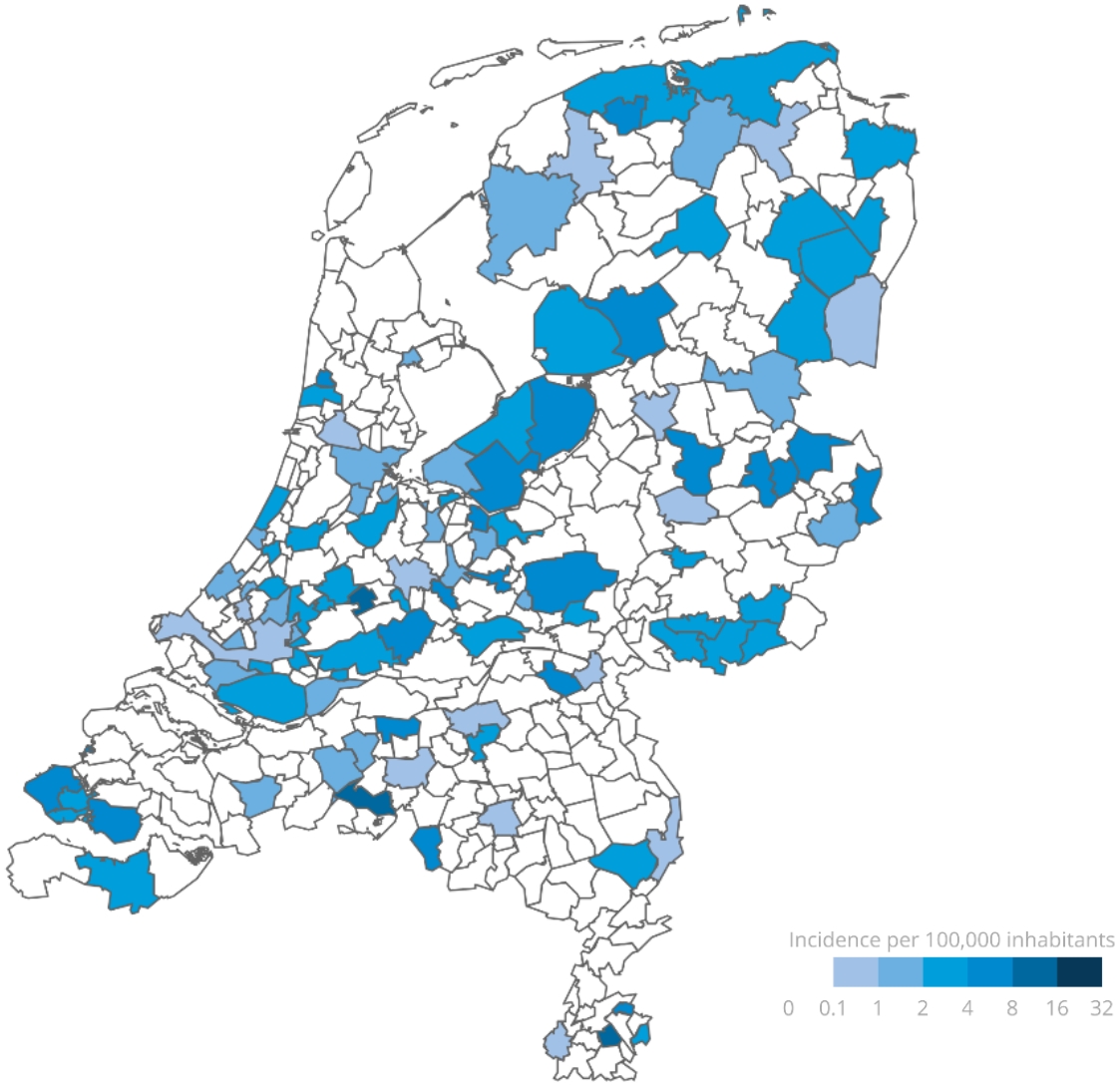


Figure 4.3 Geographical distribution of *N. meningitidis* cases based on incidence, *2019*. Incidence is calculated per municipality based on patient's place of residence.

4.4 *Serogroup and age*

Almost 20% (25 of 157) of patients with meningitis and/or meningococcemia was below the age of 5 (table 4.6). Among serogroup B cases, 65% (47/72) were between 6 and 25 years of age (table 4.6). In contrast, 62% (37/60) of the serogroup W cases were older than 50 years of age. In addition, of 72 serogroup B isolates, 41 (57%) were from CSF, while of 60 serogroup W isolates only 6 (10%) were from CSF, suggesting that the clinical presentation and population at risk for infection may be different for the serogroup B and W meningococci. Of 104 patients from whom meningococci were isolated from blood only, 11 (11%) were younger than 5 years of age, while 39 (38%) were older than 65 years of age (table 4.7). Overall, the incidence of invasive meningococcal disease is highest in the age groups 0-4 and 15-19 with predominant contribution of serogroups B and W (table 4.7). Currently, the available menB vaccines (Bexsero and Trumemba) are not included in the National Immunisation Program.

Table 4.6 Serogroups of *N. meningitidis* (isolates or PCR-positive samples from CSF and /or blood; absolute numbers) according to patient's age, 2019

	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	17	22	3	2	15	5	5	4	10	6	**72**	***45.9***
CSF	*0*	*2*	*11*	*13*	*2*	*2*	*8*	*1*	*3*	*3*	*8*	*1*	***41***	
Blood	*0*	*3*	*6*	*9*	*1*	*0*	*7*	*4*	*2*	*1*	*2*	*5*	***31***	
C	0	0	0	0	0	0	0	1	0	0	3	2	**6**	***3.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	***1***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*0*	*0*	*2*	*2*	***5***	
W	0	1	2	3	1	0	7	4	4	4	10	27	**60**	***38.2***
CSF	*0*	*0*	*1*	*1*	*0*	*0*	*2*	*0*	*1*	*0*	*0*	*2*	***6***	
Blood	*0*	*1*	*1*	*2*	*1*	*0*	*5*	*4*	*3*	*4*	*10*	*25*	***54***	
Y	0	0	0	0	1	0	1	2	0	2	3	8	**17**	***10.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*1*	***3***	
Blood	*0*	*0*	*0*	*0*	*1*	*0*	*1*	*2*	*0*	*1*	*2*	*7*	***14***	
NG*	0	0	0	0	0	0	1	1	0	0	0	0	**2**	***1.3***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*1*	*1*	*0*	*0*	*0*	*0*	***2***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Total	**0**	**6**	**19**	**25**	**5**	**2**	**24**	**13**	**9**	**10**	**26**	**43**	**157**	***100.0***
CSF	***0***	***2***	***12***	***14***	***2***	***2***	***11***	***2***	***4***	***4***	***10***	***4***	***53***	
Blood	***0***	***4***	***7***	***11***	***3***	***0***	***13***	***11***	***5***	***6***	***16***	***39***	***104***	
%	***0***	***3.8***	***12.1***	***15.9***	***3.2***	***1.3***	***15.3***	***8.3***	***5.7***	***6.3***	***16.6***	***27.4***	***100.0***	

*Non Groupable

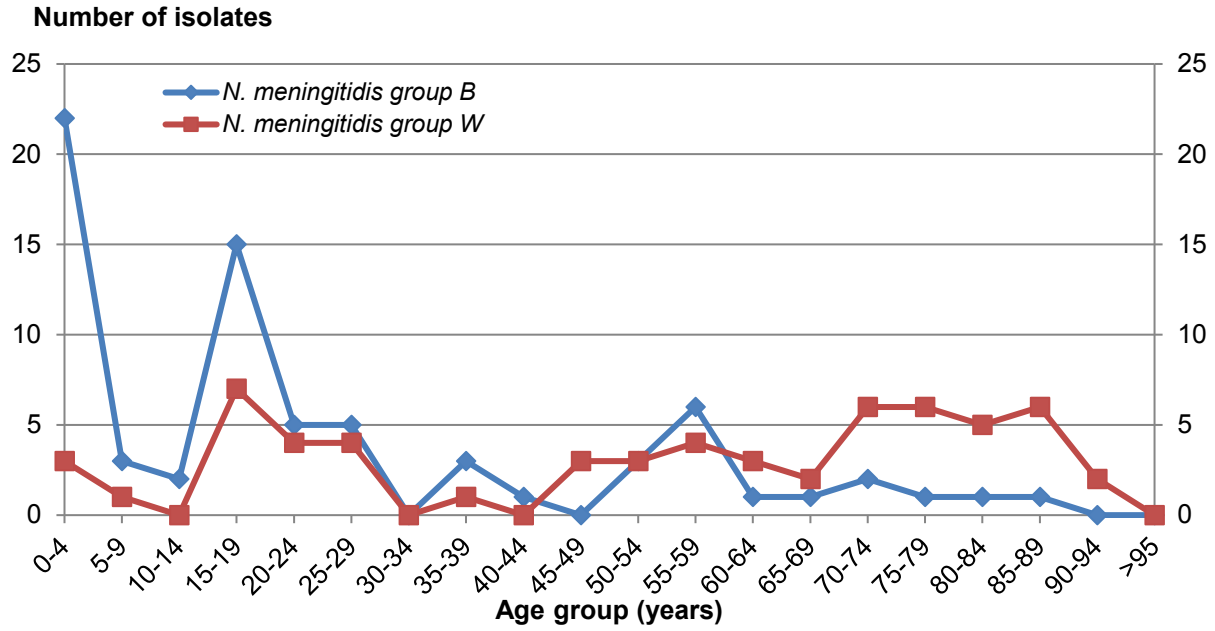
Table 4.7 Incidence of meningococcemia per 100,000 inhabitants according to different serogroups of *N. meningitidis* and patient's age, 2019

	AGE (YEARS)										TOTAL
Group	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	
B	2.97	2.44	0.33	0.21	1.43	0.46	0.45	0.09	0.28	0.18	**0.42**
CSF	*1.19*	*1.58*	*0.22*	*0.21*	*0.76*	*0.09*	*0.27*	*0.07*	*0.22*	*0.03*	***0.24***
Blood	*1.78*	*0.86*	*0.11*	*0.00*	*0.67*	*0.37*	*0.18*	*0.02*	*0.06*	*0.15*	***0.18***
C	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.08	0.06	**0.04**
CSF	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.03*	*0.00*	***0.01***
Blood	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.09*	*0.00*	*0.00*	*0.05*	*0.06*	***0.03***
W	0.59	0.29	0.11	0.00	0.67	0.37	0.36	0.09	0.28	0.81	**0.35**
CSF	*0.00*	*0.14*	*0.00*	*0.00*	*0.19*	*0.00*	*0.09*	*0.00*	*0.00*	*0.06*	***0.03***
Blood	*0.59*	*0.14*	*0.11*	*0.00*	*0.48*	*0.37*	*0.27*	*0.09*	*0.28*	*0.75*	***0.31***
Y	0.00	0.00	0.11	0.00	0.10	0.19	0.00	0.05	0.08	0.24	**0.10**
CSF	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.00*	*0.02*	*0.03*	*0.03*	***0.02***
Blood	*0.00*	*0.00*	*0.11*	*0.00*	*0.10*	*0.19*	*0.00*	*0.02*	*0.06*	*0.21*	***0.08***
NG*	0.00	0.00	0.00	0.00	0.10	0.09	0.00	0.00	0.00	0.00	**0.01**
CSF	*0.00*	*0.00*	*0.00*	*0.00*	*0.10*	*0.09*	*0.00*	*0.00*	*0.0*	*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	**3.56**	**2.72**	**0.54**	**0.21**	**2.28**	**1.20**	**0.80**	**0.23**	**0.72**	**1.30**	**0.91**
CSF	***1.19***	***1.72***	***0.22***	***0.21***	***1.05***	***0.19***	***0.36***	***0.09***	***0.28***	***0.12***	***0.31***
Blood	***2.37***	***1.00***	***0.33***	***0.00***	***1.24***	***1.02***	***0.45***	***0.14***	***0.44***	***1.18***	***0.60***

*Non Groupable

Figure 4.5 shows the age distribution of meningococcal disease caused by serogroups B and W. The age-specific incidence for serogroup B per 100,000 inhabitants in the age groups younger than 5 years and 15 - 19 years were 2.4 and 1.4, respectively (Figure 4.5B and Table 4.7; 0.4 and 0.7 for serogroup W). The age-specific incidence per 100,000 inhabitants for all age groups >19 years was below 0.5 (Table 4.7, Figure 4.5B). The age-specific incidence for serogroup W shows a different distribution compared to serogroup B (Figure 4.5B), with highest incidences for the age groups 15-19 years (0.7), ages 70-95 years (0.7-2.0) with the highest incidence in age group 85-90 years of age (2.4).

A



B

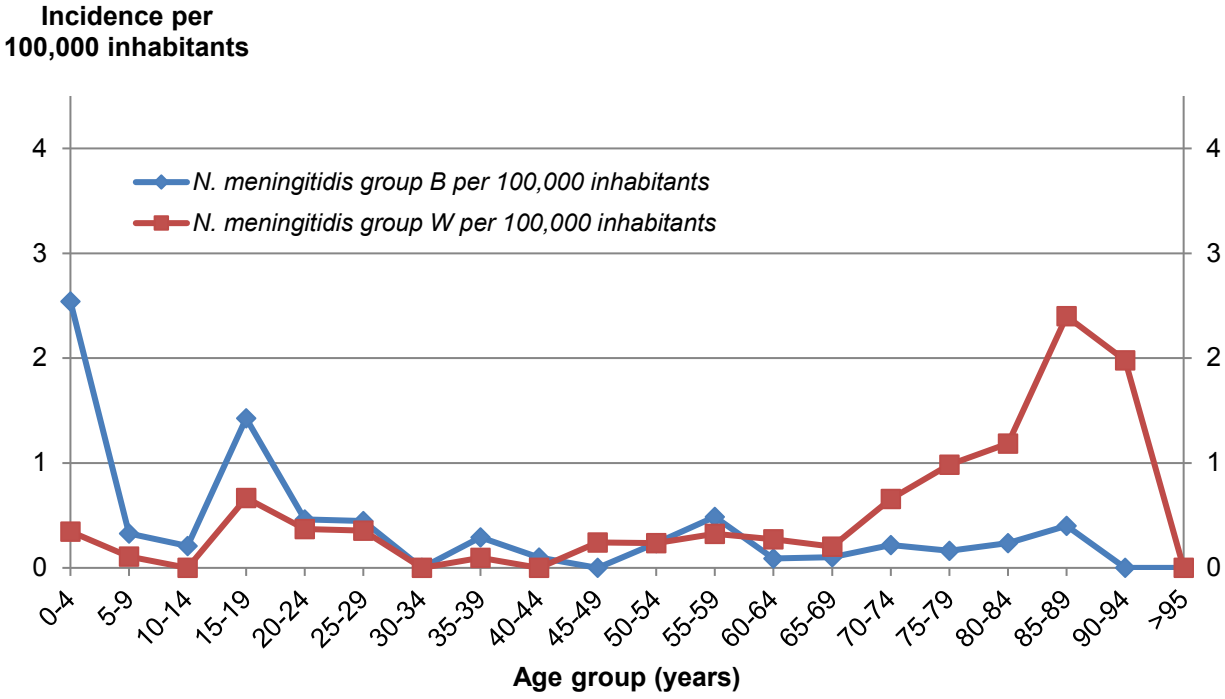


Figure 4.5 *A) Number of isolates and B) incidence of meningococcal disease* per 100,000 inhabitants *caused by serogroup B and W according to age groups, 2019*

4.5 *Distribution of PorA and FetA genosubtypes among meningococci*

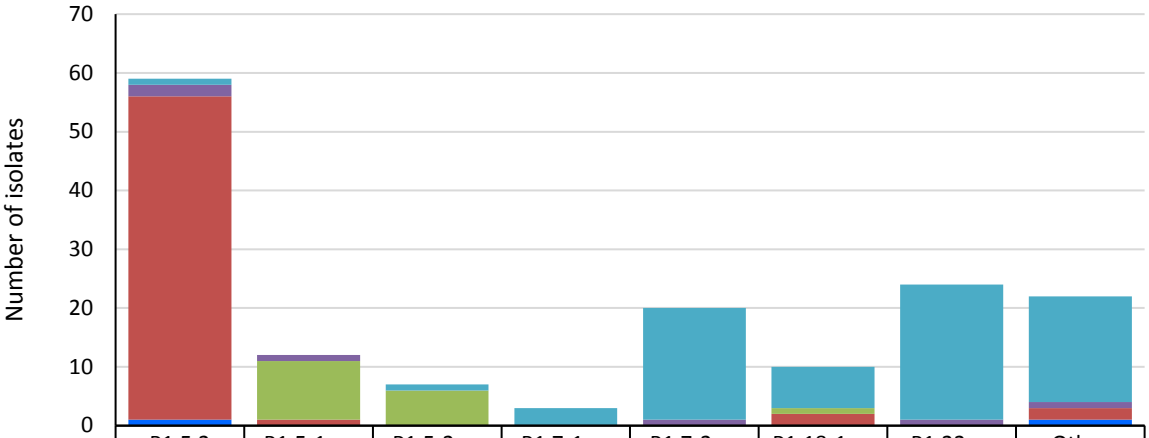
4.5.1 PorA

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

The PorA epitopes that react with the monoclonal antibodies of the subtyping scheme are encoded by the *porA* variable regions VR1 and VR2. Since 2000, we routinely sequence the DNA regions which encode VR1 and VR2 of PorA of all meningococcal isolates. The DNA sequences are translated into putative amino acid sequences and are then compared with PorA epitopes present in the database available on the website: https://pubmlst.org/neisseria/PorA/ (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 2019, the NRLBM received 21 PCR-positive samples. Sixteen samples were completely subtyped. Overall, thirty different VR1/VR2 combinations were encountered among 72 serogroup B meningococci (2016: 35 different combinations; 2018: 37 different combinations). The proportion of dominant PorA genosubtypes has shifted tremendously in the last two decades: in 2000, genosubtype P1.7-2.4 represented 40% of all serogroup B isolates and gradually declined to only 11% in 2019 (figure 4.6, table 4.8). Approximately 88% (63/72 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), wich is the highest hypothetical vaccine coverage in the past 5 years (Table 4.8).

The six serogroup C isolates had 5 different VR1/VR2 combinations.(Figure 4.6), whereas serogroup W isolates showed a strong dominance of PorA P1.5.2 (55 isolates, 92%;Figure 4.6)



	P1.5,2	P1.5-1,xx	P1.5-2;xx	P1.7-1,xx	P1.7-2,xx	P1.18-1,xx	P1.22,xx	Other
Men B	1		1	3	19	7	23	18
Men C	2	1			1		1	1
Men Y		10	6			1		
Men W	55	1				2		2
NG	1							1

Figure 4.6 *Distribution of PorA types among all received meningococcal isolates, 2019*

Table 4.8 PorA genosubtype distribution of *N. meningitidis* serogroup B isolates from 2015-2019

	VR1.VR2 combination	YEAR									
		2015		2016		2017		2018		2019	
		No.	%	No.	%	No.	%	No.	%	No.	%
Vaccine types*	1.5-1, 2-2	0	**0**	1	**1.5**	1	**1.4**	0	**0.0**	0	**0.0**
	1.5-1, other	1	**1.6**	2	**3.0**	3	**4.2**	2	**2.7**	0	**0.0**
	1.5-2.10	3	**4.8**	3	**4.4**	2	**2.9**	0	**0.0**	1	**1.4**
	1.5-2, other	2	**3.2**	1	**1.5**	2	**2.9**	1	**1.3**	0	**0.0**
	1.7,16	1	**1.6**	2	**3.0**	1	**1.4**	1	**1.3**	1	**1.4**
	1.7, other	2	**3.2**	2	**3.0**	3	**4.2**	1	**1.3**	1	**1.4**
	1.7-1, 1	3	**4.8**	2	**3.0**	0	**0**	0	**0.0**	2	**2.8**
	1.7-1, other	0	**0**	1	**1.5**	1	**1.4**	0	**0.0**	1	**1.4**
	1.7-2,4	7	**11.3**	8	**11.9**	5	**7.0**	3	**4.1**	8	**11.1**
	1.7-2, other	5	**8.2**	4	**6.0**	2	**2.9**	6	**8.2**	11	**15.2**
	1.12-1,13	0	**0**	0	**0**	0	**0.0**	0	**0.0**	0	**0.0**
	1.12-1, other	2	**3.2**	2	**3.0**	1	**1.4**	0	**0.0**	0	**0.0**
	1.18-1,3	1	**1.6**	2	**3.0**	0	**0.0**	1	**1.3**	0	**0.0**
	1.18-1, other	5	**8.2**	4	**6.0**	4	**5.6**	5	**6.8**	7	**9.7**
	1.19,15-1	2	**3.2**	1	**1.5**	1	**1.4**	1	**1.3**	1	**1.4**
	1.19, other	3	**4.8**	3	**4.4**	4	**5.6**	5	**6.8**	1	**1.4**
	1.22,14	9	**14.6**	11	**16.4**	21	**29.6**	20	**27.0**	19	**26.3**
	1.22,other	3	**4.8**	4	**6.0**	4	**5.6**	8	**10.8**	4	**5.6**
	Other, 1	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**	1	**1.4**
	Other, 14	1	**1.6**	2	**3.0**	4	**5.6**	3	**4.1**	2	**2.8**
	Other, 16	2	**3.2**	1	**1.5**	2	**2.9**	2	**2.7**	3	**4.2**
	Subtotal vaccine types	52	**83.9**	56	**83.6**	61	**86.0**	59	**79.7**	63	**87.5**
NVT**	Other **Non Vaccine Type**	10	**16.1**	11	**16.4**	10	**14.0**	15	**20.3**	9	**12.5**
	Total	62	**100.0**	67	**100.0**	71	**100.0**	74	**100.0**	72	**100.0**

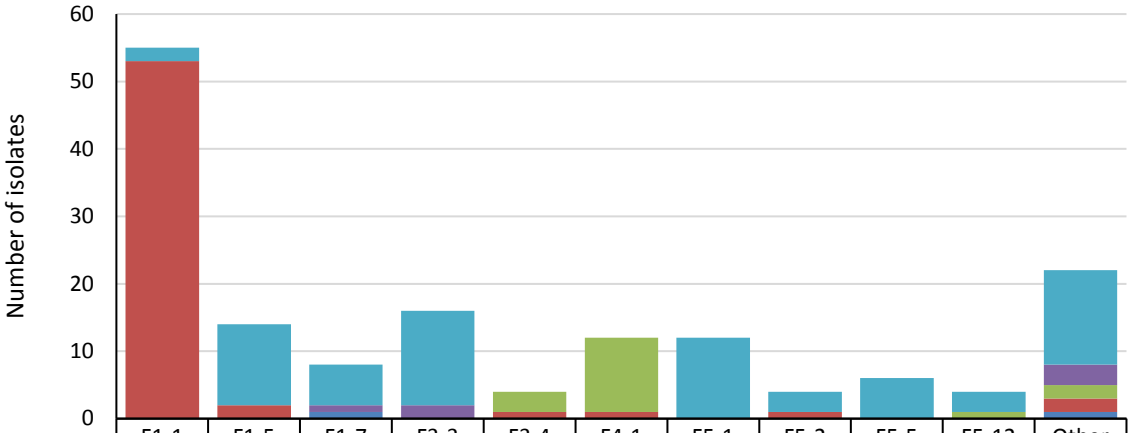
*based on a nonavalent PorA vaccine. NonaMen; serosubtypes P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1 and P1.18-1,3,6
**Non vaccine type

4.5.2 FetA

In addition to PorA epitope sequencing, meningococcal isolates are also characterized by FetA epitope sequencing. The outer membrane protein FetA is involved in iron uptake by meningococci and is considered as a potential vaccine component. Therefore, the variability of this protein has been investigated intensively. The most variable part of the protein, called VR, has been used to establish a typing scheme. Analogous to PorA typing, the VR part of *fetA* is sequenced and translated to a putative amino acid sequence. So far, approximately 270 VR sequences comprising 6 classes are identified, which are available at https://pubmlst.org/neisseria/FetA/ (PubMLST - FetA variable region typing, sd). 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 2019, 16 different FetA variants were observed among serogroup B meningococci, among wich F3-3 (19%), F1-5 and F5-1 (both 17 %) 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 4.7), whith strongly linkage to PorA VR1/VR2 P1.7-2,4. Together, these types linked to the MLST clonal complex ST41/44. In 2019, 12 isolates were of the Fet A type F1-5, of which eight were linked to P1.7-2,4 and 4 were linked to different PorA types. For the dominant FetA type F5-1, 92% (11 out of 12 isolates) expressed PorA VR1/VR2 P1.22,14. In total, 36 different PorA VR1/VR2 combinations and 22 different FetA variants were encountered among serogroup B meningococci. Frequently found combinations were P1.22,14:F5-5 (14%), P1.22,14:F5-1 (8%), and P1.18-1,30:F3-3 (4%) (Figure 4.7).

The six serogroup C meningococci had five different FetA types (Figure 4.6). In 2019, we received 60 serogroup W samples: 57 isolates and 3 PCR-positive samples. From one PCR samples complete typing was not possible. The 60 meningococcal serogroup W isolates only displayed 6 different FetA types (Figure 4.7, Table 4.9), of which F1-1 was dominant (88%) and linked to PorA VR1/VR2 P1.5,2 and MLST clonal complex 11.



	F1-1	F1-5	F1-7	F3-3	F3-4	F4-1	F5-1	F5-2	F5-5	F5-12	Other
Men B	2	12	6	14			12	3	6	3	14
Men C			1	2							3
Men Y					3	11				1	2
Men W	53	2			1	1		1			2
NG			1								1

Figure 4.7 *Distribution of meningococcal* fetA *genosubtypes, 2019*

Table 4.9 Temporal distribution in *fetA* genosubtype among *N. meningitidis* serogroups B, C and W isolates, 2015-2019

	YEARS														
	Men B					Men C					Men W				
FetA	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019	2015	2016	2017	2018	2019
F1-1	0	0	0	1	2	0	0	0	0	0	5	43	72	90	53
F1-5	10	16	12	7	12	0	0	1	0	0	0	0	0	1	2
F1-7	9	4	9	4	6	0	0	0	0	1	0	1	0	0	0
F3-3	9	7	7	9	14	7	2	3	1	2	0	0	0	1	0
F3-4	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1
F3-6	0	0	0	1	0	0	2	2	1	1	0	0	0	0	0
F3-7	0	1	0	0	0	0	0	0	0	0	0	0	2	3	1
F3-9	0	2	0	2	1	0	2	2	1	1	0	1	0	0	0
F4-1	2	0	3	2	0	0	0	0	0	0	2	0	1	0	1
F5-1	10	12	17	12	12	0	0	0	0	0	0	0	0	0	0
F5-2	2	1	1	2	3	0	0	0	0	0	0	0	0	0	1
F5-5	7	10	8	11	6	0	0	0	0	0	0	0	0	1	0
F5-8	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0
F5-12	3	3	1	1	3	0	0	0	0	0	0	0	0	0	0
F5-36	0	0	2	1	1	0	0	0	0	0	0	0	0	2	0
Other	9	10	22	20	11	0	0	1	0	1	0	0	6	2	1
Total	**62**	**67**	**82**	**74**	**72**	**7**	**6**	**9**	**3**	**6**	**8**	**46**	**81**	**102**	**60**

5 HAEMOPHILUS INFLUENZAE

5.1 *General features*

In total, 226 *Haemophilus influenzae* isolates were submitted to the NRLBM in 2019, which is a decrease compared to the 239 isolates received in 2018 (table 2.3, figure 3.3, figure 5.1). Twenty-three isolates were from CSF (or CSF and blood) (2018: 23; 2017: 30) and 203 from blood only. Thirty-nine (17.3%) of the isolates were *H. influenzae* type b (table 5.1). From 1999 to 2004, the number of *H. influenzae* type b isolates received by the NRLBM increased, but decreased after 2004 (table 5.4). The higher number of *H. influenzae* type b isolates was mainly due to an increase of *H. influenzae* type b cases among elderly people.

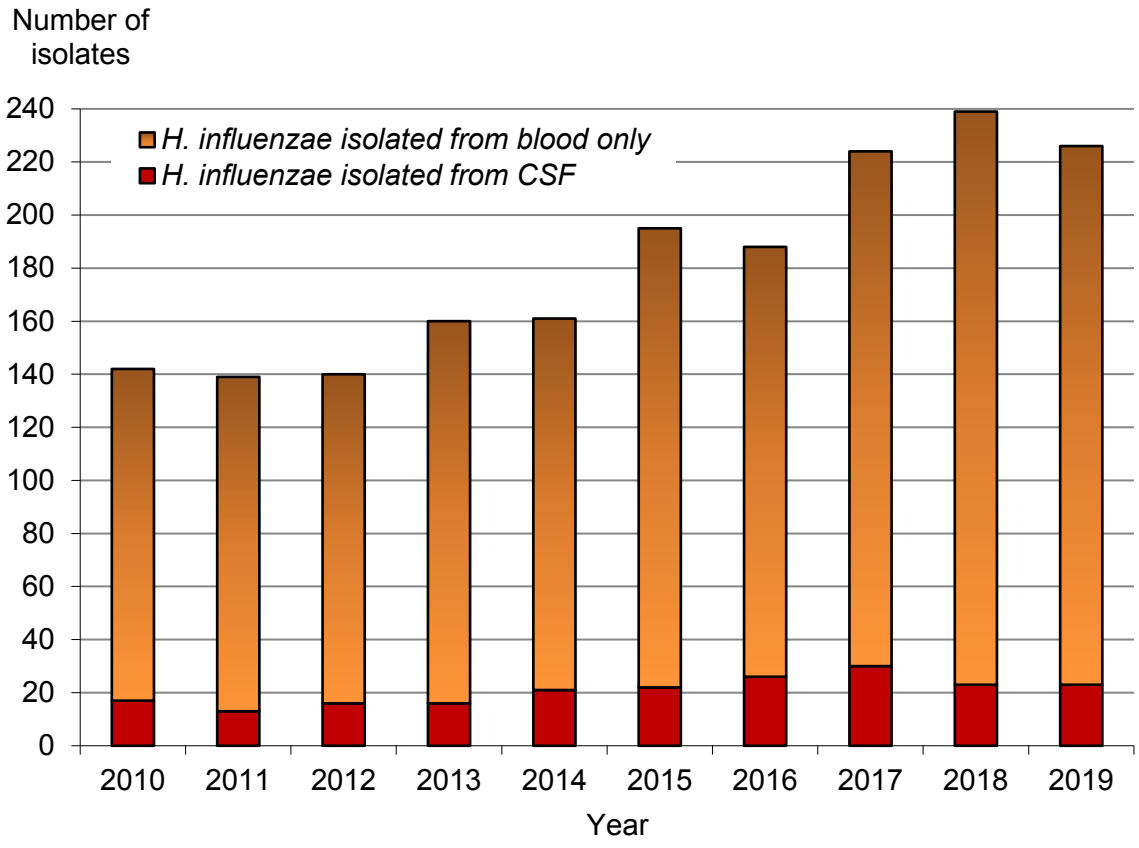


Figure 5.1 *Number of received H. influenzae isolates according to source of isolation, 2010-2019*

5.2 *Antibiotic susceptibility*

The proportion of ß-lactamase-producing invasive *H. influenzae* isolates (CSF and/or blood) was 8.0% in 2019 (Figure 5.2). In 2010, the proportion was 14.8%, which is the highest value in 25 years of surveillance. During the history of the NRLBM, the proportion of ß-lactamase-producing invasive *H. influenzae* isolates has always fluctuated for unknown reasons.

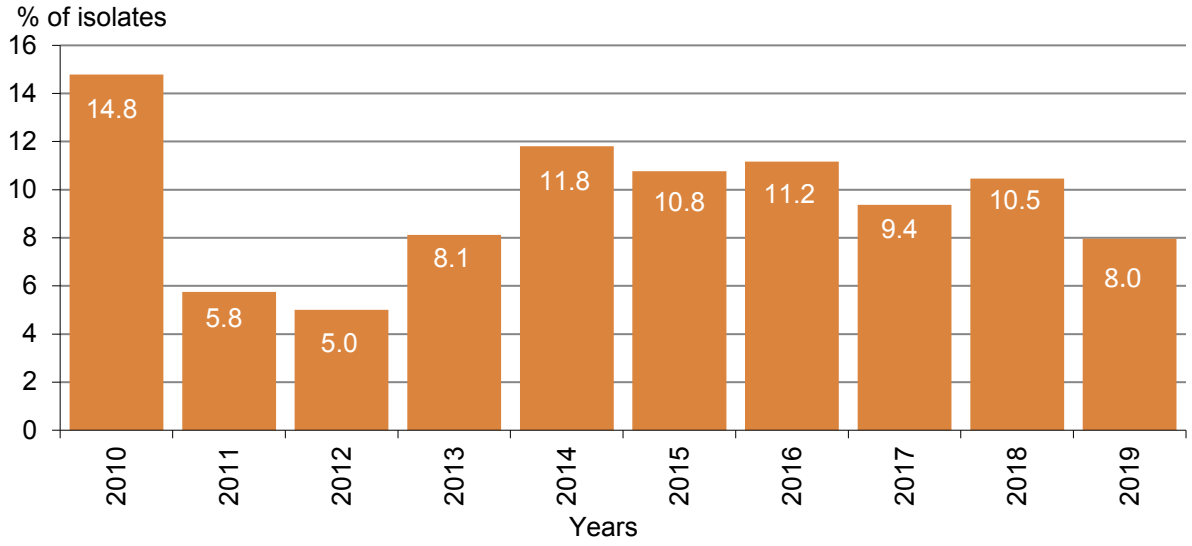


Figure 5.2 *Percentage β-lactamase producing H. influenzae strains among received isolates, 2010-2019*

5.3 *Serotype and age*

In 2019, the number of *H. influenzae* type b isolates decreased compared to the previous year which had been the highest level in the last 14 years (Figure 5.3). 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. In addition, since 2008, the number of cases due to serotype f has been slowly increasing, but are still below the numbers of *H. influenzae* b (Figure 5.3).

For the vaccine-preventable *H. influenzae* type b, we observed 11 cases of invasive disease among children younger than 2 years of age (15 in 2018; 7 in 2017; figure 5.4). Of 226 *H. influenzae* isolates, 164 were non-typeable (72.5%), 12 were isolated from CSF (or CSF and blood) and 152 were isolated from blood only (table 5.1).

Table 5.1 Serotype distribution of all received *H. influenzae isolates* according to serotype patient's age, 2019

	AGE (MONTHS)				AGE (YEARS)					TOTAL	
Group	**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	0	2	**2**	***0.9***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*2*	***2***	
Hi - b	0	10	1	6	17	0	2	5	15	**39**	***17.3***
CSF	*0*	*3*	*0*	*4*	*7*	*0*	*0*	*2*	*0*	***9***	
Blood	*0*	*7*	*1*	*2*	*10*	*0*	*2*	*3*	*15*	***30***	
Hi - e	0	0	0	0	0	0	0	0	5	**5**	***2.2***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*1*	***1***	
Blood	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*4*	***4***	
Hi - f	0	1	0	2	3	0	0	1	12	**16**	**7.1**
CSF	*0*	*0*	*0*	*1*	*1*	*0*	*0*	*0*	*0*	***1***	
Blood	*0*	*1*	*0*	*1*	*2*	*0*	*0*	*1*	*12*	***15***	
n.t.*	3	3	5	1	12	0	2	30	120	**164**	***72.5***
CSF	*0*	*0*	*1*	*0*	*1*	*0*	*0*	*1*	*10*	***12***	
Blood	*3*	*3*	*4*	*1*	*11*	*0*	*2*	*29*	*110*	***152***	
Total	**3**	**14**	**6**	**9**	**32**	**0**	**4**	**36**	**154**	**226**	***100.0***
CSF	***0***	***3***	***1***	***5***	***9***	***0***	***0***	***3***	***11***	***23***	***10.2***
Blood	***3***	***11***	***5***	***4***	***23***	***0***	***4***	***33***	***143***	***203***	***89.8***
%	***1.3***	***6.2***	***2.7***	***4.0***	***14.2***	***0***	***1.8***	***15.9***	***68.1***	***100.0***	

* non-typeable

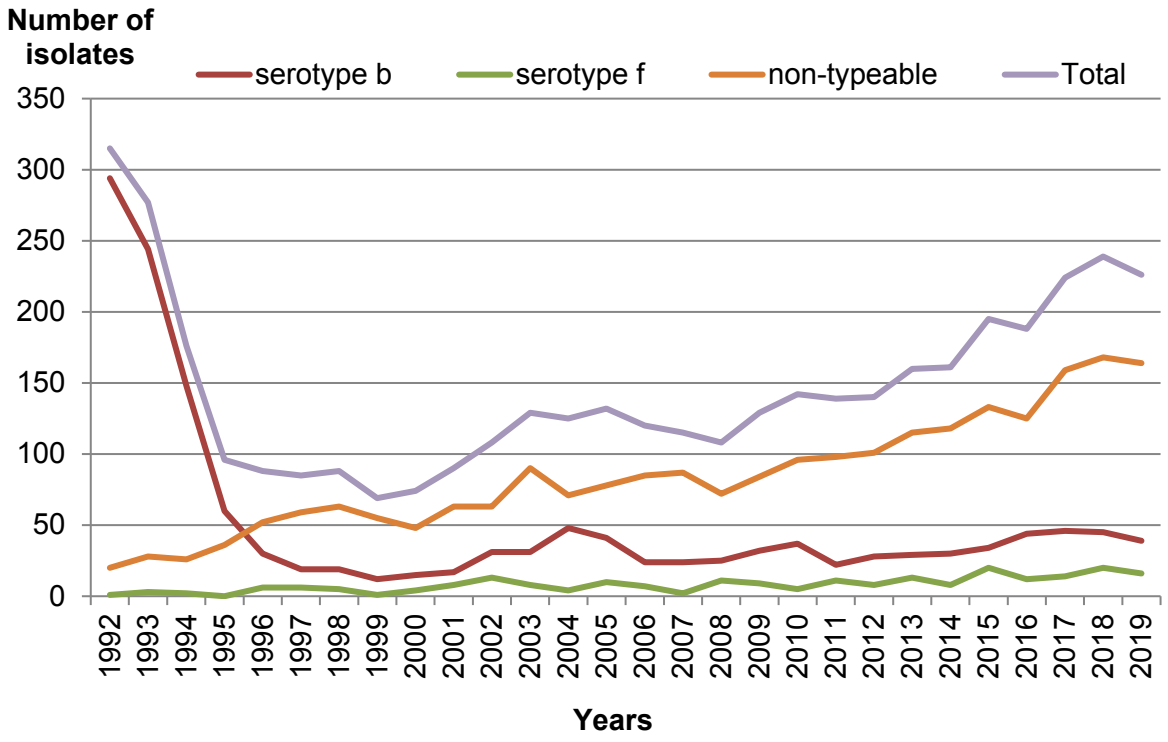


Figure 5.3 *Number of cases due to H. influenzae serotypes b,f and non-typeable H. influenzae, 1992-2019*

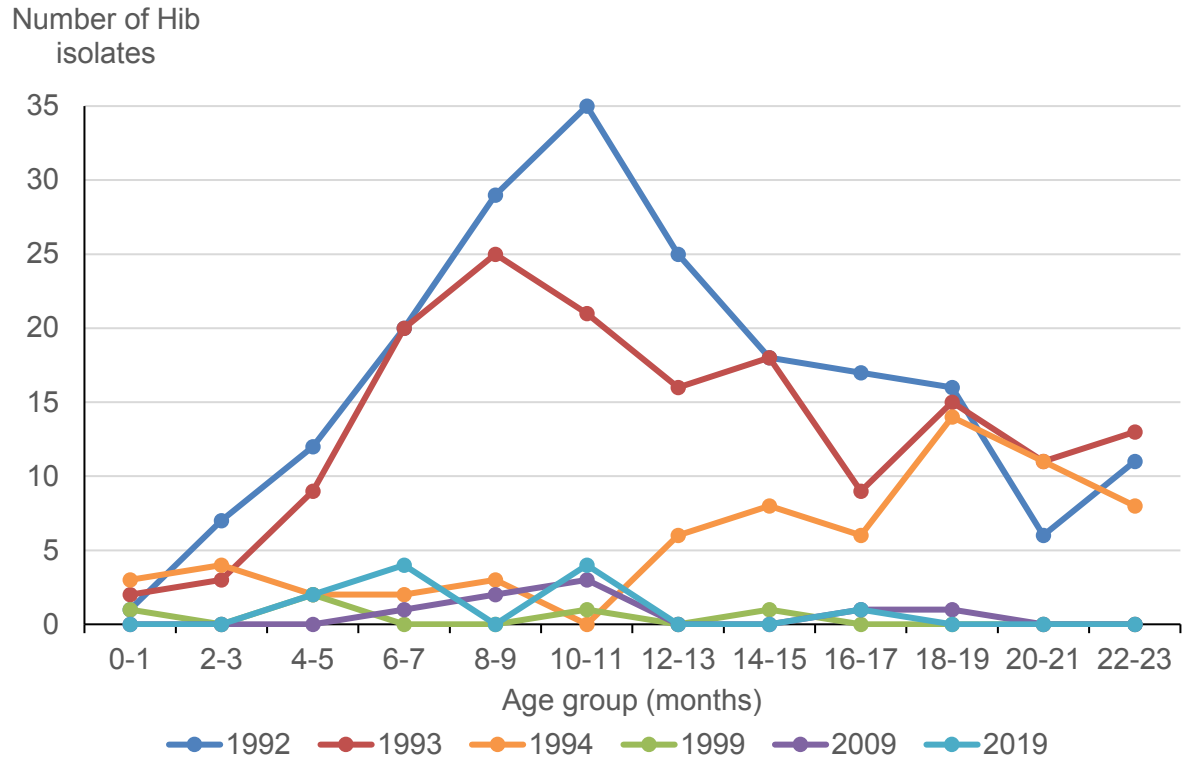


Figure 5.4 *Age distribution among children < 2 years of age of H. influenzae type b invasive disease for indicated years between 1992 - 2019. The H. influenzae type b vaccine was introduced in 1993.*

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 (table 5.2). This proportional increase is caused by an increase in absolute number of non-typeable *H. influenzae* isolates from blood, which has been steadily increasing during the period 1992 to 2019 from 15 to 164 (Table 5.2 and figure 5.5). In contrast, the absolute number of non-typeable isolates from CSF has remained stable between 1992 - 2019, with 12 non-typeable isolates from CSF in 2019 (figure 5.5). Most cases of bacteremia caused by non-typeable *H. influenzae* occur in children below the age of 4 and adults above the age of 60 (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, 2010 to 2019

n.t.*	0-4	5-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	≥80	T	%
2010	9	1	2	4	7	0	6	17	20	30	**85**	67.6
CSF	1	1	0	1	1	0	1	1	1	1	**8**	
Blood	8	0	2	3	6	0	5	16	19	29	**78**	
2011	10	3	4	5	3	5	9	14	20	14	**98**	70.5
CSF	1	0	0	0	0	0	2	2	1	0	**6**	
Blood	9	3	4	5	3	5	7	23	19	14	**92**	
2012	16	0	2	1	13	6	10	18	21	14	**101**	72.1
CSF	4	0	0	0	0	3	1	1	2	1	**12**	
Blood	12	0	2	1	13	3	9	17	19	13	**89**	
2013	8	1	2	1	7	4	5	27	29	31	**115**	71.9
CSF	2	0	0	0	1	0	0	3	0	0	**6**	
Blood	6	1	2	1	6	4	5	24	29	31	**109**	
2014	11	2	0	5	6	5	11	31	27	19	**117**	72.7
CSF	2	1	0	1	1	3	1	3	3	0	**15**	
Blood	9	1	0	4	5	2	10	28	24	19	**102**	
2015	12	3	1	5	6	9	19	34	19	24	**132**	67.7
CSF	1	1	0	0	2	3	3	4	0	0	**14**	
Blood	11	2	1	5	4	6	6	30	19	24	**118**	
2016	10	1	0	3	6	6	9	39	25	24	**123**	65.4
CSF	1	0	0	1	0	1	1	5	1	0	**10**	
Blood	9	1	0	2	6	5	8	34	24	24	**113**	
2017	18	1	3	4	8	11	16	33	37	28	**159**	71.0
CSF	7	0	1	1	0	2	3	4	2	1	**21**	
Blood	11	1	2	3	8	9	13	29	35	27	**138**	
2018	16	2	7	5	8	9	14	30	32	45	**168**	70.3
CSF	1	0	2	0	0	1	1	2	0	2	**9**	
Blood	15	2	5	5	8	8	13	28	32	43	**159**	
2019	*12*	*0*	*2*	*8*	*14*	*8*	*17*	*29*	*39*	*35*	***164***	72.6
CSF	1	0	0	0	0	1	3	2	2	0	**12**	
Blood	**11**	**0**	**2**	**8**	**14**	**7**	**14**	**23**	**37**	**35**	152	

* non-typeable

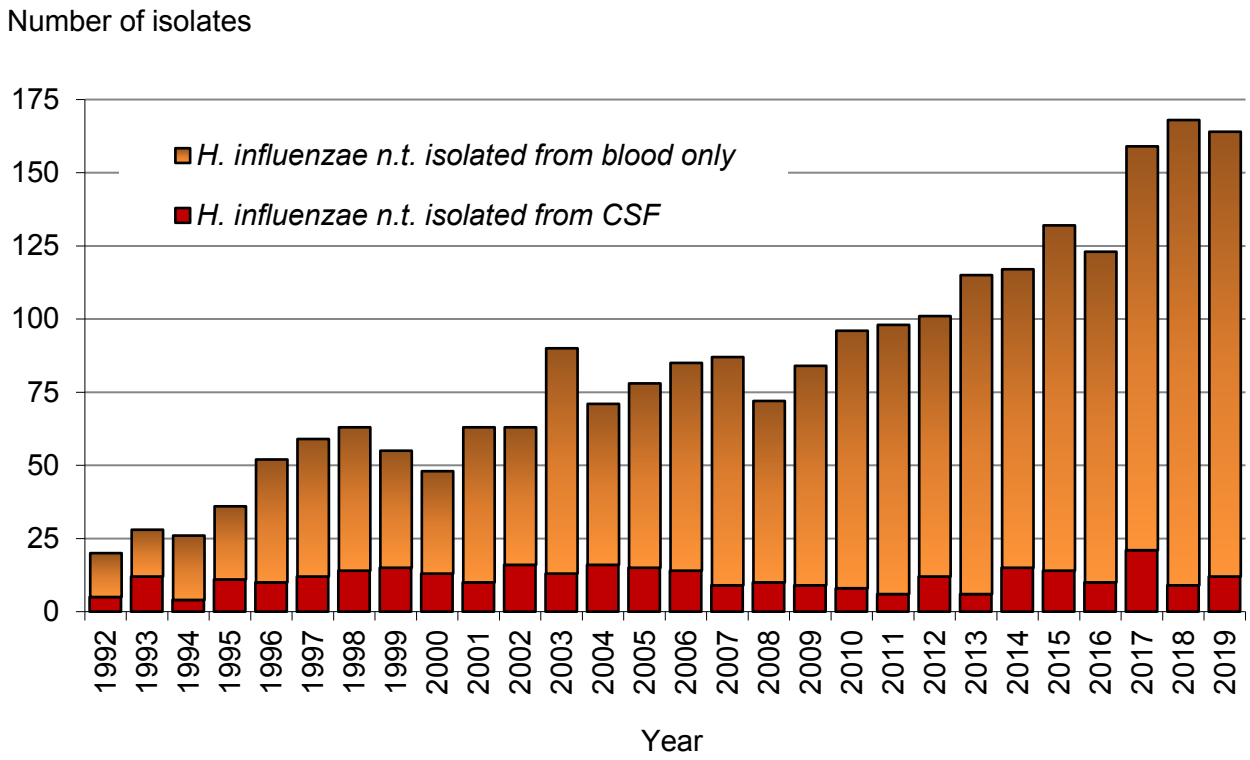


Figure 5.5 *Non-typeable H. influenzae isolates from CSF or blood received between 1992 - 2019*

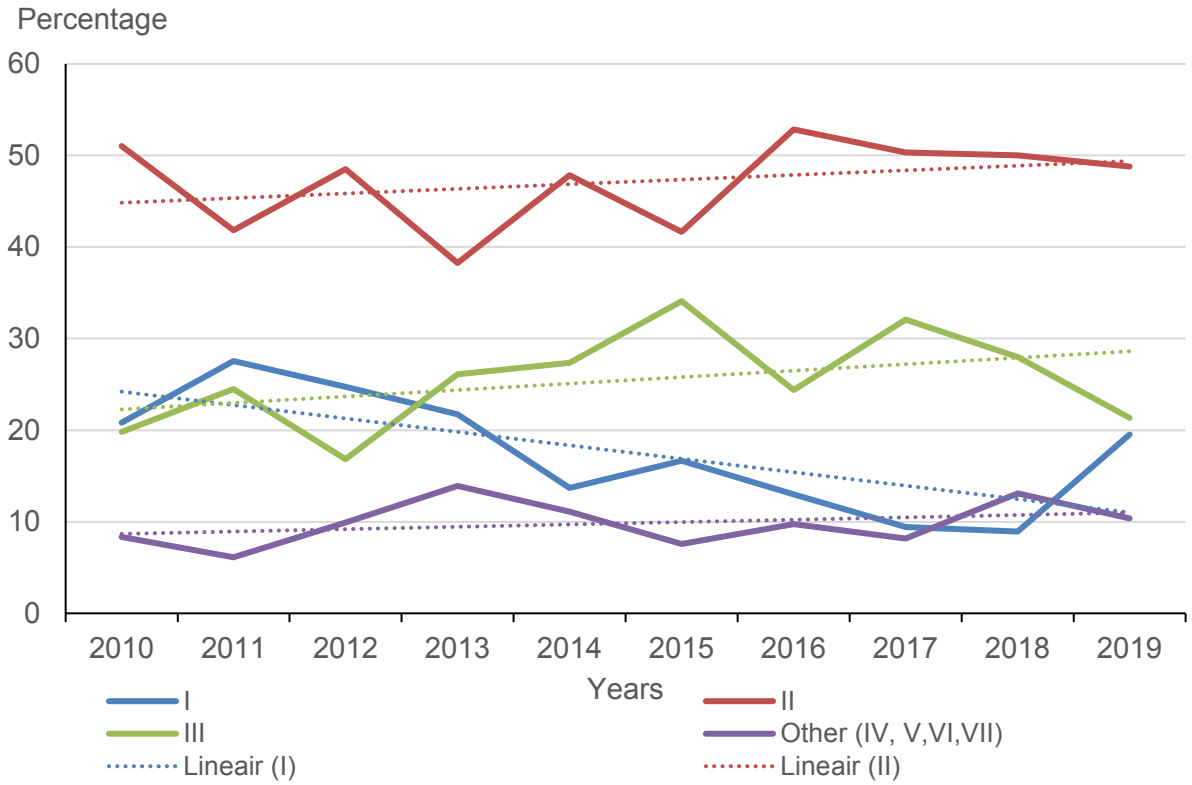
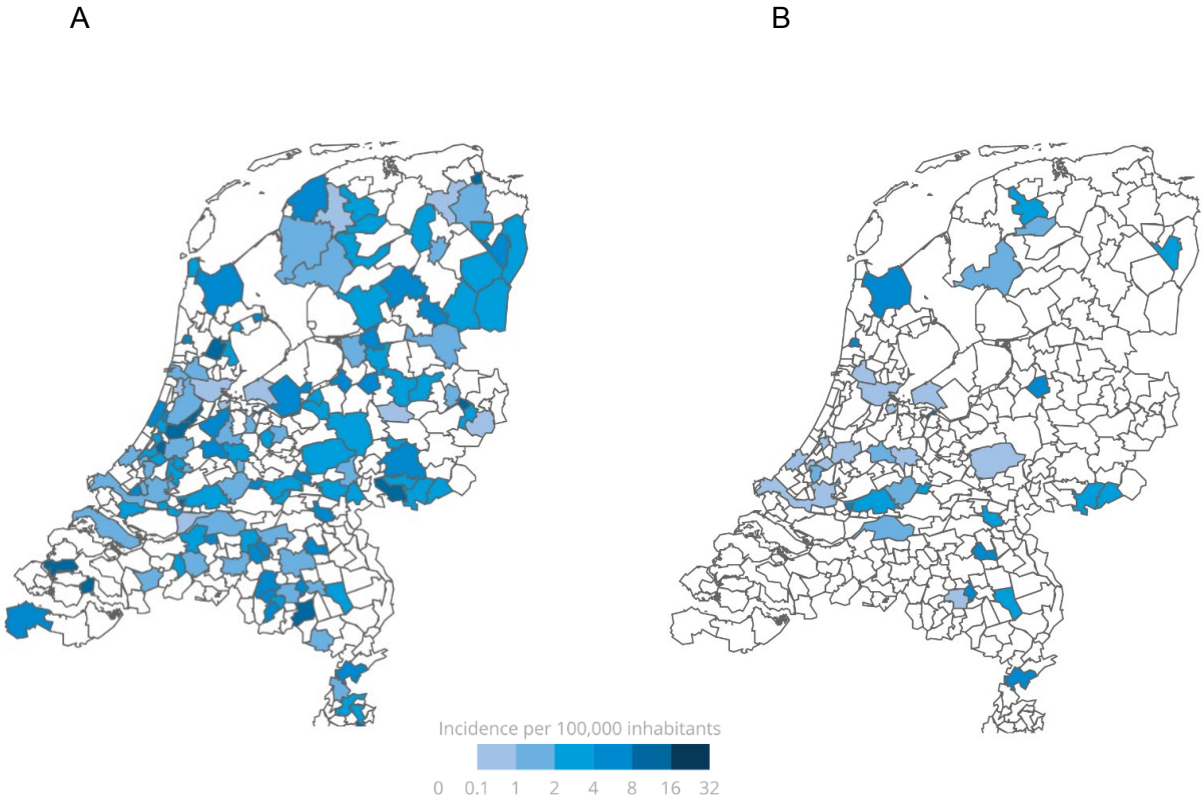


Figure 5.6 *Biotype distributions of non-typeable H. influenzae isolates from CSF and/or blood from 2010 – 2019. Dotted line indicates trend-line*

5.5 *Geographical distribution of H. influenza*

We also looked at the geographical distribution of *H. influenzae* cases (Fig. 5.5A) in general *H. influenzae* b cases (Fig. 5.5B) 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 5.5. Geographical visualization of (A) *H. influenzae* incidence and (B) *H. influenzae* b incidence per township per 100,000 inhabitants and place of residence of patient.



6 STREPTOCOCCUS PNEUMONIAE

6.1 *General features*

From 2003 onwards, the NRLBM requested nine sentinel laboratories, evenly distributed across the country and covering 25% of the Dutch population, to submit pneumococcal isolates from CSF and/or blood from patients of all ages. All medical microbiology laboratories were asked to submit pneumococcal isolates from CSF (or CSF and blood), representing meningitis patients. From 2006, the 7-valent conjugate pneumococcal polysaccharide vaccine (PCV7) was introduced in the National Immunisation Programme and all laboratories were requested to submit all invasive pneumococcal isolates from patients in the age group 0-4 years. PVC7 was replaced by the 10-valent conjugate pneumococcal polysaccharide vaccine (PVC10) from March 1, 2011 onwards. From 2017, all medical microbiology laboratories were requested to submit all invasive pneumococcal isolates without restriction to age of the patient. In 2019, the NRLBM received 1,793 isolates nationwide of which 600 pneumococcal isolates (CSF and/or blood) were received from the 9 sentinel laboratories. Of the 1,793 nationwide submitted isolates, 165 isolates were from CSF (or CSF and blood). The NRLBM also received 19 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 decreased pneumococcal meningitis incidence to 1.0 per 100,000 individuals in 2019 (Figure 6.1).

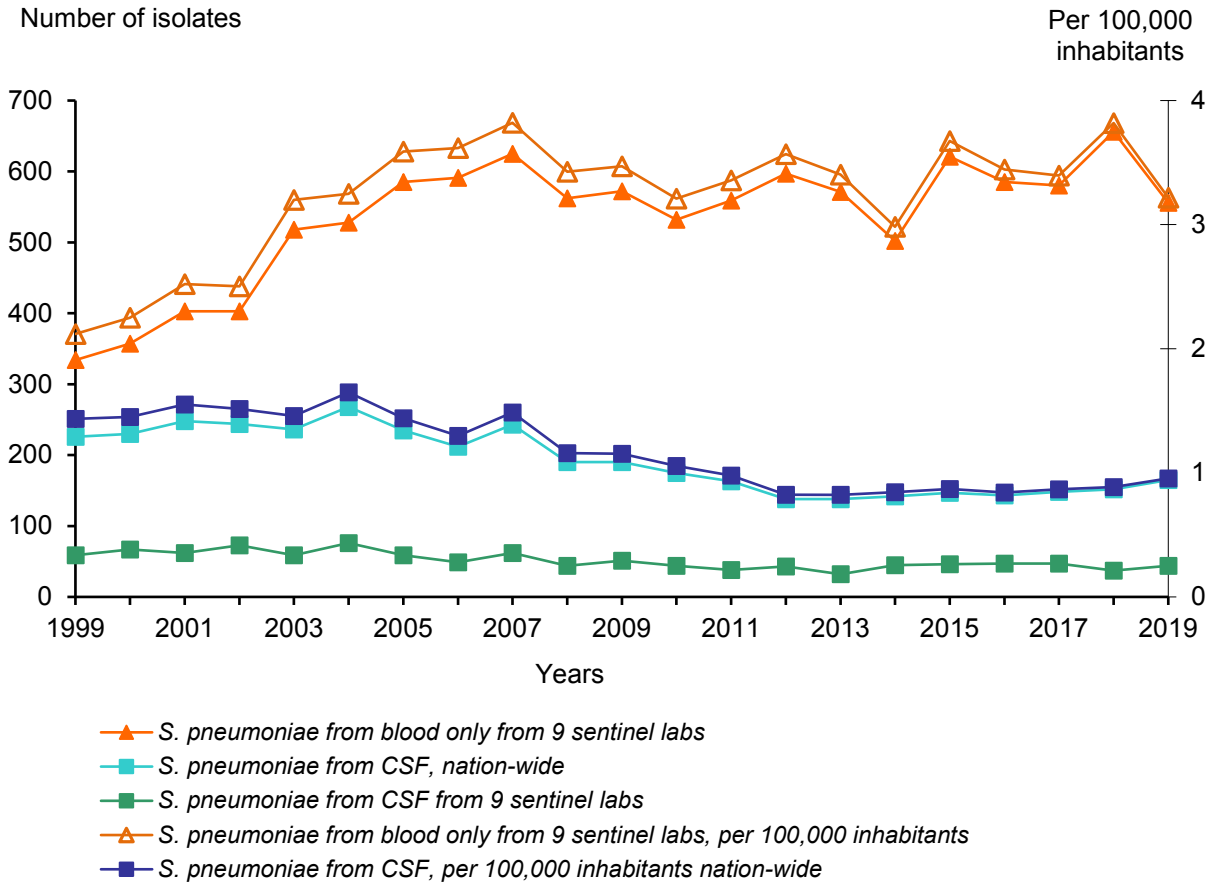


Figure 6.1 *Number of submitted S. pneumoniae isolates and invasive pneumococcal disease incidence based in isolates from blood or CSF, 1999-2019*

Figure 6.2 shows the number of *S. pneumoniae* isolates and incidence according to the patients' age. The incidence of pneumococcal meningitis is highest among patients in the age groups 0-4 and 60-64 years, whereas incidence of pneumococcal bacteremia is highest in patients 95-99. The number of isolates from patients with bacteremia is highest in the age group 70-74 years (Figure 6.2; bottom graph). Figure 6.3 shows the geographical distribution of pneumococcal disease per township based on patient's place of residence and per 100,000 inhabitants.

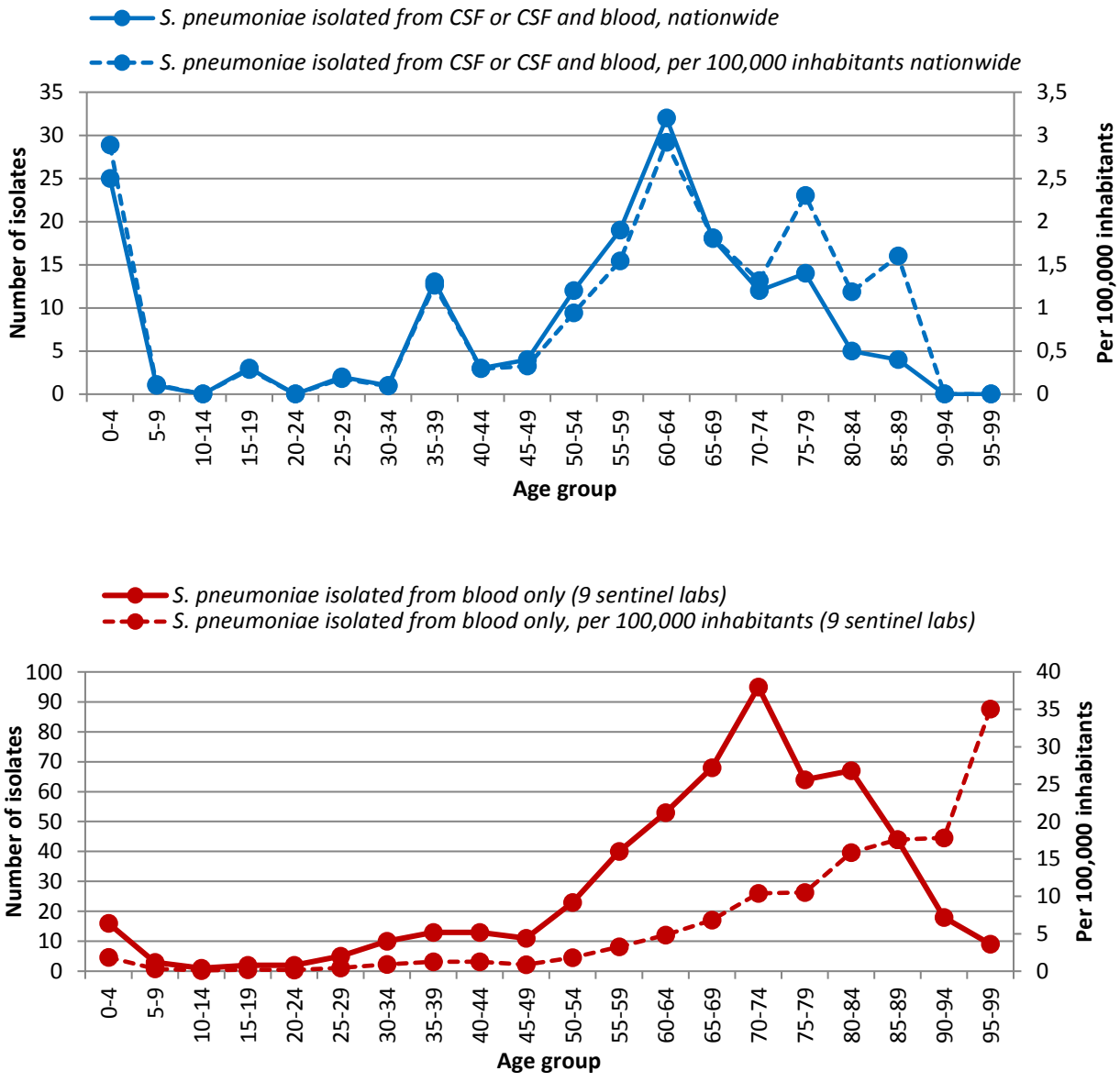


Figure 6.2 *S. pneumoniae isolates received per age group and incidence per 100,000 inhabitants according to isolation source in 2019. Top graph: isolates from CSF/CSF and blood. Bottom graph: isolates from blood only*

S. pneumonia isolated from CSF

S. pneumonia isolated from blood

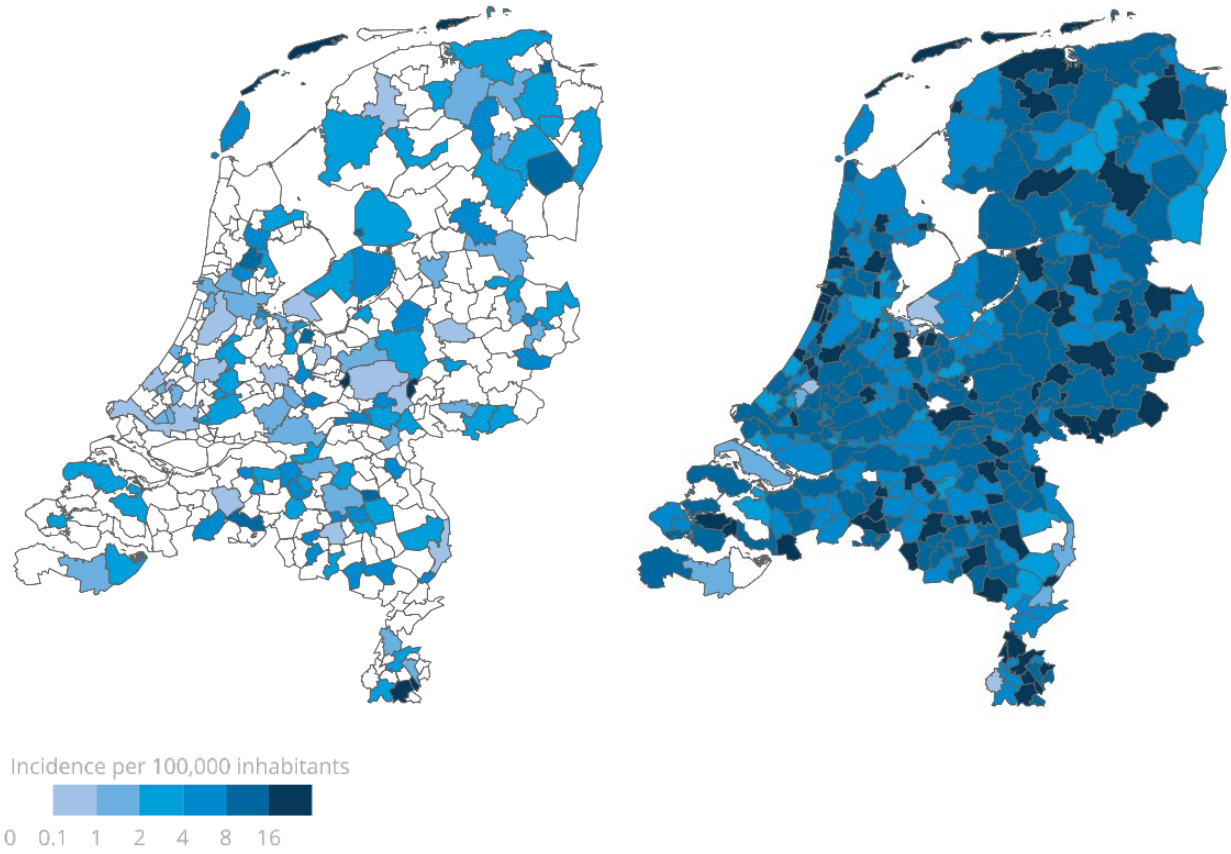


Figure 6.3 *Geographical distribution of S. pneumoniae isolates per 100,000 inhabitants, left; CSF or CS and blood, right; blood only (nationwide). Data plotted based on patient's place of residence.*

6.2 *Antibiotic susceptibility*

Among 556 isolates from blood only (9 sentinel labs), 22 (4%) isolates were intermediately susceptible to penicillin (0.06 < MIC ≤ 2.0 mg/L; table 6.1) and one isolate was resistant to penicillin (MIC > 2.0 mg/L). Among 165 nationwide patients with *S. pneumoniae* from CSF (or CSF and blood), 6 (3.6%) strains were resistant to penicillin (MIC > 0.06 mg/L). From 11 patients (10 CSF and 1 Blood) no MIC values were obtained because no *S. pneumoniae* isolate was available.

Table 6.1 Penicillin susceptibility of *S. pneumoniae* isolates, 2019

	Penicillin*				
MIC for CSF isolates (Nationwide)	**S MIC ≤ 0.06**	**I**	**R MIC > 0.06**	**ND** (PCR)**	**Total**
CSF or CSF and blood	149		6	(10)	165
MIC for blood isolates (9 sentinel labs)	**MIC ≤ 0.06**	**0.06< MIC ≤ 2.0**	**MIC >2.0**		
Blood only	532	22	1	(1)	556

* MIC values in mg/L according to EUCAST guidelines
** No MIC value known because no isolate was available (PCR-positive patient)

6.3 *Distribution according to serotype*

The distribution of serotypes, grouped by vaccine type and by age of the patient, for isolates from CSF (or CSF and blood) or blood only (submitted by the 9 sentinel labs) is presented in tables 6.2 and 6.4, respectively. Disease caused by PCV10-covered serotypes is less than 4%. Serotypes that would be covered by the PCV13 vaccine (serotypes 3, 6A and 19A) account for approximately 25% of all isolates from meningitis and bactermia patients (Tables 6.2 and 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. Despite the low number of isolates, meningitis incidence is still highest in the age group 0-11 months, followed by non-vaccinated age groups 65-79 and 50-64 (Table 6.3). As aforementioned, incidences of *S. pneumoniae* from blood only are incomplete. Effect of PCV10 introduction can be seen in tables 6.5 and 6.6, which shows a reduction of the number of vaccine-covered serotypes for the period 2010-2019. However, the overall number of invasive pneumococcal disease isolates increased 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.

Table 6.2 Serotype and age distribution of *S. pneumoniae* isolates from CSF (or CSF and blood; nationwide isolation collection), 2019. Serotypes are grouped by vaccine type.

			AGE (MONTHS)			AGE (YEARS)											
		TYPE	0	1-11	12-59	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total	%
23-valent vaccine (all above types except 6A)	10-valent vaccine	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		4	-	-	-	-	-	-	-	-	-	1	-	-	-	1	
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		7F	-	-	-	-	-	-	-	-	-	-	-	1	-	1	
		9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		18C	-	-	-	-	-	-	-	-	-	-	-	1	-	1	
		19F	-	-	-	-	-	-	-	-	-	-	1	2	-	3	
		23F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		Subtotal PCV10	-	-	-	-	-	-	-	-	-	*1*	*1*	*4*	-	*6*	***3.6***
	13-valent vaccine	3	-	1	1	2	-	-	-	-	1	1	7	10	-	21	
		6A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19A	1	3	1	5	-	-	-	-	1	-	8	5	1	20	
		Subtotal PCV13	*1*	*4*	*2*	*7*	-	-	-	-	*2*	*2*	*16*	*19*	*1*	*47*	***28.5***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		8	1	4	-	5	-	-	-	-	3	2	10	4	2	26	
		9N	-	-	-	-	-	-	-	-	-	-	1	3	-	4	
		10A	-	1	1	2	-	-	-	-	1	1	5	2	-	11	
		11A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		12F	-	1	-	1	-	-	-	-	1	1	-	1	1	5	
		15B	-	-	-	-	-	-	-	-	-	-	6	1	-	7	
		17F	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		22F	-	-	-	-	-	-	-	1	-	-	2	4	3	10	
		33F	-	-	-	-	-	-	-	-	-	-	2	-	1	3	
Subtotal PPV23			*2*	*10*	*3*	*15*	-	-	-	*1*	*7*	*6*	*42*	*34*	*8*	*113*	***68.5***
		Other	-	3	4	7	-	-	2	1	4	1	17	9	1	42	***25.5***
Type unknown			-	1	1	2	1	-	-	-	3	-	3	1	-	10	***6.0***
		Total	**2**	**14**	**8**	**24**	**1**	**-**	**2**	**2**	**14**	**7**	**62**	**44**	**9**	**165**	***100.0***

* From 10 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, 2019

	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	-	-	-	-	-	-	-	0.04	0.03	0.16	-	0.03
13-valent	2.95	0.29	-	-	-	-	0.10	0.09	0.45	0.77	0.13	0.27
23-valent	7.08	0.43	-	-	-	0.05	0.34	0.26	1.18	1.38	1.03	0.66
Other	2.95	0.72	0.11	-	0.29	0.05	0.34	0.04	0.59	0.41	0.13	0.32
Total	**10.03**	**1.15**	**0.11**	**-**	**0.29**	**0.09**	**0.67**	**0.30**	**1.76**	**1.79**	**1.16**	**0.98**

Table 6.4 Serotype and age-dependent distribution of *S. pneumoniae* isolates from blood submitted by the 9 sentinel laboratories, 2019.

			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	
		5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		6B	-	-	-	-	-	-	-	-	-	-	1	1	1	3	
		7F	-	-	-	-	-	-	-	-	-	-	3	-	1	4	
		9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		14	-	1	-	1	-	-	-	1	1	1	-	1	2	7	
		18C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		19F	-	-	-	-	-	-	-	-	-	-	1	1	2	4	
		23F	-	-	-	-	-	-	-	-	-	-	1	-	-	1	
		Subtotal PCV10	*-*	*1*	*-*	*1*	*-*	*-*	*-*	*1*	*1*	*1*	*7*	*3*	*6*	*20*	***3.6***
		3	1	-	-	1	-	-	-	-	1	2	8	16	17	45	
		6A	-	-	-	-	-	-	-	-	-	-	-	1	-	1	
		19A	-	-	4	4	1	-	-	-	3	2	16	31	27	84	
		Subtotal PCV13	*1*	*1*	*4*	*6*	*1*	*-*	*-*	*1*	*5*	*5*	*31*	*51*	*50*	*150*	***27.0***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		8	-	-	1	1	2	1	1	4	9	6	38	64	20	146	
		9N	-	-	-	-	-	-	1	-	1	1	6	13	9	31	
		10A	-	-	-	-	-	-	-	-	1	-	4	3	2	10	
		11A	-	-	-	-	-	-	-	-	-	-	2	1	2	5	
		12F	-	-	-	-	-	-	-	1	4	4	4	8	5	26	
		15B	-	-	-	-	-	-	-	-	-	-	3	4	3	10	
		17F	-	-	1	1	-	-	-	-	-	-	-	1	-	2	
		20	-	-	1	1	-	-	-	-	-	-	-	6	3	10	
		22F	-	-	-	-	-	-	-	-	-	2	8	23	14	47	
		33F	-	-	-	-	-	-	-	-	2	1	3	3	4	13	
Subtotal PPV23			*1*	*1*	*7*	*9*	*3*	*1*	*2*	*6*	*22*	*19*	*99*	*176**	*112*	*449*	***80.9***
		Other	-	3	4	7	-	-	-	1	1	5	17	49	26	1067	***19.1***
		Total	**1**	**4**	**11**	**16**	**3**	**1**	**2**	**7**	**23**	**24**	**116**	**226**	**138**	**556**	***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), 2010-2019

			TYPE	Year 2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
23-valent vaccine (all above types except 6A)	13-valent vaccine	10-valent vaccine	1	3	1	1	3	4	1	2	1	-	-
			4	3	2	4	2	2	-	1	1	2	1
			5	2	-	3	-	-	-	-	-	-	-
			6B	-	2	-	-	-	1	-	-	-	-
			7F	20	28	16	15	8	7	4	2	2	1
			9V	2	-	3	1	1	-	2	-	-	-
			14	5	2	1	-	-	1	-	-	2	-
			18C	5	5	2	2	-	1	-	1	1	1
			19F	2	6	4	2	4	2	5	6	1	3
			23F	4	2	1	-	-	1	-	1	-	-
			Subtotal PCV10	*46*	*48*	*35*	*25*	*19*	*14*	*14*	*12*	*8*	*6*
			3	20	7	13	16	13	16	25	20	20	21
			6A (not in 23 valent)	5	1	1	1	3	-	1	-	-	-
			19A	20	16	6	9	7	10	8	16	13	20
			Subtotal PCV13	*91*	*72*	*55*	*51*	*42*	*40*	*48*	*48*	*41*	*47*
			2	-	-	-	-	-	-	-	-	-	-
			8	10	17	9	16	23	24	18	21	23	26
			9N	6	7	4	2	6	6	3	6	4	4
			10A	9	7	9	7	12	5	7	7	7	11
			11A	1	5	1	1	3	2	3	2	8	-
			12F	3	7	10	9	8	9	12	8	11	5
			15B	2	3	1	-	-	-	5	7	2	7
			17F	4	3	1	1	1	-	-	1	2	-
			20	1	-	-	1	1	1	-	-	-	-
			22F	14	16	11	8	8	11	11	8	8	10
			33F	7	5	6	3	2	4	4	6	6	3
			Subtotal PPV23*	*143*	*141*	*106*	*98*	*103*	*102*	*110*	*114*	*112*	*113*
			6C	3	4	2	6	3	6	5	3	3	11
			7B	-	-	-	1	-	-	-	-	-	-
			7C	-	-	-	-	-	-	-	-	-	1
			10F	-	-	-	-	-	-	-	-	-	-
			10B	-	-	1	-	1	1	-	1	-	1
			12A	-	-	-	-	-	-	-	-	-	-
			13	-	-	-	-	-	-	-	-	-	-
			15A	1	1	1	4	6	7	2	4	3	1
			15C	2	-	3	-	-	1	-	3	1	1
			16F	5	4	-	5	2	1	3	1	5	-
			17A	-	-	-	-	-	-	-	-	-	-
			18F	-	-	-	-	-	-	-	-	-	-
			18A	-	-	-	-	-	-	-	-	-	-
			18B	-	-	1	-	-	-	-	-	-	-
			21	-	1	-	-	-	-	-	-	2	-
			22A	1	-	-	-	-	1	1	-	-	-
			23A	3	2	4	4	4	5	5	5	8	6
			23B	5	2	5	7	8	11	6	11	8	10
			24F	1	1	4	4	7	7	1	2	1	5
			24B	-	-	2	-	-	-	-	-	-	-
			25	-	-	-	-	-	-	-	-	-	-
			27	-	-	1	-	2	1	1	-	1	1
			28F	-	-	-	1	-	-	-	-	-	-
			28A	-	1	-	-	-	-	-	-	-	-
			29	-	-	1	-	-	-	-	-	-	-
			31	1	-	1	-	1	-	1	1	-	-
			33A	-	-	-	-	-	-	-	-	-	-
			34	-	1	-	-	-	1	1	1	2	2
			35F	4	1	-	2	1	2	5	1	3	3
			35B	1	-	1	3	1	1	1	-	2	-
			35D	-	-	-	-	-	-	-	-	1	1
			37	-	1	2	1	-	-	-	-	-	-
			38	1	-	2	1	-	-	-	-	-	2
			Rough (n.t.)	-	-	-	-	-	-	-	1	-	-
			Type unknown	-	-	-	-	-	-	-	-	-	10
			Total	176	163	138	138	142	147	143	148	152	165

Table 6.6 Changes in serotype distribution of *S. pneumoniae* from blood submitted by the 9 sentinel laboratories, 2010-2019. Serotypes are grouped by vaccine type.

		TYPE	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
23-valent vaccine (all above types except 6A)	13-valent vaccine	10-valent vaccine	1	53	40	50	40	41	41	22	8	8	-
			4	17	27	11	13	6	6	6	6	6	1
			5	7	11	8	9	2	1	-	1	-	-
			6B	8	3	3	3	3	4	1	-	2	3
			7F	72	91	92	75	53	56	36	27	23	4
			9V	21	5	2	4	1	5	-	2	3	-
			14	22	19	12	8	2	7	8	4	2	7
			18C	7	8	4	8	2	2	2	1	1	-
			19F	5	9	3	5	7	8	6	9	7	4
			23F	13	5	3	1	2	1	1	1	-	1
			Subtotal PCV10	*225*	*218*	*188*	*166*	*119*	*131*	*82*	*59*	*52*	*20*
			3	30	36	45	40	31	35	45	51	71	45
			6A (not in 23 valent)	9	2	6	2	-	2	-	4	4	1
			19A	57	63	78	61	44	78	75	82	101	84
			Subtotal PCV13	*321*	*319*	*317*	*269*	*194*	*246*	*202*	*196*	*228*	*150*
			2	-	-	-	-	-	-	-	-	-	-
			8	60	59	88	108	93	136	151	143	159	146
			9N	19	17	20	19	21	26	32	29	31	31
			10A	9	14	8	6	16	15	11	11	8	10
			11A	12	9	14	16	8	6	6	9	8	5
			12F	13	19	25	22	28	30	18	28	22	26
			15B	7	4	1	7	7	2	8	6	8	10
			17F	4	8	7	4	8	6	6	5	4	2
			20	3	4	-	1	4	2	3	5	6	10
			22F	29	37	41	45	34	43	28	39	45	47
			33F	10	15	22	12	12	19	18	12	28	13
			Subtotal PPV23*	*478*	*503*	*537*	*507*	*425*	*529*	*483*	*479*	*543*	*449*
			6C	9	7	10	10	7	21	20	15	24	22
			7B	-	-	-	-	-	-	-	-	1	1
			7C	-	-	-	-	-	-	-	-	1	4
			9A	-	-	1	-	1	-	1	-	-	-
			10F	-	-	-	-	1	-	-	-	-	-
			10B	-	-	-	1	-	-	-	1	2	2
			11B	-	-	-	-	-	-	-	-	-	2
			11D	-	-	-	-	-	-	-	-	-	3
			12A	-	-	-	-	-	-	-	-	1	-
			13	-	1	-	-	-	-	1	-	-	-
			15F	-	-	-	1	-	-	1	-	-	-
			15A	-	2	7	13	14	18	21	16	14	12
			15C	1	2	1	4	4	3	2	1	1	3
			16F	10	7	6	7	5	2	9	9	5	4
			17A	-	2	-	-	-	-	-	-	-	-
			18F	-	-	-	-	-	2	-	-	-	-
			18A	1	1	-	-	-	-	-	-	-	-
			18B	-	-	1	1	-	-	-	-	-	-
			21	-	-	-	2	1	-	-	1	1	-
			22A	1	1	-	1	-	1	-	-	1	-
			23A	7	2	6	6	7	7	12	15	14	11
			23B	3	9	3	6	15	5	11	17	11	17
			24F	2	3	2	4	4	7	1	6	3	7
			25F	-	-	-	-	-	1	-	1	-	-
			27	-	1	-	1	-	1	1	-	-	-
			28	-	-	-	-	-	-	-	-	-	1
			29	-	-	1	-	-	-	-	-	-	-
			31	4	2	6	2	2	4	4	3	6	1
			33A	-	-	1	-	-	-	1	-	1	-
			34	1	-	1	2	1	-	1	1	3	4
			35F	5	6	5	6	7	7	6	3	6	3
			35A	-	-	1	-	-	-	-	-	-	-
			35B	-	3	1	7	6	8	8	2	8	3
			35D	-	-	-	-	-	-	-	-	-	2
			37	1	-	-	-	1	1	-	-	-	-
			38	-	3	-	1	2	2	1	5	4	1
			40	-	-	-	1	-	-	-	-	-	-
			Rough (n.t.)	-	2	-	-	-	-	-	1	1	1
			Total	532	559	596	585	503	621	584	580	655	556

7 ESCHERICHIA COLI

The NRLBM received 96 *Escherichia coli* isolates, 18 isolated from CSF (or CSF and blood) and 78 from blood only (Figure 7.1, Table 7.1). Sixty-five percent of the *E. coli* meningitis cases occurred in the first month of life (Table 7.1). From 2010-2016, the number of received isolates was rather stable with 15-30 isolates per year. From 2017, there is a marked increase observed due to an increase in received blood isolates, which is likely explained by increased submission as result of an ongoing study on neonatal meningitis (NOGBS study; (NOGBS studie, sd))

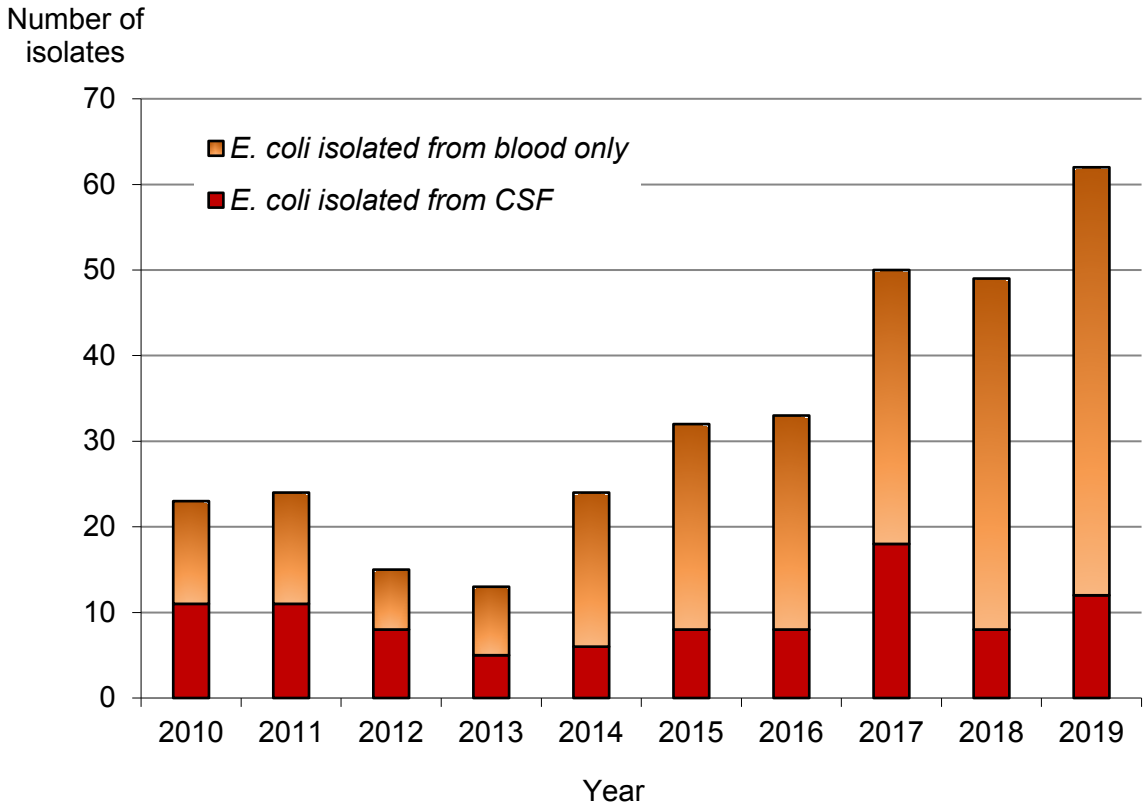


Figure 7.1 *Number of E. coli isolates received according to isolation source, 2010-2019*

Since 2016, K1 is detected by phage typing. In 2019, approximately 50% of the received *E. coli* isolates carried the K1 antigen (Table 7.1).

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, 2019

	AGE (MONTHS)			AGE (YEARS)					TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Non K1	32	19	1	52	0	0	1	1	**54**	***56***
CSF	*3*	*1*	*0*	*4*	*0*	*0*	*1*	*1*	***6***	
Blood	*29*	*18*	*1*	*48*	*0*	*0*	*0*	*0*	***48***	
K1	30	11	0	41	0	0	1	0	**42**	***44***
CSF	*9*	*2*	*0*	*11*	*0*	*0*	*1*	*0*	***12***	
Blood	*21*	*9*	*0*	*30*	*0*	*0*	*0*	*0*	***30***	
Total	**62**	**30**	**1**	*93*	*0*	*0*	*2*	*1*	**96**	***100***
CSF	*12*	*3*	*0*	*15*	*0*	*0*	*2*	*1*	*18*	
Blood	*50*	*27*	*1*	78	*0*	*0*	*0*	*0*	*78*	
%	***64.6***	***31.3***	***1.0***	***96.9***	***0***	***0***	***2.1***	***1.0***	***100***	

Since 2012, *E. coli* isolates received by the NRLBM are additionally classified 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, 52% were of H-type H7 and 17% of type H4. H-type H4 was also dominant among the non-K1 isolates (22%), with H18 and H1 accounting together for one-third of the non-K1 isolates (table 7.2)

Table 7.2 H-type distribution among K1 and non-K1 *E. coli* isolates from CSF or blood, 2015 - 2019

TYPE	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1
	2015	**2016**	**2017**	**2018**	**2019**
H1	1 / 0	0 / 2	0 / 7	1 / 6	1 / 9
H4	6 / 0	6 / 7	4 / 1	3 / 7	7 / 12
H5	1 / 2	4 / 2	1 / 4	3 / 3	5 / 9
H6	2 / 0	4 / 1	3 / 1	2 / 2	1 / 1
H7	6 / 0	7 / 3	9 / 2	17 / 1	22 / 3
H9	-	0 / 2	0 / 4	0 / 1	-
H18	2 / 0	0 / 5	0 / 3	0 / 6	3 / 10
H31	2 / 0	-	0 / 2	-	0 / 3
Other	5 / 1	3 / 4	2 / 7	4 / 6	3 / 7
Total	**25 / 3**	**24 / 26**	**19 / 30**	**30 / 32**	**42 / 54**
%	***89/11***	***48/52***	***39/61***	***48/52***	***44/56***

The types O4 (9%), O15 (9%) and O25 (15%) are most prevalent among non-K1 isolates, while the type O1 (12%) and O75 (17%) are most frequent among K1 isolates. The 10 isolates showed in the group 'Other' were all differt O-types (Figure 7.2)

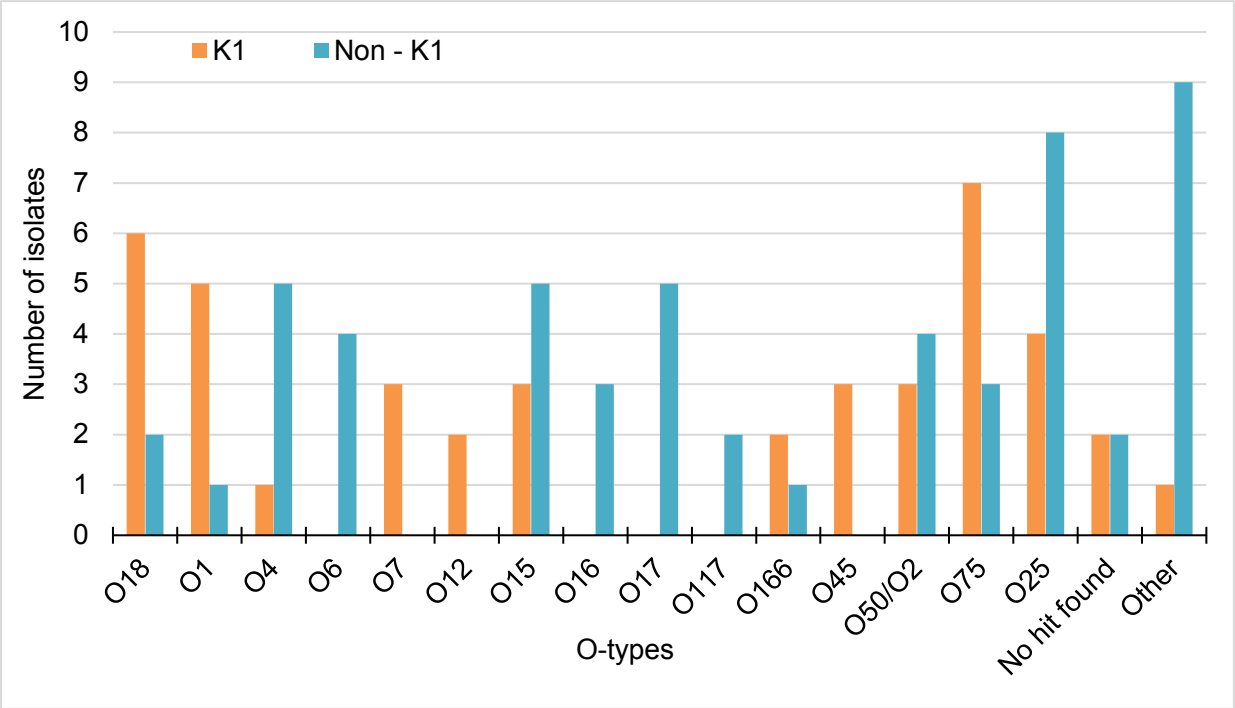


Figure 7.2 Distribution of O-types among K1 and non-K1 *E. coli* isolates from CSF and/or blood, 2019

Among K1 isolates, the O/H combination O75:H5 was most prevalent while among non-K1 isolates, O25:H4 was dominant (Figure 7.3).

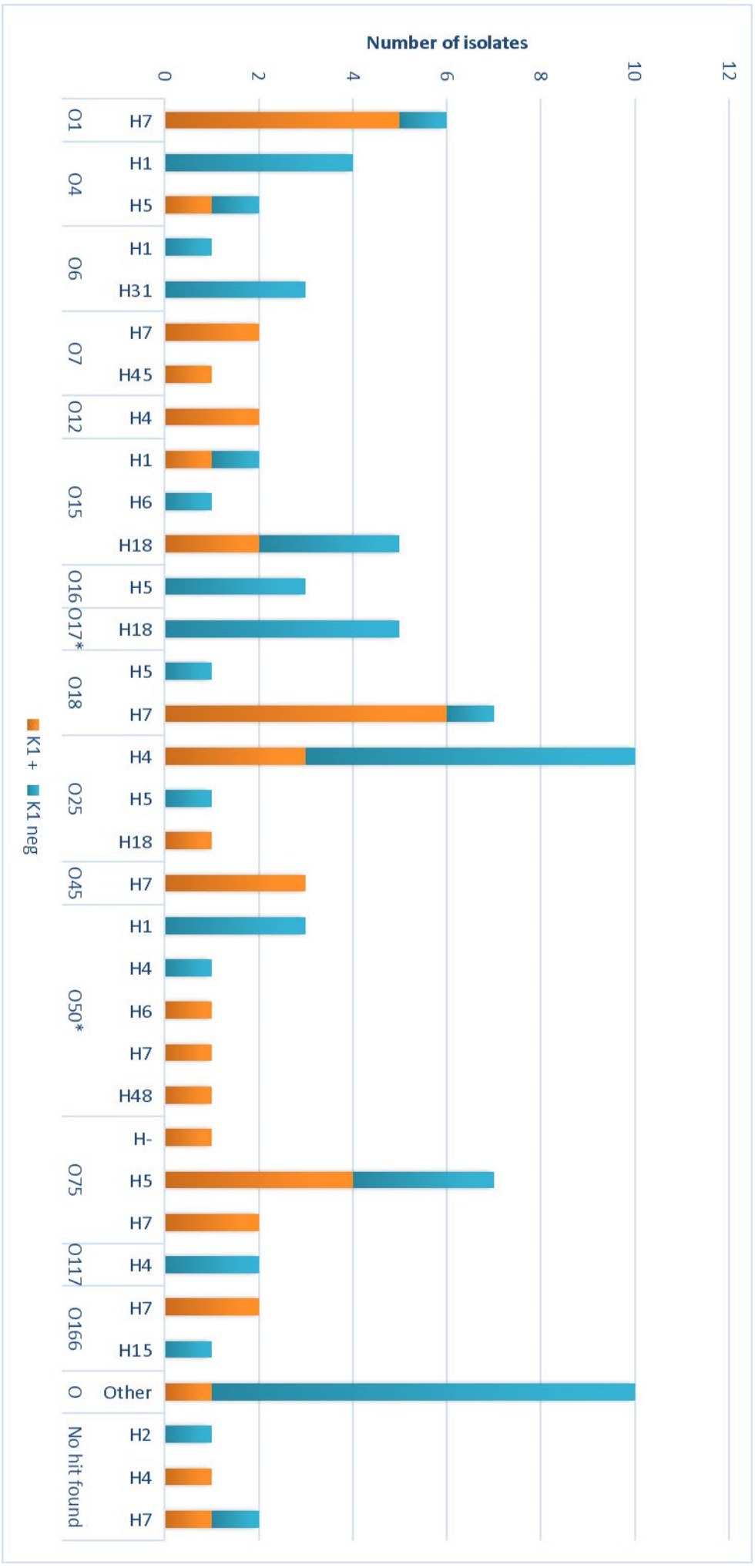


Figure 7.3 Distribution of O- and H-type combinations among K1 and non-K1 *E. coli* isolates from CSF and/or blood, 2019

*O17 = O17/O77
O50 = O50/O2

8 STREPTOCOCCUS AGALACTIAE – (group B)

In 2019, the NRLBM received 120 *Streptococcus agalactiae* isolates, which is an increase compared to the 106 isolates in 2018 and 87 isolates in 2017 (figure 8.1). Twenty-three (19%) *S. agalactiae* isolates were from CSF (or CSF and blood) and 97 (81%) from blood only (table 8.1). Sixty-four percent of all the cases occurred in the first month of life (83% for CSF cases). As in previous years, Serotype III was most prevalent (table 8.1).

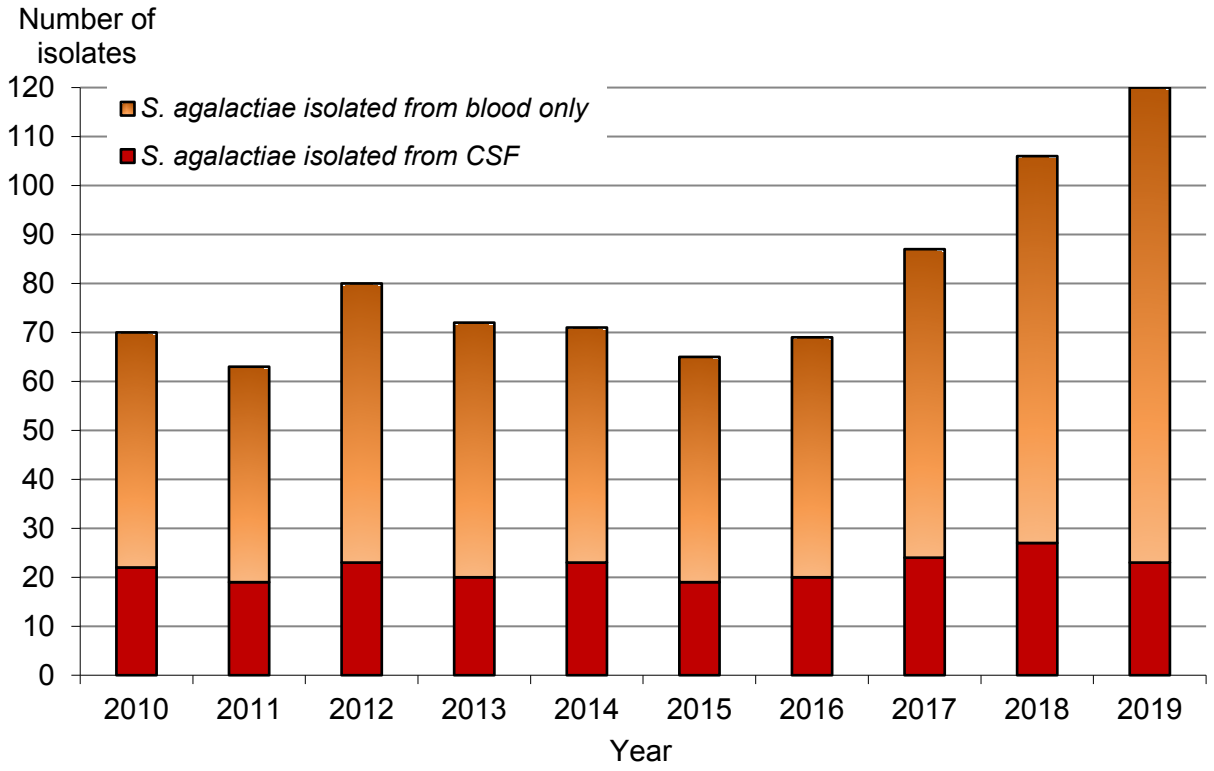


Figure 8.1 *Distribution of S. agalactiae isolates, 2010 - 2019*

Table 8.1 Serotypes of *S. agalactiae* isolates from CSF and/or blood, by age of patients, 2019

	AGE (MONTHS)			AGE (YEARS)					TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**T**	**%**
Ia	9	11	1	21	0	0	1	1	**23**	***19.2***
CSF	*3*	*1*	*0*	*4*	*0*	*0*	*0*	*0*	***4***	
Blood	*6*	*10*	*1*	*17*	*0*	*0*	*1*	*1*	***19***	
Ib	7	4	0	11	0	0	0	0	**11**	***9.2***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*7*	*4*	*0*	*11*	*0*	*0*	*0*	*0*	***11***	
II	7	2	0	9	0	0	0	0	**9**	***7.5***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*7*	*2*	*0*	*9*	*0*	*0*	*0*	*0*	***9***	
III	47	16	0	63	0	0	0	0	**63**	***52.5***
CSF	*16*	*1*	*0*	*17*	*0*	*0*	*0*	*0*	***17***	
Blood	*31*	*15*	*0*	*46*	*0*	*0*	*0*	*0*	***46***	
V	4	5	0	9	0	0	1	1	**11**	***9.2***
CSF	*0*	*1*	*0*	*1*	*0*	*0*	*0*	*1*	***2***	
Blood	*4*	*4*	*0*	*8*	*0*	*0*	*1*	*0*	***9***	
VI	1	0	0	1	0	0	0	0	**1**	***0.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	***1***	
VIII	1	0	0	1	0	0	0	0	**1**	***0.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	***1***	
IX	1	0	0	1	0	0	0	0	**1**	***0.8***
CSF	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	***0***	
Blood	*1*	*0*	*0*	*1*	*0*	*0*	*0*	*0*	***1***	
Total	**77**	**38**	**1**	**116**	**0**	**0**	**2**	**2**	120	***100.0***
CSF	***19***	***3***	***0***	***22***	***0***	***0***	***0***	***1***	***23***	
Blood	***58***	***35***	***1***	***94***	***0***	***0***	***2***	***1***	***97***	
%	***64.2***	***31.7***	***0.8***	***96.6***	***0***	***0***	***1.7***	***1.7***	***100.0***	

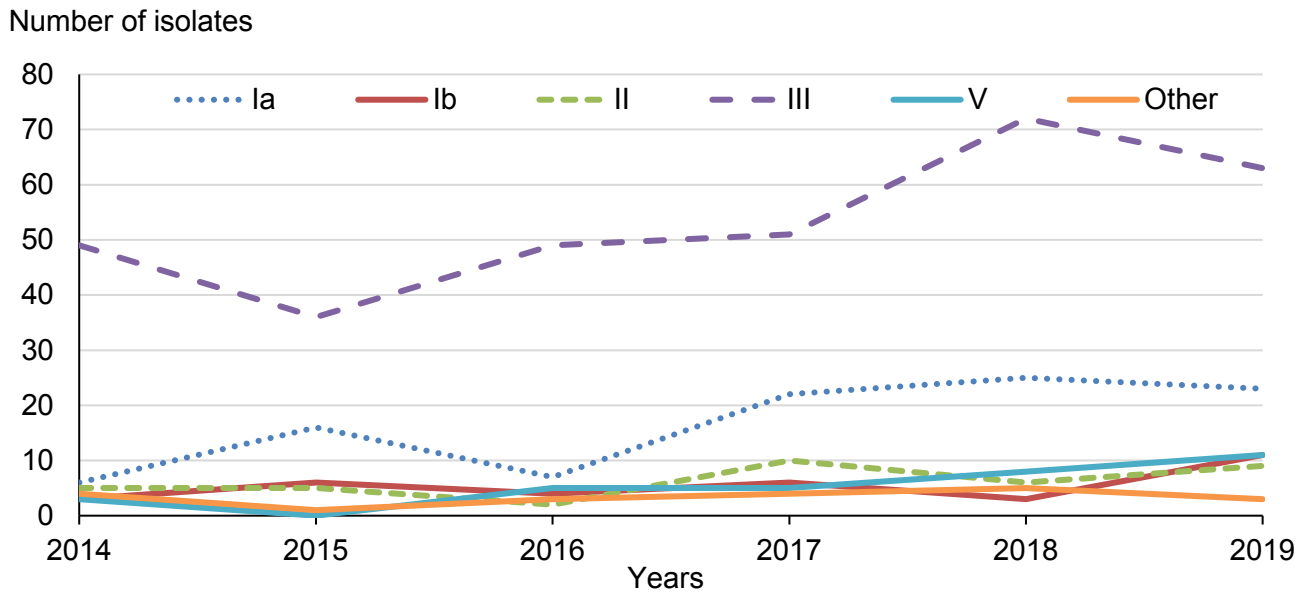


Figure 8.2 *Distribution of S. agalactiae serotypes, 2014 - 2019*

9 LISTERIA MONOCYTOGENES

Onehundred seven *Listeria monocytogenes* isolates were submitted to the NRLBM. Of these, 26 (24%) were from CSF (or CSF and blood) and 81 (76%) from blood only (Figure 9.1). The large majority (94%) occurred among individuals over 50 years of age. Similar to previous years, serotypes 1/2a and 4b were most prevalent in 2019 (Table 9.1). This slight increase in isolates compared to 2018 is likely explained by a food-related outbreak linked to ingestion of contaminated meats from one factory (RIVM, Vleeswaren waarschijnlijk bron 20 patiënten met Listeria, sd). The distribution of serotypes is similar to previous years (Figure 9.2)

Table 9.1 Total number of *L. monocytogenes* isolates from CSF and/or blood grouped according to age of patient and serotype, 2019

	AGE (YEARS)					TOTAL	
Group	**0-4**	**5-19**	**20-49**	**50-79**	**≥80**	**T**	**%**
1/2a All	1	0	1	29	13	***44***	***41.1***
CSF	*0*	*0*	*0*	*8*	*2*	***10***	
Blood	*1*	*0*	*1*	*21*	*11*	***34***	
1/2b All	1	0	0	2	2	**5**	***4.7***
CSF	*0*	*0*	*0*	*0*	*1*	***1***	
Blood	*1*	*0*	*0*	*2*	*1*	***4***	
1/2c All	0	0	0	4	0	**4**	***3.7***
CSF	*0*	*0*	*0*	*1*	*0*	***1***	
Blood	*0*	*0*	*0*	*3*	*0*	***3***	
4b All	2	0	1	30	21	**54**	***50.5***
CSF	*0*	*0*	*1*	*8*	*5*	***14***	
Blood	*2*	*0*	*0*	*22*	*16*	***40***	
Total	**4**	**0**	**2**	**65**	**36**	**107**	***100.0***
CSF	***0***	***0***	***1***	***17***	***8***	***26***	***24.3***
Blood	***4***	***0***	***1***	***48***	***28***	***81***	***57.7***
%	***3.7***	***0***	***1.9***	***60.8***	***33.6***	***100.0***	

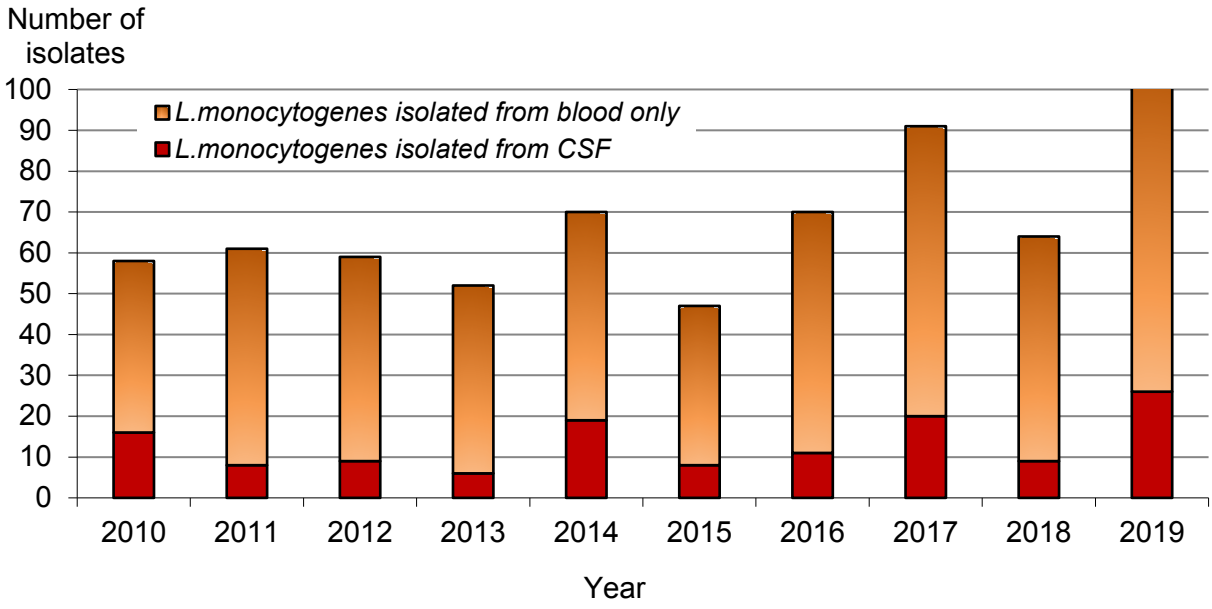


Figure 9.1 *Number of L. monocytogenes isolates grouped by isolation source, 2010-2019*

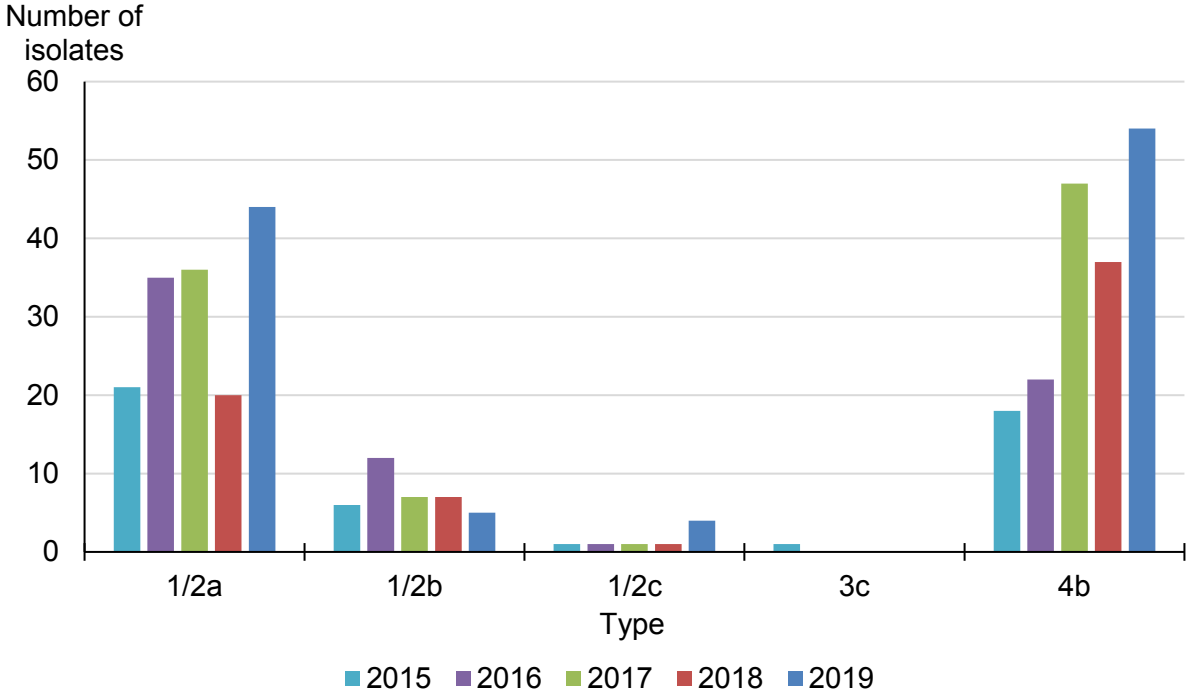


Figure 9.2 *Number of L. monocytogenes isolates grouped by serotype, 2015-2019*

10 STREPTOCOCCUS PYOGENES – Group A

Until 2019, the NRLBM received *Streptococcus pyogenes* isolates associated with meningitis only. Since 2015, the NRLBM performs group A *emm*-typing based on sequencing of the hypervariable part of the *emm* gene (CDC - Streptococcus Laboratory, sd), which encodes the surface expressed M protein. Currently, over 220 different *emm* genotypes are recognized. In 2014, an *emm*-cluster based system was proposed, which clusters related M proteins based on shared binding and functional properties (Sanderson-Smith, 2014).

In 2019, 243 *S. pyogenes* isolates were submitted to the NRLBM, 14 isolated from CSF (or CSF and blood) and 122 from blood only and 107 from other sites (Table 10.3). The increase in submitted isolates is explained by the participation of the NRLBM in a 2-year pilot study to gain insight into invasive *S. pyogenes* isolates from puerperal sepsis/fever at the national level (pGAS). In addition, the NRLBM receives *S. pyogenes* isolates from other invasive infections that are submitted through 9 sentinel laboratories that cover approximately 25% of the Dutch population from April 2019 (iGAS). The *emm* typing data of all isolates is displayed in Table 10.2 and the emm-cluster based data in Table 10.3.

Table 10.1 *S. pyogenes* isolates from CSF and/or blood according to patient's age, 2019.

TYPE	AGE (YEARS)					TOTAL	
	0-4	5-9	10-19	20-49	≥50	T	%
CSF	2	1	2	0	9	**14**	***10.3***
No studie	1	1	2	0	9	**13**	
PGAS	0	0	0	0	0	**0**	
IGAS	1	0	0	0	0	**1**	
Blood	11	5	4	38	64	**122**	***89.3***
No studie	3	1	0	6	13	**23**	
PGAS	2	1	0	20	4	**27**	
IGAS	6	3	4	12	47	**72**	
Total	**13**	**6**	**6**	**38**	**73**	**136**	***100***
%	***9.6***	***4.4***	***4.4***	***27.9***	***53.7***	***100***	

Table 10.2 *emm*-type and *emm*-cluster distribution of *S. pyogenes* isolates from CSF, blood or other sites, 2019

Cluster	*emm* type	CSF	Blood	Other *	*1*	*2*	*3*	*4*	Total
E1	4.0	1	7	3	*0*	*1*	*2*	*0*	11
Total E1		**1**	**7**	**3**	***0***	***1***	***2***	***0***	**11**
E2	76.0	0	1	0	*0*	*0*	*0*	*0*	1
	168.0	0	1	0	*0*	*0*	*0*	*0*	1
	104.0	0	0	1	*0*	*0*	*1*	*0*	1
Total E2		**0**	**2**	**1**	***0***	***0***	***1***	***0***	**3**
E3	44.0	0	2	3	*0*	*0*	*3*	*0*	5
	49.0	0	1	0	*0*	*0*	*0*	*0*	1
	82.0	0	1	0	*0*	*0*	*0*	*0*	1
	87.0	0	8	5	*1*	*0*	*4*	*0*	13
	118.0	0	0	1	*0*	*0*	*1*	*0*	1
Total E3		**0**	**12**	**9**	***1***	***0***	***8***	***0***	**21**
E4	8.0	0	1	0	*0*	*0*	*0*	*0*	1
	22.0	0	10	5	*0*	*0*	*4*	*1*	15
	28.0	1	7	13	*1*	*0*	*10*	*2*	21
	28.14	0	1	0	*0*	*0*	*0*	*0*	1
	73.0	0	0	1	*1*	*0*	*0*	*0*	1
	77.0	0	12	5	*0*	*0*	*5*	*0*	17
	89.0	0	10	15	*1*	*0*	*13*	*1*	25
	102.2	0	0	1	*0*	*0*	*1*	*0*	1
Total E4		**1**	**41**	**40**	***2***	***0***	***33***	***4***	**82**
E5	170.0	0	1	0	*0*	*0*	*0*	*0*	1
Total E5		**0**	**1**	**0**	***0***	***0***	***0***	***0***	**1**
E6	65.4	0	1	0	*0*	*0*	*0*	*0*	1
	11.0	0	2	5	*1*	*0*	*4*	*0*	7
	75.0	1	9	12	*2*	*1*	*8*	*1*	22
	81.0	0	0	1	*0*	*0*	*1*	*0*	1
	94.0	0	0	1	*0*	*0*	*1*	*0*	1
Total E6		**1**	**12**	**19**	***3***	***1***	***14***	***1***	**32**
D4	53.1	0	0	1	*0*	*0*	*0*	*1*	1
	56.2	0	0	1	*1*	*0*	*0*	*0*	1
Total D4		**0**	**0**	**2**	***1***	***0***	***0***	***1***	**2**
Clade Y	14.15	0	1	0	*0*	*0*	*0*	*0*	1
Total Clade Y		**0**	**1**	**0**	***0***	***0***	***0***	***0***	**1**
A-C3	1.0	2	25	14	*1*	*4*	*6*	*3*	41
	1.15	0	1	0	*0*	*0*	*0*	*0*	1
	1.25	0	1	0	*0*	*0*	*0*	*0*	1
	1.29	1	0	0	*0*	*0*	*0*	*0*	1
	1.3	0	1	1	*0*	*0*	*1*	*0*	2
Total A-C3		**3**	**28**	**15**	***1***	***4***	***7***	***3***	**46**

A-C4	12.0	1	6	3	*1*	*0*	*2*	*0*	10
	12.37	0	1	1	*0*	*0*	*1*	*0*	2
Total A-C4		**1**	**7**	**4**	***1***	***0***	***3***	***0***	**12**
M5	5.44	1	0	0	*0*	*0*	*0*	*0*	1
Total M5		**1**	**0**	**0**	***0***	***0***	***0***	***0***	**1**
M6	6.0	3	7	10	*4*	*0*	*5*	*1*	20
	6.4	3	1	1	*0*	*1*	*0*	*0*	5
	6.98	0	0	1	*0*	*0*	*1*	*0*	1
Total M6		**6**	**8**	**12**	***4***	***1***	***6***	***1***	**26**
	29.2	0	1	0	*0*	*0*	*0*	*0*	1
Total M29		**0**	**1**	**0**	***0***	***0***	***0***	***0***	**1**
	95.0	0	1	0	*0*	*0*	*0*	*0*	1
Total M95		**0**	**1**	**0**	***0***	***0***	***0***	***0***	**1**
	1.127	0	0	1	*0*	*0*	*0*	*1*	1
	145.1	0	1	0	*0*	*0*	*0*	*0*	1
	Unknown	0	0	1	*1*	*0*	*0*	*0*	1
Total Other		**0**	**1**	**2**	***1***	***0***	***0***	***1***	**3**
Total		**14**	**122**	**107**	*15*	*7*	*74*	*11*	**243**

*1: isolates from an abscess or pus; 2: Throat, nose, ear, BAL and sputum; 3: Cervix, fluor, vagina, lochia and urine; 4: Synovial fluid and wound

11 ANTIGEN AND DNA DETECTION

The NRLBM received 156 culture-negative specimens of CSF, serum or other bodily fluids for antigen or DNA detection (Table 2.1). The Cryptococcal Antigen Lateral Flow Assay (CrAg LFA) assay was used to detect *C. neoformans*. PCR was performed with primers and probes specific for *N. meningitidis* (targeted on the *ctrA* gene) for *S. pneumoniae* (targeted on the *pia* gene) and for *H. influenzae* (*siaT* gene). When CSF was positive in the meningococcal PCR, the same sample was subjected to serogroup-specific PCR.

Of 156 culture-negative samples, 50 (32%) were positive for one of the target species by PCR. Of these, 28 (18%) (26 CSF, 2 sera or DNA isolated from a skin biopsy) were positive for *N. meningitidis* and 19 (12%) were positive for *S. pneumoniae*.

Table 11.1 CSF and serum samples tested for antigens or DNA, 2019

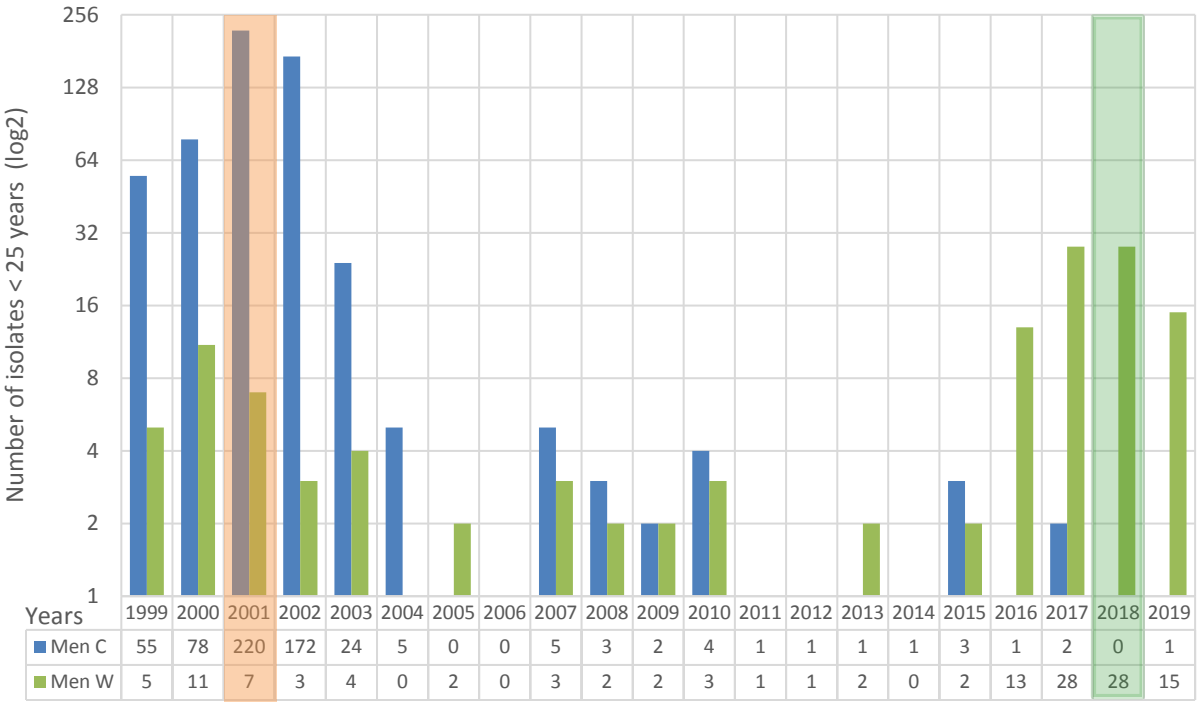
	CSF * (or DNA from CSF)	SERA or other fluids	TOTAL
***C. neoformans* (LFA)**	6	1	**7**
DNA of			
N. meningitidis	1	0	**1**
N. meningitidis group B	21	1	**22**
N. meningitidis group W	3	1	**4**
N. meningitidis group Y	1	0	**1**
S. pneumoniae	18	1	19
H. influenzae	3	0	3
Sub Total	**53**	**4**	**57**
Antigen and PCR negative	90	9	**99**
Total	**143**	**13**	**156**

*The 7 samples with a positive LFA test, were from 4 different patient. Of those patients also an isolate was received, the further CSF samples were follow up.
From 8 patients with *S. pneumoniae* isolated from blood, the CSF was culture-negative but PCR-positive for pneumococcal DNA. Those were counted as CSF patients.
From 6 patients with *N. meningitidis* isolated from blood, the CSF was culture-negative but PCR-positive for meningococcal DNA. Those were counted as CSF patients.
Of all 3 patients with positive Hi PCR an blood isolate was received. Those patients were counted as CSF patients.

12 VACCINATION PROSPECTS

12.1 *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 recent years, the number of cases of Meningococcal W disease has been increasing in the Netherlands. In response, the Meningococcal C vaccine has been replaced by one that protects against meningococcal types A, C, W and Y as of 1 May 2018. Because meningococcal type W is also hazardous for older children, the vaccination is also be offered to teenagers in the year they turn 14, as of 1 October 2018. In 2019, 6 cases of meningococcal disease (1.5% of all cases; table 4.4) were due to serogroup C meningococci. The six patients had not been vaccinated because of age.



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

Figure 12.1 *Number of N.meningitidis serogroup C and W isolates in patients < 25 years of age, 1999-2019. Start of vaccination with MenC and MenACWY vaccine is indicated in orange and green color, respectively.*

Two meningococcal group B vaccines are registrered in the Netherlands but not included in the National Immunization program (RIVM, Meningokokken B vaccinatie, sd)

12.2 ***H. influenzae***

The existing *H. influenzae* vaccine consists of the type b polysaccharide conjugated to the tetanus toxoid protein. Since July 1993, children born after the 1st of April 1993 are vaccinated with the PRP-T vaccine, at the ages of 3, 4, 5, and 11 months, and since 1999 at the age of 2, 3, 4 and 11 months. The effect of vaccination on the frequency of *H. influenzae* meningitis cases is shown in figure 12.2. The number of *H. influenzae* meningitis cases caused by *H. influenzae* type b gradually decreased since the introduction of the vaccine, while the number of meningitis cases caused by *H. influenzae* non-type b remained similar. In 2019, seven *H. influenzae* type b isolates were received from meningitis patients that should have been vaccinated (<26 years of age) (2018: 9; 2017: 7; 2016: 11) (figures 12.2 and 12.3). Of those 7 patients, two patients were not vaccinated at all and from five patients, vaccination status was unknown.

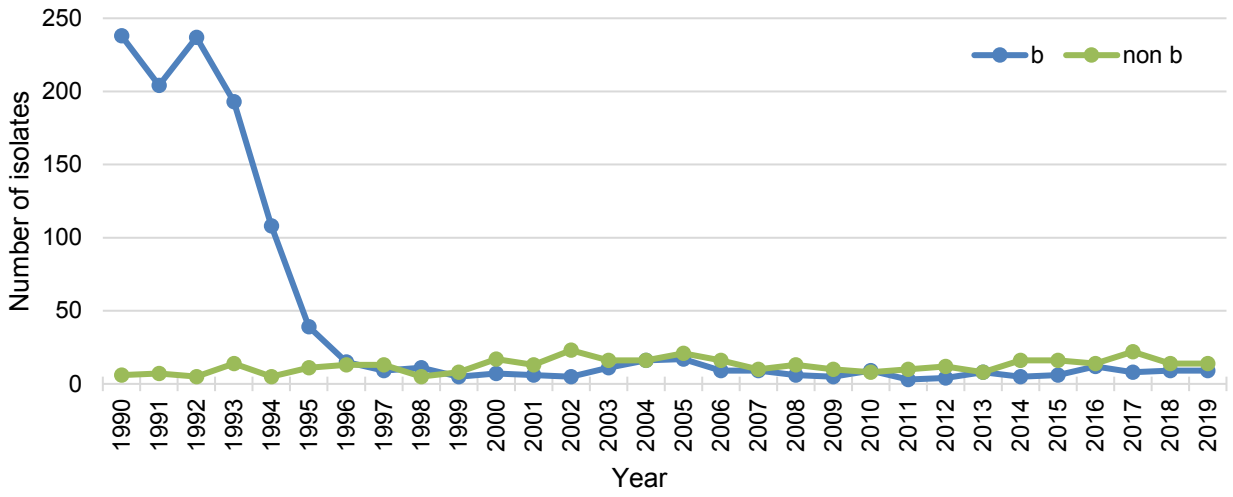


Figure 12.2 *The number of H. influenzae type b and non-type b meningitis cases, 1990 - 2019*

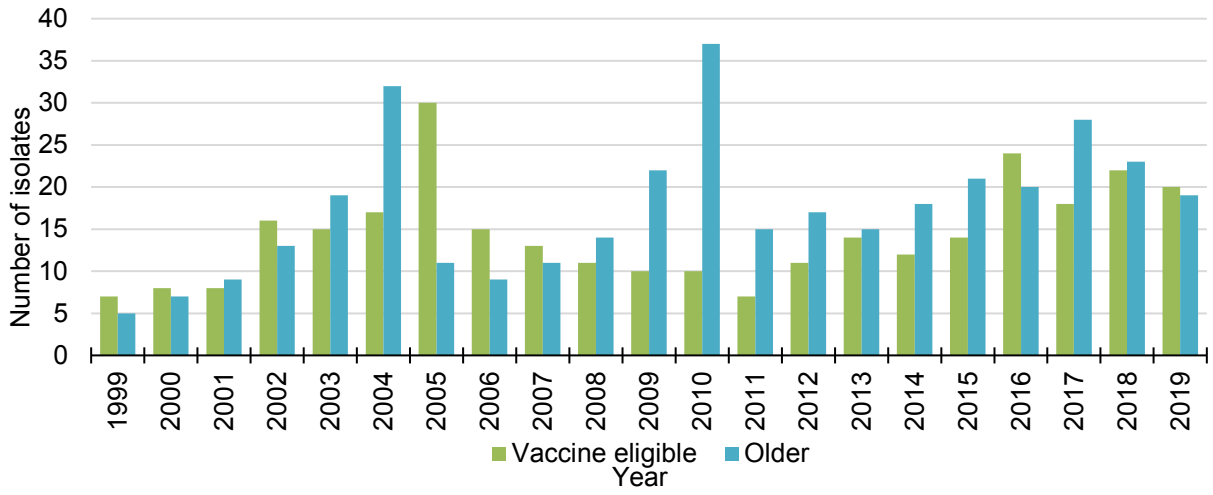


Figure 12.3 *The number of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 1999 –2019*

12.3 *S. pneumoniae*

The first pneumococcal conjugated polysaccharide vaccine contained 7 serotype-specific polysaccharides linked to inactive diphtheria toxin (7-valent polysaccharide conjugate vaccine. PCV7). Since July 2006, children born after the 1st April 2006 are vaccinated with PCV7 at the age of 2, 3, 4 and 11 months. In April 2011, the 10-valent vaccine (PCV10) was introduced for all newborns born from March 1st 2011. In 2019, 3.6% of the CSF isolates were of a serotype covered by the PCV10 vaccine (table 6.2). There were 6 patients with pneumococcal meningitis due to pneumonococci with a PCV-10 vaccine serotype (4, 7F, 18C and 19F; Table 6.5). All of these 6 (older) patients (49,61,68,72,and 78 years of age) were not vaccinated because of age. The beneficial effect of vaccination is partly countered by an increase of the number of cases due to non-vaccine types (figure 12.4).

The pneumococcal polysaccharide vaccine covers 23 serotypes (PPV23). Seventy-four percent of the CSF isolates were of a serotype, which is covered by this vaccine (table 6.5). (2019: 69%; 2007: 90%). In 2020, pneumococcal vaccination (PPV23) will be offered to all 60-, 65-, 70- and 75-year-olds in the Netherlands.

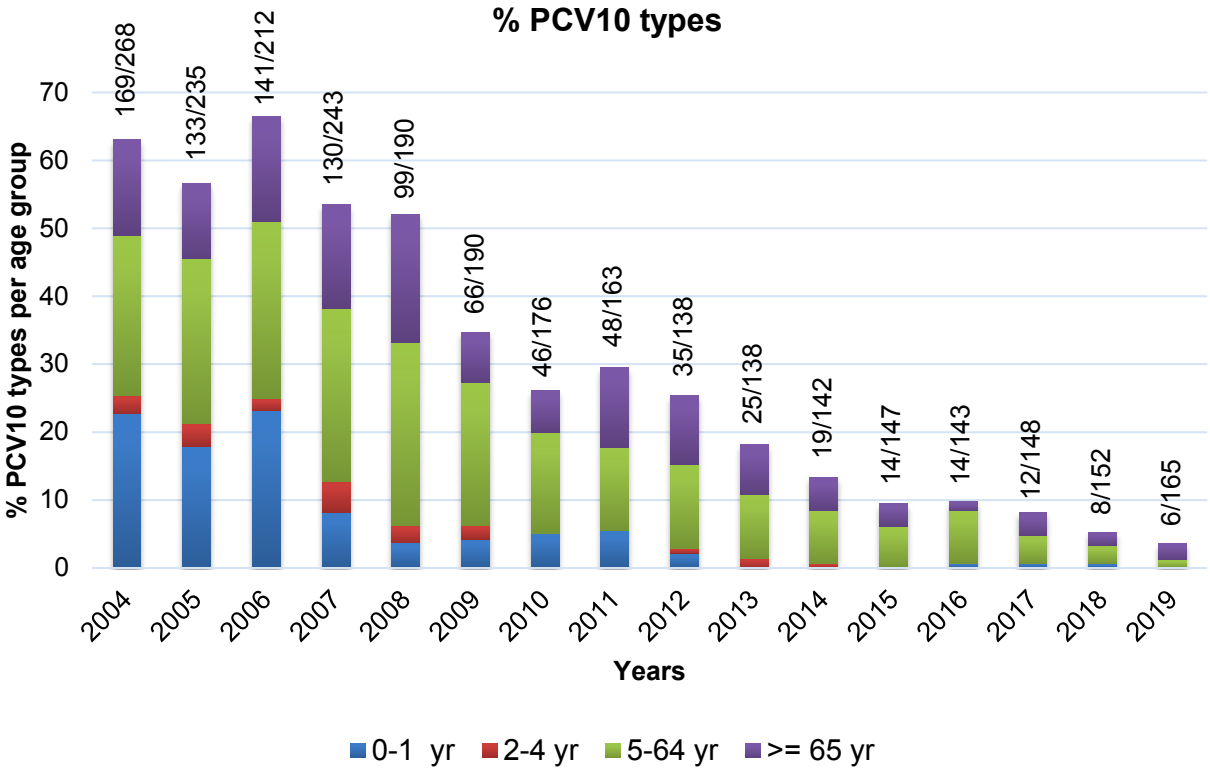


Figure 12.4 *The age distribution of S.pneumoniae invasive disease due to pneumococci of serotypes included in PCV-10, 2004-2019. (n PCV-10 type / n total CSF isolates)*

13 PUBLICATIONS

1. B Brandwagt DAH, van der Ende A, Ruijs WLM, de Melker HE, Knol MJ. Evaluation of the surveillance system for invasive meningococcal disease (IMD) in the Netherlands, 2004-2016. BMC Infect Dis. 2019;19:860.
2. Costerus JM, Brouwer MC, Sprengers MES, Roosendaal SD, van der Ende A, de Beek DV. CT-hersenen, lumbaalpunctie en klinische achteruitgang bij bacteriële meningitis [Cranial computed tomography, lumbar puncture and clinical deterioration in bacterial meningitis: a nationwide cohort study]. Ned Tijdschr Geneeskd. 2019;163:D3328.
3. de Gier B, van Kassel MN, Sanders EAM, van de Beek D, Hahné SJM, van der Ende A, Bijlsma MW. Disease burden of neonatal invasive Group B Streptococcus infection in the Netherlands. PLoS One. 2019;14:e0216749
4. Ferwerda B, Maury MM, Brouwer MC, Hafner L, van der Ende A, Bentley S, Lecuit M, van de Beek D. Residual Variation Intolerance Score Detects Loci Under Selection in Neuroinvasive *Listeria monocytogenes*. Front Microbiol. 2019;10:2702.
5. Figueiredo AHA, Brouwer MC, Bijlsma MW, van der Ende A, van de Beek D. Community-acquired pneumonia in patients with bacterial meningitis: a prospective nationwide cohort study. Clin Microbiol Infect. 2019:S1198-743X(19)30486-0.
6. Garcia Garrido HM, Mak AMR, Wit FWNM, Wong GWM, Knol MJ, Vollaard A, Tanck MWT, Van Der Ende A, Grobusch MP, Goorhuis A. Incidence and Risk Factors for Invasive Pneumococcal Disease and Community-acquired Pneumonia in Human Immunodeficiency Virus-Infected Individuals in a High-income Setting. Clin Infect Dis. 2019;21:ciz728
7. Hanquet G, Krizova P, Valentiner-Branth P, Ladhani SN, Nuorti JP, Lepoutre A, Mereckiene J, Knol M, Winje BA, Ciruela P, Ordobas M, Guevara M, McDonald E, Morfeldt E, Kozakova J, Slotved HC, Fry NK, Rinta-Kokko H, Varon E, Corcoran M, van der Ende A, Vestrheim DF， Munoz-Almagro C, Latasa P, Castilla J, Smith A, Henriques-Normark B, Whittaker R, Pastore Celentano L, Savulescu C; SpIDnet/I-MOVE+ Pneumo Group. Effect of childhood pneumococcal conjugate vaccination on invasive disease in older adults of 10 European countries: implications for adult vaccination. Thorax. 2019;74:473-482.
8. Kasanmoentalib ES, Valls Serón M, Engelen-Lee JY, Tanck MW, Pouw RB, van Mierlo G, Wouters D, Pickering MC, van der Ende A, Kuijpers TW, Brouwer MC, van de Beek D. Complement factor H contributes to mortality in humans and mice with bacterial meningitis. J Neuroinflammation. 2019;16:279.
9. Krone M, Gray S, Abad R, Skoczyńska A, Stefanelli P, van der Ende A, Tzanakaki G, Mölling P, João Simões M, Křížová P, Emonet S, Caugant DA, Toropainen M, Vazquez J, Waśko I, Knol MJ, Jacobsson S, Rodrigues Bettencourt C, Musilek M, Born R, Vogel U, Borrow R. Increase of invasive meningococcal serogroup W disease in Europe, 2013 to 2017. Euro Surveill. 2019;24:1800245.
10. Lees JA, Ferwerda B, Kremer PHC, Wheeler NE, Serón MV, Croucher NJ, Gladstone RA, Bootsma HJ, Rots NY, Wijmega-Monsuur AJ, Sanders EAM, Trzciński K, Wyllie AL, Zwinderman AH, van den Berg LH, van Rheenen W, Veldink JH, Harboe ZB, Lundbo LF, de Groot LCPGM, van Schoor NM, van der Velde N, Ängquist LH, Sørensen TIA, Nohr EA, Mentzer AJ, Mills TC, Knight JC, du Plessis M, Nzenze S, Weiser JN, Parkhill J, Madhi S, Benfield T, von Gottberg A, van der Ende A, Brouwer MC, Barrett JC, Bentley SD, van de Beek D. Joint sequencing of human and pathogen genomes reveals the genetics of pneumococcal meningitis. Nat Commun. 2019;10:2176.
11. Loenenbach AD, van der Ende A, de Melker HE, Sanders EAM, Knol MJ. The Clinical Picture and Severity of Invasive Meningococcal Disease Serogroup W Compared

With Other Serogroups in the Netherlands, 2015-2018. Clin Infect Dis. 2019;26:ciz578.
12. Monge S, Mollema L, de Melker H, Sanders E, van der Ende A, Knol M. Clinical Characterization of Invasive Disease Caused by Haemophilus influenzae Serotype b in a High Vaccination Coverage Setting. J Pediatric Infect Dis Soc. 2019 Jul 1;8(3):261-264.
13. Pouw RB, Brouwer MC, de Gast M, van Beek AE, van den Heuvel LP, Schmidt CQ, van der Ende A, Sánchez-Corral P, Kuijpers TW, Wouters D. Potentiation of complement regulator factor H protects human endothelial cells from complement attack in aHUS sera. Blood Adv. 2019;3:621-632.
14. Taha MK, Deghmane AE, Knol M, van der Ende A. Whole genome sequencing reveals Trans-European spread of an epidemic *Neisseria meningitidis* serogroup W clone. Clin Microbiol Infect. 2019;25:765-767.
15. Ter Horst L, Brouwer MC, van der Ende A, van de Beek D. Community-Acquired Bacterial Meningitis In Adults With Cerebrospinal Fluid Leakage. Clin Infect Dis. 2019;12:ciz649.
16. Vestjens SMT, Sanders EAM, Vlaminckx BJ, de Melker HE, van der Ende A, Knol MJ. Twelve years of pneumococcal conjugate vaccination in the Netherlands: Impact on incidence and clinical outcomes of invasive pneumococcal disease. Vaccine. 2019;37:6558-6565.
17. Willemse N, van der Ende A, Schultsz C. Reinfection with *Streptococcus suis* analysed by whole genome sequencing. Zoonoses Public Health. 2019;66:179-183.

14 ACKNOWLEDGEMENTS

Many have contributed to the work of the NRLBM and to this report. We would like to thank:

- the National Institute of Public Health and the Environment (RIVM Bilthoven. dr. J.T. van Dissel) for ongoing financial support and scientific interaction (Dr. Mirjam Knol).
- Mrs. A. Arends. Mrs. W.C.M Bril - Keijzers. Mrs. M.M. Feller and Mrs. I.G.A. de Beer for their outstanding technical laboratory assistance
- Mrs. I.G.A. de Beer for preparing data from the computer files and layout of this report

15 Bibliography

CBS - Statline Statistics Netherland. From www.cbs.nl: www.cbs.nl

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

NOGBS studie. From Neuroinfecties Amsterdam: https://meningitisamc.nl/professionals/wetenschappelijk-onderzoek-professionals/nogbs-studie

PubMLST - FetA variable region typing. From Public databases for molecular typing: https://pubmlst.org/neisseria/FetA/

PubMLST - PorA typing. From Public databases for molecular typing: https://pubmlst.org/neisseria/PorA/

RIVM. *Meningokokken B vaccinatie*. Opgehaald van https://lci.rivm.nl/richtlijnen/meningokokken-b-vaccinatie

RIVM. *Vleeswaren waarschijnlijk bron 20 patiënten met Listeria*. From RIVM: https://www.rivm.nl/nieuws/vleeswaren-waarschijnlijk-bron-20-patienten-met-listeria

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