NETHERLANDS REFERENCE LABORATORY FOR BACTERIAL MENINGITIS

BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2017

AMC
Academic Medical Center
University of Amsterdam

RIVM
National Institute of Public Health
and
Environmental Protection

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

1 INTRODUCTION

This is the **46th** Annual Report of the Netherlands Reference Laboratory for Bacterial Meningitis of the Academic Medical Center (AMC) and the National Institute of Public Health and the Environment (RIVM). The Reference Laboratory is located within the Department of Medical Microbiology of the AMC in Amsterdam. Nearly all clinical microbiology laboratories of the Netherlands collaborate by submitting bacterial isolates and/or cerebrospinal fluid samples from patients with meningitis and we are most grateful to our colleagues for their cooperation.

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

In the archives of the Reference Laboratory data from approximately 72.870 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 Reference Laboratory are:
- to perform surveillance of bacterial meningitis;
- to describe the epidemiology of bacterial meningitis in the Netherlands;
- to provide keys 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 would appreciate receiving your opinion and suggestions on this report.

Amsterdam, november, 2018

dr. A. van der Ende PhD, biochemist
dr. W. Freudenburg, medical microbiologist

2 ISOLATES, CSF SPECIMENS AND SERA RECEIVE

The Netherlands Reference Laboratory for Bacterial Meningitis 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, and a bacteraemia patient, respectively. Incidences have been calculated by dividing the number of isolates collected over one year (in a certain patient's age group) by the number of inhabitants over one year (in that age group) multiplied by 100,000. Population figures were obtained from Statistics Netherlands (Centraal Bureau voor de Statistiek, http://www.cbs.nl) using StatLine. By estimation, the Reference Laboratory receives about 90% of the isolates of Dutch meningitis patients, hence incidences presented in this report are likely to be underestimated.

In 2017, the Reference Laboratory received isolates from CSF and/or blood from 2118 patients, and 45 specimens of CSF and/or serum wich were positive in PCR. (table 2.1/table 11.1). Of these patients, 328 were confirmed cases of bacterial meningitis.

Table 2.1

	Number of specimens
Isolate (CSF and/or blood)	2118
Samples of CSF, Sera and other fluid, without isolate	93
Total	**2211**

In 2017, 51 clinical microbiology laboratories submitted isolates to the Reference Laboratory.

Table 2.2 shows the 2118 isolates according to species and to laboratory where cases were diagnosed.

Table 2.2 Number of isolates from CSF and/or blood received in 2017, according to laboratory

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Alkmaar	MCA lab. Med. Microbiologie	2	10	68	1	2	3	-	-	-	-	1	-	**87**
Amersfoort	Meander Medisch Centrum	5	-	31	-	1	1	-	-	-	-	-	-	**38**
Amsterdam	Academisch Medisch Centrum	3	6	31	4	6	1	-	-	5	-	12	-	**68**
	Academisch ziekenhuis VU	5	1	5	-	-	1	-	-	-	2	-	-	**14**
	Onze Lieve Vrouwe Gasthuis	9	2	9	-	4	1	1	-	-	3	-	-	**29**
	Slotervaart	-	2	26	-	-	5	-	-	-	-	-	-	**33**
Apeldoorn	Gelre Ziekenhuizen	6	7	43	-	2	2	-	1	-	1	1	-	**63**
Arnhem	Rijnstate	2	5	50	-	1	1	1	1	-	-	-	-	**61**
Breda	Amphia Ziekenhuis	3	15	20	4	11	4	1	-	-	2	-	-	**60**
Capelle ad IJssel	IJsselland Ziekenhuis	1	2	3	-	1	-	-	-	-	-	-	-	**7**
Delft	Reinier Haga MDC	6	-	2	2	-	2	-	-	-	-	-	-	**12**
Den Bosch	Regionaal laboratorium Den Bosch	7	10	81	-	2	4	-	-	-	-	-	1	**105**
Den Haag	Haga Ziekenhuis, loc. Leyenburg	14	6	42	-	2	-	-	-	-	-	-	-	**64**
	MA Haaglanden, loc Westeinde	2	1	1	-	1	-	-	-	-	-	1	-	**6**
Deventer	Deventer Ziekenhuis	3	3	19	-	1	3	-	-	-	-	-	-	**29**
Doetinchem	Slingeland Ziekenhuis	2	1	17	-	2		-	-	-	-	-	-	**22**
Dordrecht	RLM Dordrecht / Gorinchem	2	8	52	-	1	1	1	-	-	-	-	-	**65**
Ede	Gelderse Vallei	2	9	10	-	3	3	1	2	1	1	1	-	**33**
Goes	Lab. v. Med.Microb. & Imm., ADRZ	5	2	3	-	-	-	1	-	-	-	-	-	**11**
Gouda	Groene Hart Ziekenhuis	-	5	29	-	-	2	1	-	-	-	-	-	**37**
Groningen	Certe, Lab. v. Infectieziekten	4	16	55	-	2	7	-	-	-	-	-	-	**84**
	UMCG	2	1	-	3	-	-	-	-	-	-	-	-	**6**
Haarlem	St. Streeklab voor de Volksgezondheid	2	6	92	2	3	2	-	-	-	-	-	-	**107**
Harderwijk	St. Jansdal Ziekenhuis	4	-	3	-	-	1*	-	-	-	-	-	-	**8**
Hengelo	LabMicTa	11	7	113	1	4	3	1	-	-	-	-	-	**140**
Hilversum	Centraal Bact. Ser. Lab.	3	-	2	-	1	1	-	-	-	-	-	-	**7**
Hoorn	Westfries gasthuis	4	4	21	-	3	1	1	-	-	-	-	1	**35**
Leeuwarden	Izore, centrum infectieziekten Friesland	10	13	96	-	4	4	-	-	-	-	-	-	**127**
Leiden	Alrijne ziekenhuis	5	5	60	-	-	3	-	-	-	-	-	-	**73**
	LUMC, KML, Lab.voor Bacteriologie	8	7	29	2	5	2	1	-	-	-	2	-	**56**
Maastricht	Acad. Ziekenhuis Maastricht	2	3	-	-	-	-	-	-	-	-	-	-	**5**
Nieuwegein	St. Antonius Ziekenhuis	7	11	58	-	1	3	2	-	-	-	-	-	**82**
Nijmegen	Canisius Wilhelmina Zknhs	5	3	16	-	-	5	-	-	-	-	-	-	**29**
	UMC St. Radboud	2	4	33	14	3	2	-	-	-	-	-	-	**58**
Roermond	St. Laurentius Ziekenhuis	1	2	-	-	-	1	-	-	-	-	-	-	**4**

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Roosendaal	St. Fransiscus Ziekenhuis	2	4	2	-	-	1	-	-	-	-	-	-	**9**
Rotterdam	Erasmus MC Med. Microbiologie	6	6	22	-	2	-	-	-	-	-	-	1	**37**
	Ikazia Ziekenhuis	3	1	7	-	-	2	-	-	-	-	1	-	**14**
	Maasstad Ziekenhuis	3	1	6	-	-	-	-	-	-	-		-	**10**
	St.Franciscus Gasthuis	12	6	48	-	8	1	4	-	-	-	1	-	**80**
Sittard	Zuyderland Medisch Centrum	2	1	31	1	2	4	-	-	-	-	2	-	**43**
Terneuzen	Zorgsaam Zeeuws-Vlaanderen	2	1	3	-	-	-	1	-	-	-	-	-	**7**
Tilburg	Streeklab. Tilburg	1	4	5	-	1	4	-	-	-	-	-	-	**15**
Utrecht	Diakonessenhuis	3	8	1	-	-	2	-	-	-	-	-	-	**14**
	UMC Med. Microbiologie	3	1	30	12	-	2	-	1	-	-	-	-	**49**
Veldhoven	PAMM, Lab. Med. Microbiologie	5	8	105	-	2	2	1	-	-	-	-	-	**123**
Vredenburg	Medical Microbology, Curacao/St.Maarten	1	1	4	-	-	-	-	-	-	-	-	-	**6**
Venlo	Vie Curie medisch centrum	4	-	2	-	1	-	-	-	-	-	-	-	**7**
Weert	St. Jans gasthuis	-	-	2	-	-	1	-	-	-	-	-	-	**3**
Woerden	Zuwe Hofpoort Ziekenhuis	2	-	3	-	-	-	-	-	-	-	-	-	**5**
Zwolle	Isala Klinieken LMMI	3	5	12	3	5	3	-	-	-	-	-	-	**31**
Total		**201**	**224**	**1403**	**49**	**87**	**91**	**18**	**5**	**6**	**9**	**22**	**3**	**2118**

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; **nv:** non-viable

* One *L. monocytogenes* was send bij Utrecht UMC and St Antonius NIeuwegein,

The distribution of the isolates received in the 5 year period 2013 through 2017 is presented in table 2.3. The number of total isolates increased from 1243 in 2014 to 2118 in 2017. The number of cases of meningococcal disease increased from 73 in 2014 to 201 in 2017. Since June 2006, children born after the first of April 2006 are vaccinated with a conjugated polysaccharide vaccine against *Streptococcus pneumoniae*. The number of *S. pneumoniae* isolates from CSF decreased from more than 200 yearly before 2007 to 148 in 2017. The number of *Listeria monocytogenes* isolates increased from 47 in 2015 to 91 in 2017. The number of *Haemophilus influenzae* isolates increased, 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 2013 – 2017

Species	2013			2014			2015			2016			2017		
	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total
N. meningitidis*	39	72	111	31	42	73	33	51	84	36	100	136	67	134	201
H. influenzae	16	144	160	21	140	161	22	173	195	26	162	188	30	194	224
S. pneumoniae	138	768	906	142	627	769	147	754	901	143	762	905	148	1255	1403
E. coli	8	18	26	8	24	32	8	20	28	18	32	50	8	41	49
S. agalactiae	20	52	72	23	48	71	19	46	65	20	49	69	24	63	87
L. monocytogenes	6	46	52	19	51	70	8	39	47	11	59	70	20	71	91
S. pyogenes	9	22	31	2	6	8	3	13	16	5	5	10	7	11	18
S. aureus	5	18	23	13	10	23	8	8	16	10	1	11	5	0	5
Coag.neg.Staph.	6	0	6	2	0	2	2	0	2	2	0	2	6	0	6
C. neoformans	6	2	8	4	3	7	7	2	9	7	3	10	7	2	9
others	14	6	20	22	4	26	30	10	40	19	18	37	9	13	22
non viable	0	1	1	0	1	1	0	1	1	0	2	2	0	3	3
Total	**267**	**1149**	**1416**	**287**	**956**	**1243**	**287**	**1117**	**1404**	**297**	**1193**	**1490**	**331**	**1787**	**2118**

*Including PCR positive patients
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 2013 to 2017 is shown in table 2.4. The incidence of *H. influenzae* infection was 38% lower than in the years before vaccination was introduced (2.1 in 1992; 1.3 in 2017). The incidence *of H. influenzae* infection increased from 2010 until now, mainly caused by an increase in the number of cases of bacteraemia due to unencapsulated *H. influenzae*.

Table 2.4 Number of isolates from CSF and/or blood per 100,000 inhabitants, 2013 - 2017

Species	2013	2014	2015	2016	2017
N. meningitidis	0.66	0.43	0.50	0.80	1.18
H. influenzae	0.95	0.96	1.15	1.11	1.31
S. pneumoniae	5.40	4.57	5.33	5.33	8.21
E. coli	0.15	0.19	0.17	0.29	0.29
S. agalactiae	0.43	0.42	0.38	0.41	0.51
L. monocytogenes	0.31	0.42	0.28	0.41	0.53
S. pyogenes	0.18	0.05	0.09	0.06	0.11
S. aureus	0.14	0.14	0.09	0.07	0.03
Coag. neg. Staph.	0.04	0.01	0.01	0.01	0.04
C. neoformans	0.05	0.04	0.05	0.06	0.05
others	0.12	0.15	0.24	0.22	0.13
non viable	0.01	0.01	0.01	0.01	0.02
Total	**8.44**	**7.39**	**8.31**	**8.78**	**12.40**

Table 2.5 Total number of isolates from CSF and/or blood received in 2017, according to bacterial species and specimen source

Species		CSF or CSF and blood	Blood only	Total	%
***Neisseria meningitidis*[1]**		67	134	201	**9.5**
***Haemophilus influenzae* [2]**		30	194	224	**10.6**
***Streptococcus pneumoniae* [3]**		148	1255	1403	**66.3**
Escherichia coli		8	41	49	**2.3**
Streptococcus agalactiae		24	63	87	**4.1**
Listeria monocytogenes		20	71	91	**4.3**
Streptococcus pyogenes		7	11	18	**0.9**
Staphylococcus aureus		5	0	5	**0.2**
Coagulase-negative staphylococcus [4]		6	0	6	**0.3**
Cryptococcus neoformans		7	2	9	**0.4**
Others total		9	13	22	**1.0**
Others	*Klebsiella pneumoniae*	*1*	*0*	*1*	
	Neisseria cinerea	*0*	*1*	*1*	
	Neisseria gonorrhoeae	*0*	*1*	*1*	
	Neisseria mucosa	*0*	*1*	*1*	
	Streptococcus gallolyticus ssp gallolyticus	*0*	*3*	*3*	
	Streptococcus gallolyticus ssp pasteurianus	*0*	*2*	*2*	
	Streptococcus infantis	*1*	*1*	*2*	
	Streptococcus intermedius	*1*	*0*	*1*	
	Streptococcus massiliensis	*0*	*1*	*1*	
	Streptococcus mitis	*1*	*1*	*2*	
	Streptococcus oralis	*2*	*0*	*2*	
	Streptococcus oralis subsp tigurinus	*1*	*0*	*1*	
	Haemophilus parainfluenzae	*0*	*1*	*1*	
	Haemophilus haemolyticus	*0*	*1*	*1*	
	Enterococcus faecalis	*1*	*0*	*1*	
	Rothia mucilaginosa	*1*	*0*	*1*	
Non viable		0	3	3	**0.1**
Total %		**331**	**1787**	**2118**	**100.0**

1 In one patient *Neisseria meningitidis* and *Streptococcus pneumoniae* were isolated from the blood.

2 In two patients *Haemophilus influenza and* and *Streptococcus pneumoniae* were isolated from the blood.

3 In one patient *Streptococcus pneumoniae* and *Streptococcus oralis subsp tigurinus* were isolated from the CSF.

4 6 Coagulase-negative staphylococcus were isolated from CSF. *Staphylococcus epidermidis* (3), *Staphylococcus hominis (2) and one Staphylococcus schleiferi*

Table 2.5 shows the distribution of isolates according to the specimen from which they were cultured. The predominant species were *N. meningitidis, H. influenzae* and *S. pneumoniae*. Patients with two different isolates were counted twice. Example, patients mentioned in footnote nr 1 were counted once for *N. meningitidis* and once for *S. pneumoniae*.

3 BACTERIAL MENINGITIS - general data

In 2017, the Reference Laboratory received CSF isolates from 310 patients. Furthermore, 30 culture-negative CSF samples appeared to be positive by antigen detection or PCR (Table 11.1). Of these CSF samples, 21 were positive for *N. meningitidis* and 9 for *S. pneumoniae.* Including these cases, the total number of patients with confirmed meningitis amounted to 340. The proportion of meningococcal and pneumococcal cases among meningitis patients was 20% and 46%, respectively (Figure 3.1).

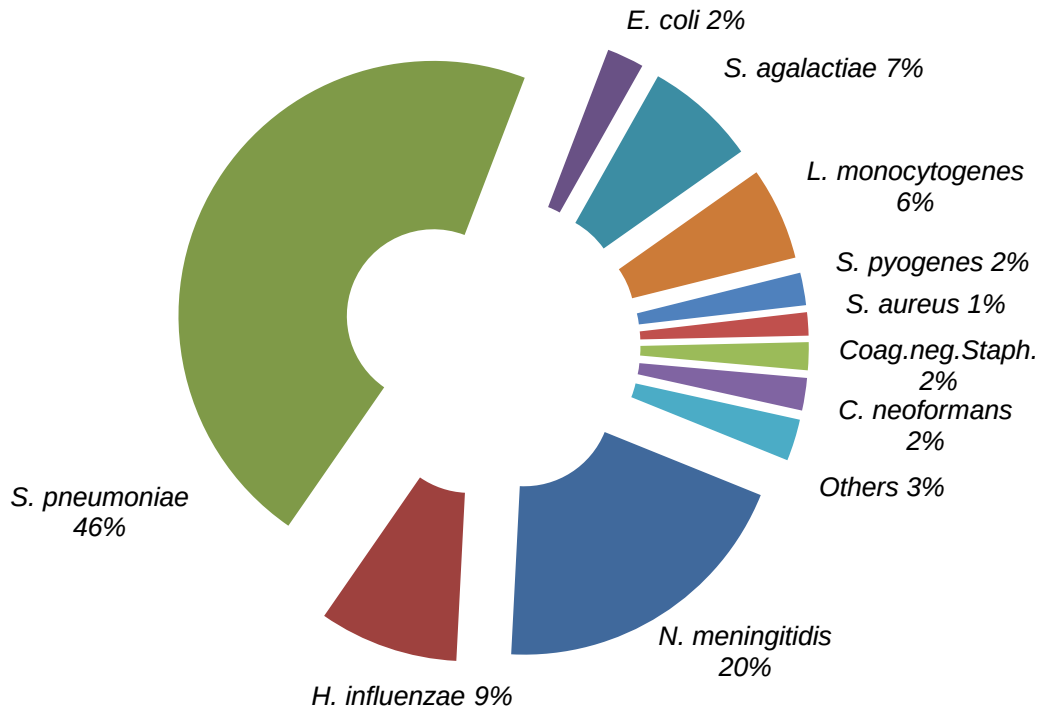

Figure 3.1 *Proportional distribution of CSF isolates and CSF positive samples, 2017*

Figure 3.2 shows the annual total number of bacterial isolates from CSF during the period 2008-2017. The 10 years trend line indicates a decrease over the last decade. The incidence per 100,000 inhabitants also shows a decreasing trend and varied between 2.1 and 1.6 during the period 2008-2017 (Figure 3.2). Since 2013, the downwards trend has stopped and a slight increase has been noted.
Data concerning *N. meningitidis, H. influenzae* and *S. pneumoniae* during the same period are presented in figure 3.3. Since the introduction of vaccination against *H. influenzae* type b in 1993, the incidence of *Haemophilus* meningitis decreased to 0.18 per 100,000 and remained at this low level. The number of cases of meningococcal meningitis (with an isolate) decreased from 480 cases (incidence of 3.1) in 1993 to 36 cases (incidence of 0.21) in 2016, mainly due to a decline in the number of cases of serogroup B and C meningitis. Nationwide vaccination against serogroup C meningococci was started in 2002. The year 2003 was the first year, since two decades, in which *N. meningitidis* was not the main cause of bacterial meningitis in the Netherlands. In 2017 the number of meningococcal meningitis cases doubled to 67 (46 isolates and 21 PCR positive CSF samples). Pneumococcal meningitis was slowly increasing since 1991 as the annual incidence rose from 1.0 to 1.6 per 100,000 inhabitants in 2004, but had decreased to 0.87 in 2017 due to vaccination against pneumococci introduced in June 2006 in the National Immunisation Programme.

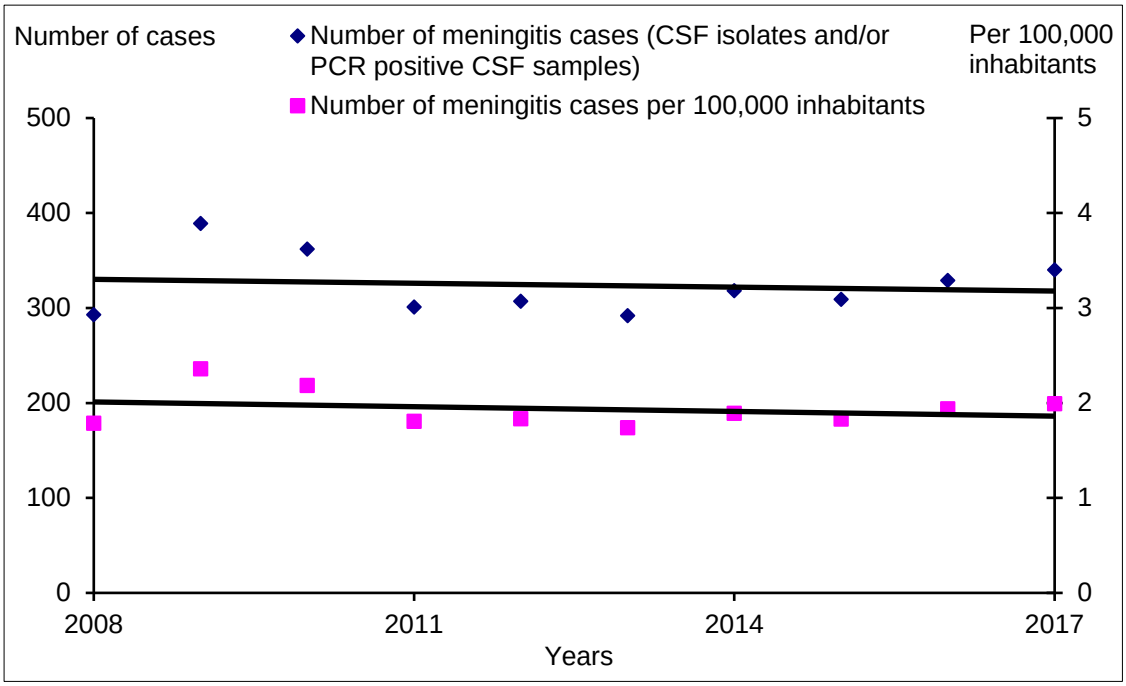

Figure 3.2 *Meningitis cases, 2008-2017*

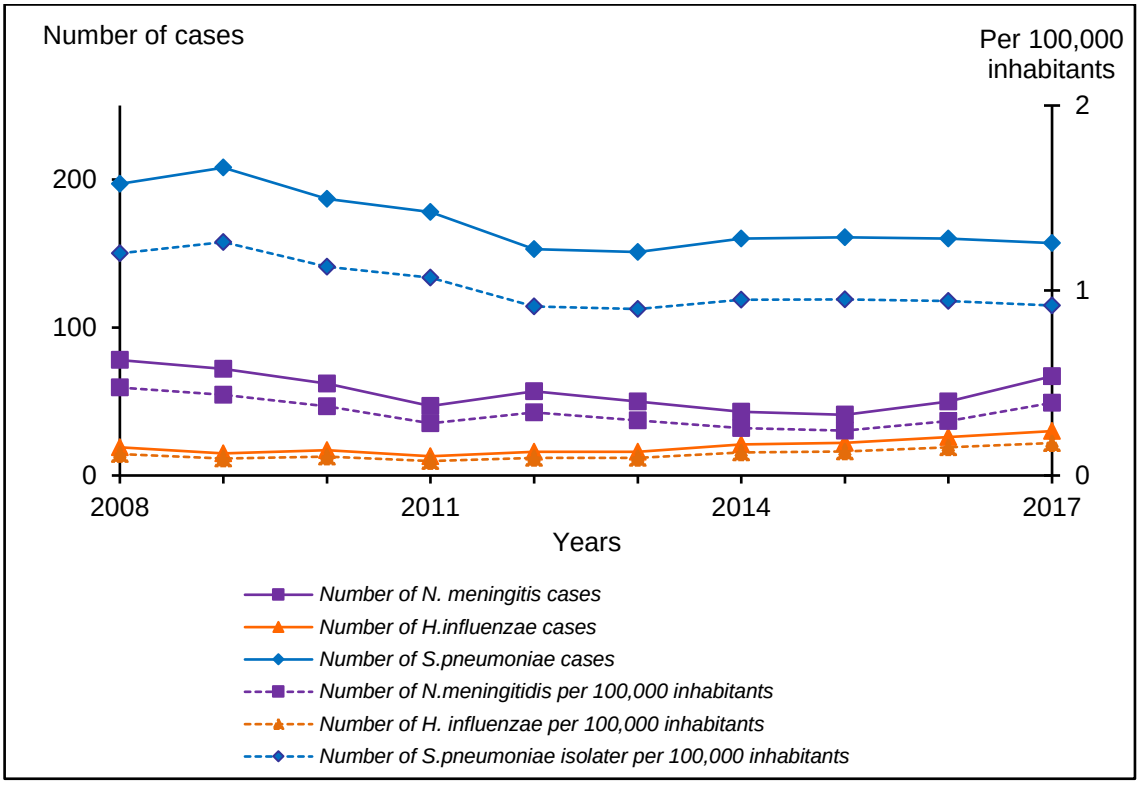

Figure 3.3 *Meningococcal, Haemophilus and pneumococcal meningitis (isolates and/or positive PCR from CSF), 2008-2017*

Table 3.1 shows the frequency of isolation of the different bacterial species from CSF by annual quarter. 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, 2017

	ANNUAL QUARTER					
SPECIES	**First**	**Second**	**Third**	**Fourth**	**Total**	**%**
N. meningitidis	27	10	9	21	**67**	***19.7***
H. influenzae	12	11	4	3	**30**	***8.8***
S. pneumoniae	71	39	13	34	**157**	***46.2***
E. coli	0	2	3	3	**8**	***2.3***
S. agalactiae	7	7	5	5	**24**	***7.0***
L. monocytogenes	1	4	8	7	**20**	***5.9***
S. pyogenes	5	2	0	0	**7**	***2.1***
S. aureus	1	2	1	1	**5**	***1.5***
Coag.neg.Staph.	3	1	1	1	**6**	***1.8***
C. neoformans	2	3	1	1	**7**	***2.1***
Others	4	0	1	4	**9**	***2.6***
non viable	0	0	0	0	**0**	***0.0***
Total	**133**	**81**	**46**	**80**	**340**	***100.0***
%	***39.2***	***23.8***	***13.5***	***23.5***	***100.0***	

Tables 3.2 and 3.3 show the distribution of the bacterial species isolated from CSF according to the age of the patient and the age-specific incidence per 100,000, respectively. *Streptococcus agalactiae* is still the predominant species isolated in neonates (younger than 1 month), and represented 67% of the isolates in this age group, whereas in the age group 1-11 months the predominant species were *S. pneumoniae* and *N. meningitidis* (together 70%). Since the introduction of the vaccine against *H.influenzae* type b, the number of cases of *H.influenzae* meningitis in the age group 0-4 year has strongly decreased (1992: 231; 2015: 7). The number of Hi isolates increased to 13 in this agegroup, two times higher than in 2015.

Table 3.2 Isolates and PCR positive samples from CSF grouped according to patients' age, 2017

	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. meningitidis	0	9	13	22	2	3	18	5	3	2	7	4	1	**67**	***19.7***
H. influenzae	0	3	10	13	1	0	1	1	0	2	6	5	1	**30**	***8.8***
S. pneumoniae	1	12	8	21	1	1	1	3	7	15	46	53	9	**157**	***46.2***
E. coli	6	0	0	6	0	0	0	0	0	0	0	2	0	**8**	***2.3***
S. agalactiae	16	6	0	22	0	0	0	0	1	0	1	0	0	**24**	***7.0***
L. monocytogenes	0	0	0	0	0	0	1	0	0	1	1	11	6	**20**	***5.9***
S. pyogenes	0	0	2	2	0	0	0	0	0	3	1	1	0	**7**	***2.1***
S. aureus	0	0	0	0	0	0	0	1	0	1	1	0	2	**5**	***1.5***
Coag.neg.Staph.	1	0	0	1	0	1	0	0	0	0	2	2	0	**6**	***1.8***
C. neoformans	0	0	0	0	0	0	0	0	2	0	3	1	1	**7**	***2.1***
Others	0	0	0	0	1	0	0	2	1	1	2	2	0	**9**	***2.6***
non viable	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
Total	*24*	*30*	*33*	*87*	*5*	*5*	*21*	*12*	*14*	*25*	*70*	*81*	*20*	**340**	***100***
%	**7.0**	**8.8**	**9.7**	**25.5**	**1.5**	**1.5**	**6.2**	**3.5**	**4.1**	**7.4**	**20.6**	**23.8**	**5.9**	***100***	

* From one patient day of birth is unknown.

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

Table 3.3 Age-specific incidence of bacterial meningitis per 100,000 inhabitants grouped according to species, 2017

SPECIES	AGE (YEARS)											Total
	0	1-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	
N. meningitidis	5.22	1.86	0.22	0.31	1.74	0.23	0.15	0.08	0.20	0.17	0.13	0.39
H. influenzae	1.74	1.43	0.11	0.00	0.10	0.05	0.00	0.08	0.17	0.21	0.13	0.18
S. pneumoniae	7.55	1.14	0.11	0.10	0.10	0.14	0.34	0.64	1.30	2.21	1.18	0.92
E. coli	3.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.05
S. agalactiae	12.77	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.03	0.00	0.00	0.14
L. monocytogenes	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.04	0.03	0.46	0.79	0.12
S. pyogenes	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.13	0.03	0.04	0.00	0.04
S. aureus	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.04	0.03	0.00	0.26	0.03
Coag.neg.Staph.	0.58	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.06	0.08	0.00	0.04
C. neoformans	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.08	0.04	0.13	0.04
Others	0.00	0.00	0.11	0.00	0.00	0.09	0.05	0.04	0.06	0.08	0.00	0.05
non viable	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	**31.34**	**4.71**	**0.54**	**0.41**	**1.26**	**0.46**	**0.59**	**1.06**	**1.86**	**3.17**	**2.49**	**1.81**

Table 3.4 shows the frequency of the isolates per species from CSF according to gender of the patients. For most species the Male/Female ratio varied between 0.7 and 1.5. The overall M/F ratio was 1.0.

Table 3.4 Isolates and PCR positive samples from CSF according to patients' gender. 2017

SPECIES	M	F	M/F – ratio	Sex not known	Total	%
N. meningitidis	31	34	**0.7**	2	**67**	***19.7***
H. influenzae	13	15	**0.9**	2	**30**	***8.8***
S. pneumoniae	79	77	**1.0**	1	**157**	***46.2***
E. coli	4	4	**1.0**	0	**8**	***2.3***
S. agalactiae	10	14	**0.7**	0	**24**	***7.0***
L. monocytogenes	10	10	**1.0**	0	**20**	***5.9***
S. pyogenes	3	4	**0.8**	0	**7**	***2.1***
S. aureus	3	2	**1.5**	0	**5**	***1.5***
Coag.neg.Staph.	3	3	**1.0**	0	**6**	***1.8***
C. neoformans	6	1	**6.0**	0	**7**	***2.1***
Others	8	1	**8.0**	0	**9**	***2.6***
non viable	0	0	**0.0**	0	**0**	***0.0***
Total	**170**	**165**	**1.0**	**5**	**340**	***100.0***
%	***50.0***	***48.5***		***1.5***	***100***	

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2017 the Reference Laboratory received 175 *Neisseria meningitidis* isolates of which 46 were isolated from CSF (or CSF and blood) (36 in 2016) and 129 from blood only (100 in 2016). In addition, 21 culture negative CSF and 5 blood samples were tested positive for meningococcoi by PCR. In total we received meningococcal isolates or PCR positive CSF or blood from 201 patients The distribution of isolates according to month of receipt shows the highest number of isolates in the first quarter (figure 4.1).

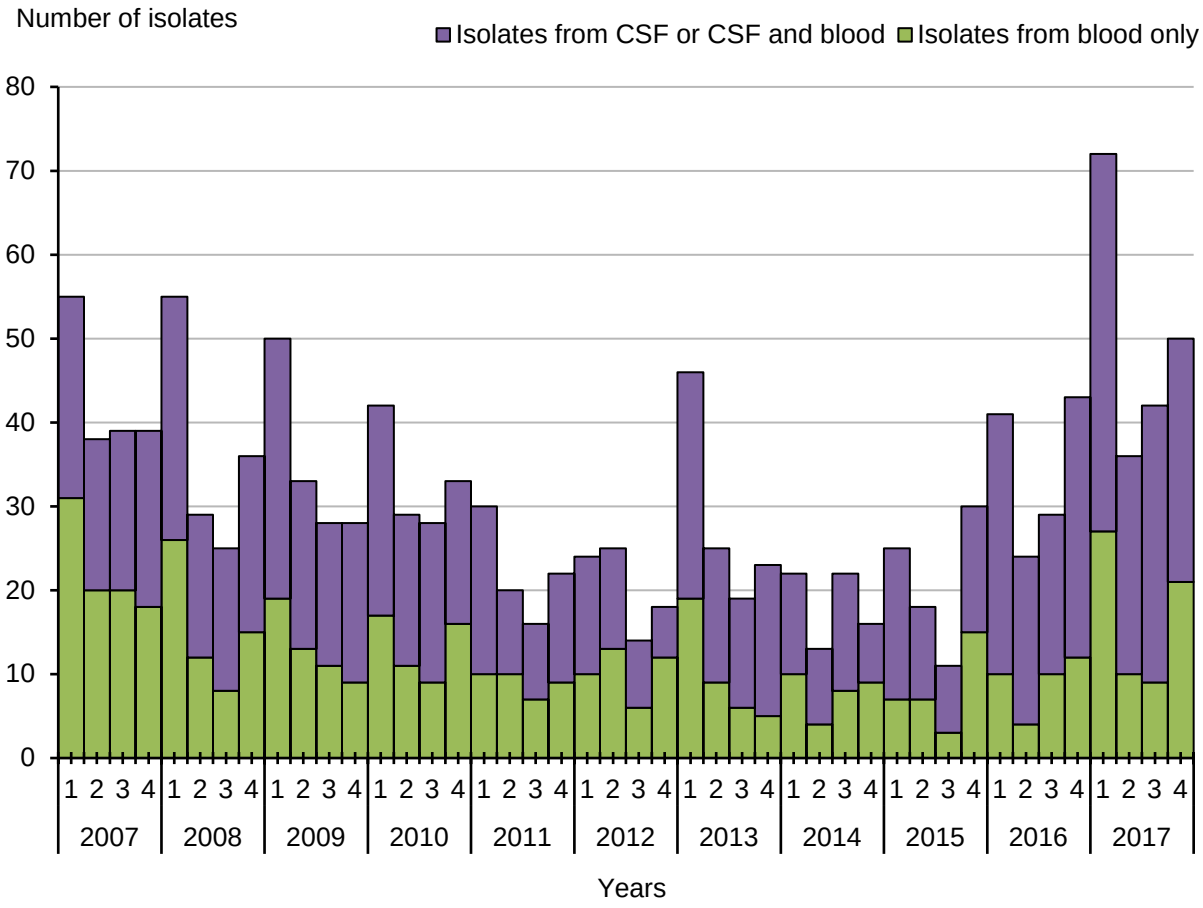

Figure 4.1 *Seasonal distribution of meningococcal disease, 2007-2017*

4.2 Antibiotic susceptibility

Eighty-one percent of all isolates (141/175) were susceptible to penicillin (MIC ≤ 0.064 µg/ml). This is lower than in previous years. The proportion of susceptible isolates varied between 65% in 2012 to 95% in 2015. In general, mutations in *penA* encoding a penicillin binding protein confers the meningococcus to reduced penicillin susceptibility. Nucleotide sequence analyses of *penA* confirmed the increase of the number of reduced penicillin susceptible meningococcal isolates. All isolates were susceptible to Rifampicine.

Table 4.1 Susceptibility of *N. meningitidis* CSF and/or blood isolates to penicillin, 2017

	Penicillin*					
	MIC ≤ 0.064 sensitive	0.064< MIC≤0.25	0.25< MIC≤1.0	MIC >1.0	Total	%
CSF or CSF and blood	37	9	0	0	**46**	**26**
Blood only	104	24	1	0	**129**	**74**
Total	**141**	**33**	**1**	**0**	**175**	**100**
%	**80**	**19**	**1**	**0**	**100**	

* MIC values in µg/ml

Table 4.2 Susceptibility of *N. meningitidis* from CSF/ CSF and blood to penicillin, 2010-2017

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064< MIC≤0.25		0.25< MIC≤1.0		MIC >1.0		Total
	N	%	N	%	N	%	N	%	
2010	43	**81.1**	10	**18.9**	0	**0.0**	0	**0.0**	**53**
2011	29	**78.4**	8	**21.6**	0	**0.0**	0	**0.0**	**37**
2012	24	**58.5**	16	**39.0**	1	**2.4**	0	**0.0**	**41**
2013	35	**89.7**	3	**7.7**	1	**2.6**	0	**0.0**	**39**
2014	26	**83.9**	5	**16.1**	0	**0.0**	0	**0.0**	**31**
2015	32	**97.0**	1	**3.0**	0	**0.0**	0	**0.0**	**33**
2016	32	**88.0**	4	**12.0**	0	**0.0**	0	**0.0**	**36**
2017	37	**80.4**	9	**19.6**	0	**0.0**	0	**0.0**	**46**

* MIC values in µg/ml

Table 4.3 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2010-2017

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064< MIC≤0.25		0.25< MIC≤1.0		MIC >1.0		Total
	N	%	N	%	N	%	N	%	
2010	67	**84.8**	12	**15.2**	0	**0.0**	0	**0.0**	79
2011	34	**64.2**	19	**35.9**	0	**0.0**	0	**0.0**	53
2012	27	**67.5**	13	**32.5**	0	**0.0**	0	**0.0**	40
2013	53	**73.6**	18	**25.0**	1	**1.4**	0	**0.0**	72
2014	37	**88.1**	5	**11.9**	0	**0.0**	0	**0.0**	42
2015	48	**94.1**	3	**5.9**	0	**0.0**	0	**0.0**	51
2016	88	**88.0**	12	**12.0**	0	**0.0**	0	**0.0**	100
2017	104	**80.6**	24	**18.6**	1	**0.8**	0	**0.0**	129

* MIC values in µg/ml

4.3 Serogroups

Serogroup B accounted for 41% (2016: 49%; 2015: 74%; 2014: 73%) of all isolates. Group C and Y are responsible for 5% and 14%, respectively. The proportion group W increased to 41% (was 34% in 2016; 10% in 2015) (table 4.4). The serogroup distribution observed during the whole collection period 1959 - 2017 (figure 4.2) shows that in 2014 the number of group B isolates (53 cases) was the lowest since 1976. In 2017, the number of group B isolates (81) was slightly higher than the previous year. The proportion of group C isolates was 24% in 1991, decreased to about 10% in 1994 and was since then increasing with a sharp rise from 19% (105 cases) in 2000 to 40% (276 cases) in 2001 (figure 4.2 and figure 4.2.1). In June 2002, vaccination against serogroup C was included in the National Immunisation Programme. Since then, the number of serogroup C isolates received by the Reference Laboratory rapidly decreased to only a few isolates per year; in 2017 there was a slight increase from 3 group C isolates in 2014 to 9 in 2017 (figure 4.3). Since November 2015, the proportion of group W increased, similar to what was observed in England and Wales since 2009 (Ladhani SN et al. Increase in endemic *Neisseria meningitidis* capsular group W sequence type 11 complex associated with severe invasive disease in England and Wales. Clin Infect Dis. 2015;60:578-85).

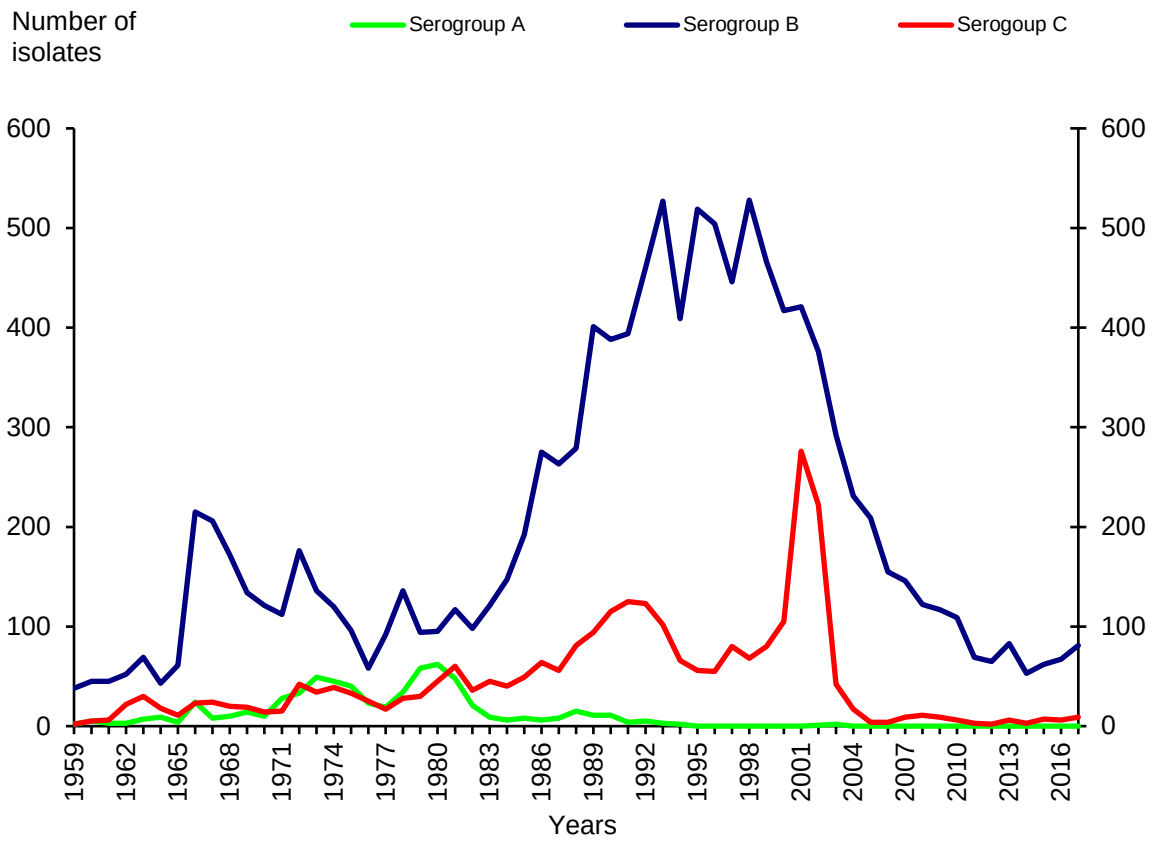

Figure 4.2. *Distribution of meningococcal serogroups A, B and C, 1959-2017*

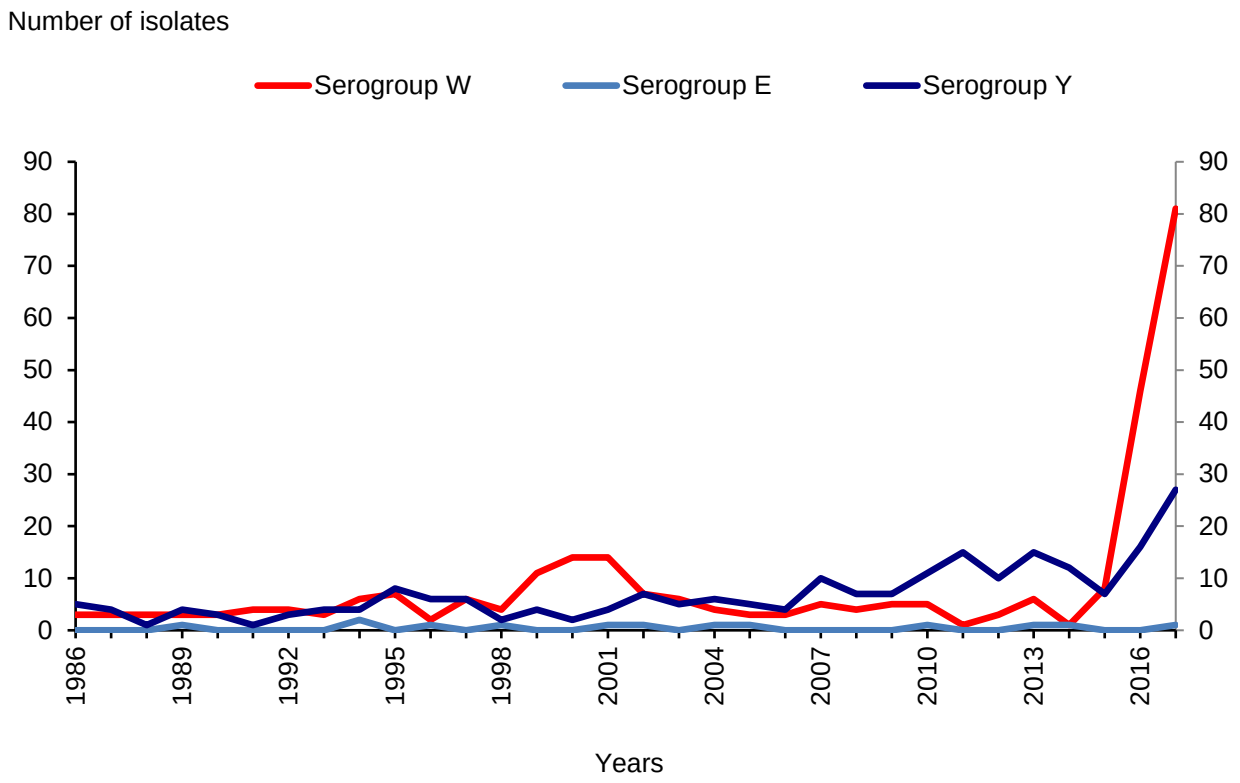

Figure 4.3. *Distribution of meningococcal serogroups Y, W and E, 1986-2017*

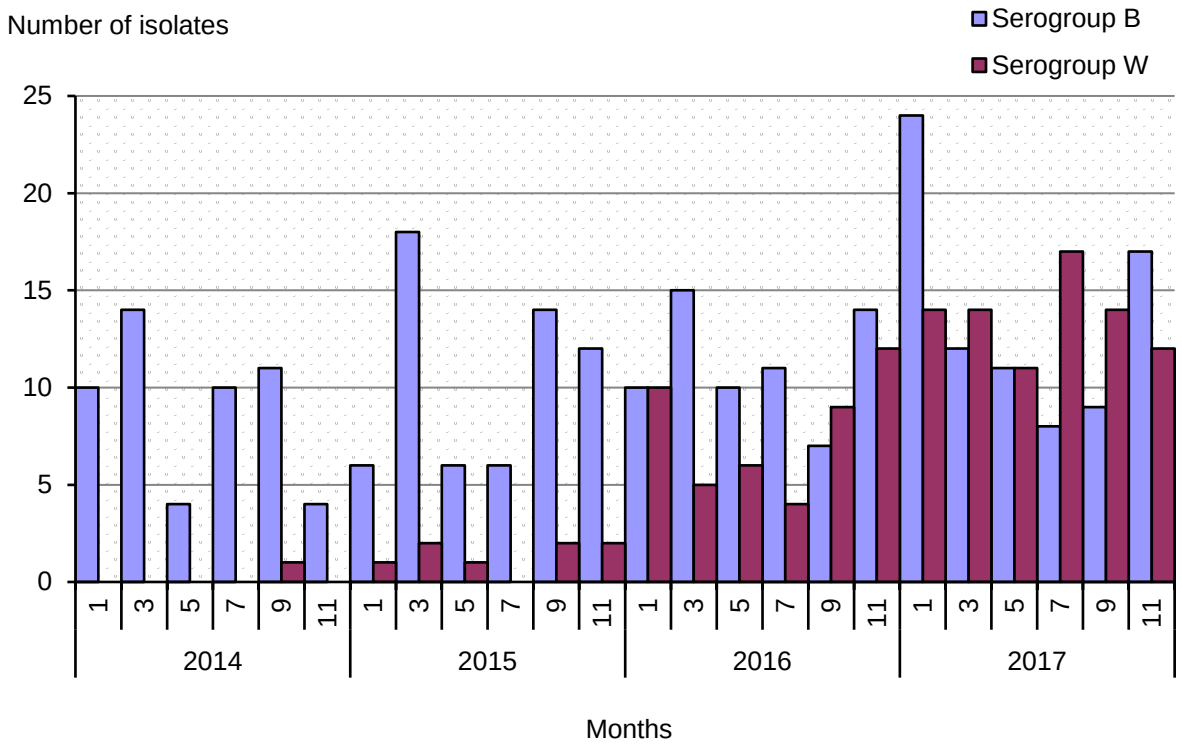

Figure 4.4 *Bimonthly distribution of meningococcal serogroups B and W, 2014-2017*

4.4 Serogroup and age

The age distribution of patients with meningitis and/or meningococcemia shows that 19% (38 of 201) of the patients was younger than 5 years (table 4.4. figure 4.4). Of 134 patients from whom meningococci were isolated from blood only,16 (12%) were younger than 5 years of age, while 43 (32%) were older than 65 years of age (table 4.7). Among serogroup B cases, 74% (61/82) were younger than 25 years of age. In contrast, 88% (71/81) of the serogroup W cases were older than 15 years of age. In addition, of 81 serogroup B isolates, 49 (60%) were from CSF, while of 81 serogroup W isolates only 10 (12%) were from CSF.

Table 4.4 Serogroups of *N. meningitidis* (isolates or PCR positieve samples from CSF and /or blood; absolute numbers) by patient age, 2017

	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	T	%
B	0	11	18	29	3	5	18	6	3	6	5	7	**82**	***40.8***
C	0	1	0	1	0	0	1	0	0	1	2	4	**9**	***4.5***
E	0	0	0	0	0	0	1	0	0	0	0	0	**1**	***0.5***
W	0	4	4	8	0	2	13	5	2	11	22	18	**81**	***40.3***
Y	0	0	0	0	0	0	1	1	0	0	6	19	**27**	***13.4***
NG*	0	0	0	0	0	0	0	1	0	0	0	0	**1**	***0.5***
Total	**0**	**16**	**22**	**38**	**3**	**7**	**34**	**13**	**5**	**18**	**35**	**48**	**201**	***100.0***
%	***0***	***8***	***10.9***	***18.9***	***1.5***	***3.5***	***16.9***	***6.5***	***2.5***	***8.9***	***17.4***	***23.9***	***100.0***	

*Non Groupable

Table 4.5 Serogroups of *N. meningitidis* (isolates or PCR positieve samples from CSF, or CSF* and blood; absolute numbers) by patient age, 2017

	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	T	%
B	0	7	12	19	2	3	13	2	2	3	2	3	**49**	***73.1***
C	0	1	0	1	0	0	1	0	0	0	1	1	**4**	***6.0***
E	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0***
W	0	1	1	2	0	0	3	1	0	2	2	0	**10**	***14.9***
Y	0	0	0	0	0	0	1	0	0	0	2	1	**4**	***6.0***
NG**	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0***
Total	**0**	**9**	**13**	**22**	**2**	**3**	**18**	**3**	**2**	**5**	**7**	**5**	**67**	***100.0***
%	***0.0***	***13.4***	***19.4***	***32.8***	***3.0***	***4.5***	***26.8***	***4.5***	***3.0***	***7.5***	***10.4***	***7.5***	***100***	

* From 1 patient with a meningococci isolated from skin biopsy. CSF was culture-negative but CSF-PCR positive for meningococcal DNA.
**Non Groupable

Table 4.6 Age distribution of meningitis (incidence per 100.000 inhabitants) by different serogroups of *N. meningitidis* (isolates or PCR positive samples from CSF, or CSF and blood), 2017

	AGE (YEARS)										TOTAL
Group	0	1-4	5-9	10-14	15-19	20-24	25-29	30-49	50-64	≥65	T
B	4.06	1.71	0.22	0.31	1.26	0.19	0.18	0.07	0.06	0.09	**0.29**
C	0.58	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.03	0.03	**0.02**
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
W	0.58	0.14	0.00	0.00	0.29	0.09	0.00	0.05	0.06	0.00	**0.06**
Y	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.06	0.03	**0.02**
NG*	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Total	**5.22**	**1.86**	**0.22**	**0.31**	**1.74**	**0.28**	**0.18**	**0.11**	**0.20**	**0.16**	**0.39**

*Non Groupable

Table 4.7 Serogroups of *N. meningitidis* (isolates or PCR positive samples from blood only; absolute numbers) by patient age, 2017

	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	T	%
B	0	4	6	10	1	2	5	4	1	3	3	4	**33**	***24.6***
C	0	0	0	0	0	0	0	0	0	1	1	3	**5**	***3.7***
E	0	0	0	0	0	0	1	0	0	0	0	0	**1**	***0.8***
W	0	3	3	6	0	2	10	4	2	9	20	18	**71**	***52.9***
Y	0	0	0	0	0	0	0	1	0	0	4	18	**23**	***17.2***
NG*	0	0	0	0	0	0	0	1	0	0	0	0	**1**	***0.8***
Total	**0**	**7**	**9**	**16**	**1**	**4**	**16**	**10**	**3**	**13**	**28**	**43**	**134**	***100.0***
%	***0.0***	***5.2***	***6.7***	***11.9***	***0.8***	***3.0***	***11.9***	***7.5***	***2.2***	***9.7***	***20.9***	***32.1***	***100.0***	

*Non Groupable

Table 4.8 Age distribution of meningococcemia (incidence per 100.000 inhabitants) by different serogroups of *N. meningitidis* (isolates from blood only), 2017

	AGE (YEARS)										TOTAL
Group	0	1-4	5-9	10-14	15-19	20-24	25-29	30-49	50-64	≥65	T
B	2.32	0.86	0.11	0.20	0.48	0.38	0.09	0.07	0.08	0.13	**0.19**
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.09	**0.03**
E	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	**0.01**
W	1.74	0.43	0.00	0.20	0.97	0.38	0.18	0.20	0.56	0.57	**0.42**
Y	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.11	0.57	**0.13**
NG*	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	**0.01**
Total	**4.06**	**1.29**	**0.11**	**0.41**	**1.55**	**0.94**	**0.28**	**0.29**	**0.79**	**1.36**	**0.78**

*Non Groupable

4.5 Group B meningococci

Figure 4.4 shows the age distribution of group B and W meningococcal disease. The age-specific incidences for serogroup B per 100.000 inhabitants in the age groups younger than 5 years and 15 - 19 years were 3.2 and 1.7 respectively (0.9 and 1.3 for serogroup W). The age-specific incidences per 100.000 inhabitants in the age groups >19 years was less than 0.5. For serogroup W a small peak appeared in agegroup 50-60 (incidence of 0.8 and 0.6). At age 85-94 the incidence of serogroup W meningococcal disease was 2.9 and 2.0.

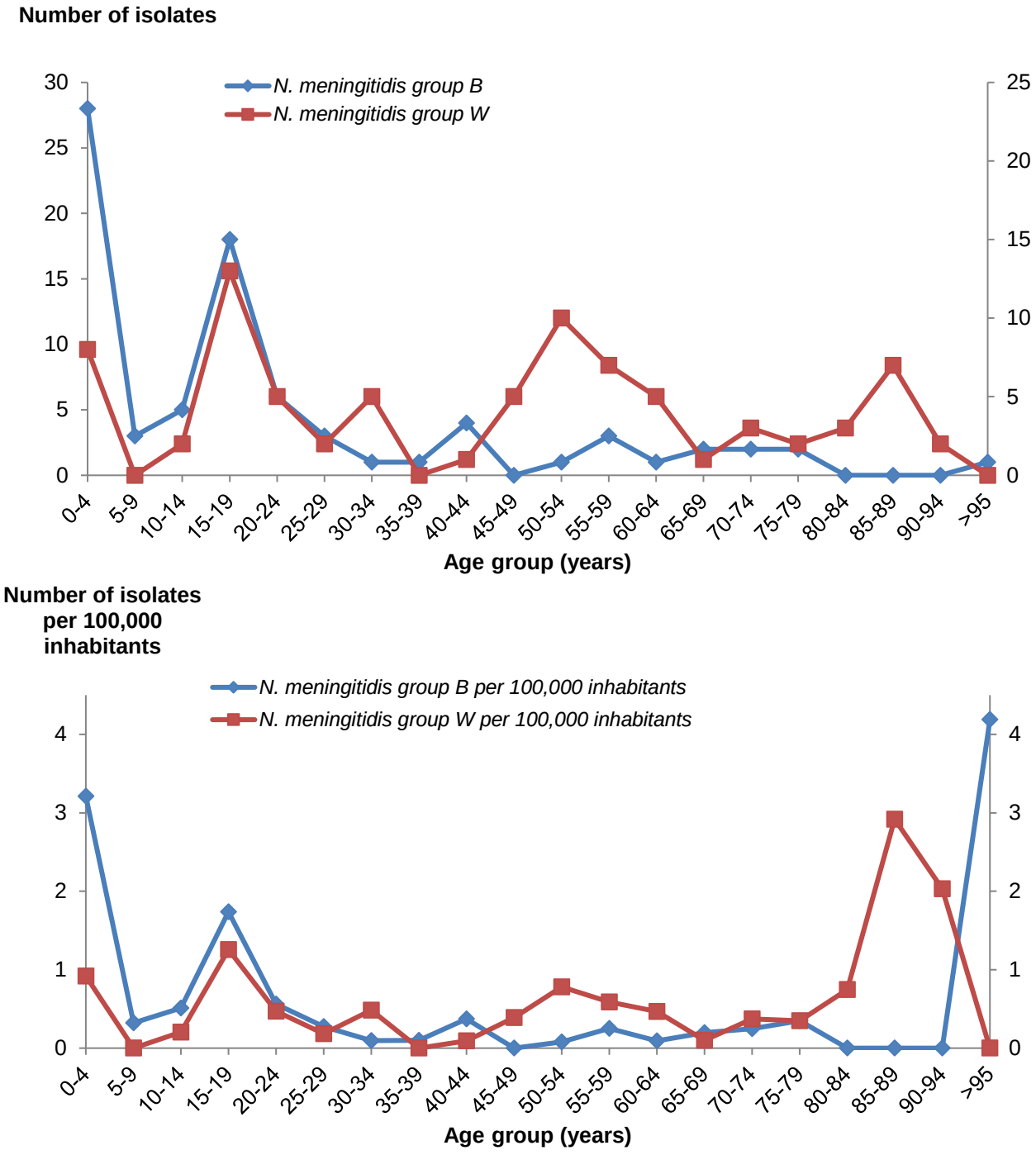

Figure 4.4 *Age distribution of serogroup B and W meningococcal disease in 2017*

4.6 Distribution of PorA genosubtypes among serogroup B, C and W meningococci

The monoclonal antibodies used for (sub)typing of meningococci are no longer available. Therefore, from January 1, 2005 onwards, typing of meningococcal isolates using monoclonal antibodies is not performed anymore by the Reference Laboratory. Instead, epitopes of PorA and FetA are determined by sequencing of their DNA coding regions.

The epitopes of PorA that react with the monoclonal antibodies of the subtyping scheme are encoded by the variable regions VR1 and VR2 of *porA*. encoding the outer membrane protein PorA. 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. which are then compared with the PorA epitopes present in the database available on the website: http://neisseria.org/nm/typing/pora/

In 2017, 11 PCR positive samples were not typeable. Among 71 serogroup B meningococci, 36 different VR1/VR2 combinations were encountered (2014: 28; 2015: 32; 2016: 35). The proportion of the dominant PorA genosubtype P1.7-2.4 decreased from 40% of all serogroup B isolates in 2000 to 7% in 2017 (figure 4.5, figure 4.7; table 4.9). Eighty-six percent (61/71) of the serogroup B isolates had at least one of the PorA epitopes present in the NonaMen vaccine currently in development (table 4.9).

The nine serogroup C isolates had 5 different VR1/VR2 combinations. P1.5.2 (3), P1.5-1.10-8, P1.18-1.deletion, P1.7-1.1 and P1.17.16-4.

In 2017, we received 8 PCR positive samples, of which only three complete typing was possible. Of 74 serogroup W cases, 74 (91%) had P1.5.2, one P1.18-1.3 and one type P1.22.26.

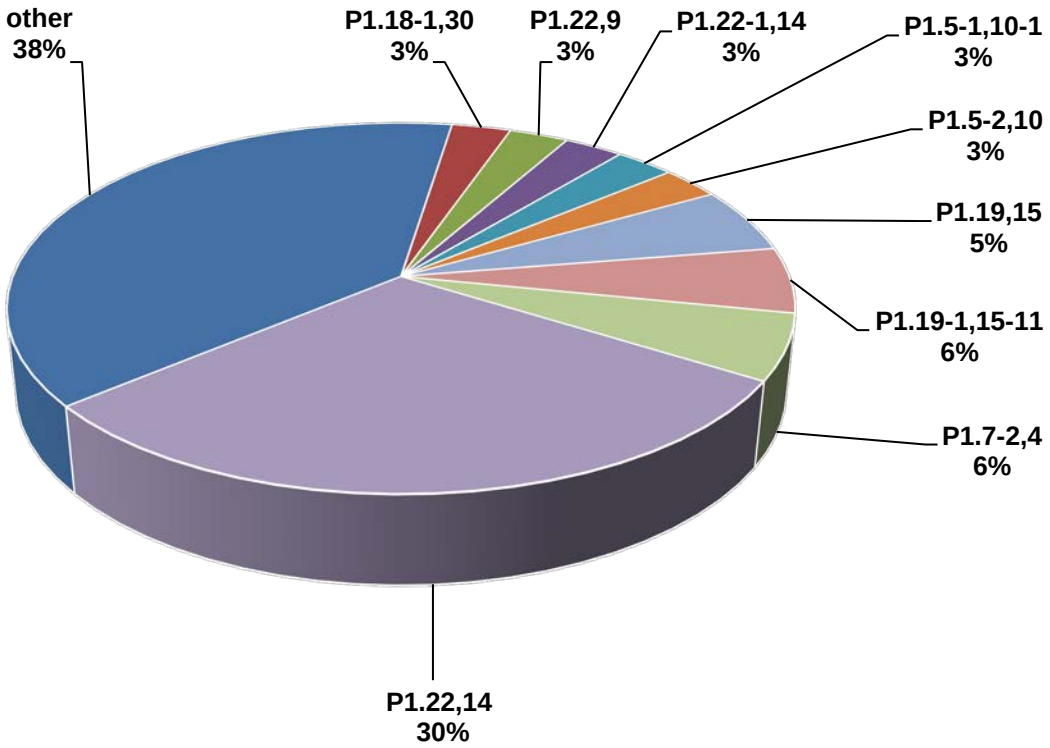

Figure 4.5 *Distribution of group B meningococcal PorA types, 2017*

Table 4.9 *N. meningitidis* serogroup B isolates according to PorA genosubtype, 2013-2017

	VR1.VR2 combination	YEAR									
		2013		2014		2015		2016		2017	
		No.	%	No.	%	No.	%	No.	%	No.	%
Vaccine types*	1.5-1. 2-2	0	**0**	0	**0**	0	**0**	1	**1.5**	1	**1.4**
	1.5-1. other	1	**1.2**	1	**1.9**	1	**1.6**	2	**3.0**	3	**4.2**
	1.5-2.10	7	**8.4**	4	**7.5**	3	**4.8**	3	**4.4**	2	**2.9**
	1.5-2. other	0	**0**	1	**1.9**	2	**3.2**	1	**1.5**	2	**2.9**
	1.7.16	1	**1.2**	0	**0**	1	**1.6**	2	**3.0**	1	**1.4**
	1.7. other	5	**6.0**	1	**1.9**	2	**3.2**	2	**3.0**	3	**4.2**
	1.7-1. 1	0	**0**	1	**1.9**	3	**4.8**	2	**3.0**	0	**0**
	1.7-1. other	0	**0**	1	**1.9**	0	**0**	1	**1.5**	1	**1.4**
	1.7-2.4	7	**8.4**	8	**15.0**	7	**11.3**	8	**11.9**	5	**7.0**
	1.7-2. other	13	**15.7**	3	**5.7**	5	**8.2**	4	**6.0**	2	**2.9**
	1.12-1.13	0	**0**	0	**0**	0	**0**	0	**0**	0	**0.0**
	1.12-1. other	1	**1.2**	1	**1.9**	2	**3.2**	2	**3.0**	1	**1.4**
	1.18-1.3	3	**3.6**	0	**0**	1	**1.6**	2	**3.0**	0	**0.0**
	1.18-1. other	3	**3.6**	9	**17.0**	5	**8.2**	4	**6.0**	4	**5.6**
	1.19.15-1	3	**3.6**	2	**3.7**	2	**3.2**	1	**1.5**	1	**1.4**
	1.19. other	3	**3.6**	3	**5.7**	3	**4.8**	3	**4.4**	4	**5.6**
	1.22.14	9	**10.9**	9	**17.0**	9	**14.6**	11	**16.4**	21	**29.6**
	1.22.other	6	**7.3**	3	**5.7**	3	**4.8**	4	**6.0**	4	**5.6**
	Other. 14	2	**2.4**	1	**1.9**	1	**1.6**	2	**3.0**	4	**5.6**
	Other. 16	3	**3.6**	1	**1.9**	2	**3.2**	1	**1.5**	2	**2.9**
	Subtotal vaccine types	67	**80.7**	49	**92.5**	52	**83.9**	56	**83.6**	61	**86.0**
NVT**	Other **Non Vaccine Type**	16	**19.3**	4	**7.5**	10	**16.1**	11	**16.4**	10***	**14.0**
	Total	83	**100.0**	53	**100.0**	62	**100.0**	67	**100.0**	71	**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

***10 isolates with a Non vaccine type; 11 PCR positive samples were not typeable.

4.7 Distribution of FetA genosubtypes among serogroup B, C and W meningococci

In addition to sequencing of PorA epitopes, meningococcal isolates are also characterized by sequencing of an epitope of FetA. This outer membrane protein 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 aminoacid sequence. So far, about 270 VR sequences comparising 6 classes. are identified. available at
http://neisseria.org/perl/agdbnet/agdbnet.pl?file=fetavr.xml
As an example of a type designation: F5-2. in which the first digit indicates the class and the second digit the variant of this class.

In 2017, 18 different FetA variants were observed among serogroup B meningococci. The dominant types were F1-5 and F 5-1, accounting for respectively 17% and 24% of group B meningococci (figure 4.6 and 4.7; table 4.10). In previous years the dominant type was F1-5 wich was strongly linked to PorA VR1/VR2 P1.7-2.4 and together to the MLST clonal complex ST41/44. In 2017, Fet A type F1-5 of 11 isolates was linked with 9 different PorA types. FetA type F5-1 (17) was 12 times linked with PorA VR1/VR2 P1.22.14.

The eight serogroup C meningococci had four different FetA types: F1-5. F3-3. F3-6 and F3-9. In 2017, we received 81 serogroup W samples: 73 isolates and 8 PCR positive samples. From only three PCR samples complete typing was possible. Of 76 serogroup W cases, 74 (91%) had P1.5.2. one P1.18-1.3 and one type P1.22.26.
The 81 meningococci W cases had only 4 different FetA types (F1-1. F3-7, F4-1 and F5-9). Seventy-two isolates (89%) had FetA type F1-1, linked to PorA VR1/VR2 P1.5.2 and MLST clonal complex 11.

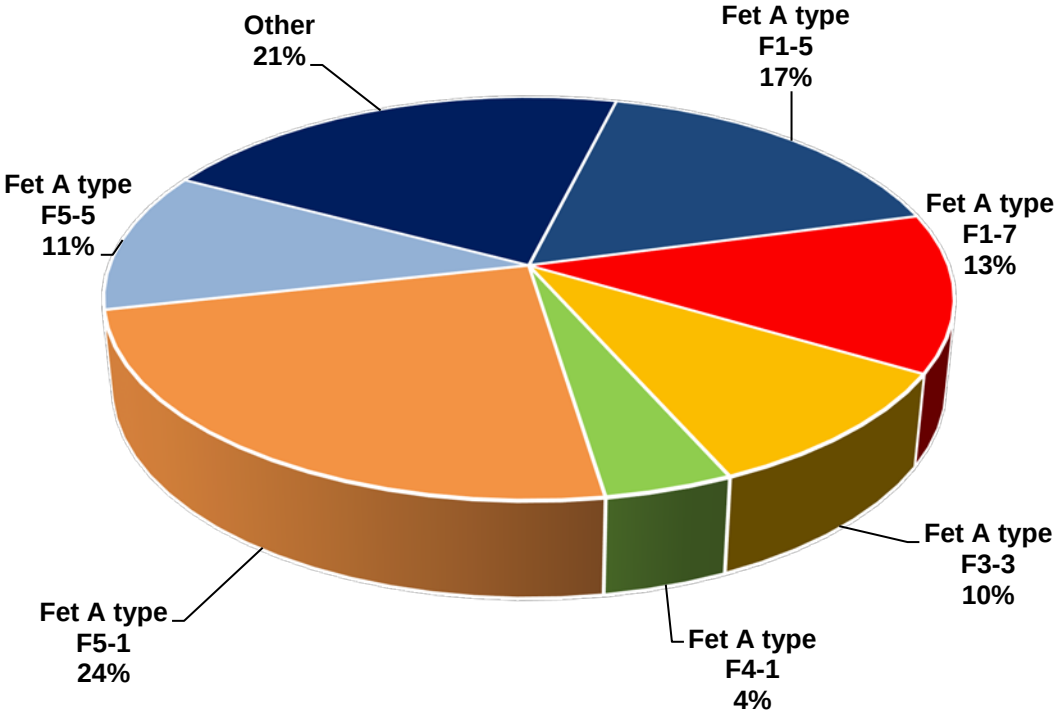

Figure 4.6 *Distribution of group B meningococcal FetA genosubtypes, 2017*

Table 4.10 *N. meningitidis* serogroup B isolates according to FetA genosubtype, 2013-2017

FetA type	YEARS									
	2013		2014		2015		2016		2017	
	No.	%	No.	%	No.	%	No.	%	No.	%
F1-5	17	20.5	8	15.1	10	16.1	16	23.9	12	16.9
F1-7	6	7.2	5	9.4	9	14.5	4	6.0	9	12.7
F1-15	1	1.2	1	1.9	1	1.6	0	0.0	1	1.4
F3-3	6	7.2	10	18.9	9	14.5	7	10.4	7	9.9
F3-7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
F3-9	2	2.4	1	1.9	0	0.0	2	3.0	0	0.0
F4-1	2	2.4	1	1.9	2	3.2	0	0.0	3	4.2
F5-1	14	16.9	14	26.4	10	16.2	12	17.9	17	23.9
F5-2	2	2.4	0	0.0	2	3.2	0	0.0	1	1.4
F5-5	8	9.7	4	7.5	7	11.3	10	14.9	8	11.3
F5-8	0	0.0	1	1.9	1	1.6	0	0.0	0	0.0
F5-12	0	0.0	1	1.9	3	4.8	3	4.5	1	1.4
Other	25	30.1	7	13.2	8	13.0	13	19.4	12	16.9
Total	**83**	**100.0**	**53**	**100.0**	**62**	**100.0**	**67**	**100.0**	**71**	**100.0**

In 2017, 36 different VR1/VR2 combinations and 18 different FetA variants were encountered among serogroup B meningococci. Among the dominant FetA type F5-1, accounting for 24% of group B meningococci, one was of P1.5-2.10:F5-1, 12 were of P1.22.14:F5-1, two were of type P1.19.15:F5-1 and two had another combination. Frequently found combinations were P1.22.14:F5-1 (17%) , P1.22.14:F5-5 (9%) and P1.7-2.4:F1-5 (5%) (Figure 4.7).

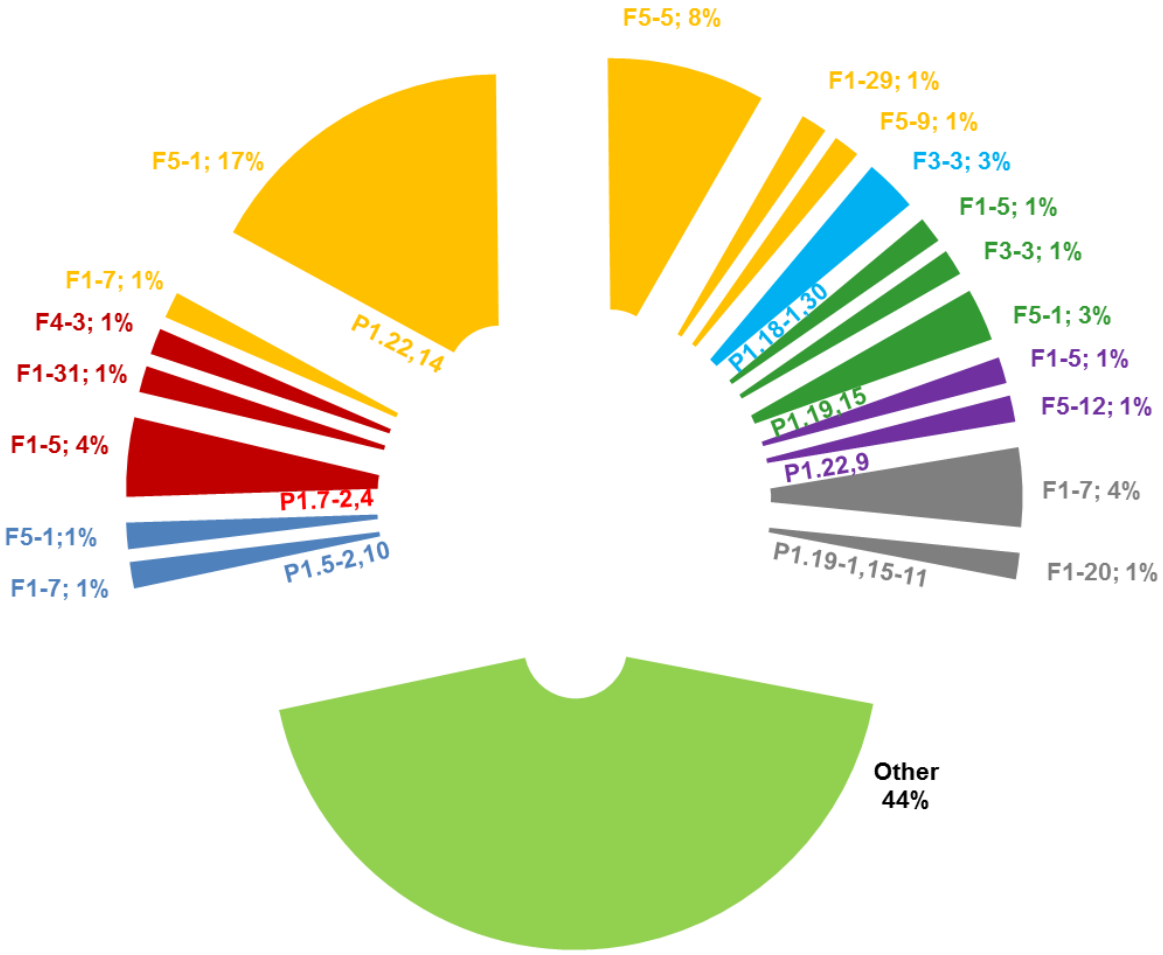

Figure 4.7 *Distribution of group B meningococcal PorA and FetA geno(sub)types, 2017*

5 *HAEMOPHILUS INFLUENZAE*

5.1 General features

In total, 224 *Haemophilus influenzae* isolates were submitted to the Reference Laboratory. This number is higher than that in previous years (table 2.3, figure 3.3, figure 5.1). Thirty isolates were from CSF (or CSF and blood) (2016: 26; 2015: 22; 2014: 21). and 194 from blood only. Fourty-six (20%) 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 Reference Laboratory 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.

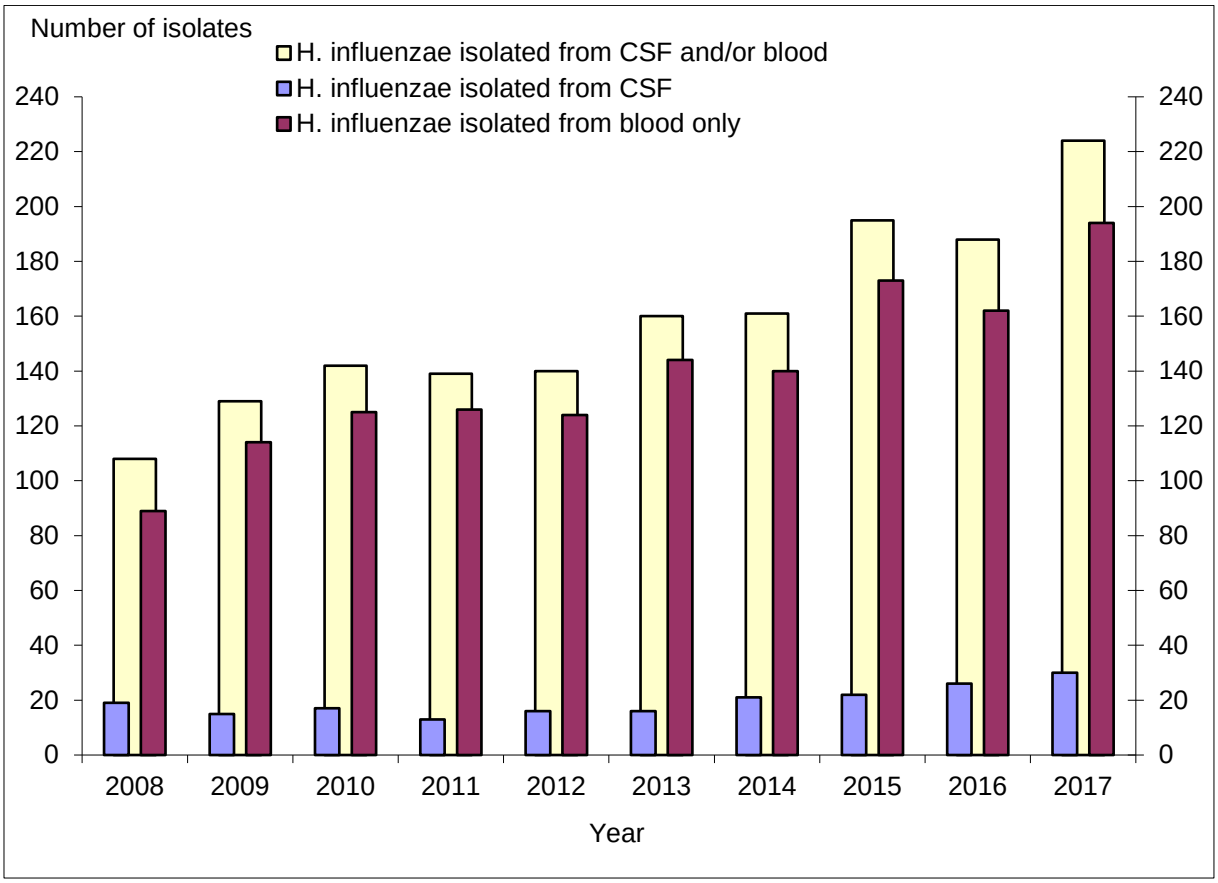

Figure 5.1 *Distribution of H. influenzae, 2008-2017*

5.2 Antibiotic susceptibility

The proportion of ß-lactamase producing invasive *H. influenzae* isolates (CSF and/or blood) was decreasing since 2004 and reached a remarkable low value of less than 1% in 2008. 2010 shows the highest value (14.8%) in 25 years. During the history of the Reference Laboratory the proportion has always fluctuated. The reason for this fluctuation is unknown.

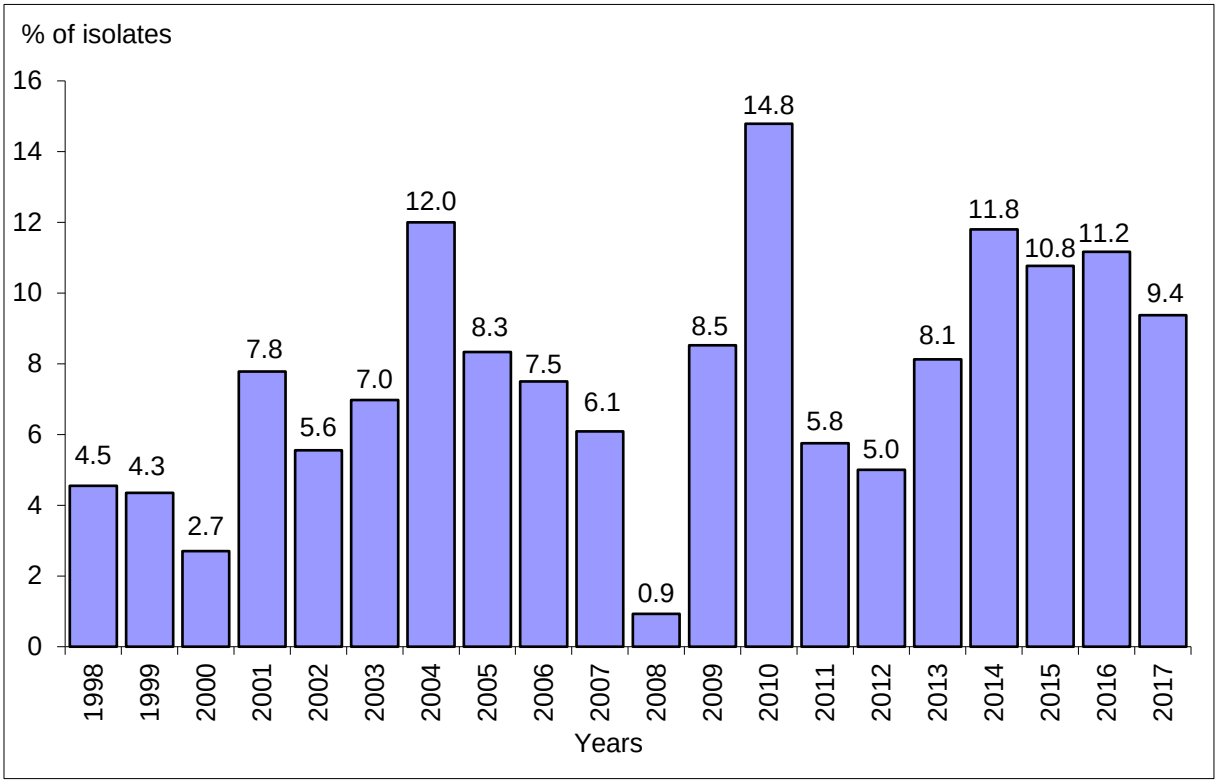

Figure 5.2 *Percentage β-lactamase producing H. influenzae, 1998-2017*

5.3 Serotype and age

Seven cases of *H. influenzae* type b invasive disease were observed among children younger than 2 years of age (13 in 2016; 8 in 2015; 6 in 2014) (figure 5.3). Of 224 *H. influenzae* isolates, 159 were non-typeable; 10 isolated from CSF (or CSF and blood) and 138 isolated from blood only (table 5.1. 5.2 and 5.3). Non-typable strains were isolated more frequently than type b isolates (table 5.1).

Table 5.1 Total number of *H.influenzae isolates* from CSF and/or blood, according to serotype and age, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
a	0	0	0	0	0	0	1	0	**1**	***1***
b	0	4	10	14	4	0	7	21	**46**	***20***
e	0	0	0	0	0	0	1	3	**4**	***2***
f	0	0	0	0	1	0	2	11	**14**	***6***
n.t.*	5	3	10	18	1	3	23	114	**159**	***71***
Total	**5**	**7**	**20**	**32**	**6**	**3**	**34**	**149**	**224**	***100***
%	***2***	***3***	***9***	***14***	***3***	***1***	***15***	***67***	***100***	

* non-typeable

Table 5.2 *H.influenzae* isolates from CSF (or CSF and blood), according to serotype and age, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
a	0	0	0	0	0	0	0	0	**0**	***0***
b	0	2	4	6	1	0	0	1	**8**	***27***
e	0	0	0	0	0	0	0	0	**0**	***0***
f	0	0	0	0	0	0	0	1	**1**	***3***
n.t.*	0	1	6	7	0	1	3	10	**21**	***70***
Total	0	3	10	13	1	1	3	12	**30**	***100.0***
%	***0.0***	***10***	***33***	***43***	***3***	***3***	***10***	***41***	***100.0***	

* non-typeable

Table 5.3 *H. influenzae* isolates from blood only, according to serotype and age, 2017.

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
a	0	0	0	0	0	0	1	0	**1**	***1***
b	0	2	6	8	3	0	7	20	**38**	***19***
e	0	0	0	0	0	0	1	3	**4**	***2***
f	0	0	0	0	1	0	2	10	**13**	***7***
n.t.*	5	2	4	11	1	2	20	104	**138**	***71***
Total	**5**	**4**	**10**	**19**	**5**	**2**	**31**	**137**	**194**	***100.0***
%	***3***	***2***	***5***	***10***	***3***	***1***	***16***	***70***	***100.0***	

* non-typeable

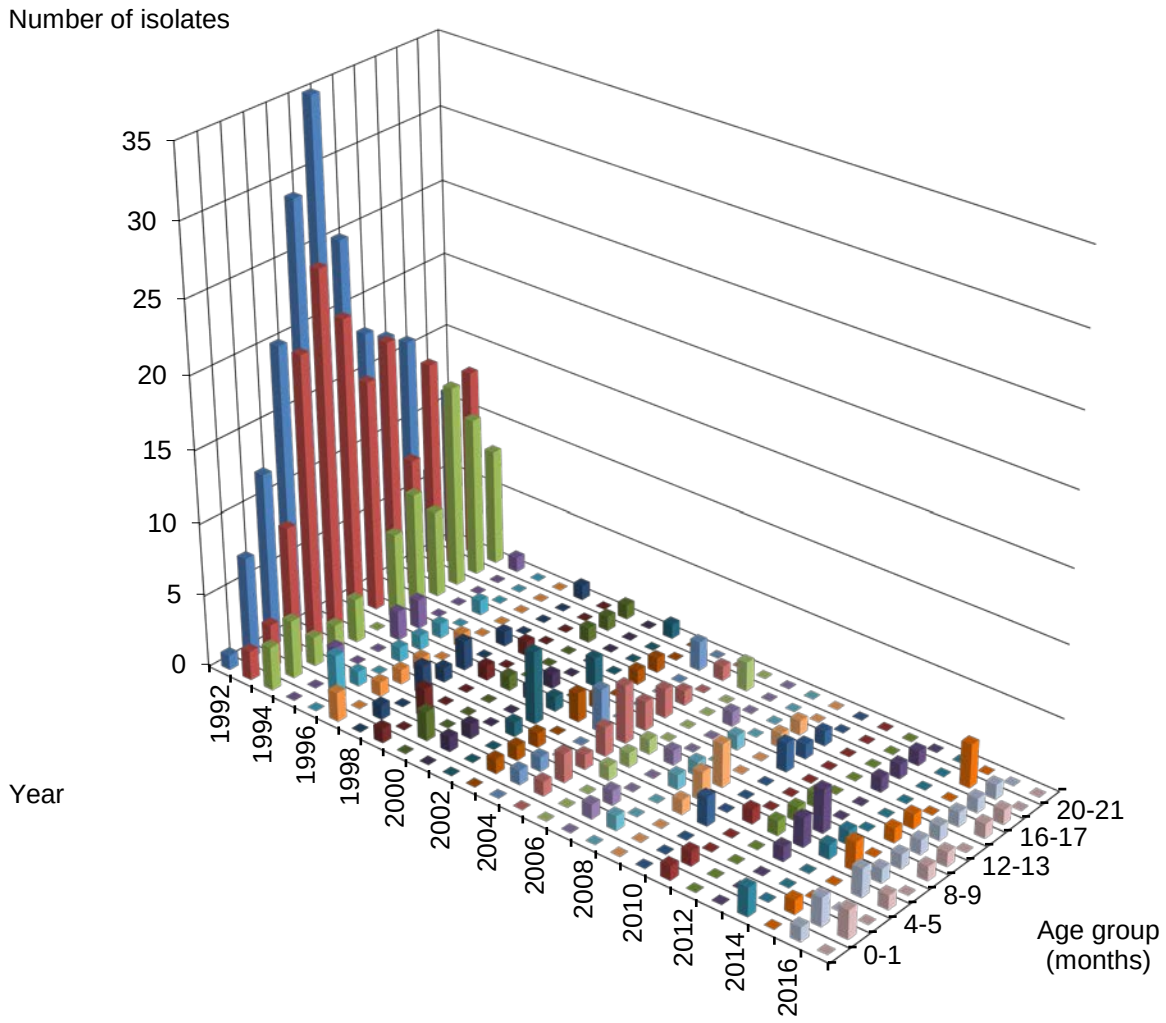

Figure 5.3 *Age distribution of H. influenzae type b invasive disease in the first two years of life, 1992-2017*

5.4 Distribution of non-typable H. influenzae

The proportion of non-typable isolates increased from 6% in 1992 to about 70% from 1997 onwards (table 5.4). In 2017 the proportion of non-typable isolates was 71%.

Table 5.4 *H. influenzae* isolates from CSF and/or blood received from 1992 to 2017 according to year and serotype.

YEAR	SEROTYPE						TOTAL		CSF (or CSF and blood)	Blood Only
	a	b	d	e	f	n.t.*	Total	% n.t.*		
1992	-	294	-	-	1	20	315	**6.3**	241	74
1993	-	244	1	1	3	28	277	**10.1**	204	73
1994	-	148	-	-	2	26	176	**14.8**	112	64
1995	-	60	-	-	-	36	96	**37.5**	50	46
1996	-	30	-	-	6	52	88	**59.1**	28	60
1997	-	19	-	1	6	59	85	**69.4**	22	63
1998	-	19	1	-	5	63	88	**71.6**	31	57
1999	-	12	-	1	1	55	69	**79.7**	23	46
2000	4	15	1	2	4	48	74	**64.9**	24	50
2001	-	17	-	2	8	63	90	**70.0**	19	71
2002	-	31	-	1	13	63	108	**58.3**	28	79
2003	-	31	-	-	8	90	129	**69.8**	27	102
2004	-	48	-	2	4	71	125	**56.8**	32	93
2005	1	41	-	2	10	78	132	**59.1**	37	95
2006	-	24	-	4	7	85	120	**70.8**	25	95
2007	-	24	-	2	2	87	115	**75.7**	19	97
2008	-	25	-	-	11	72	108	**66.7**	19	89
2009	-	32	1	3	9	84	129	**65.1**	15	114
2010	1	37	-	3	5	96	142	**67.6**	17	125
2011	-	22	-	8	11	98	139	**70.5**	13	126
2012	1	28	-	2	8	101	140	**72.1**	16	124
2013	-	29	-	3	13	115	160	**71.9**	16	144
2014	1	30	1	3	8	118	161	**72.7**	21	140
2015	-	34	-	8	20	133	195	**67.7**	22	173
2016	1	44	1	5	12	125	188	**65.4**	26	162
2017	1	46	-	4	14	159	224	**71.0**	30	194

* non-typeable

In 2017, the number of *H. influenzae* type b increased compared to the previous year and was the highest since the last 13 years (Table 5.4, Figure 5.5). Since 2000, the number of cases of nontypeable and serotype b increased. In addition, since 2008, the number of cases due to serotype f is increasing, albeit slowly (Figure 5.5). The absolute number of non-typable isolates from CSF remained stable during the period 1992 to 2006, but decreased somewhat from then on as shown in figure 5.4. In 2017, 21 non-typable isolates from CSF were received; 2 times more than in 2016. The number of non-typable *H. influenzae* isolates from blood increased during the period 1992 to 2017 from 15 to 138 (figure 5.4).

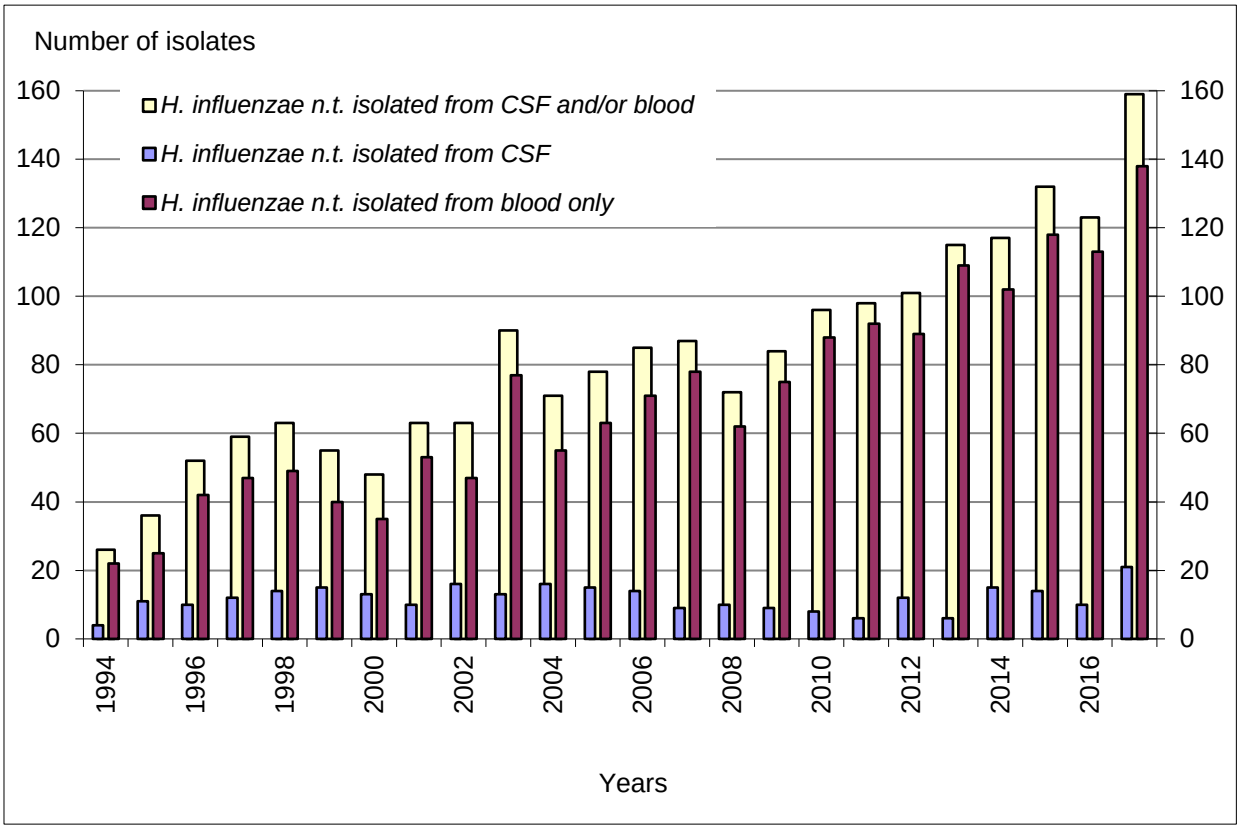

Figure 5.4 *Non-typable H. influenzae isolates from CSF and/or blood received from 1994 - 2017*

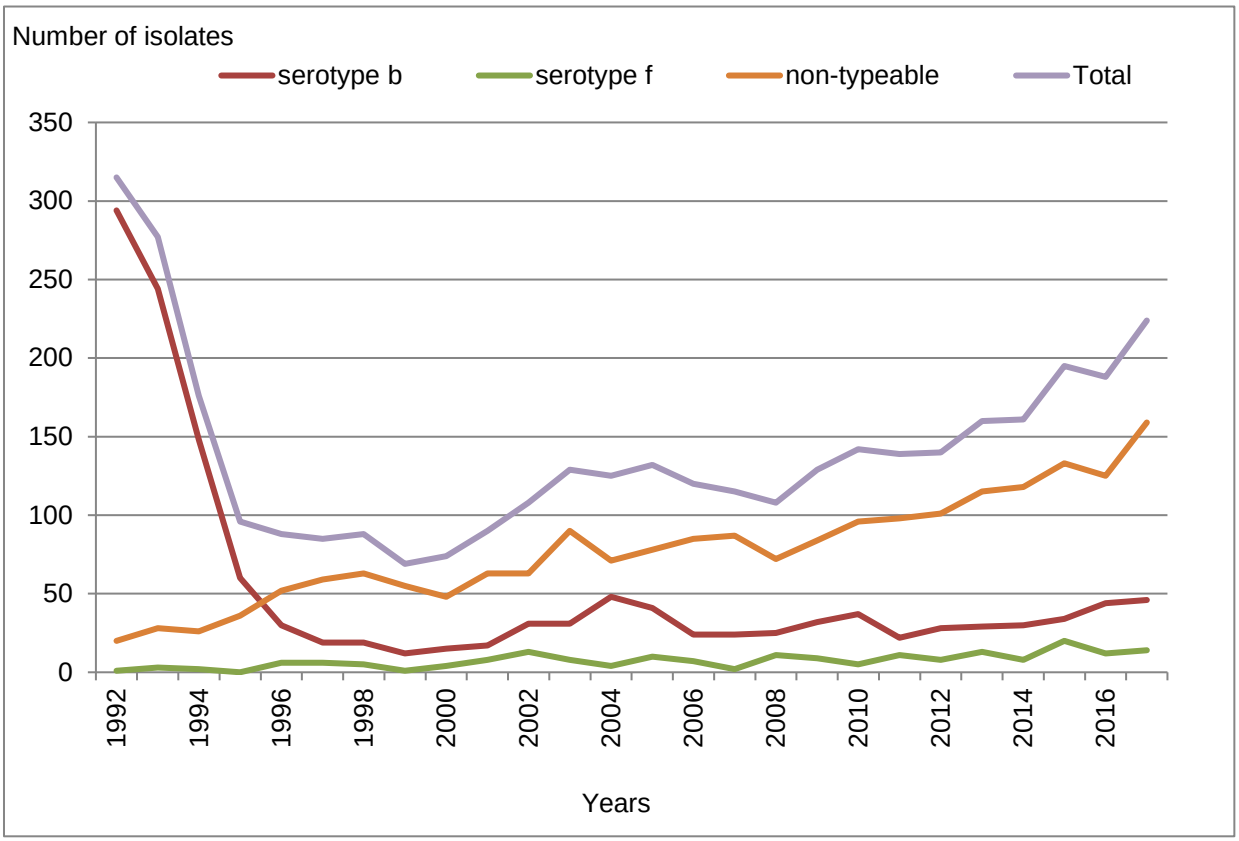

Figure 5.5 *Number of cases due to H. influenza serotype b and f and non-typeable H. influenza, 1992-2017*

Table 5.5 Non-typable *H. influenzae* isolates from CSF and/or blood received from 2008 to 2017 according to year and biotype.

	Biotype							Total
	I	II	III	IV	V	VI	VII	
2008	16	29	18	3	5	1	-	**72**
2009	28	30	12	10	3	1	-	**84**
2010	20	49	19	2	6	-	-	**96**
2011	27	41	24	3	2	1	-	**98**
2012	25	49	17	2	6	1	1	**101**
2013	25	44	30	7	7	2	-	**115**
2014	16	56	32	1	9	3	-	**117**
2015	22	55	45	1	8	-	1	**132**
2016	16	65	30	6	5	-	1	**123**
2017	15	80	51	1	9	3	-	**159**

*non-typable

Among non-serotypable *H. influenzae* isolates biotype II was the predominant biotype during the last ten years. (Table 5.5).

6 *STREPTOCOCCUS PNEUMONIAE*

6.1 General features

From 2003 onwards, the Reference Laboratory asked nine sentinel laboratories, evenly distributed over the country and covering 25% of the population to submit pneumococcal isolates from CSF and/or blood. All medical microbiology laboratories were asked to submit pneumococcal isolates CSF (or CSF and blood). From 2006 all laboratories are requested to submit all invasive pneumococcal isolates from patients in the age group 0-4 year. In 2006, the 7-valent conjugate pneumococcal polysaccharide vaccine was introduced in the National Immunization Programme This vaccine was replaced by the 10-valent vaccine from March 1, 2011 onwards. From 2017, all laboratories are requested to submit all invasive pneumococcal isolates. In 2017, the Reference Laboratory received 1401 isolates nationwide; 627 pneumococcal isolates (from CSF and/or blood) were received from the 9 sentinal laboratories, while 148 isolates from CSF (or CSF and blood) were nationwide submitted to the Reference Laboratory and 9 PCR positive culture negative (CSF or blood) samples were received. The incidence of pneumococcal meningitis gradually increased from 1.0 in 1990 to 1.6 in 2004; due to vaccination with the heptavalent polyscaccharide conjugate vaccine it slightly decreased to 0.9 in 2017.

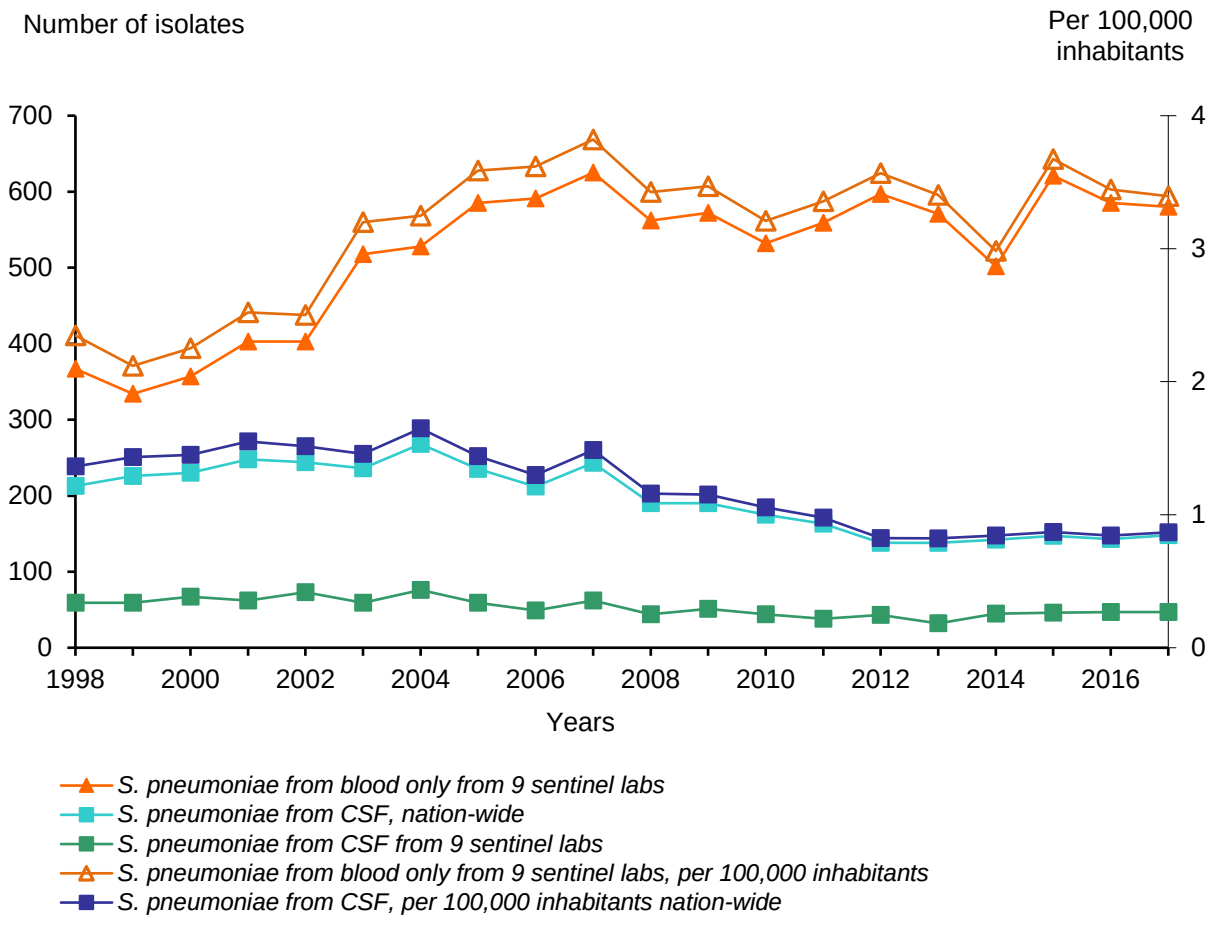

Figure 6.1 *Distribution of S. pneumoniae isolates, 1998-2017*

6.2 Antibiotic susceptibility

Among 148 isolates from CSF (or CSF and blood, nationwide) and 580 isolates from the blood only (9 sentinel labs). 29 isolates from the blood (4%) were intermediately susceptible to penicillin (0.06< MIC≤2.0 mg/L. table 6.1). Eight (5.7% from all CSF isolates) strains isolated from CSF were resistant to penicillin (MIC > 0.06 mg/L).

Table 6.1 Susceptibility of *S. pneumoniae* isolates to penicillin. 2017

	Penicillin*				
	S	I	R	Total	%
MIC for CSF (Nationwade)	MIC ≤ 0.06		MIC > 0.06		
CSF or CSF and blood	140	-	8	**148**	***20.3***
MIC for blood (9 sentinel labs)	MIC ≤ 0.06	0.06< MIC ≤ 2.0	MIC >2.0		
Blood only	551	29	-	**580**	***79.7***
Total	**681**	**29**	**8**	**728**	***100.0***
%	***93.5***	***4.0***	***1.1***	***100.0***	

* MIC values in µg/ml according to EUCAST guidelines

Figure 6.2 shows the distribution of *S. pneumoniae* isolates according to the patients' age. The incidence of pneumococcal meningitis is highest among patients in the age group 70 – 75 year (Table 6.4).

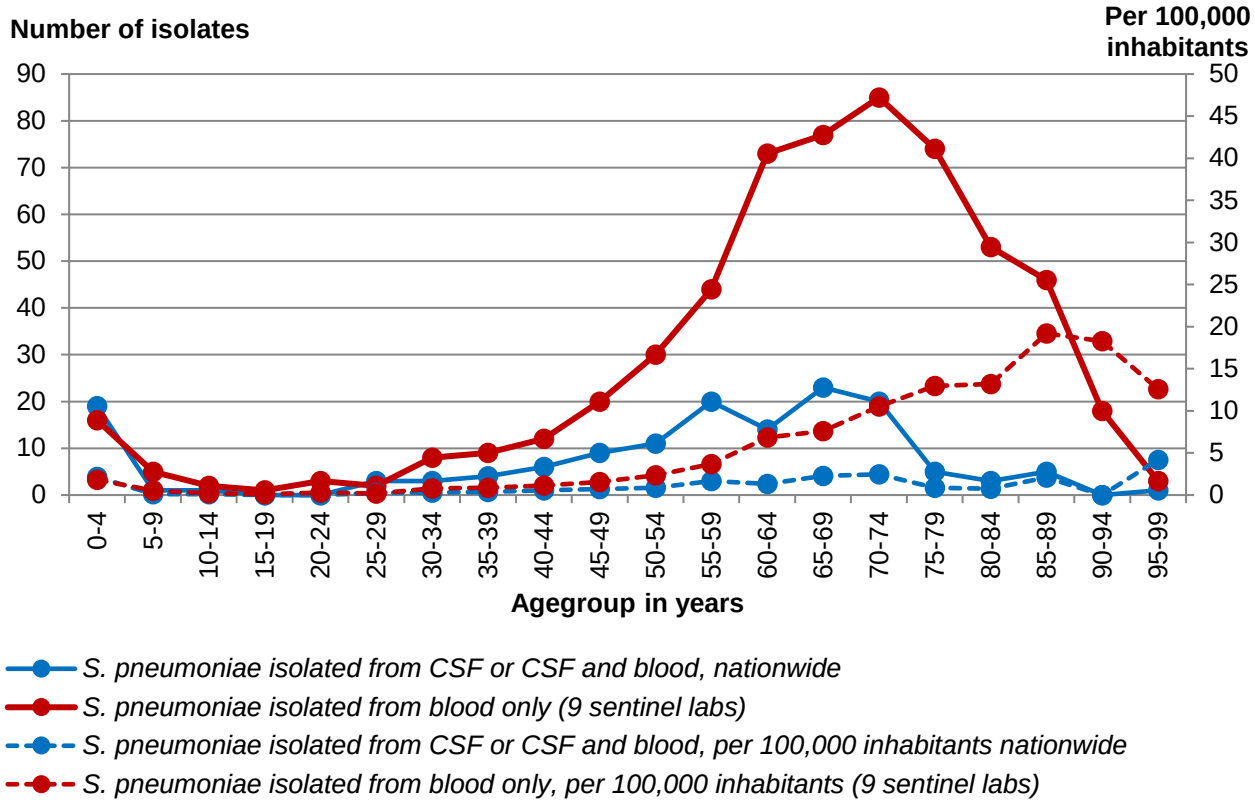

Figure 6.2 *Distribution of S. pneumoniae isolates received in 2017 according to age*

6.3 Distribution according to serotype

The relationship between age and major types of all isolates (received from the 9 sentinel laboratories) is shown in table 6.2. For isolates from CSF (or CSF and blood), the distribution of serotypes by age of the patient is presented in table 6.3. while the incidence of *S. pneumoniae* meningitis per serotype per 100.000 inhabitants is shown in table 6.4. The distribution of serotypes by age of the patient for pneumococcal isolates from blood only is shown in table 6.5. As aforementioned, incidences of *S. pneumoniae* from blood only are incomplete. Effect of the 10-valent vaccine can been seen in table 6.6 and table 6.7. showing a reduction of the number of isolates with vaccine types. However, the overall number of invasive pneumococcal disease isolates increased due to an increase of the number of isolates with non-vaccine types.

Table 6.2 *S. pneumoniae* isolates from CSF and/or blood (from the 9 sentinel laboratories), by serotype and age of patients, 2017

			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	10-valent vaccine	7-valent vaccine 4	-	-	-	-	-	-	-	-	1	-	2	2	1	6	***1.0***
		6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
		9V	-	-	-	-	-	-	-	-	-	-	1	-	1	2	***0.3***
		14	-	-	-	-	-	-	-	-	-	1	-	1	2	4	***0.6***
		18C	-	-	1	1	-	-	-	1	-	-	-	-	-	2	***0.3***
		19F	-	-	-	-	-	-	-	-	-	-	-	7	4	11	***1.7***
		23F	-	-	-	-	-	-	-	-	-	-	1	-	-	1	***0.2***
		Subtotal	-	-	*1*	*1*	-	-	-	*1*	*1*	*1*	*4*	*10*	*8*	*26*	***4.1***
		1	-	-	-	-	-	-	-	-	-	2	4	2	1	9	***1.4***
		5	-	-	-	-	-	-	-	1	-	-	-	-	-	1	***0.2***
		7F	-	-	-	-	1	-	-	-	2	3	5	12	5	28	***4.5***
		Subtot	-	-	1	1	1	-	-	2	3	6	13	24	14	64	***10.2***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
		3	-	2	-	2	-	1	-	1	1	2	17	27	7	58	***9.2***
		8	-	-	1	1	-	-	1	-	7	10	39	62	26	146	***23.3***
		9N	-	-	-	-	1	-	-	-	-	2	6	17	6	32	***5.1***
		10A	-	-	-	-	-	-	-	-	1	-	4	7	2	14	***2.2***
		11A	-	-	1	1	-	-	-	-	-	1	3	2	3	10	***1.6***
		12F	-	-	-	-	-	-	-	-	3	8	6	10	6	33	***5.3***
		15B	-	-	-	-	-	-	-	-	-	-	-	3	3	6	***1.0***
		17F	-	-	-	-	-	-	-	-	-	-	4	2	-	6	***1.0***
		19A	1	4	-	5	1	-	-	1	1	1	27	31	20	87	***13.9***
		20	-	-	-	-	-	-	-	-	-	-	1	2	2	5	***0.8***
		22F	-	-	1	1	-	-	-	-	-	-	9	22	9	41	***6.5***
		33F	-	2	-	2	-	-	-	-	1	1	3	5	3	15	***2.4***
Subtotal 23-valent vaccine			*1*	*8*	*4*	*13*	*3*	*1*	*1*	*4*	*17*	*31*	*132*	*214*	*101*	*517*	***82.5***
		Other	-	3	6	9	2	2	-	4	2	3	30	36	22	110	***17.5***
		Total	**1**	**11**	**10**	**22**	**5**	**3**	**1**	**8**	**19**	**34**	**162**	**250**	**123**	**627**	***100.0***

Table 6.3 Distribution of *S. pneumoniae* isolates from CSF (or CSF and blood) nation-wide. by serotype and age of patients, 2017.

			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	10-valent vaccine	7-valent vaccine	4	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.7
			6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			9V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			18C	-	-	-	-	-	-	1	-	-	-	-	-	1	1	0.7
			19F	-	-	-	-	-	-	-	-	1	1	3	1	6	4.0
			23F	-	-	-	-	-	-	-	-	1	-	-	-	1	1	0.7
		Subtotal	-	-	-	-	-	-	-	*1*	-	*2*	*1*	*3*	*2*	*9*	**6.1**
			1	-	-	-	-	-	-	-	-	-	-	-	1	-	1	0.7
			5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			7F	-	1	-	1	-	-	-	-	-	-	1	-	-	2	1.4
		Subtotal	-	*1*	-	*1*	-	-	-	*1*	-	*2*	*2*	*4*	*2*	*12*	**8.2**
			2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			3	-	-	1	1	-	1	-	1	3	1	7	5	1	20	13.5
			8	-	1	-	1	-	-	-	-	1	4	7	8	-	21	14.2
			9N	-	-	-	-	-	-	-	-	-	-	3	3	-	6	4.0
			10A	-	1	-	1	-	-	-	-	-	-	1	4	1	7	4.7
			11A	-	-	-	-	-	-	-	-	-	-	1	-	1	2	1.4
			12F	-	-	-	-	-	-	-	-	1	2	3	2	-	8	5.4
			15B	-	2	3	5	-	-	-	-	-	-	1	1	-	7	4.7
			17F	-	-	-	-	-	-	-	-	-	-	1	-	-	1	0.7
			19A	1	3	-	4	1	-	-	-	-	3	4	4	-	16	10.8
			20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			22F	-	-	1	1	-	-	-	-	1	-	4	1	1	8	5.4
			33F	-	2	-	2	-	-	-	-	-	1	1	2	-	6	4.0
Subtotal 23-valent vaccine			*1*	*10*	*5*	*16*	*1*	*1*	-	*2*	*6*	*13*	*35*	*34*	*6*	*114*	**77.0**
		Other		1	2	3	-	-	-	1	1	2	10	14	3	34	23.0
		Total	**1**	**11**	**7**	**19**	**1**	**1**	**-**	**3**	**7**	**15**	**45**	**48**	**9**	**148**	**100.0**

* From 6 patients with a pneumococcus isolated from blood. CSF was culture-negative but PCR was positive for pneumococcal DNA. Cases were in age groups 40-49 years (1). 50-64 years (3) and 65-79 years (2)

Table 6.4 Age-specific incidence of pneumococcal meningitis nation-wide (isolates from CSF or CSF and blood) per 100.000 inhabitants according to type, 2017

	AGE (YEARS)										
TYPE	0	1-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total
7-valent	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.08	0.03	0.13	0.26	0.05
10-valent	0.58	0.00	0.00	0.00	0.00	0.05	0.00	0.08	0.06	0.17	0.26	0.07
23-valent	6.38	0.71	0.11	0.10	0.00	0.09	0.29	0.55	0.99	1.42	0.79	0.67
Other	0.58	0.29	0.00	0.00	0.00	0.05	0.05	0.08	0.28	0.58	0.39	0.20
Total	**6.97**	**1.00**	**0.11**	**0.10**	**0.00**	**0.14**	**0.34**	**0.64**	**1.27**	**2.00**	**1.18**	**0.87**

Table 6.5 Distribution of *S. pneumoniae* isolates from Blood only (from the 9 sentinel laboratories) by serotype and age of patients, 2017.

		TYPE	AGE (MONTHS) 0	1-11	12-59	AGE (YEARS) 0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total	%
23-valent vaccine	10-valent vaccine	4	-	-	-	-	-	-	-	-	1	-	2	2	1	6	***1***
	7-valent vaccine	6B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		9V	-	-	-	-	-	-	-	-	-	-	1	-	1	2	***0.3***
		14	-	-	-	-	-	-	-	-	-	1	-	1	2	4	***0.7***
		18C	-	-	1	1	-	-	-	-	-	-	-	-	-	1	***0.2***
		19F	-	-	-	-	-	-	-	-	-	-	-	6	3	9	***1.6***
		23F	-	-	-	-	-	-	-	-	-	-	1	-	-	1	***0.2***
		Subtotal	-	-	*1*	*1*	-	-	-	-	*1*	*1*	*4*	*9*	*7*	*23*	***4.0***
		1	-	-	-	-	-	-	-	-	-	2	4	1	1	8	***1.4***
		5	-	-	-	-	-	-	-	1	-	-	-	-	-	1	***0.2***
		7F	-	-	-	-	1	-	-	-	2	3	4	12	5	27	***4.6***
		Subtotal	-	-	-	*1*	*1*	-	-	*1*	*3*	*6*	*12*	*22*	*13*	*59*	***10.2***
		2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		3	-	2	-	2	-	-	-	-	1	2	15	25	6	51	***8.8***
		8	-	-	1	1	-	-	1	-	7	9	39	60	26	143	***24.6/7***
		9N	-	-	-	-	1	-	-	-	-	2	5	15	6	29	***5.0***
		10A	-	-	-	-	-	-	-	-	1	-	4	5	1	11	***1.9***
		11A	-	-	1	1	-	-	-	-	-	1	2	2	3	9	***1.6***
		12F	-	-	-	-	-	-	-	-	2	7	4	9	6	28	***4.8***
		15B	-	-	-	-	-	-	-	-	-	-	-	3	3	6	***1.0***
		17F	-	-	-	-	-	-	-	-	-	-	3	2	-	5	***0.9***
		19A	-	2	-	2	1	-	-	1	1	1	25	31	20	82	***14.1***
		20	-	-	-	-	-	-	-	-	-	-	1	2	2	5	***0.9***
		22F	-	-	1	1	-	-	-	-	-	-	7	22	9	39	***6.7***
		33F	-	1	-	1	-	-	-	-	1	1	3	3	3	12	***2.1***
Subtotal 23-valent vaccine			-	*5*	*4*	*9*	*3*	-	*1*	*2*	*16*	*29*	*120*	*201*	*98*	*479*	***82.6***
		Other	-	2	6	8	2	2	-	3	1	3	27	34	21	101	***17.4***
		Total	**-**	**7**	**10**	**17**	**5**	**2**	**1**	**5**	**17**	**32**	**147**	**235**	**119**	**580**	***100.0***

Table 6.6 Distribution of pneumococcal CSF isolates according to serotype nation-wide, 2008-2017

		TYPE	Year 2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
23-valent vaccine	10-valent vaccine	7-valent vaccine	4	8	4	3	2	4	2	2	-	1	1
			6B	11	3	-	2	-	-	-	1	-	-
			9V	7	2	2	-	3	1	1	-	2	-
			14	8	3	5	2	1	-	-	1	-	-
			18C	8	6	5	5	2	2	-	1	-	1
			19F	7	10	2	6	4	2	4	2	5	6
			23F	17	5	4	2	1	-	-	1	-	1
			***Subtotal* 7-valent vaccine**	*66*	*33*	*21*	*19*	*15*	*7*	*7*	*6*	*8*	*9*
			1	8	8	3	1	1	3	4	1	2	1
			5	-	-	2	-	3	-	-	-	-	-
			7F	25	25	20	28	16	15	8	7	4	2
			***Subtotal* 10-valent vaccine**	*99*	*66*	*46*	*48*	*35*	*25*	*19*	*14*	*14*	*12*
			2	-	-	-	-	-	-	-	-	-	-
			3	17	24	20	7	13	16	13	16	25	20
			8	9	10	10	17	9	16	23	24	18	21
			9N	1	3	6	7	4	2	6	6	3	6
			10A	7	10	9	7	9	7	12	5	7	7
			11A	2	8	1	5	1	1	3	2	3	2
			12F	2	2	3	7	10	9	8	9	12	8
			15B	4	8	2	3	1	-	-	-	5	7
			17F	-	-	4	3	1	1	1	-	-	1
			19A	8	6	20	16	6	9	7	10	8	16
			20	1	-	1	-	-	1	1	1	-	-
			22F	10	13	14	16	11	8	8	11	11	8
			33F	6	6	7	5	6	3	2	4	4	6
			***Subtotal* 23-valent vaccine**	*166*	*156*	*143*	*141*	*106*	*98*	*103*	*102*	*110*	*114*
			6A	4	6	5	1	1	1	3	-	1	-
			6C	-	-	3	4	2	6	3	6	5	3
			7B	-	-	-	-	-	1	-	-	-	-
			10F	-	-	-	-	-	-	-	-	-	-
			10B	-	-	-	-	1	-	1	1	-	1
			12A	-	-	-	-	-	-	-	-	-	-
			13	-	1	-	-	-	-	-	-	-	-
			15A	1	-	1	1	1	4	6	7	2	4
			15C	3	1	2	-	3	-	-	1	-	3
			16F	2	-	5	4	-	5	2	1	3	1
			17A	-	1	-	-	-	-	-	-	-	-
			18F	-	-	-	-	-	-	-	-	-	-
			18A	-	-	-	-	-	-	-	-	-	-
			18B	1	-	-	-	1	-	-	-	-	-
			21	-	-	-	1	-	-	-	-	-	-
			22A	-	1	1	-	-	-	-	1	1	-
			23A	1	3	3	2	4	4	4	5	5	5
			23B	3	7	5	2	5	7	8	11	6	11
			24F	2	6	1	1	4	4	7	7	1	2
			24B	-	-	-	-	2	-	-	-	-	-
			25	1	-	-	-	-	-	-	-	-	-
			27	2	-	-	-	1	-	2	1	1	-
			28F	-	-	-	-	-	1	-	-	-	-
			28A	-	-	-	1	-	-	-	-	-	-
			29	-	-	-	-	1	-	-	-	-	-
			31	-	1	1	-	1	-	1	-	1	1
			33A	-	-	-	-	-	-	-	-	-	-
			34	1	1	-	1	-	-	-	1	1	1
			35F	2	2	4	1	-	2	1	2	5	1
			35B	-	-	1	-	1	3	1	1	1	-
			37	-	1	-	1	2	1	-	-	-	-
			38	1	3	1	-	2	1	-	-	-	-
			Rough (n.t.)	-	-	-	-	-	-	-	-	-	1
			Total	190	190	176	163	138	138	142	147	143	148

Table 6.7 Distribution of *S. pneumoniae* from blood only submitted by the 9 sentinel laboratories, according to serotype, 2008-2017

			TYPE	Year 2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
23-valent vaccine	10-valent vaccine	7-valent	4	30	26	17	27	11	13	6	6	6	6
			6B	25	12	8	3	3	3	3	4	1	-
			9V	42	26	21	5	2	4	1	5	-	2
			14	54	34	22	19	12	8	2	7	8	4
			18C	15	15	7	8	4	8	2	2	2	1
			19F	9	10	5	9	3	5	7	8	6	9
			23F	13	12	13	5	3	1	2	1	1	1
			Subtotal 7-valent vaccine	*188*	*135*	*93*	*85*	*38*	*42*	*23*	*33*	*24*	*23*
			1	64	65	53	40	50	40	41	41	22	8
			5	2	6	7	11	8	9	2	1	-	1
			7F	65	86	72	91	92	75	53	56	36	27
			Subtotal 10-valent vaccine	*319*	*292*	*225*	*227*	*188*	*166*	*119*	*131*	*82*	*59*
			2	-	-	-	-	-	-	-	-	-	-
			3	31	34	30	36	45	40	31	35	45	51
			8	46	52	60	59	88	108	93	136	151	143
			9N	19	18	19	17	20	19	21	26	32	29
			10A	7	9	9	14	8	6	16	15	11	11
			11A	3	12	12	9	14	16	8	6	6	9
			12F	6	5	13	19	25	22	28	30	18	28
			15B	4	6	7	4	1	7	7	2	8	6
			17F	1	7	4	8	7	4	8	6	6	5
			19A	33	30	57	63	78	61	44	78	75	82
			20	3	3	3	4	-	1	4	2	3	5
			22F	24	24	29	37	41	45	34	43	28	39
			33F	10	11	10	15	22	12	12	19	18	12
			Subtotal 23-valent vaccine	*506*	*503*	*478*	*503*	*537*	*507*	*425*	529	483	479
			6A	18	11	9	2	6	2	-	2	-	4
			6C	1	7	9	7	10	10	7	21	20	15
			7C	-	-	-	-	-	-	-	-	-	-
			9A	-	-	-	-	1	-	1	-	1	-
			10F	1	-	-	-	-	-	1	-	-	-
			10B	-	-	-	-	-	1	-	-	-	1
			13	-	-	-	1	-	-	-	-	1	-
			15F	-	-	-	-	-	1	-	-	1	-
			15A	1	1	-	2	7	13	14	18	21	16
			15C	2	2	1	2	1	4	4	3	2	1
			16F	9	8	10	7	6	7	5	2	9	9
			17A	-	-	-	2	-	-	-	-	-	-
			18F	-	-	-	-	-	-	-	2	-	-
			18A	-	1	1	1	-	-	-	-	-	-
			18B	-	-	-	-	1	1	-	-	-	-
			21	-	-	-	-	-	2	1	-	-	1
			22A	1	-	1	1	-	1	-	1	-	-
			23A	3	9	7	2	6	6	7	7	12	15
			23B	3	6	3	9	3	6	15	5	11	17
			24F	7	-	2	3	2	4	4	7	1	6
			25F	1	-	-	-	-	-	-	1	-	1
			27	1	1	-	1	-	1	-	1	1	-
			28A	-	-	-	-	-	-	-	-	-	-
			29	-	-	-	-	1	-	-	-	-	-
			31	3	1	4	2	6	2	2	4	4	3
			33A	-	-	-	-	1	-	-	-	1	-
			34	-	1	1	-	1	2	1	-	1	1
			35F	2	4	5	6	5	6	7	7	6	3
			35A	-	-	-	-	1	-	-	-	-	-
			35B	-	4	-	3	1	7	6	8	8	2
			37	-	-	1	-	-	-	1	1	-	-
			38	3	5	-	3	-	1	2	2	1	5
			40	-	-	-	-	-	1	-	-	-	-
			Rough (n.t.)	-	-	-	2	-	-	-	-	-	1
			Total	562	564	532	559	596	585	503	621	584	580

7 *ESCHERICHIA COLI*

The Reference Laboratory received 49 *Escherichia coli* strains, 8 isolated from CSF (or CSF and blood) and 41 from blood only (table 7.1, 7.2 and 7.3). Sixty-nine percent of the cases of *E. coli* meningitis occurred in the first month of life.

Table 7.1 Serotypes of *E. coli* isolates from CSF and/or blood. by age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	19	10	-	29	-	-	-	1	**30**	***61***
K1	15	3	-	18	-	-	-	1	**19**	***39***
Total	**34**	**13**	**-**	**47**	**-**	**-**	**-**	**2**	**49**	***100***
%	***69***	***27***	***0***	***96***	***0***	***0***	***0***	***4***	***100***	

Table 7.2 Serotypes of *E. coli* isolates from CSF (or CSF and blood), by age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	1	-	-	1	-	-	-	1	**2**	***25***
K1	5	-	-	5	-	-	-	1	**6**	***75***
Total	**6**	**-**	**-**	**6**	**-**	**-**	**-**	**2**	**8**	***100***
%	***75***	***0***	***0***	***75***	***0***	***0***	***0***	***25***	***100***	

Table 7.3 Serotypes of *E. coli* isolates from blood only by age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	18	10	-	28	-	-	-	-	**28**	***88***
K1	10	3	-	13	-	-	-	-	**13**	***12***
Total	**28**	**13**	**-**	**41**	**-**	**-**	**-**	**-**	**41**	***100***
%	***88***	***12***	***0***	***100***	***0***	***0***	***0***	***0***	***100***	

Since 2012 all isolates were tested for the H-type. Eighty-six percent of all K1 isolates were of type H4 and H7, while 37% of the non-K1 isolates were H1 and H5 (table 7.4)

Table 7.4 H-type versus K-type of *E. coli* isolates from CSF and/or blood, 2013 - 2017

TYPE	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1	K1 / Non K1
	2013	**2014**	**2015**	**2016**	**2017**
H-	4 / 3	1 / 1	2 / 1	-	1 / 0
H1	-	1 / 1	1 / 0	0 / 2	0 / 7
H2	1 / 0	-	-	-	1 / 0
H4	5 / 0	5 / 0	6 / 0	6 / 7	4 / 1
H5	1 / 0	-	1 / 2	4 / 2	1 / 4
H6	0 / 1	0 / 3	2 / 0	4 / 1	3 / 1
H7	9 / 0	11 / 0	6 / 0	7 / 3	9 / 2
H8	-	-	-	-	0 / 1
H9	-	-	-	0 / 2	0 / 4
H10	-	0 / 3	-	1 / 1	-
H11	-	-	1 / 0	-	-
H15	-	-	1 / 0	-	-
H16	-	-	-	0 / 1	-
H18	1 / 0	1 / 1	2 / 0	0 / 5	0 / 2
H18/H34	-	-	-	-	0 / 1
H19	-	0 / 2	-	-	-
H21	-	-	-	0 / 1	-
H25	-	-	-	0 / 1	-
H28	-	-	-	-	0 / 1
H31	1 / 0	1 / 0	2 / 0	-	0 / 2
H33	-	-	1 / 0	1 / 0	0 / 2
H38	-	0 / 1	-	-	-
H41	-	-	-	-	0 / 1
H42	-	-	-	1 / 0	-
H45	-	-	-	-	0 / 1
Total	**22 / 4**	**20 /12**	**25 / 3**	**24 / 26**	**19 / 30**
%					

Since 2016, K1 is detected with Fage typing. O and H typing was done with Whole Genome Sequencing. The types O non typable, O1, O4, O6 and O169/O183 are most prevalent among non-K1 isolates, while the type O75 was found among K1 isolates but numbers are small (Table 7.5).

Table 7.5 O-type versus K-type of *E. coli* isolates from CSF and/or blood, 2017

O type	K1	Non K1	Total
O-	0	3	**3**
O-/O15	0	1	**1**
O1	2	1	**3**
O2	2	0	**2**
O2/O50	2	0	**2**
O4	0	3	**3**
O6	0	7	**7**
O7	1	1	**2**
O9	0	1	**1**
O13/O135	1	0	**1**
O15	0	2	**2**
O16	1	1	**2**

O type	K1	Non K1	Total
O18	2	0	**2**
O45	2	0	**2**
O75	4	0	**4**
O86	1	1	**2**
O88	0	1	**1**
O117	0	1	**1**
O120	1	0	**1**
O126	0	1	**1**
O137	0	1	**1**
O148	0	1	**1**
O169/O183	0	3	**3**
O179	0	1	**1**
Total	**19**	**30**	**49**

Ammong K1 isolates, the O/H combinations O75 H7, O18 H7 and O1 H7 were found each 2 times.
Ammong non-K1 isolates, O6H1, O4H5 and O169/O183 H5 were dominant (5 respectivily 3 and 3 times)

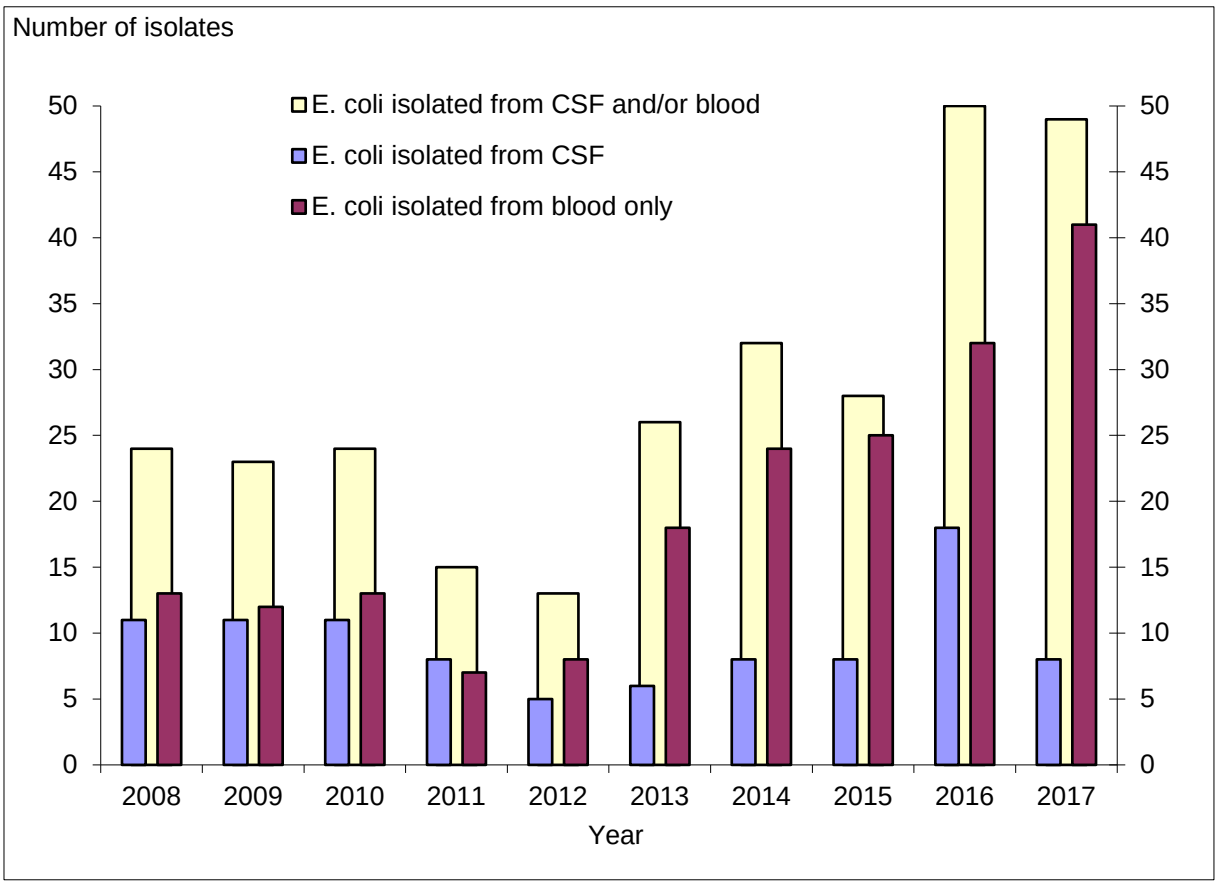

Figure 7.1 *Distribution of E. coli. 2008-2017*

8 *STREPTOCOCCUS AGALACTIAE* – (group B)

In 2017 the Reference Laboratory received 87 *Streptococcus agalactiae* isolates (2016: 69; 2015: 65. figure 8.1). Twenty-four *S. agalactiae* isolates were from CSF (or CSF and blood) and 63 from blood only (table 8.1. 8.2 and 8.3). Eighty-three percent of the cases occurred in the first month of life. Serotype III was the most prevalent (table 8.1). In addition, compared to 2014, the absolute number as well as the proportion of serotype Ia isolates increased in 2015 (2014: 6 (8.5%); 2015: 16 (25%). In 2017. Ia was increased to 18 (21%) cases.

Table 8.1 Serotypes of *S. agalactiae* isolates from CSF and/or blood, by age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	13	5	0	18	0	0	0	0	**18**	***21***
Ib	4	0	0	4	0	0	1	0	**5**	***6***
II	6	0	0	6	0	0	0	0	**6**	***7***
III	41	8	0	49	0	0	0	0	**49**	***56***
IV	2	0	0	2	0	0	0	0	**2**	***2***
V	5	0	0	5	0	0	0	0	**5**	***6***
VI	1	0	0	1	0	0	0	0	**1**	***1***
VII	0	0	0	0	0	0	0	1	**1**	***1***
Total	**72**	**13**	**0**	**85**	**0**	**0**	**1**	**1**	**87**	***100***
%	***83***	***15***	***0***	***98***	***0***	***0***	***1***	***1***	***100***	

Table 8.2 Serotypes of *S. agalactiae* isolates from CSF (or CSF and blood), by age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	1	3	0	4	0	0	0	0	**4**	***17***
Ib	1	0	0	1	0	0	1	0	**2**	***8***
II	2	0	0	2	0	0	0	0	**2**	***8***
III	12	3	0	15	0	0	0	0	**15**	***63***
VII	0	0	0	0	0	0	0	1	**1**	***4***
Total	**16**	**6**	**0**	**22**	**0**	**0**	**1**	**1**	**24**	***100***
%	***67***	***25***	***0***	***92***	***0***	***0***	***4***	***4***	***100***	

Table 8.3 Serotypes of *S. agalactiae* isolates from blood only, by age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	12	2	0	14	0	0	0	0	14	***22***
Ib	3	0	0	3	0	0	0	0	3	***5***
II	4	0	0	4	0	0	0	0	4	***6***
III	29	5	0	34	0	0	0	0	34	***54***
IV	2	0	0	2	0	0	0	0	2	***3***
V	5	0	0	5	0	0	0	0	5	***8***
VI	1	0	0	1	0	0	0	0	1	***2***
Total	**56**	**7**	**0**	**63**	**0**	**0**	**0**	**0**	**63**	***100***
%	***89***	***11***	***0***	***100***	***0***	***0***	***0***	***0***	***100***	

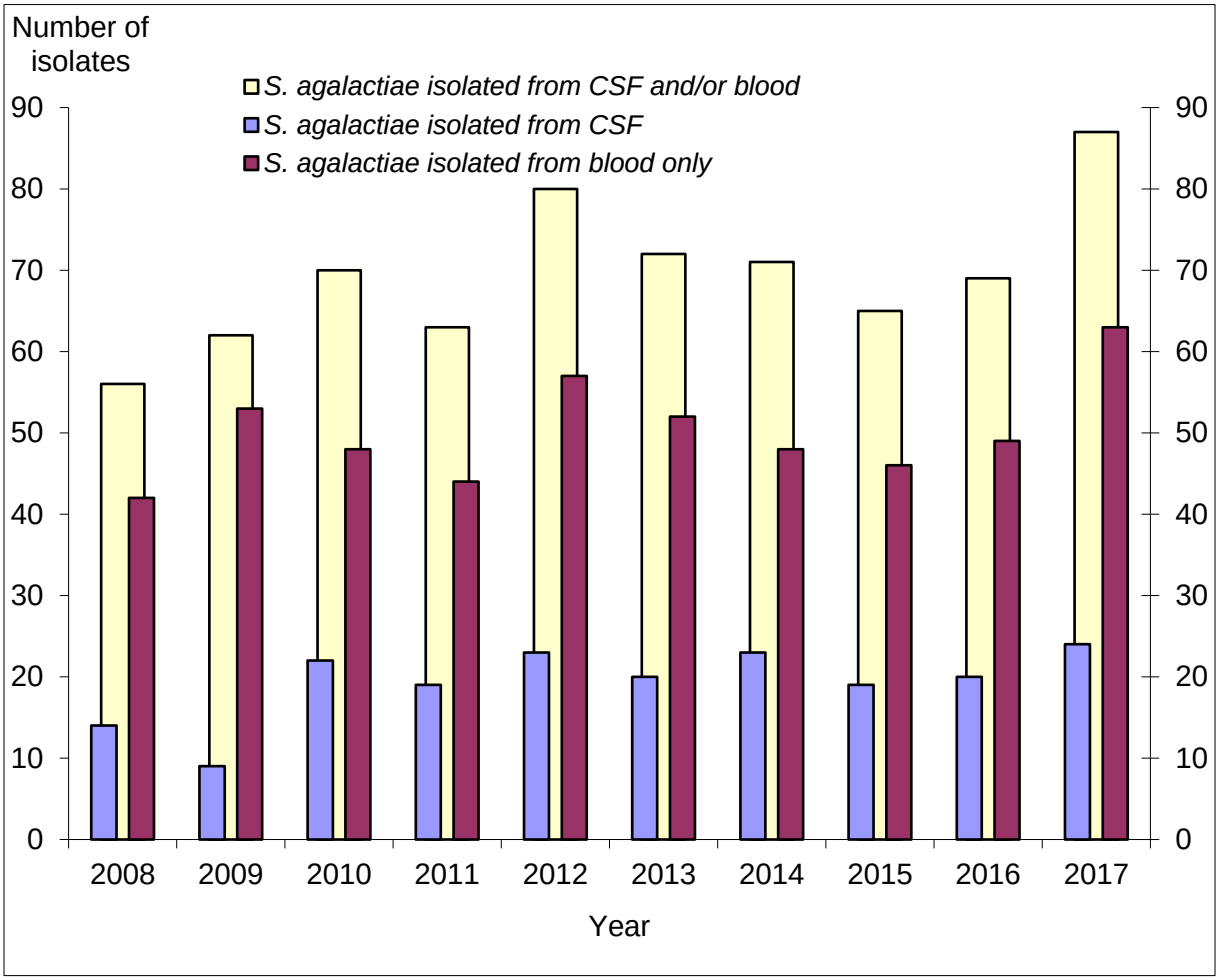

Figure 8.1 *Distribution of S. agalactiae, 2008-2017*

9 *LISTERIA MONOCYTOGENES*

Ninty-one *Listeria monocytogenes* isolates were submitted to the Reference Laboratory. Of these, 20 were from CSF (or CSF and blood) and 71 from blood only (figure 9.1). (2016: 11 CSF and 59 blood only; 2015: 8 CSF and 39 blood only; 2014: 19 CSF and 51 blood only). Most cases (95%) occurred among persons older than 50 years. In 2017 (as in previous years) serotypes 1/2a and 4b were most prevalent (table 9.1).

Table 9.1 *L. monocytogenes* isolates from CSF/blood, by type and age of patients, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2a	0	0	0	0	0	0	1	35	**36**	***39***
1/2b	0	0	0	0	0	0	0	7	**7**	***8***
1/2c	0	0	0	0	0	0	0	1	**1**	***1***
4b	0	0	0	0	0	0	4	43	**47**	***52***
Total	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**86**	**91**	***100***
%	***0***	***0***	***0***	***0***	***0***	***0***	***5***	***95***	***100***	

Table 9.2 *L. monocytogenes* isolates from CSF (or CSF and blood), by type and age, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2a	0	0	0	0	0	0	0	5	5	***25***
1/2b	0	0	0	0	0	0	0	1	**1**	***5***
1/2c	0	0	0	0	0	0	0	0	**0**	***0***
4b	0	0	0	0	0	0	2	12	**14**	***70***
Total	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**18**	**20**	***100***
%	***0***	***0***	***0***	***0***	***0***	***0***	***10***	***90***	***100***	

Table 9.3 *L. monocytogenes* isolates from blood only, by serotype and age, 2017

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2a	0	0	0	0	0	0	1	30	**31**	***44***
1/2b	0	0	0	0	0	0	0	6	**6**	***8***
1/2c	0	0	0	0	0	0	0	1	**1**	***1***
4b	0	0	0	0	0	0	2	31	**33**	***47***
Total	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**68**	**71**	***100***
%	***0***	***0***	***0***	***0***	***0***	***0***	***4***	***96***	***100***	

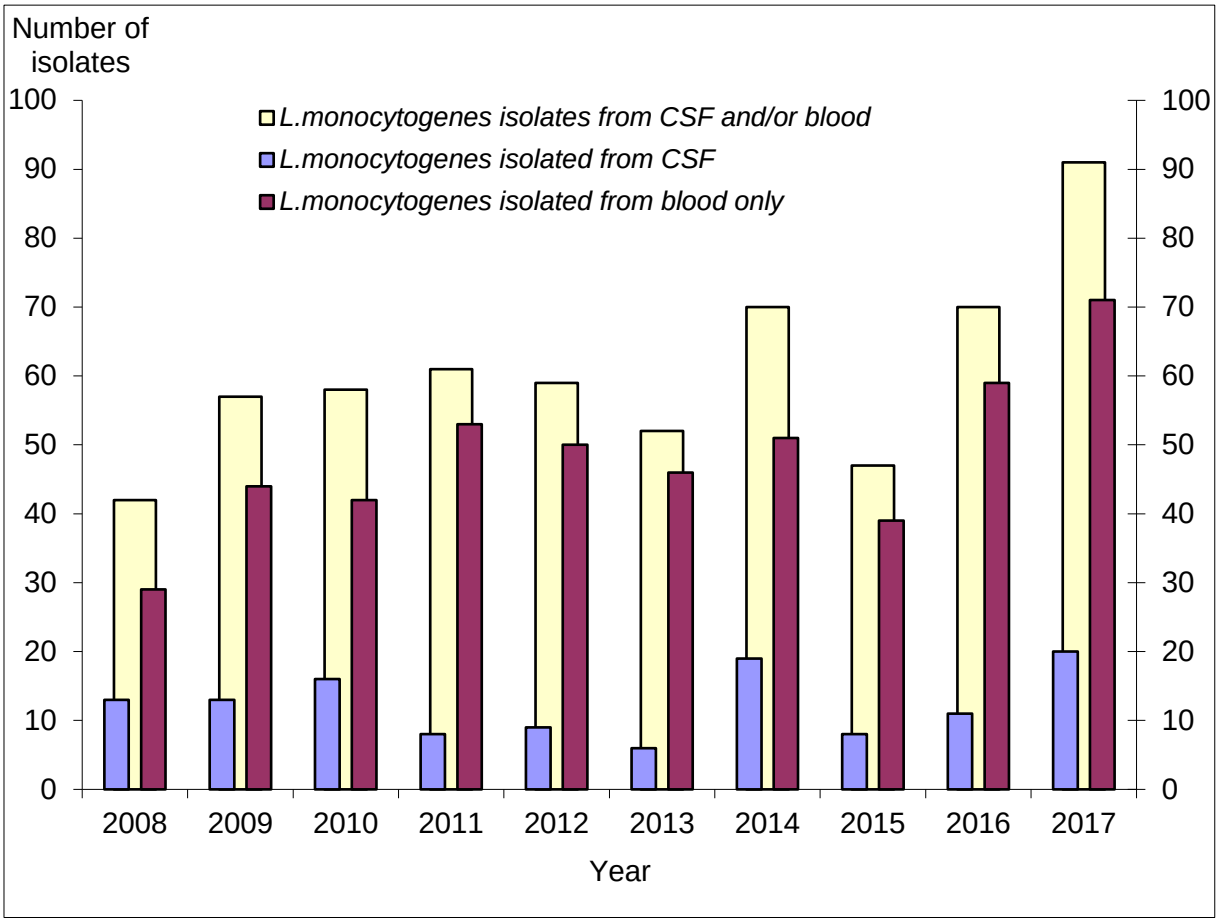

Figure 9.1 *Distribution of L. monocytogenes, 2008-2017*

10 *STREPTOCOCCUS PYOGENES*

Eighteen *Streptococcus pyogenes* isolates were submitted to the Reference Laboratory. 7 isolated from CSF (or CSF and blood) and 11 from blood only.

Since 2015, the Reference Laboratory assesses group A M-type by sequencing of the hypervariable part of the gene encoding the M protein. The M protein gene (EMM) encodes the cell surface M virulence protein and is responsible for over 100 *S. pyogenes* M serotypes.

Table 10.1 *S. pyogenes* isolates from CSF and/or blood received in 2017 according to source of isolation and age.

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
CSF	0	0	2	2	0	0	3	2	***7***	***39***
Blood	0	0	2	2	0	0	4	5	**11**	***61***
Total	**0**	**0**	**4**	**4**	**0**	**0**	**7**	**7**	**18**	***100***
%	***0***	***0***	***22***	***22***	***0***	***0***	***39***	***39***	***100***	

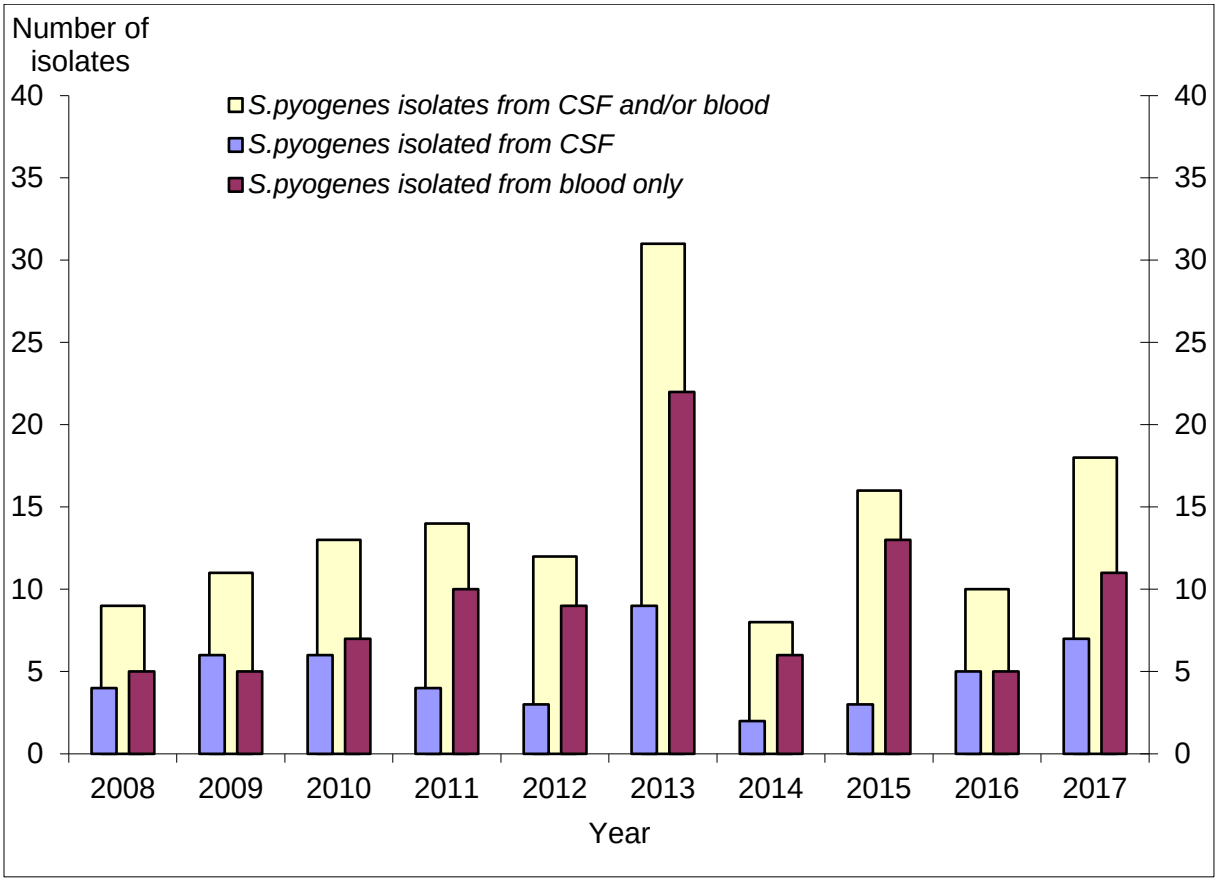

Figure 10.1 *Distribution of S. pyogenes, 2008-2017*

Table 10.2 S. pyogenes isolates from CSF and/or blood according to EMM- type, 2017

EMM-TYPE	CSF	BLOOD	TOTAL	
			T	%
1.0 Cluster A-C3	5	6	**11**	***61***
12.0 Cluster A-C4	1	2	**3**	***15***
3.93 Cluster A-C5	1	0	**1**	***6***
49.3	0	1	**1**	***6***
102.2	0	1	**1**	***6***
113.0 Cluster E3	0	1	**1**	***6***
Total	***7***	**11**	**18**	***100***
%	***39***	***61***	***100***	

11 *ANTIGEN AND DNA DETECTION*

The Reference Laboratory received 93 culture-negative specimens of CSF, serum or other body fluids for antigen or DNA detection. Polyclonal antibodies were used in latex-agglutination. PCR was performed with primers and probes specific for *N. meningitidis* (targeted on the *ctrA* gene) and for *S. pneumoniae* (targeted on the *pia* gene). When CSF was positive in the meningococcal PCR, it was then subjected to serogroup-specific PCR.

Of 93 specimens, 45 (48%) were positive by PCR. Of these, 29 (31%) (24 CSF, 5 sera or DNA isolated from a skinbiopsy) were positive for *N. meningitidis* and 16 (17%) were positive for *S. pneumoniae*.

Thus, in 2017, PCR-positive, culture-negative CSF samples accounted for 36% (24/67) of cases of meningococcal meningitis registered in the database of the Reference Laboratory. For *S. pneumoniae* this percentage was 10% (15/157).

Table 11.1 CSF and serum samples. tested for antigens or DNA, 2017

Antigen of	CSF * (or DNA from CSF)	SERA	TOTAL
C. neoformans	0	0	**0**
H. influenzae type b	0	0	**0**
DNA of			
N. meningitidis group B	14	1	**15**
N. meningitidis group C	1	0	**1**
N. meningitidis group W	5	4	**9**
N. meningitidis group Y	4	0	**4**
S. pneumoniae	15	1	16
Sub Total	**39**	**6**	**45**
Antigen and PCR negative	45	3	**48**
Total	**84**	**9**	**93**

* From 6 patients with a *S. pneumoniae* isolated from blood. the CSF was culture-negative but PCR-positive for pneumococcal DNA. From 3 patients with a N. meningitidis isolated from blood. the CSF was culture-negative but PCR-positive for meningococcal DNA.

12 *VACCINATION PROSPECTS*

12.1 N. meningitidis

In the Netherlands, vaccination against serogroup C meningococcal disease has been 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 have been vaccinated. In 2017, 9 cases of meningococcal disease (4.5% of all cases; table 4.4) were due to serogroup C meningococci (2016: 4.4%; 2015: 8.3%; 2014: 4.1%). All nine patients were not vaccinated because of age. This indicates that the vaccination programme is successful. (figure 12.1)

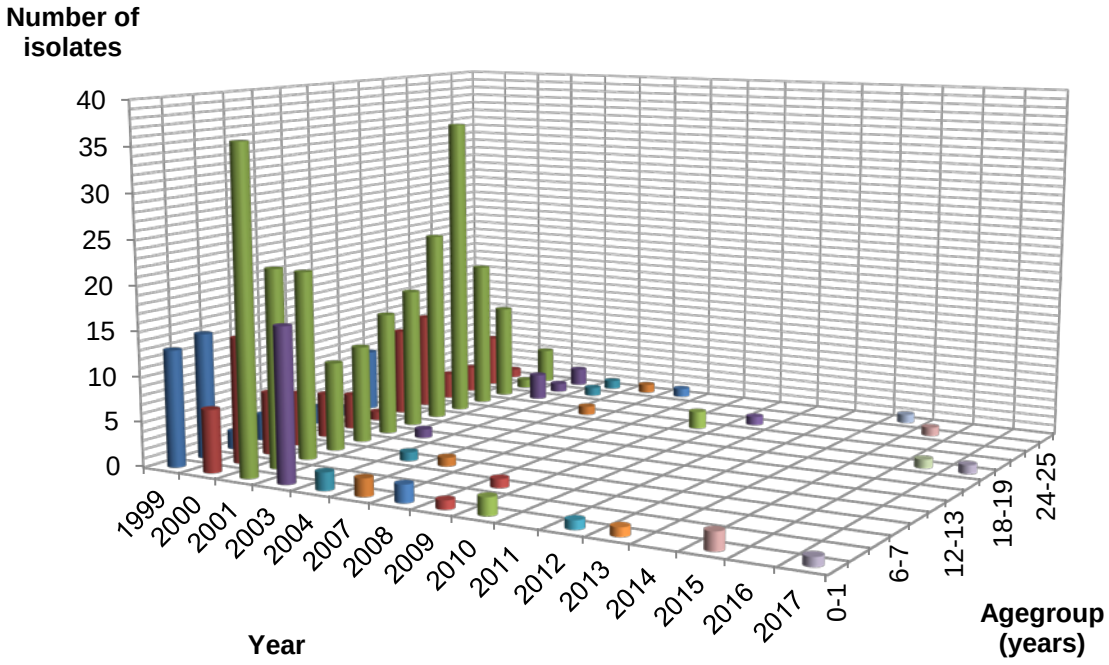

Figure 12.1 *Age distribution of N.meningitidis serogroup C invasive disease in the first 25 years of life, 1999-2017.*

A PorA-based protein vaccine composed of nine different genosubtypes (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), If available, would have prevented 61 cases (86%; table 4.9) of serogroup B meningococcal disease and 93 (46%) of all 201 cases of meningococcal disease. The latter proportion is lower than in the previous years due to the increase of serogroup W meningococcal disease. The vast majorty of these cases is due to meningococci with PorA P1.5.2.

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 first of April 1993 are vaccinated with the PRP-T vaccine, at first at the age 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 gradually decreased since the introduction of the vaccine, while the number of meningitis cases caused by *H. influenzae* non-type b did not alter. In 2017, the number of invasive meningitis isolates of *H. influenzae* type b, received from patients that should have been vaccinated (<24 years of age) decreased from 11 to 7 (2016: 11; 2015: 14; 2014: 12) (figure 12.2 and 12.3). Of those 7 patients. Four had received all doses and one received only one dose of the vaccine. Two patients were not vaccinated.

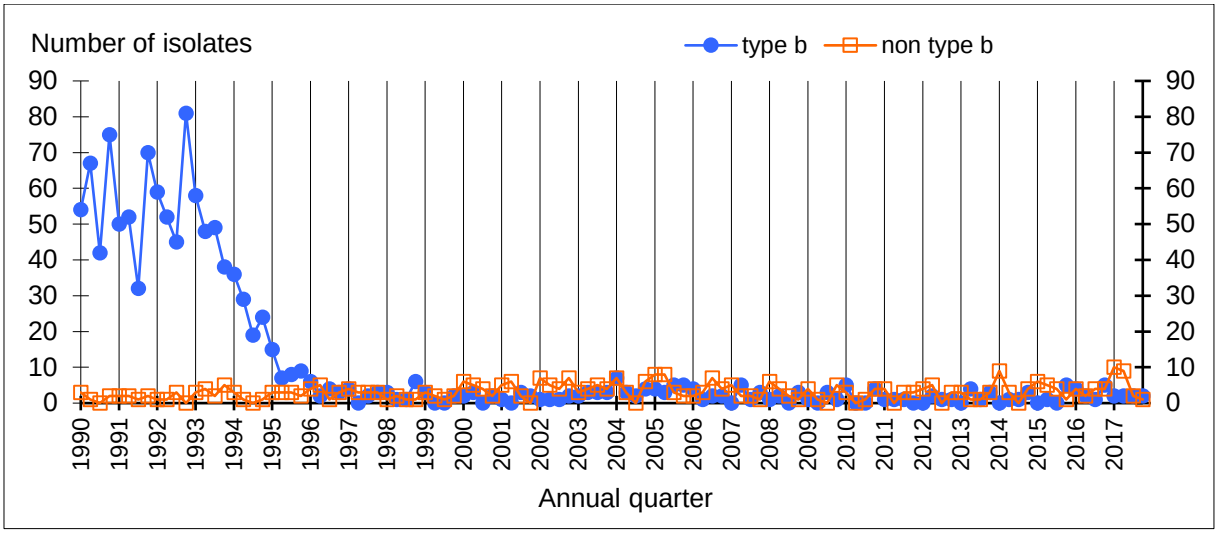

Figure 12.2 *The distribution of H. influenzae type b and non-type b meningitis cases according to annual quarter, 1990 –2017*

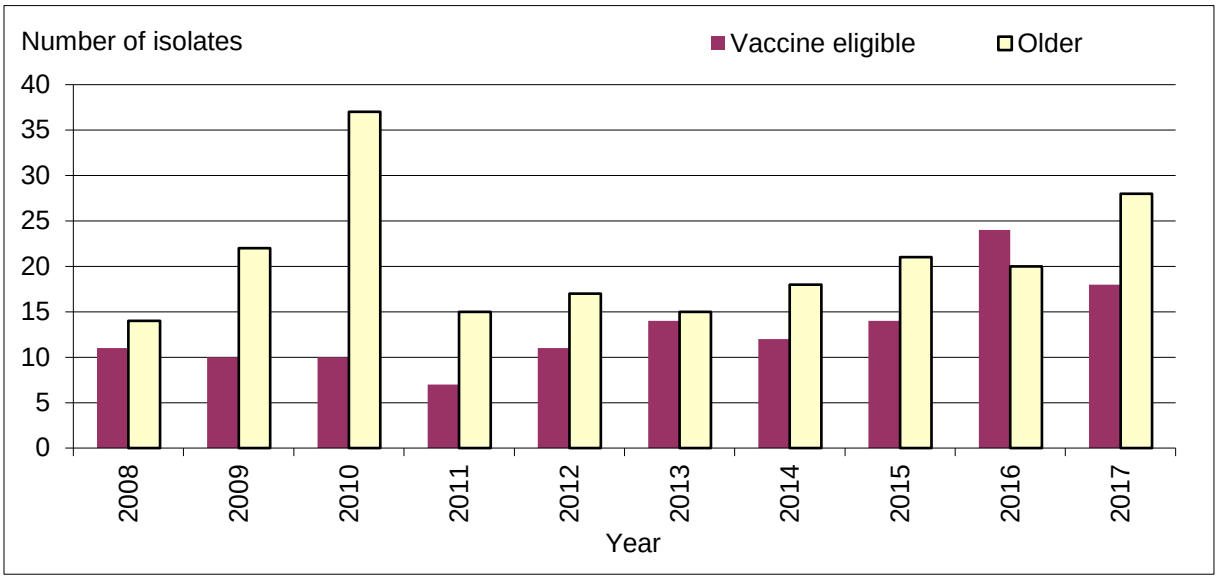

Figure 12.3 *The distribution of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 2008 –2017*

12.3 S. pneumoniae

The pneumococcal conjugated polysaccharide vaccine contains 7 serotype-specific polysaccharides linked to inactive diphtheria toxin (7-valent polysaccharide conjugate vaccine. PCV7). Since July 2006, children born after the first of April 2006 are vaccinated with this vaccine at age of 2, 3, 4 and 11 months. In April 2011, the 10-valent vaccine (PCV10) was introduced for all newborns born since March 1, 2011. In 2017, 6.1% of the CSF isolates were of a serotype covered by this hepta-valent conjugate polysaccharide vaccine, while 8.1% of the isolates were covered by the 10-valent vaccine (table 6.6). In 2017 the proportion of CSF isolates with a PVC7 serotype was ten times lower than about ten years ago (2006: 56%) as a result of the vaccination. There were 9 patients with invasive pneumococcal disease due to pneumonococci with a vaccine (PVC7) serotype (4, 18C, 19F and 23F) and 3 patients with invasive pneumococcal disease due to pneumonococci with a vaccine (PVC10 - 7) serotype (1 time type 1 and 2 times 7F). Those 12 patients were not vaccinated because of age. The beneficial effect of vaccination is partly abrogated by an increase of the number of cases due to non-vaccine types (figure 12.4).

The pneumococcal non-conjugated polysaccharide vaccine contains 23 serotype-specific polysaccharides. Seventy-seven percent of the CSF isolates were of a serotype which is represented in this vaccine (table 6.6) (2017: 77%; 2007: 90%).

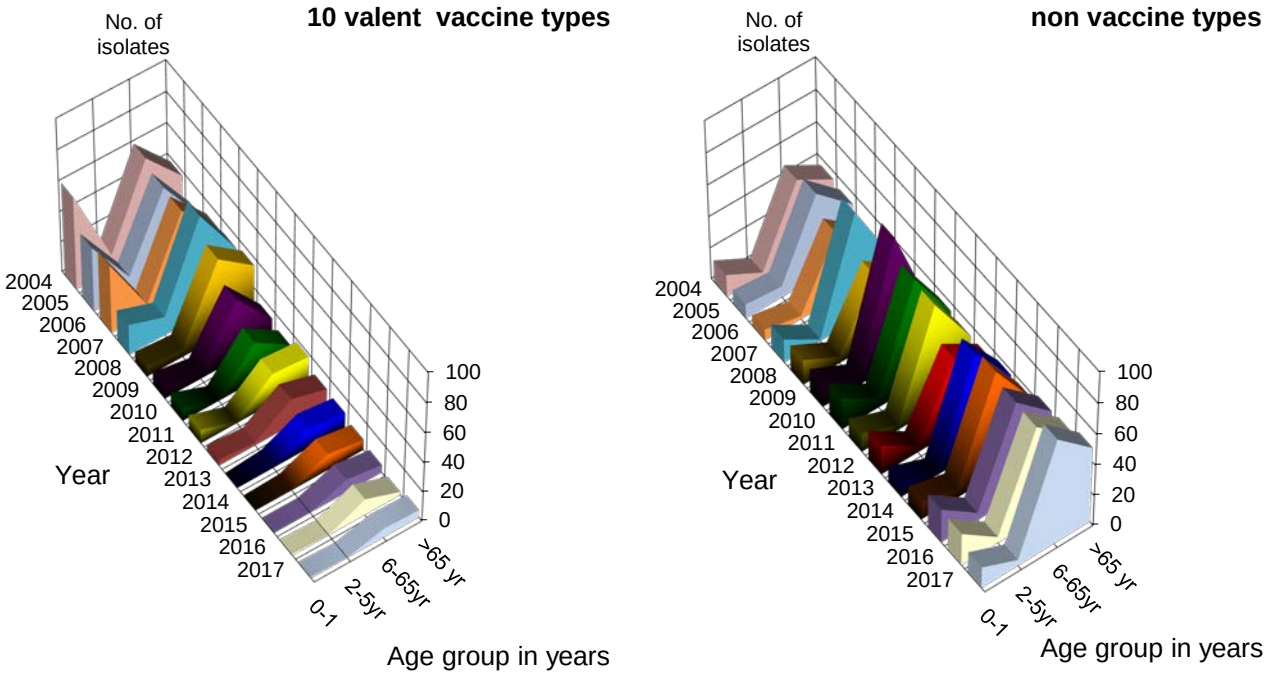

Figure 12.4 *The age distribution of S.pneumoniae invasive disease due to pneumococci of serotypes included in the hepta-valent conjugated polysaccharide vaccine. 2004-2016. Left: vaccine types. Right: types not included in this 10 valent vaccine*

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