BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2016

AMC
Academic Medical Center
University of Amsterdam

RIVM
National Institute of Public Health
and
Environmental Protection

BACTERIAL MENINGITIS IN THE NETHERLANDS
ANNUAL REPORT 2016

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

The contents of this report may be quoted, provided the source be given:

Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM)
Bacterial meningitis in the Netherlands; annual report 2016
Amsterdam: University of Amsterdam, 2017

CONTENTS

1 INTRODUCTION

This is the **45th** 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 70.750 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, Oktober, 2017

dr. A. van der Ende PhD, biochemist
dr. W. Freudenberg, 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 2016, the Reference Laboratory received isolates from CSF and / or blood from 1490 patients, and 39 specimens of CSF and/or serum wich were positive in PCR (or crypt. agglutination.) (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)	1490
CSF samples (without isolate)	97
Sera (and other fluid, without isolate)	12
Total	**1599**

In 2016, 53 clinical microbiology laboratories submitted isolates to the Reference Laboratory.

Table 2.2 shows the 1490 isolates according to species and to laboratory where cases were diagnosed.

Table 2.2 Number of isolates from CSF and/or blood received in 2016, according to laboratory

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Alkmaar	MCA lab. Med. Microbiologie	7	8	20	4	5	3	-	2	-	-	-	-	**49**
Amersfoort	Meander Medisch Centrum	2	4	11	-	1	2	1	-	-	-	-	-	**21**
Amsterdam	Academisch Medisch Centrum	3*	5	26	2	3	2	1	1	1	-	19	-	**63**
	Academisch ziekenhuis VU	2	1	3	-	-	-	-	-	-	-	2	-	**8**
	Onze Lieve Vrouwe Gasthuis	4	2	4	-	-	3	-	1	-	1	-	-	**15**
	Slotervaart	1	2	13	-	-	1	-	-	-	-	-	-	**17**
Apeldoorn	Gelre Ziekenhuizen	1	8	18	-	2	-	-	-	-	-	1	-	**30**
Arnhem	Rijnstate	3	8	50	1	1	4	-	-	-	-	1	-	**68**
Breda	Amphia Ziekenhuis	-	11	6*	1	5	1	-	-	-	1	1	-	**26**
Capelle ad IJssel	IJsselland Ziekenhuis	-	1	2	1	2	-	-	-	-	-	-	-	**6**
Delft	Reinier de Graaf groep	1	2	3	-	-	-	-	-	-	-	-	-	**6**
Den Bosch	Regionaal laboratorium Den Bosch	1	6	38	1	3	1	-	-	-	-	-	-	**50**
Den Haag	Haga Ziekenhuis, loc. Leyenburg	5	1	8	1	1	1	-	-	-	-	-	-	**17**
	MA Haaglanden, loc Westeinde	-	1	-	-	-	-	-	1	-	1	-	-	**3**
Deventer	Deventer Ziekenhuis	2	2	6	-	1	1	-	-	-	-	-	-	**12**
Doetinchem	Slingeland Ziekenhuis	1	2	-	-	1	-	-	-	-	-	-	-	**4**
Dordrecht	RLM Dordrecht / Gorinchem	4	7	56	2	8	2	-	-	-	-	-	-	**79**
Ede	Gelderse Vallei	3	4	1	-	1	-	2	1	1	-	1	-	**14**
Etten Leur	Stichting Huisartsen Laboratorium	-	-	-	-	-	1	-	-	-	-	-	-	**1**
Gouda	Groene Hart Ziekenhuis	-	1	1	-	-	1	-	-	-	-	2	-	**5**
Goes		3	-	2	-	1	-	-	-	-	-	-	-	**6**
Groningen	Certe, Lab. v. Infectieziekten	6	11	10	-	3	2	-	1	-	-	-	-	**33**
	UMCG	1	3	7	3	-	-	-	-	-	1	-	-	**15**
Haarlem	St. Streeklab voor de Volksgezondheid	1	4	88	-	2	4	-	-	-	1	-	-	**100**
Harderwijk	St. Jansdal Ziekenhuis	2	-	1	-	1	-	-	-	-	-	-	-	**4**
Hengelo	LabMicTa	7	15	104	1	4	8	2	-	-	-	-	-	**141**
Hilversum	Centraal Bact. Ser. Lab.	2	-	2	-	-	1	1	-	-	1	1	-	**8**
Hoorn	Westfries gasthuis	7	2	11	-	2	2	-	-	-	-	-	-	**24**
Leeuwarden	Izore, centrum infectieziekten Friesland	9	8	100	-	5	-	-	-	-	-	1	-	**123**
Leiden	Alrijne ziekenhuis	5	1	14	-	2	1	-	-	-	-	-	-	**23**
	LUMC, KML, Lab.voor Bacteriologie	1	2	8	3	3	3*	-	2	-	-	1	-	**23**
Maastricht	Acad. Ziekenhuis Maastricht	1	4	-	-	-	-	-	-	-	2	-	-	**7**
Nieuwegein	St. Antonius Ziekenhuis	6	9	70	-	-	2	-	-	-	-	1	-	**88**
Nijmegen	Canisius Wilhelmina Zknhs	1	1	2	3	-	1	-	-	-	-	-	1	**9**
	UMC St. Radboud	4	3	23	8	-	4	-	-	-	-	1	-	**43**

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Roermond	St. Laurentius Ziekenhuis	1	-	2	-	-	-	1	1	-	-	-	-	**5**
Roosendaal	St. Fransiscus Ziekenhuis	2	2	2	-	-	-	-	-	-	-	-	-	**6**
Rotterdam	Erasmus MC Med. Microbiologie	6	5	7	2	2	1	-	-	-	-	-	-	**23**
	Ikazia Ziekenhuis	3	2	-	-	1	2	-	-	-	-	-	-	**8**
	Maasstad Ziekenhuis	5	2	-	-	-	1	1	-	-	-	-	-	**9**
	St.Franciscus Gasthuis	3	1	2	-	2	-	1	-	-	-	-	1	**10**
Schiedam	Vlietland ziekenhuis	2	1	-	-	-	2	-	-	-	-	1	-	**6**
Sittard	Zuyderland Medisch Centrum	3	4	32	-	-	1	-	-	-	1	2	-	**43**
Terneuzen	Zorgsaam Zeeuws-Vlaanderen	-	-	1	-	-	1	-	-	-	-	-	-	**2**
Tilburg	Streeklab. Tilburg	4	10	17	4	-	3	-	1	-	-	-	-	**39**
Utrecht	Diakonessenhuis	5	2	1	1	-	-	-	-	-	1	-	-	**10**
	UMC Med. Microbiologie	1	6	13	11	1	1*	-	-	-	-	-	-	**33**
Veldhoven	PAMM, Lab. Med. Microbiologie	3	10	105	-	4	5	-	-	-	-	1	-	**128**
Vredenburg	Medical Microbology, Curacao	-	-	2	-	-	-	-	-	-	-	-	-	**2**
Venlo	Vie Curie medisch centrum	-	-	5	1	-	1	-	-	-	-	-	-	**7**
Weert	St. Jans gasthuis	-	-	1	-	-	-	-	-	-	-	-	-	**1**
Woerden	Zuwe Hofpoort Ziekenhuis	2	-	2	-	-	-	-	-	-	-	-	-	**4**
Zwolle	Isala Klinieken LMMI	-	4	5	-	2	1	-	-	-	-	1	-	**13**
Total		136	188	905	50	69	70	10	11	2	10	37	2	**1490**

\# **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 *N. meningitidis* was send bij Amsterdam AMC and CoMicro, Hoorn
One *S. pneumoniae* was send bij Breda, Amphia and Dordrecht RLM
One *L. monocytogenes* was send bij Utrecht UMC and St Antonius NIeuwegein,

The distribution of the isolates received in the 5 year period 2012 through 2016 is presented in table 2.3. The number of total isolates increased from 1243 in 2014 to 1490 in 2016. The number of cases of meningococcal disease was higher compared to the number of cases in 2015 (2016: 136; 2015: 84; 2014: 73). 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 143 in 2016. The number of *Listeria monocytogenes* was high in 2005 (81), most likely due to an intensified surveillance performed by the RIVM. In 2016, the number of *L. monocytogenes* isolates was 70. 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 2012 – 2016

Species	**2012**			**2013**			**2014**			**2015**			**2016**		
	CSF	**Blood**	**Total**	**CSF**	**Blood**	**Total**	**CSF**	**Blood**	**Total**	**CSF**	**Blood**	**Total**	**CSF**	**Blood**	**Total**
N. meningitidis	41	40	81	39	72	111	31	42	73	33	51	84	36	100	136
H. influenzae	16	124	140	16	144	160	21	140	161	22	173	195	26	162	188
S. pneumoniae	138	731	869	138	768	906	142	627	769	147	754	901	143	762	905
E. coli	5	8	13	8	18	26	8	24	32	8	20	28	18	32	50
S. agalactiae	23	57	80	20	52	72	23	48	71	19	46	65	20	49	69
L. monocytogenes	9	50	59	6	46	52	19	51	70	8	39	47	11	59	70
S. pyogenes	3	9	12	9	22	31	2	6	8	3	13	16	5	5	10
S. aureus	7	1	8	5	18	23	13	10	23	8	8	16	10	1	11
Coag.neg.Staph.	6	0	6	6	0	6	2	0	2	2	0	2	2	0	2
C. neoformans	9	1	10	6	2	8	4	3	7	7	2	9	7	3	10
others	17	8	25	14	6	20	22	4	26	30	10	40	19	18	37
non viable	0	1	1	0	1	1	0	1	1	0	1	1	0	2	2
Total	**274**	**1030**	**1304**	**267**	**1149**	**1416**	**287**	**956**	**1243**	**287**	**1117**	**1404**	**297**	**1193**	**1490**

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 2012 to 2016 is shown in table 2.4. The incidence of *H. influenzae* infection was 50% lower than in the years before vaccination was introduced (2.1 in 1992; 1.1 in 2016). 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, 2012 - 2016

Species	**2012**	**2013**	**2014**	**2015**	**2016**
N. meningitidis	0.48	0.66	0.43	0.50	0.80
H. influenzae	0.84	0.95	0.96	1.15	1.11
S. pneumoniae	5.19	5.40	4.57	5.33	5.33
E. coli	0.08	0.15	0.19	0.17	0.29
S. agalactiae	0.48	0.43	0.42	0.38	0.41
L. monocytogenes	0.35	0.31	0.42	0.28	0.41
S. pyogenes	0.07	0.18	0.05	0.09	0.06
S. aureus	0.05	0.14	0.14	0.09	0.07
Coag. neg. Staph.	0.04	0.04	0.01	0.01	0.01
C. neoformans	0.06	0.05	0.04	0.05	0.06
others	0.15	0.12	0.15	0.24	0.22
non viable	0.01	0.01	0.01	0.01	0.01
Total	**7.79**	**8.44**	**7.39**	**8.31**	**8.78**

Table 2.5 Total number of isolates from CSF and/or blood received in 2016, according to bacterial species and specimen source

Species		CSF or CSF and blood	Blood only	Total	%
Neisseria meningitidis		36	100	136	**9.1**
Haemophilus influenzae [1]		26	162	188	**12.6**
Streptococcus pneumoniae		143	762	905	**60.8**
Escherichia coli		18	32	50	**3.4**
Streptococcus agalactiae		20	49	69	**4.6**
Listeria monocytogenes		11	59	70	**4.7**
Streptococcus pyogenes		5	5	10	**0.7**
Staphylococcus aureus		10	1	11	**0.7**
Coagulase-negative staphylococcus [2,3]		2	-	2	**0.1**
Cryptococcus neoformans		7	3	10	**0.7**
Others total		19	18	37	**2.5**
Others	*Pseudomonas aeruginosa*	*1*	*0*	*1*	
	Serratia marcescens	*1*	*0*	*1*	
	Aggregatibacter segnis	*0*	*1*	*1*	
	Klebsiella oxytoca	*1*	*0*	*1*	
	Klebsiella pneumoniae	*1*	*0*	*1*	
	Moraxella catarrhalis	*1*	*0*	*1*	
	Moraxella osloensis	*2*	*0*	*2*	
	Micrococcus luteus	*1*	*0*	*1*	
	Streptococcus equinus	*0*	*1*	*1*	
	Streptococcus gallolyticus ssp gallolyticus	*0*	*2*	*2*	
	Streptococcus gallolyticus ssp pasteurianus	*0*	*4*	*4*	
	Streptococcus gordonii	*0*	*1*	*1*	
	Streptococcus infantis	*0*	*2*	*2*	
	Streptococcus intermedius	*2*	*0*	*2*	
	Streptococcus lutetiensis	*0*	*1*	*1*	
	Streptococcus massiliensis	*0*	*1*	*1*	
	Streptococcus mitis	*2* [4]	*2*	*4*	
	Streptococcus oralis	*1*	*0*	*1*	
	Streptococcus parasanguinis	*0*	*1*	*1*	
	Streptococcus salivarius	*2*	*1*	*3*	
	Haemophilus parainfluenzae	*0*	*1*	*1*	
	Enterococcus faecalis	*1*	*0*	*1*	
	Enterococcus faecium	*1*	*0*	*1*	
	Rhizobium radiobacter	*1*	*0*	*1*	
	Rothia mucilaginosa	*1*	*0*	*1*	
Non viable		0	2 [5]	2	**0.1**
Total **%**		**297**	**1193**	**1490**	**100.0**

1 In one patient *Haemophilus influenzae* and *Staphylococcus aureus* were islated from the CSF.
2 In one patient *Staphylococcus epidermidis, Serratia marcescens* and *Klebsiella oxytoca* were islated from the CSF.
3 2 Coagulase-negative staphylococcus were *Staphylococcus epidermidis*
4 In one patient *Streptococcus salivarius* and *Streptococcus mitis* were islated from the CSF.
5 Non viable, in two patients a Streptococcus pneumoniar was isolated from the blood. Those isolates were non viable.

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 *S. aureus* and once for *H. influenzae*.

3 BACTERIAL MENINGITIS - general data

In 2016, the Reference Laboratory received CSF isolates from 297 patients. Furthermore, 24 culture-negative CSF samples appeared to be positive by antigen detection or PCR (Table 11.1). Of these CSF samples, 14 were positive for *N. meningitidis* and 7 for *S. pneumoniae.* Including these cases, the total number of patients with confirmed meningitis amounted to 328. The proportion of meningococcal and pneumococcal meningitis among these patients was 16% and 47%, respectively (Figure 3.1).

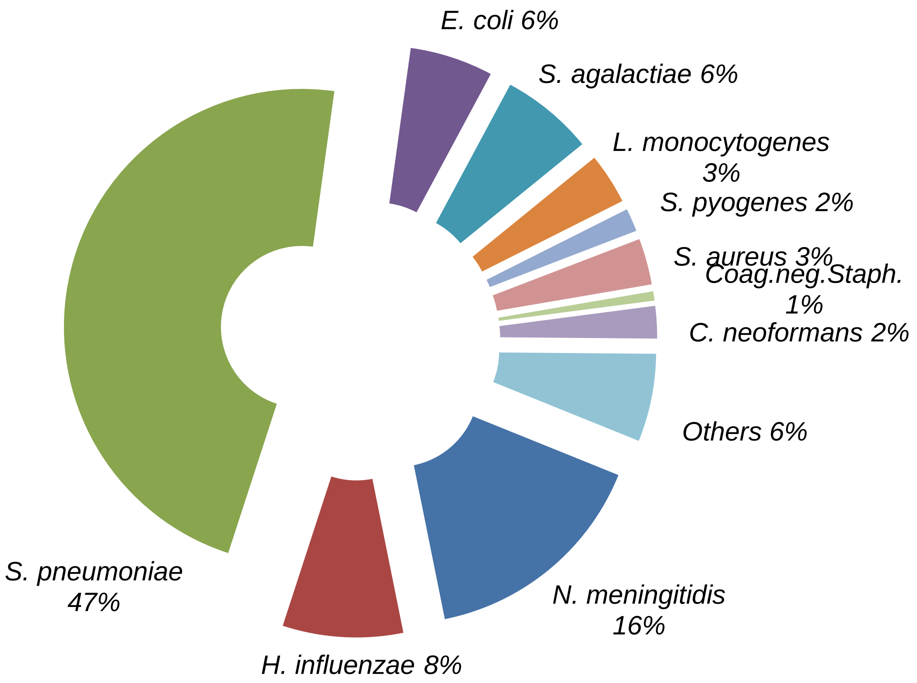

Figure 3.1 *Proportional distribution of CSF isolates and CSF positive samples, 2016*

Figure 3.2 shows the annual total number of bacterial isolates from CSF during the period 2007-2016. 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 2.8 and 1.8 during the period 2007-2016 (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 0.12 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. 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 0.84 in 2016 due to vaccination against pneumococci introduced in June 2006 in the National Immunisation Programme.

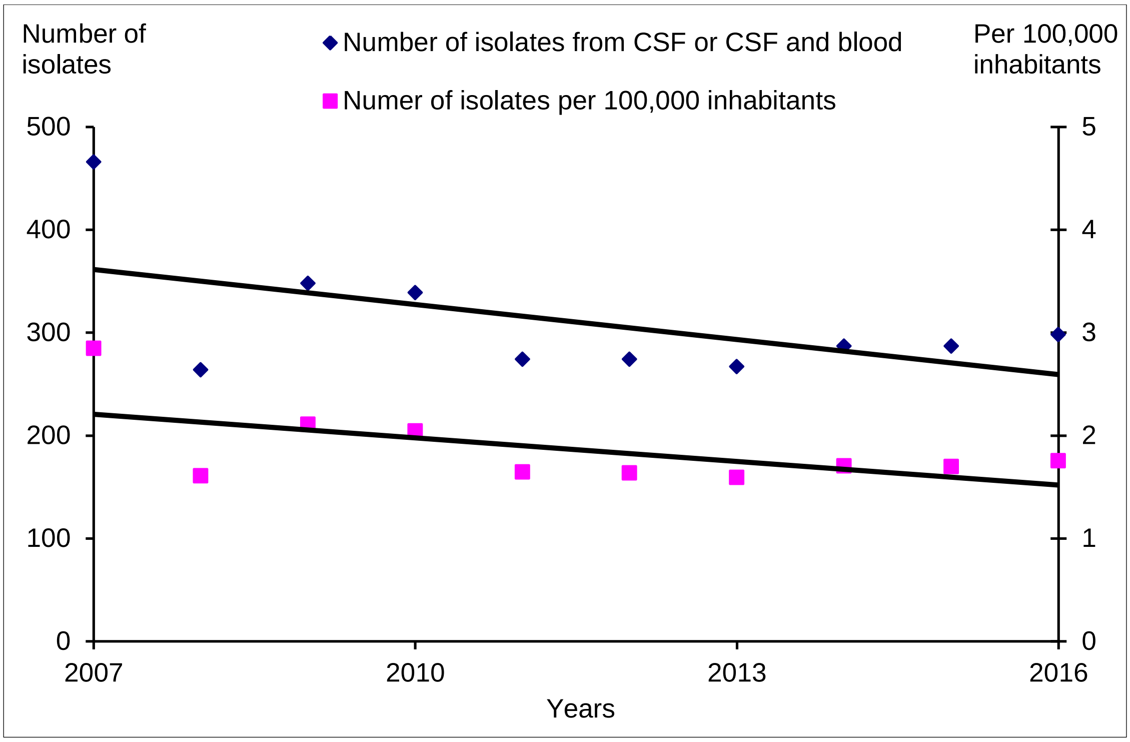

Figure 3.2 *Isolates from CSF, 2007-2016*

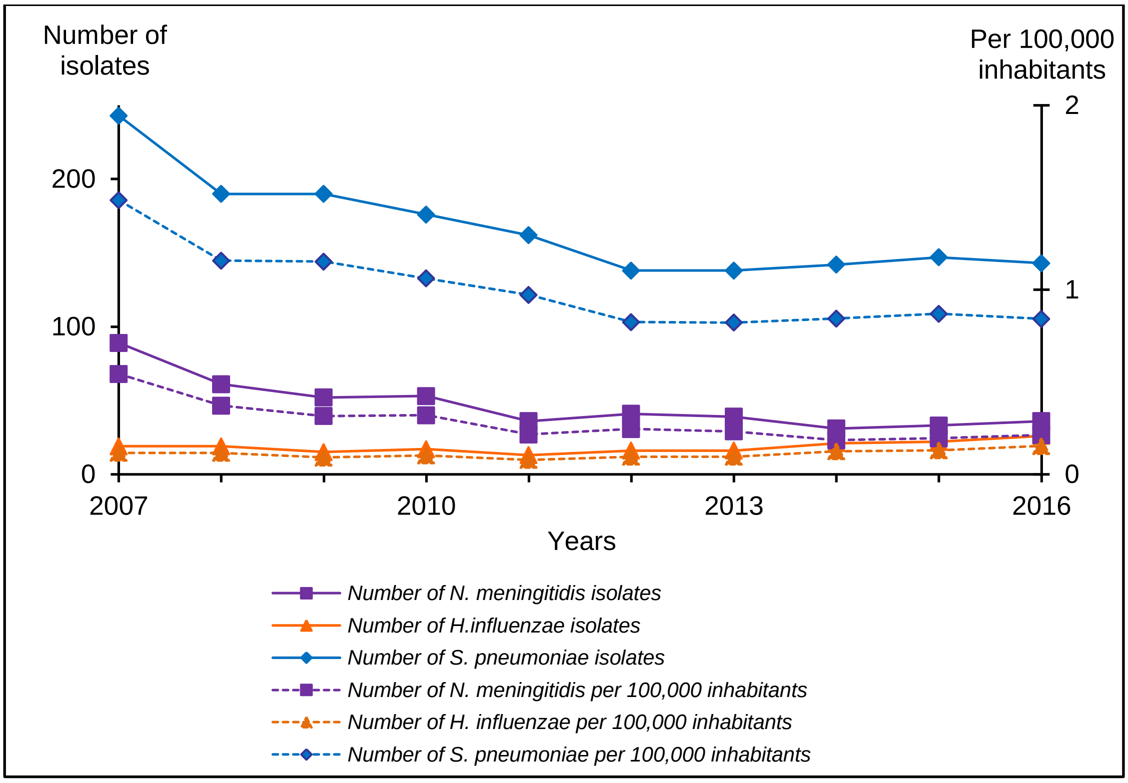

Figure 3.3 *Meningococcal, Haemophilus and pneumococcal meningitis, 2007-2016*

Table 3.1 shows the frequency of isolation of the different bacterial species from CSF by annual quarter. As in previous years, most strains were received during the first quarter of the year.

Table 3.1 Isolates from CSF by annual quarter, 2016

	ANNUAL QUARTER					
SPECIES	**First**	**Second**	**Third**	**Fourth**	**Total**	**%**
N. meningitidis	10	4	10	12	**36**	*12.1*
H. influenzae	8	4	5	9	**26**	*8.8*
S. pneumoniae	50	41	12	40	**143**	*48.1*
E. coli	3	2	7	6	**18**	*6.1*
S. agalactiae	5	2	9	4	**20**	*6.7*
L. monocytogenes	3	1	6	1	**11**	*3.7*
S. pyogenes	2	3	0	0	**5**	*1.7*
S. aureus	2	3	2	3	**10**	*3.4*
Coag.neg.Staph.	0	0	1	1	**2**	*0.7*
C. neoformans	1	3	2	1	**7**	*2.3*
Others	4	5	8	2	**19**	*6.4*
non viable	0	0	0	0	**0**	*0.0*
Total	**88**	**68**	**62**	**79**	**297**	***100.0***
%	***29.6***	***22.9***	***20.9***	***26.6***	**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 43% 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 86%). 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 14 in this agegroup, two times higher than in 2015.

Table 3.2 Isolates from CSF grouped according to patients' age, 2016

Group	**AGE (MONTHS)**			**AGE (YEARS)**										**TOTAL**	
	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	6	6	12	3	0	10	4	1	3	2	0	1	**36**	***12.1***
H. influenzae	0	4	10	14	0	0	0	1	0	2	4	5	0	**26**	***8.8***
S. pneumoniae	1	19	5	25	5	1	1	3	9	7	41	40	11	**143**	***48.1***
E. coli	7	3	0	10	0	0	0	0	1	0	2	3	1	**18**	***6.1***
S. agalactiae	10	5	0	15	0	0	0	0	0	0	1	3	1	**20**	***6.7***
L. monocytogenes	0	0	0	0	0	0	0	0	0	0	3	7	1	**11**	***3.7***
S. pyogenes	0	0	0	0	1	0	0	0	0	1	0	3	0	**5**	***1.7***
S. aureus	0	2	0	2	0	0	0	1	2	1	3	1	0	**10**	***3.4***
Coag.neg.Staph.	1	0	0	1	0	0	0	0	0	0	0	0	1	**2**	***0.7***
C. neoformans	0	0	0	0	0	0	0	1	0	2	2	1	1	**7**	***2.3***
Others	4	1	1	6	0	1	1	0	2	1	4	3	0	**19***	***6.4***
non viable	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
Total	**23**	**29**	**14**	**66**	**11**	**6**	**8**	**8**	**19**	**23**	**74**	**55**	**17**	**297**	*100*
%	***8.0***	***10.1***	***4.9***	***23.0***	***3.8***	***2.1***	***2.8***	***2.8***	***6.6***	***8.0***	***25.8***	***19.2***	***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, 2016

SPECIES	AGE (YEARS)											
	0	1-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total
N. meningitidis	3.52	0.85	0.32	0.00	0.98	0.19	0.05	0.12	0.06	0.00	0.13	**0.21**
H. influenzae	2.35	1.42	0.00	0.00	0.00	0.05	0.00	0.08	0.11	0.21	0.00	**0.15**
S. pneumoniae	11.74	0.71	0.54	0.10	0.10	0.14	0.44	0.29	1.17	1.71	1.47	**0.84**
E. coli	5.87	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.06	0.13	0.13	**0.11**
S. agalactiae	8.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.13	0.13	**0.12**
L. monocytogenes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.30	0.13	**0.06**
S. pyogenes	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.04	0.00	0.13	0.00	**0.03**
S. aureus	1.17	0.00	0.00	0.00	0.00	0.05	0.10	0.04	0.09	0.04	0.00	**0.06**
Coag.neg.Staph.	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	**0.01**
C. neoformans	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.08	0.06	0.04	0.13	**0.04**
Others	2.94	0.14	0.00	0.10	0.10	0.00	0.10	0.04	0.11	0.13	0.00	**0.11**
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	**30.53**	**1.98**	**1.18**	**0.60**	**0.79**	**0.37**	**0.94**	**0.96**	**2.11**	**2.35**	**2.27**	**1.75**

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 0.3 and 1.8. The M/F ratio among patients infected with *S. aureus, C. neoformans* or *E. coli were* 2.0, 2.5 and 3.5 respectivily. The overall M/F ratio was 1.2.

Table 3.4 Isolates from CSF according to patients' gender, 2016

SPECIES	M	F	M/F – ratio	Sex not known	Total	%
N. meningitidis	16	20	**0.8**	0	**36**	*12.1*
H. influenzae	14	12	**1.2**	0	**26**	*8.8*
S. pneumoniae	81	61	**1.3**	1	**143**	*48.1*
E. coli	14	4	**3.5**	0	**18**	*6.1*
S. agalactiae	7	12	**0.6**	1	**20**	*6.7*
L. monocytogenes	7	4	**1.8**	0	**11**	*3.7*
S. pyogenes	1	4	**0.3**	0	**5**	*1.7*
S. aureus	6	3	**2.0**	1	**10**	*3.4*
Coag.neg.Staph.	2	0	**-**	0	**2**	*0.7*
C. neoformans	5	2	**2.5**	0	**7**	*2.3*
Others	10	9	**1.2**	0	**19**	*6.4*
non viable	0	0	**0.0**	0	**0**	*0.0*
Total	**163**	**131**	**1.2**	**3**	**297**	***100***
%	***54.9***	***44.1***		***1.0***	***100***	

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2016, the Reference Laboratory received 136 *Neisseria meningitidis* isolates, of which 36 were isolated from CSF (or CSF and blood) (33 in 2015) and 100 from blood only (51 in 2015). Twenty DNA positive samples were received,14 from CSF, 4 from blood and two from other body fluids wich were neglected, because not al details are available. From those 20 DNA samples 14 were of group B, one Y and five W. This means that 13% of invasive meningococcal disease concerned patients with a positive blood culture only, either because no meningitis was present or because no CSF specimen was obtained. The distribution of isolates according to month of receipt shows the highest number of isolates in the fourth quarter, while in previous years the highest number of isolates was received in the first quarter of the year (figure 4.1).

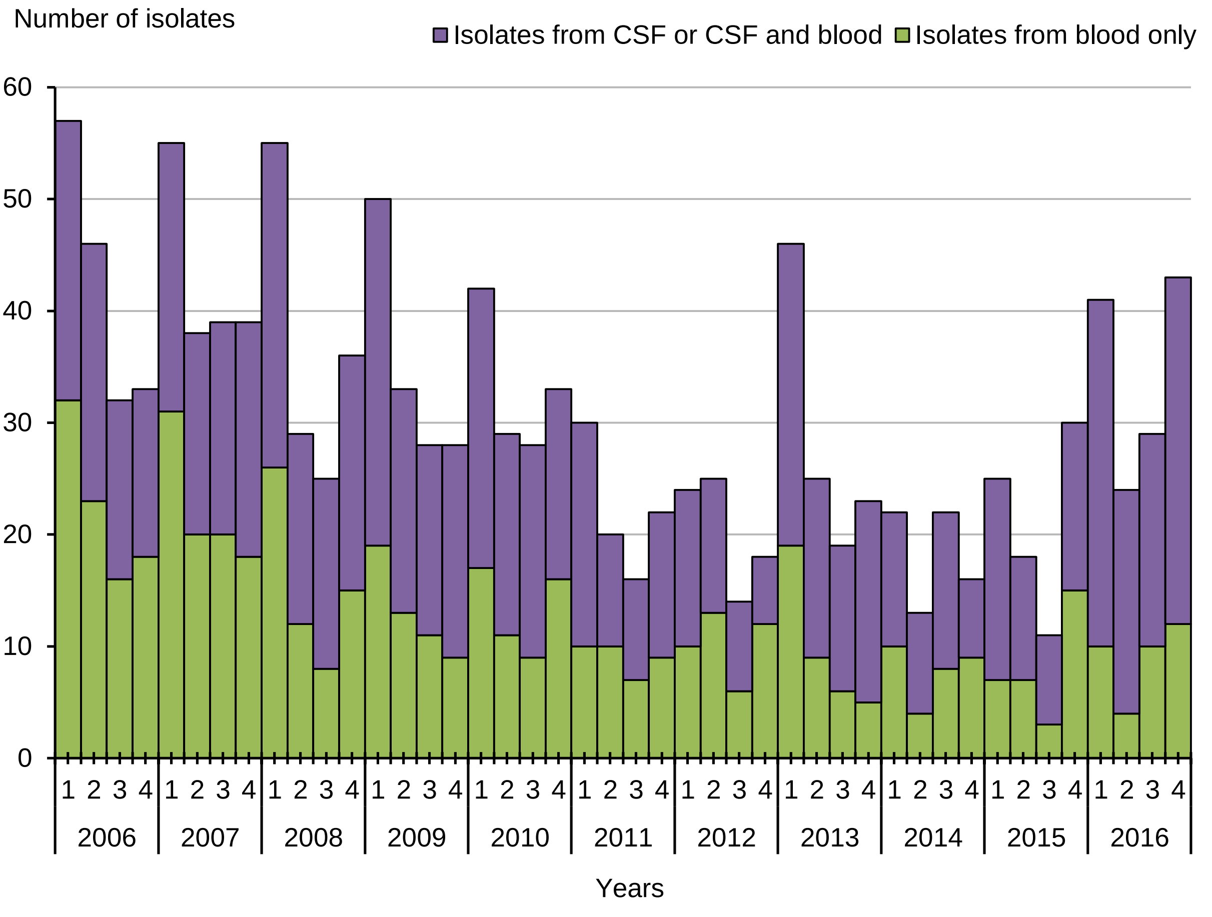

Figure 4.1 *Seasonal distribution of meningococcal disease, 2006-2016*

4.2 Antibiotic susceptibility

Eighty-eight percent of all isolates (120/136) were susceptible to penicillin (MIC ≤ 0.064 µg/ml; CSF isolates 89%, isolates from blood only 88%). This is lower as in the previous year. 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 decrease of the number of reduced penicillin susceptible meningococcal isolates. Two isolates were resistant to Rifampicine, which means that 98.5% of all isolates were susceptible to Rifampicin.

Table 4.1 Susceptibility of *N. meningitidis* CSF and/or blood isolates to penicillin, 2016

	Penicillin*					
	MIC ≤ 0.064 sensitive	0.064< MIC≤0.25	0.25< MIC≤1.0	MIC >1.0	Total	%
CSF or CSF and blood	32	4	0	0	**36**	**26**
Blood only	88	12	0	0	**100**	**74**
Total	**120**	**16**	**0**	**0**	**136**	**100**
%	**88**	**12**	**0**	**0**	**100**	

* MIC values in µg/ml

Table 4.2 Susceptibility of *N. meningitidis* from CSF/ CSF and blood to penicillin, 2009-2016

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064< MIC≤0.25		0.25< MIC≤1.0		MIC >1.0		Total
	N	%	N	%	n	%	n	%	
2009	51	**98.1**	1	**1.9**	0	**0.0**	0	**0.0**	**52**
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**

* MIC values in µg/ml

Table 4.3 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2009-2016

	Penicillin*								
	MIC ≤ 0.064 sensitive		0.064< MIC≤0.25		0.25< MIC≤1.0		MIC >1.0		Total
	N	%	n	%	n	%	n	%	
2009	77	**88.5**	10	**11.5**	0	**0.0**	0	**0.0**	87
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

* MIC values in µg/ml

4.3 Serogroups

Serogroup B accounted for 49% (2015: 74%; 2014: 73% 2013: 75%) of all isolates. Group C and Y are responsible for 4% and 12%, respectively. The proportion group W increased to 34% (was 10% in 2015) (table 4.4). The serogroup distribution observed during the whole collection period 1959 - 2016 (figure 4.2) shows that in 2014 the number of group B isolates (53 cases) was the lowest since 1976. In 2016, the number of group B isolates (67) 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 2016 there was a slight increase from 3 group C isolates in 2014 to 6 in 2016 (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).

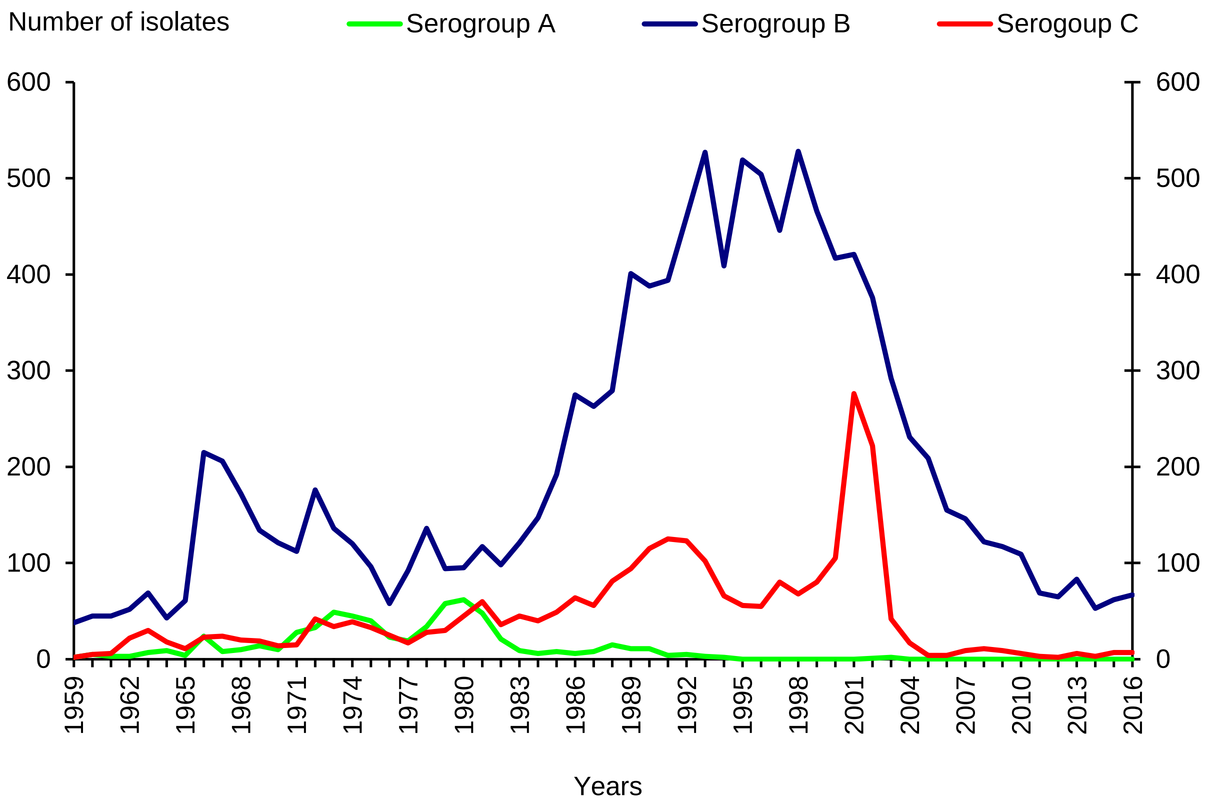

Figure 4.2. *Distribution of meningococcal serogroups A, B and C, 1959-2016*

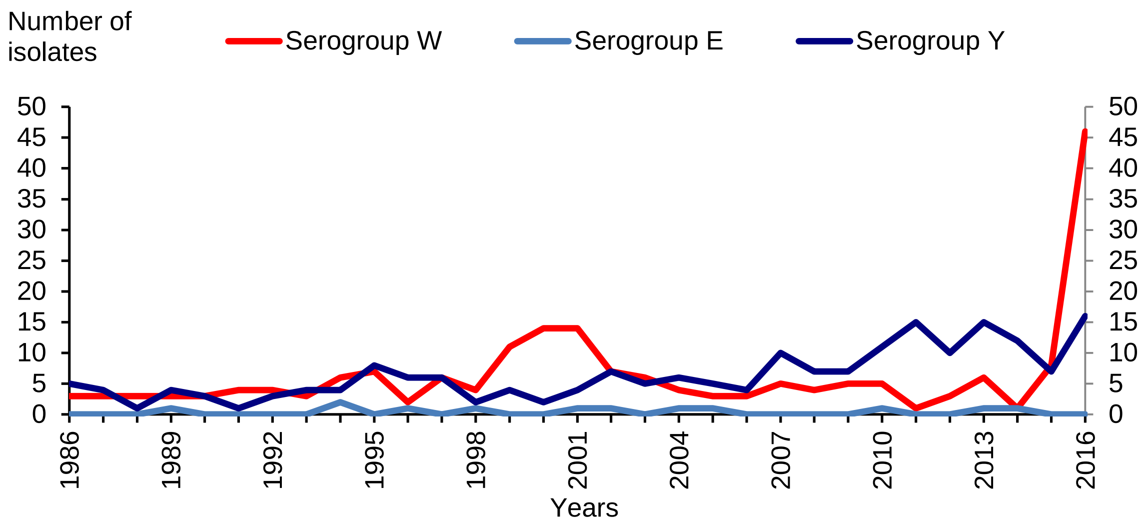

Figure 4.2.1. *Distribution of meningococcal serogroups Y, W and E, 1986-2016*

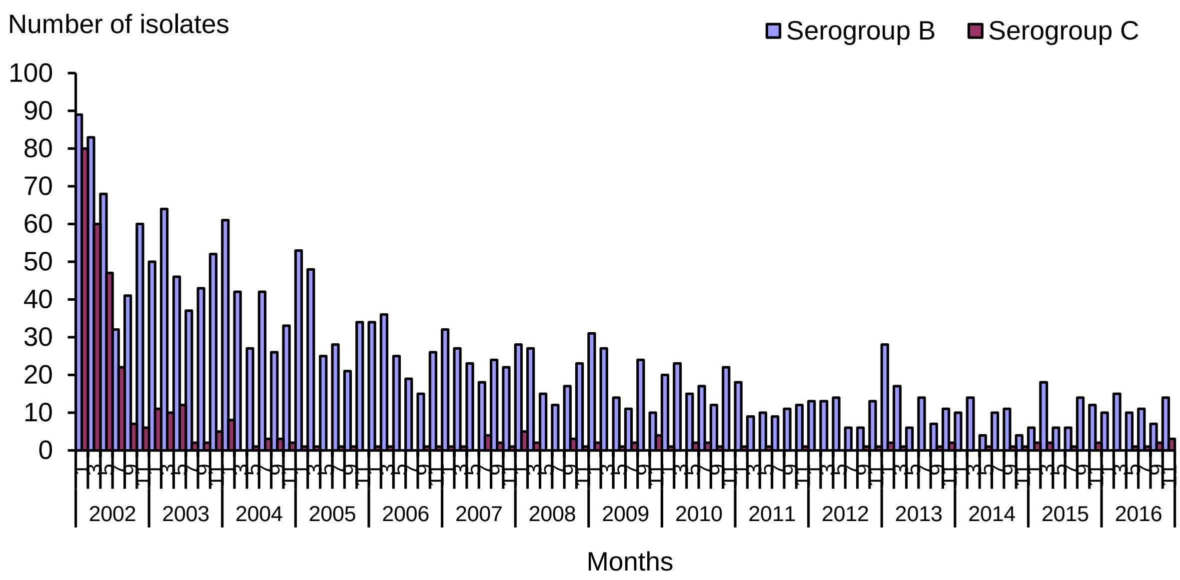

Figure 4.3 *Bimonthly distribution of meningococcal serogroups B and C, 2002-2016*

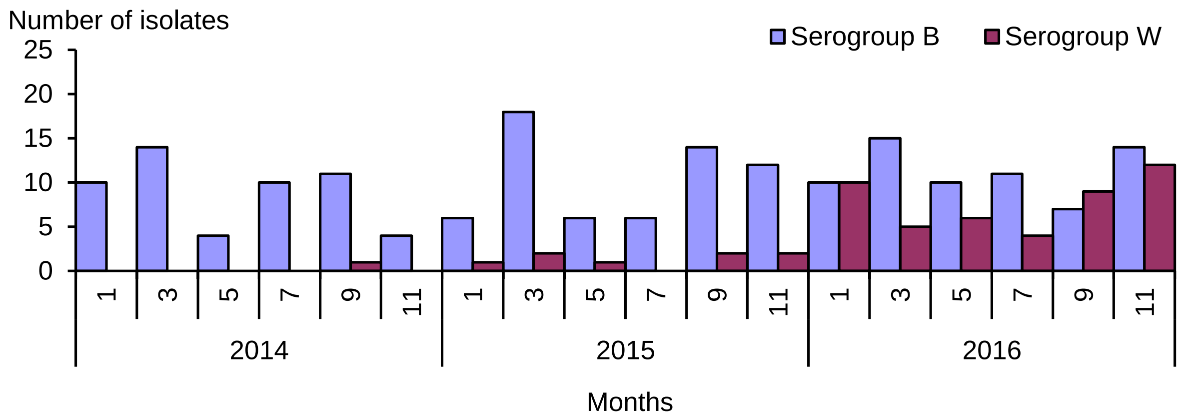

Figure 4.4 *Bimonthly distribution of meningococcal serogroups B and W, 2014-2016*

4.4 Serogroup and age

The age distribution of patients with meningitis and/or meningococcemia shows that 18% (25 of 136) of the patients was younger than 5 years (table 4.4, figure 4.4). Among patients from whom meningococci were isolated from blood only, only 13% was younger than 5 years, while almost 40% was older than 65 years of age (table 4.7). Among serogroup B cases, 64% (43/67) were younger than 25 years of age. In contrast, 65% (30/46) of the serogroup W cases were older than 50 years of age. In addition, of 67 serogroup B isolates, 24 (36%) were from CSF, while of 46 serogroup W isolates, only 5 (11%) were from CSF.

Table 4.4 Serogroups of *N. meningitidis* (all isolates: from CSF and /or blood, absolute numbers) by patient age, 2016

	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	10	12	22	6	2	8	5	2	8	8	6	**67**	***49.3***
C	0	0	0	0	0	0	1	0	1	1	0	3	**6**	***4.4***
Y	0	0	0	0	0	0	2	1	0	2	2	9	**16**	***11.8***
W	0	0	3	3	1	1	4	4	2	1	9	21	**46**	***33.8***
X	0	0	0	0	0	0	0	0	0	0	0	1	**1**	***0.7***
Total	**0**	**10**	**15**	**25**	**7**	**3**	**15**	**10**	**5**	**12**	**19**	**40**	**136**	***100.0***
%	***0.0***	***7.4***	***11.0***	***18.4***	***5.1***	***2.2***	***11.0***	***7.4***	***3.7***	***8.8***	***14.0***	***29.4***	***100.0***	

Table 4.5 Serogroups of *N. meningitidis* (isolates from CSF, or CSF* and blood; absolute numbers) by patient age, 2016

	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	6	4	10	2	0	7	2	0	2	1	0	**24**	***66.7***
C	0	0	0	0	0	0	1	0	0	1	0	1	**3**	***8.3***
Y	0	0	0	0	0	0	2	1	0	1	0	0	**4**	***11.1***
W	0	0	2	2	1	0	0	1	0	0	1	0	**5**	***13.9***
Total	**0**	**6**	**6**	**12**	**3**	**0**	**10**	**4**	**0**	**4**	**2**	**1**	**36**	***100.0***
%	***0.0***	***16.7***	***16.7***	***33.3***	***8.3***	***0.0***	***27.8***	***11.1***	***0.0***	***11.1***	***5.6***	***2.8***	***100***	

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

Table 4.6 Age distribution of meningitis (incidence per 100,000 inhabitants) by different serogroups of *N. meningitidis* (isolates from CSF, or CSF and blood), 2016

	AGE (YEARS)										TOTAL
Group	0	1-4	5-9	10-14	15-19	20-24	25-29	30-49	50-64	≥65	T
B	3.52	1.13	0.22	0.00	0.69	0.19	0.00	0.05	0.03	0.00	**0.14**
C	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.02	0.00	0.03	**0.02**
Y	0.00	0.00	0.00	0.00	0.20	0.09	0.00	0.02	0.00	0.00	**0.02**
W	0.00	0.28	0.11	0.00	0.00	0.09	0.00	0.00	0.03	0.00	**0.03**
Total	**3.25**	**0.85**	**0.32**	**0.00**	**0.98**	**0.38**	**0.00**	**0.09**	**0.06**	**0.03**	**0.21**

Table 4.7 Serogroups of *N. meningitidis* (isolates from blood only, absolute numbers) by patient age, 2016

	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	8	12	4	2	1	3	2	6	7	6	**43**	***43.0***
C	0	0	0	0	0	0	0	0	1	0	0	2	**3**	***3.0***
Y	0	0	0	0	0	0	0	0	0	1	2	9	**12**	***12.0***
W	0	0	1	1	0	1	4	3	2	1	8	21	**41**	***41.0***
X	0	0	0	0	0	0	0	0	0	0	0	1	**1**	***1.0***
Total	**0**	**4**	**9**	**13**	**4**	**3**	**5**	**6**	**5**	**8**	**17**	**39**	**100**	***100.0***
%	***0.0***	***4.0***	***9.0***	***13.0***	***4.0***	***3.0***	***5.0***	***6.0***	***5.0***	***8.0***	***17.0***	***39.0***	***100.0***	

Table 4.8 Age distribution of meningococcemia (incidence per 100,000 inhabitants) by different serogroups of *N. meningitides* (isolates from blood only), 2016

	AGE (YEARS)										TOTAL
Group	0	1-4	5-9	10-14	15-19	20-24	25-29	30-49	50-64	≥65	T
B	2.35	1.13	0.43	0.20	0.10	0.28	0.19	0.14	0.20	0.19	**0.25**
C	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.06	**0.02**
Y	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.06	0.29	**0.07**
W	0.00	0.14	0.00	0.10	0.39	0.28	0.19	0.02	0.23	0.68	**0.24**
X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	**0.01**
Total	**2.35**	**1.27**	**0.43**	**0.30**	**0.49**	**0.56**	**0.47**	**0.18**	**0.49**	**1.26**	**0.59**

4.5 Group B meningococci

Figure 4.4 shows the age distribution of group meningococcal disease. The age-specific incidences per 100,000 inhabitants in the age groups younger than 5 years and 15 - 19 years were 2.5 and 0.8 respectively. The age-specific incidences per 100,000 inhabitants in the age groups >19 years was less than 0.5 except for the age group 85-95 years (incidence of 0.9-1.0).

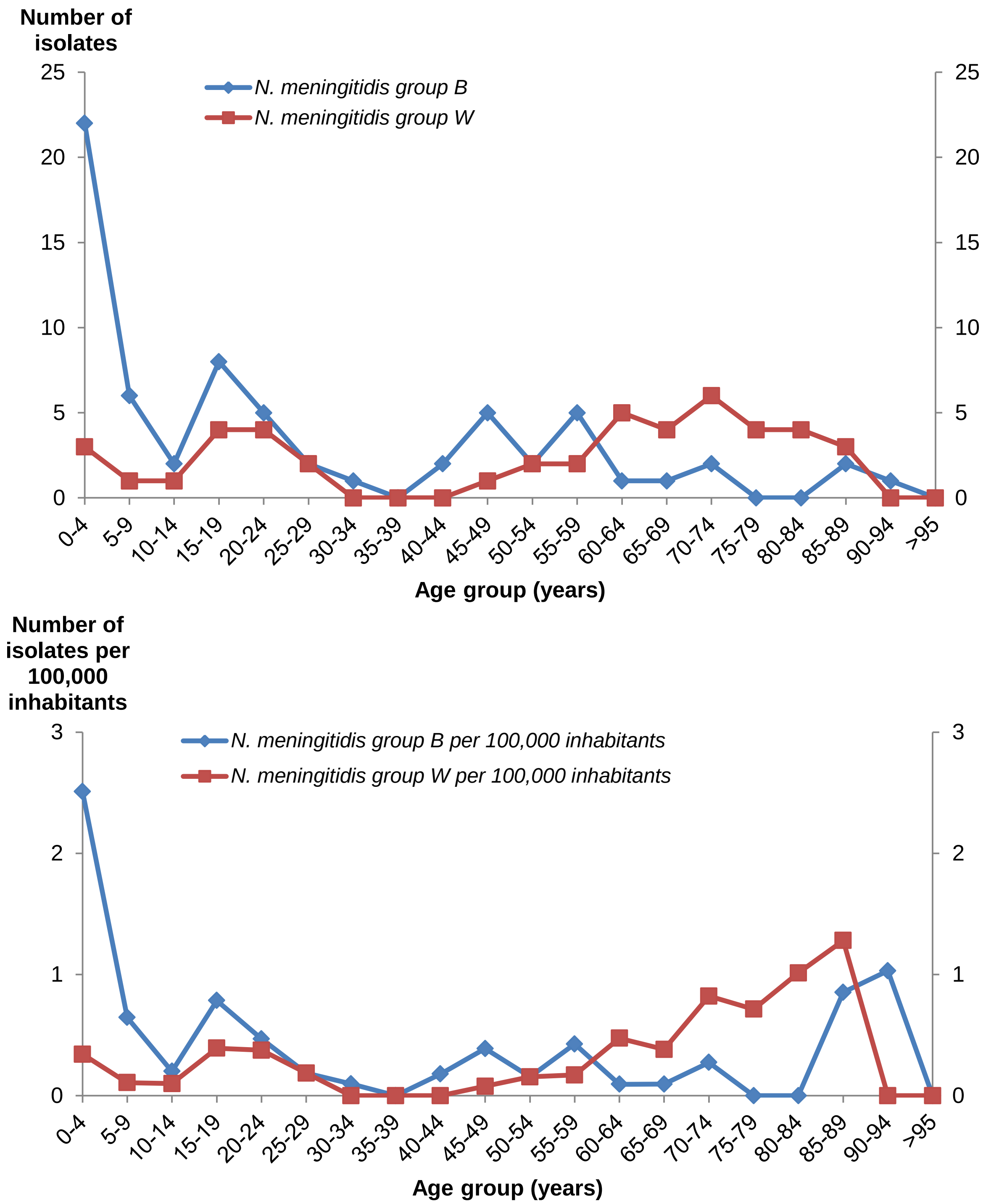

Figure 4.4 *Age distribution of serogroup B and W meningococcal disease in 2016*

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 on, 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 2016, 35 different VR1/VR2 combinations were encountered among serogroup B meningococci (2014: 28; 2015: 32). The proportion of the dominant PorA genosubtype P1.7-2,4 decreased from 40% of all serogroup B isolates in 2000 to 12% in 2016 (figure 4.5, figure 4.7; table 4.9). Eighty-four percent 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 six serogroup C isolates had 4 different VR1/VR2 combinations, P1.5,2, P1.5-1,10-8, P1.18-1,3 and P1.NT,30-3. Of the 46 serogroup W isolates, 43 (74%) had P1.5,2 and three had P1.18-1,3.

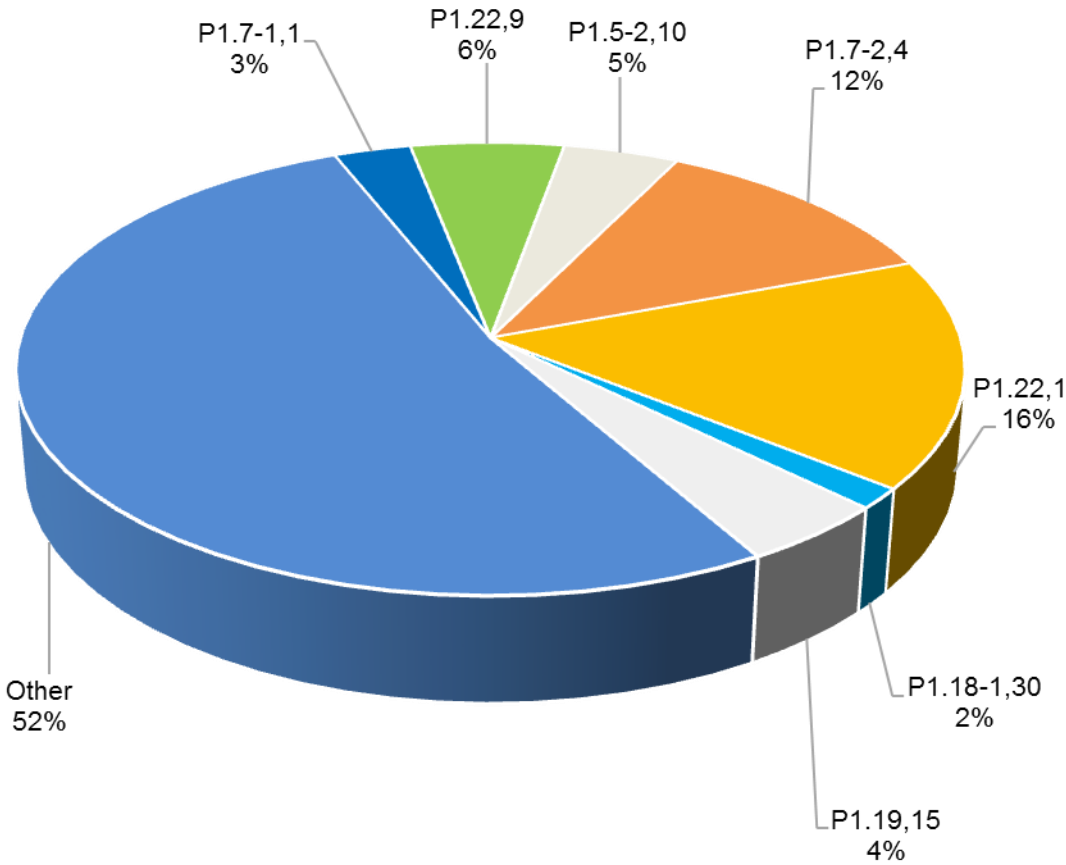

Figure 4.5 *Distribution of group B meningococcal PorA types, 2016*

Table 4.9 *N. meningitidis* serogroup B isolates according to PorA genosubtype, 2012-2016

	VR1,VR2 combination	2012 No.	2012 %	2013 No.	2013 %	2014 No.	2014 %	2015 No.	2015 %	2016 No.	2016 %
Vaccine types*	1.5-1, 2-2	0	**0**	0	**0**	0	**0**	0	**0**	1	**1.5**
	1.5-1, other	3	**4.6**	1	**1.2**	1	**1.9**	1	**1.6**	2	**3.0**
	1.5-2,10	4	**6.2**	7	**8.4**	4	**7.5**	3	**4.8**	3	**4.4**
	1.5-2, other	0	**0**	0	**0**	1	**1.9**	2	**3.2**	1	**1.5**
	1.7,16	0	**0**	1	**1.2**	0	**0**	1	**1.6**	2	**3.0**
	1.7, other	1	**1.5**	5	**6.0**	1	**1.9**	2	**3.2**	2	**3.0**
	1.7-1, 1	2	**3.1**	0	**0**	1	**1.9**	3	**4.8**	2	**3.0**
	1.7-1, other	0	**0**	0	**0**	1	**1.9**	0	**0**	1	**1.5**
	1.7-2,4	6	**9.2**	7	**8.4**	8	**15.0**	7	**11.3**	8	**11.9**
	1.7-2, other	8	**12.3**	13	**15.7**	3	**5.7**	5	**8.2**	4	**6.0**
	1.12-1,13	0	**0**	0	**0**	0	**0**	0	**0**	0	**0**
	1.12-1, other	1	**1.5**	1	**1.2**	1	**1.9**	2	**3.2**	2	**3.0**
	1.18-1,3	1	**1.5**	3	**3.6**	0	**0**	1	**1.6**	2	**3.0**
	1.18-1, other	5	**7.7**	3	**3.6**	9	**17.0**	5	**8.2**	4	**6.0**
	1.19,15-1	0	**0**	3	**3.6**	2	**3.7**	2	**3.2**	1	**1.5**
	1.19, other	4	**6.2**	3	**3.6**	3	**5.7**	3	**4.8**	3	**4.4**
	1.22,14	12	**18.5**	9	**10.9**	9	**17.0**	9	**14.6**	11	**16.4**
	1.22,other	8	**12.3**	6	**7.3**	3	**5.7**	3	**4.8**	4	**6.0**
	Other, 14	0	**0**	2	**2.4**	1	**1.9**	1	**1.6**	2	**3.0**
	Other, 16	2	**3.1**	3	**3.6**	1	**1.9**	2	**3.2**	1	**1.5**
	Subtotal vaccine types	57	**87.7**	67	**80.7**	49	**92.5**	52	**83.9**	56	**83.6**
NVT**	Other **Non Vaccine Type**	8	**12.3**	16	**19.3**	4	**7.5**	10	**16.1**	11	**16.4**
	Total	65	**100.0**	83	**100.0**	53	**100.0**	62	**100.0**	67	**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

4.7 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 2016, 20 different FetA variants were observed among serogroup B meningococci. The dominant types were F1-5 and F 5-1, accounting for respectively 24 and 18% 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 2016, Fet A type F1-5 of 16 isolates was linked with 9 different PorA types. FetA type F1-5 was 6 times linked with PorA VR1/VR2 P1.7-2,4 and 8 times with P1.7-2 (7 in 2015; 7 in 2014; 5 in 2013).

The six serogroup C meningococci had three different FetA types, F3-3, F3-6 and F3-9 F3-3.The 46 meningococci W isolates had only 4 different FetA types (F1-1, F1-7, F3-4 and F3-9). Fourty-three isolates had FetA type F1-1, linked to PorA VR1/VR2 P1.5,2 and MLST clonal complex 11.

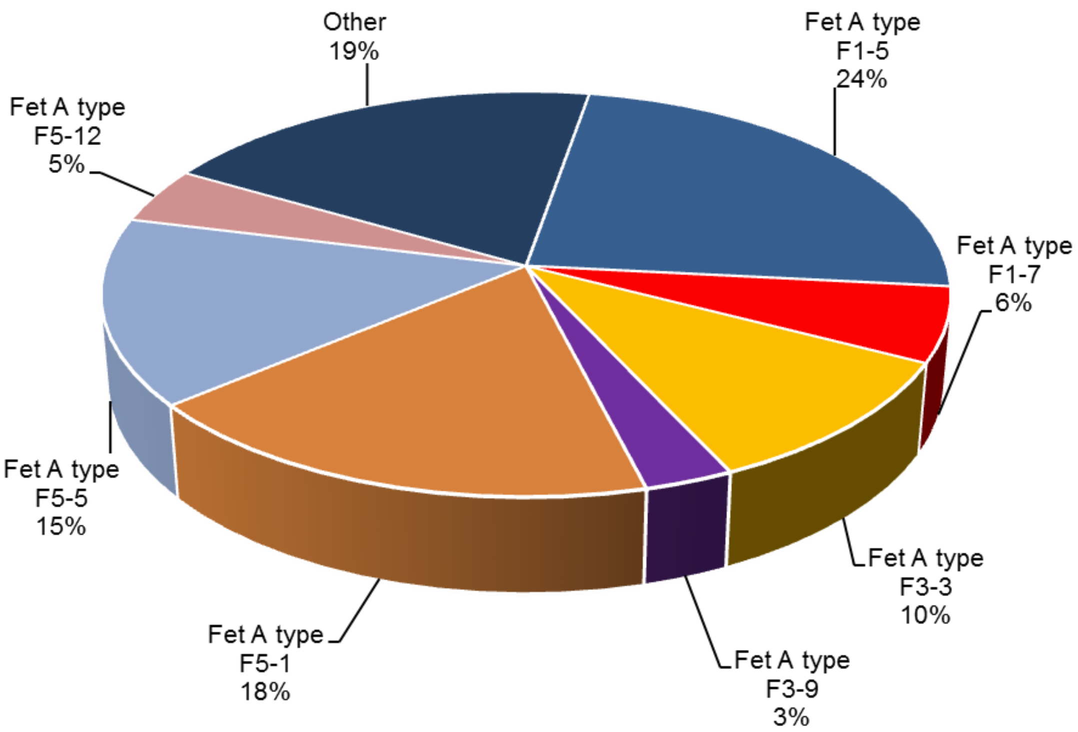

Figure 4.6 *Distribution of group B meningococcal FetA genosubtypes, 2016*

Table 4.10 *N. meningitidis* serogroup B isolates according to FetA genosubtype, 2012-2016

	YEARS									
FetA type	**2012**		**2013**		**2014**		**2015**		**2016**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
F1-5	23	35.4	17	20.5	8	15.1	10	16.1	16	23.9
F1-7	2	3.1	6	7.2	5	9.4	9	14.5	4	6.0
F1-15	1	1.5	1	1.2	1	1.9	1	1.6	0	0.0
F3-3	4	6.2	6	7.2	10	18.9	9	14.5	7	10.4
F3-7	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
F3-9	3	4.6	2	2.4	1	1.9	0	0.0	2	3.0
F4-1	2	3.1	2	2.4	1	1.9	2	3.2	0	0.0
F5-1	7	10.8	14	16.9	14	26.4	10	16.2	12	17.9
F5-2	0	0.0	2	2.4	0	0.0	2	3.2	0	0.0
F5-5	11	16.9	8	9.7	4	7.5	7	11.3	10	14.9
F5-8	1	1.5	0	0.0	1	1.9	1	1.6	0	0.0
F5-12	2	3.1	0	0.0	1	1.9	3	4.8	3	4.5
Other	9	13.8	25	30.1	7	13.2	8	13.0	13	19.4
Total	**65**	**100.0**	**83**	**100.0**	**53**	**100.0**	**62**	**100.0**	**67**	**100.0**

In 2016, 35 different VR1/VR2 combinations and 20 different FetA variants were encountered among serogroup B meningococci. Among the dominant FetA type F5-1, accounting for 18% of group B meningococci, 3 were of P1.5-2,10:F5-1, 3 were of P1.22,14:F5-1 and 3 were of type P1.19,15:F5-, three had another combination. Frequently found combinations are P1.22,14:F5-5 (10.4%) and P1.7-2,4:F1-5 (8.9%) (Figure 4.7).

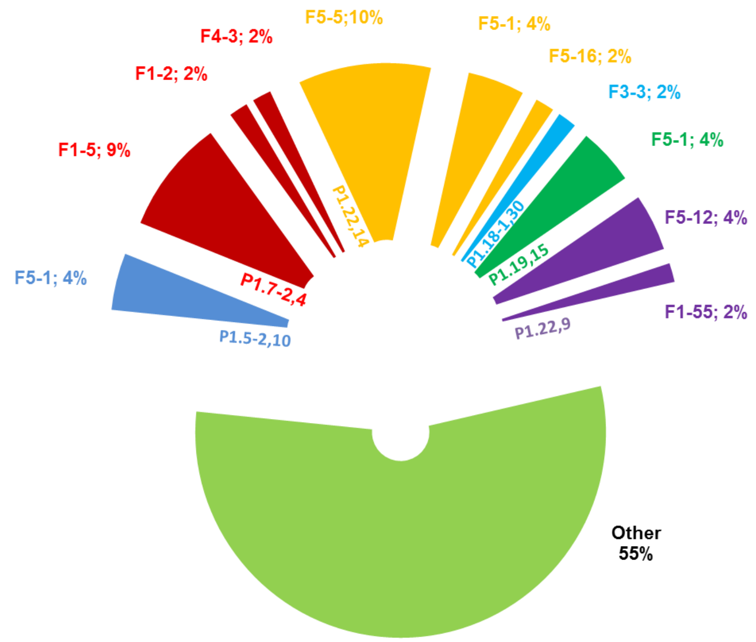

Figure 4.7 *Distribution of group B meningococcal PorA and FetA geno(sub)types, 2016*

5 *HAEMOPHILUS INFLUENZAE*

5.1 General features

In total, 188 *Haemophilus influenzae* isolates were submitted to the Reference Laboratory. This number is similar to that in previous years (table 2.3, figure 3.3, figure 5.1). Twenty-six strains were isolated from CSF (or CSF and blood) (2015: 22; 2014: 21; 2013: 16; 2012: 16; 2011: 13), and 162 from blood only. Thirty-four (23%) 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.

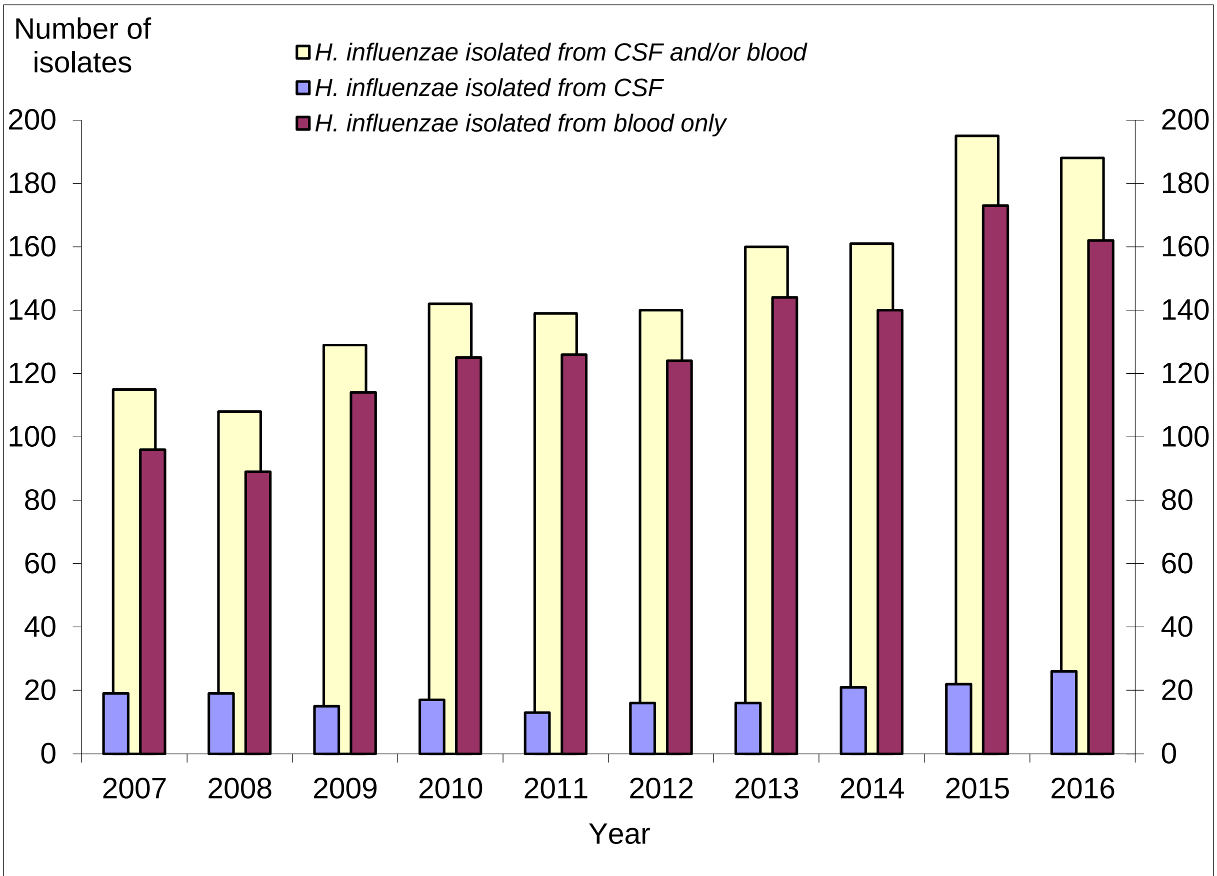

Figure 5.1 *Distribution of H. influenzae, 2007-2016*

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.

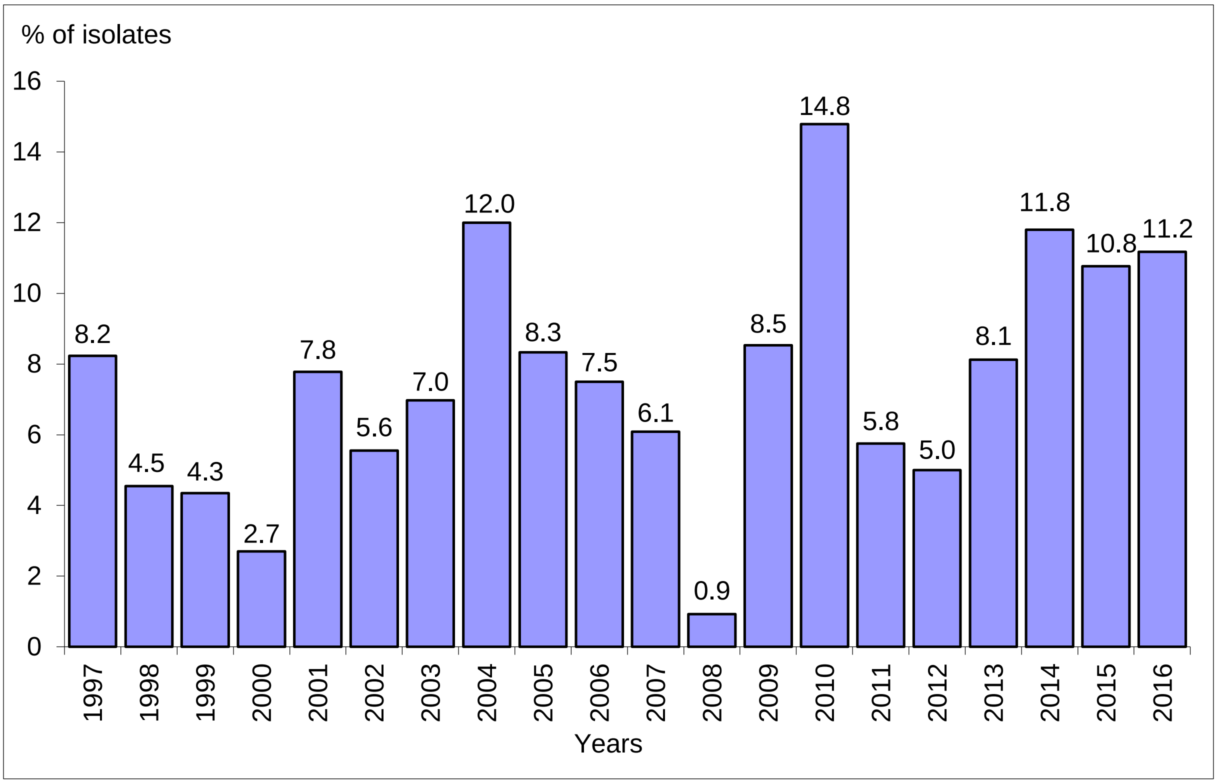

Figure 5.2 *Percentage β-lactamase producing H. influenzae, 1997-2016*

5.3 Serotype and age

Thirteen cases of *H. influenzae* type b invasive disease were observed among children younger than 2 years of age (8 in 2015; 6 in 2014; 9 in 2013; 3 in 2012) (figure 5.3). Of 188 *H. influenzae* isolates, 123 were non-typeable; 10 isolated from CSF (or CSF and blood) and 113 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, 2016

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	2	**3**	***1.6***
b	0	7	14	21	1	1	4	17	**44**	***23.4***
d	0	0	0	0	0	0	0	1	**1**	***0.5***
e	0	0	0	0	0	0	1	4	**5**	***2.7***
f	0	2	2	4	0	0	1	7	**12**	***6.4***
n.t.*	3	7	0	10	1	0	15	97	**123**	***65.4***
Total	**3**	**16**	**16**	**35**	**2**	**1**	**22**	**128**	**188**	***100***
%	***1.6***	***8.5***	***8.5***	***18.6***	***1.0***	***0.5***	***11.7***	***68.1***	***100***	

* non-typable

Table 5.2 *H.influenzae* isolates from CSF (or CSF and blood), according to serotype and age, 2016

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**	***0.0***
b	0	2	9	11	0	0	0	1	**12**	***46.1***
d	0	0	0	0	0	0	0	0	**0**	***0.0***
e	0	0	0	0	0	0	0	0	**0**	***0.0***
f	0	1	1	2	0	0	1	1	**4**	***15.4***
n.t.*	0	1	0	1	0	0	2	7	**10**	***38.5***
Total	0	**4**	**10**	**14**	**0**	**0**	**3**	**9**	**26**	***100.0***
%	***0.0***	***15.4***	***38.5***	***53.9***	***0.0***	***0.0***	***11.5***	***34.6***	***100.0***	

* non-typable

Table 5.3 *H. influenzae* isolates from blood only, according to serotype and age, 2016.

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	2	**3**	***1.9***
b	0	5	5	10	1	1	4	16	**32**	***19.8***
d	0	0	0	0	0	0	0	1	**1**	***0.6***
e	0	0	0	0	0	0	1	4	**5**	***3.1***
f	0	1	1	2	0	0	0	6	**8**	***4.9***
n.t.*	3	6	0	9	1	0	13	90	**113**	***69.7***
Total	**3**	**12**	**6**	**21**	**2**	**1**	**19**	**119**	**162**	***100.0***
%	***1.9***	***7.4***	***3.7***	***13.0***	***1.2***	***0.6***	***11.7***	***73.5***	***100.0***	

* non-typable

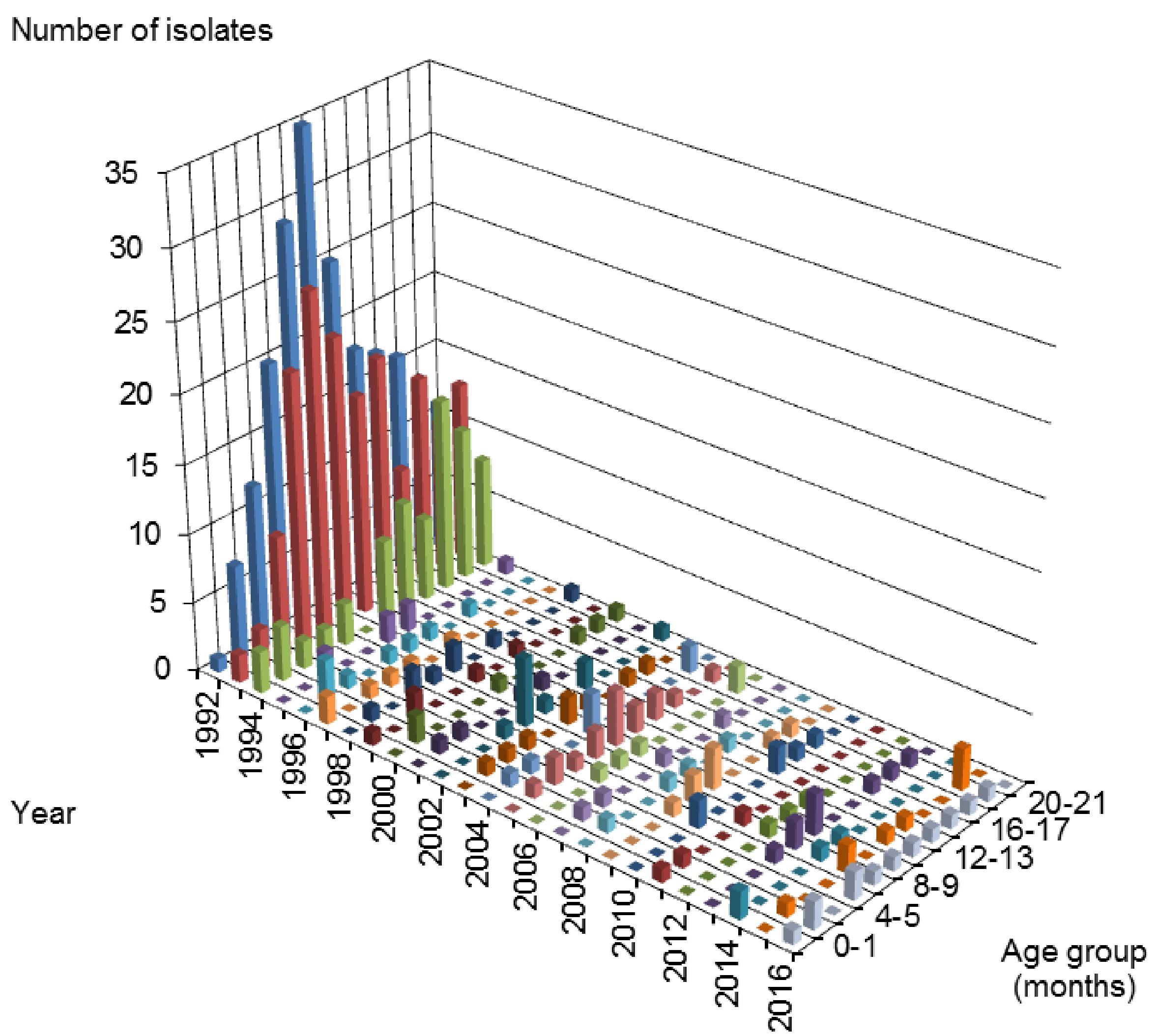

Figure 5.3 *Age distribution of H. influenzae type b invasive disease in the first two years of life, 1992-2016*

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 2016 the proportion of non-typable isolates was 65.4%.

Table 5.4 *H. influenzae* isolates from CSF and/or blood received from 1992 to 2016 according to year and serotype.

	SEROTYPE						TOTAL		CSF (or CSF and blood)	Blood Only
YEAR	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	2	30	1	3	8	117	161	**72.7**	21	**140**
2015	1	34	-	8	20	132	195	**67.7**	22	**173**
2016	3	44	1	5	12	123	188	**65.4**	26	**162**

* non-typable

In 2016, the number of *H. influenzae* type b increased compared to the previous year and was the highest since the last 10 years. 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 2016 10 non-typable isolates from CSF were received; 2 times more than in 2013. The number of non-typable *H. influenzae* isolates from blood increased during the period 1992 to 2015 from 15 to 118 (figure 5.4).

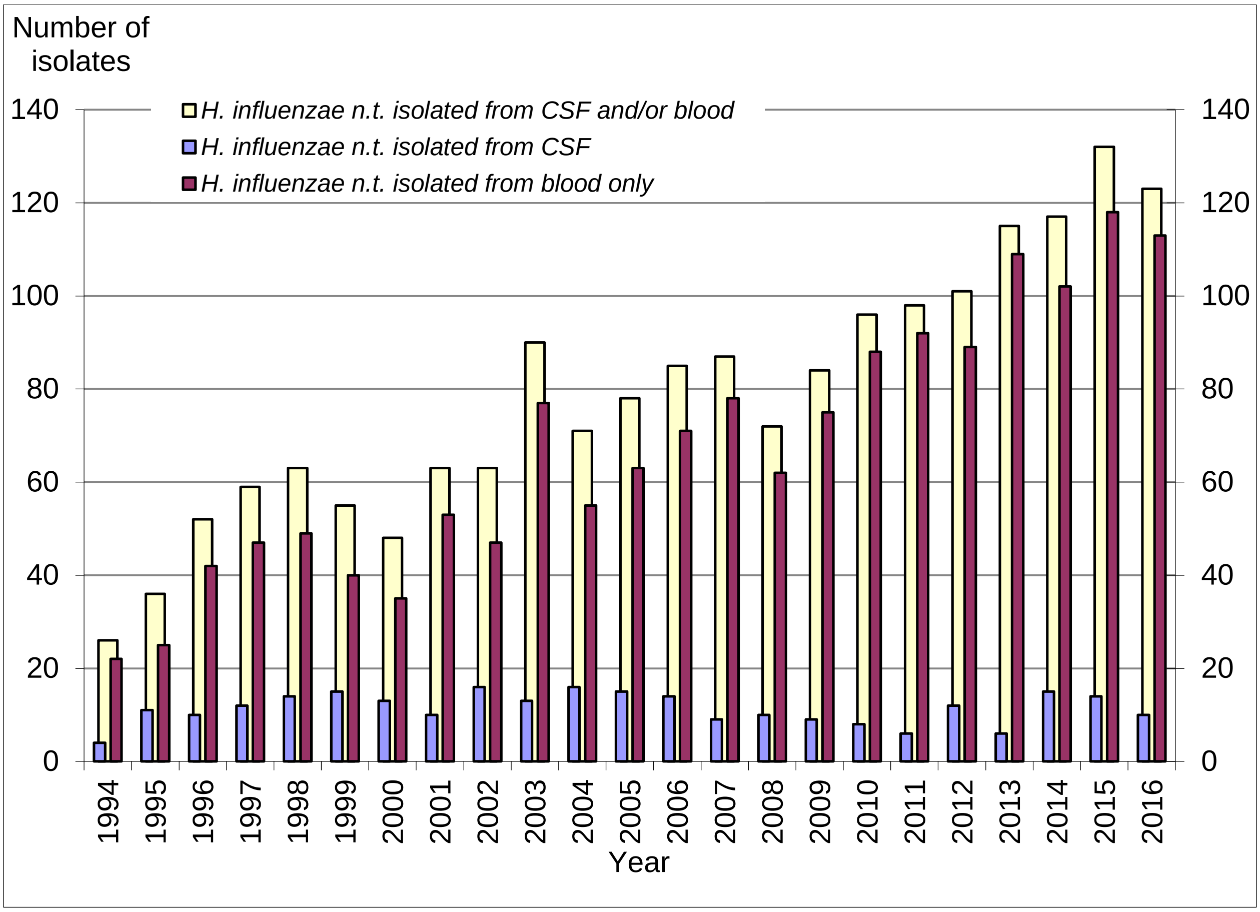

Figure 5.4 *Non-typable H. influenzae isolates from CSF and/or blood received from 1994-2016*

Table 5.5 Non-typable *H. influenzae* isolates from CSF and/or blood received from 2007 to 2016 according to year and biotype.

	Biotype							Total
	I	II	III	IV	V	VI	VII	
2007	12	47	19	1	7	1	-	**87**
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**

*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

The Reference Laboratory received 905 *S. pneumoniae* isolates and also 7 culture negative (CSF or blood) samples. Those were posititive in the Pia PCR. Of the 905 isolates, 143 were isolated from CSF or from CSF and blood (table 2.3; figure 6.1). The incidence of pneumococcal meningitis gradually rose from 1.0 in 1990 to 1.6 in 2004; due to vaccination with the hepta-valent polyscaccharide conjugate vaccine it slightly decreased to 0.8 in 2016. A steep increase in the number of pneumococcal blood isolates had occurred between 1994 (312 isolates) and 2003 (1471 isolates). This increase can be explained by the increasing use of automated blood culture devices by the contributing laboratories and by a real increase in the number of cases of pneumococcal bacteremia due to pneumonia among patients of the increasing cohort of the elderly (figure 6.1) and by a more complete submission of isolates by the laboratories.
The number of isolates from blood sent to the Reference Laboratory decreased from 1471 in 2003 to 762 in 2016. This was due to a change in policy: from 2003 onwards, we asked only nine sentinel laboratories, evenly distributed over the country, to submit pneumococcal blood isolates. Thus, the numbers of *S. pneumoniae* from blood only are incomplete.

This policy has been changed to monitor the effect of the introduction of the 7-valent conjugate pneumococcal polysaccharide vaccine by June 1st, 2006. In April 2011 the 10-valent vaccine was introduced for all newborns born March 1, 2011. From 2006 onwards, all laboratories are requested to send all invasive pneumococcal isolates from patients in the age group 0-4 year, while from patients older than 4 year only isolates from CSF are requested. Again, from nine sentinel laboratories we ask all invasive pneumococcal isolates from all patients.

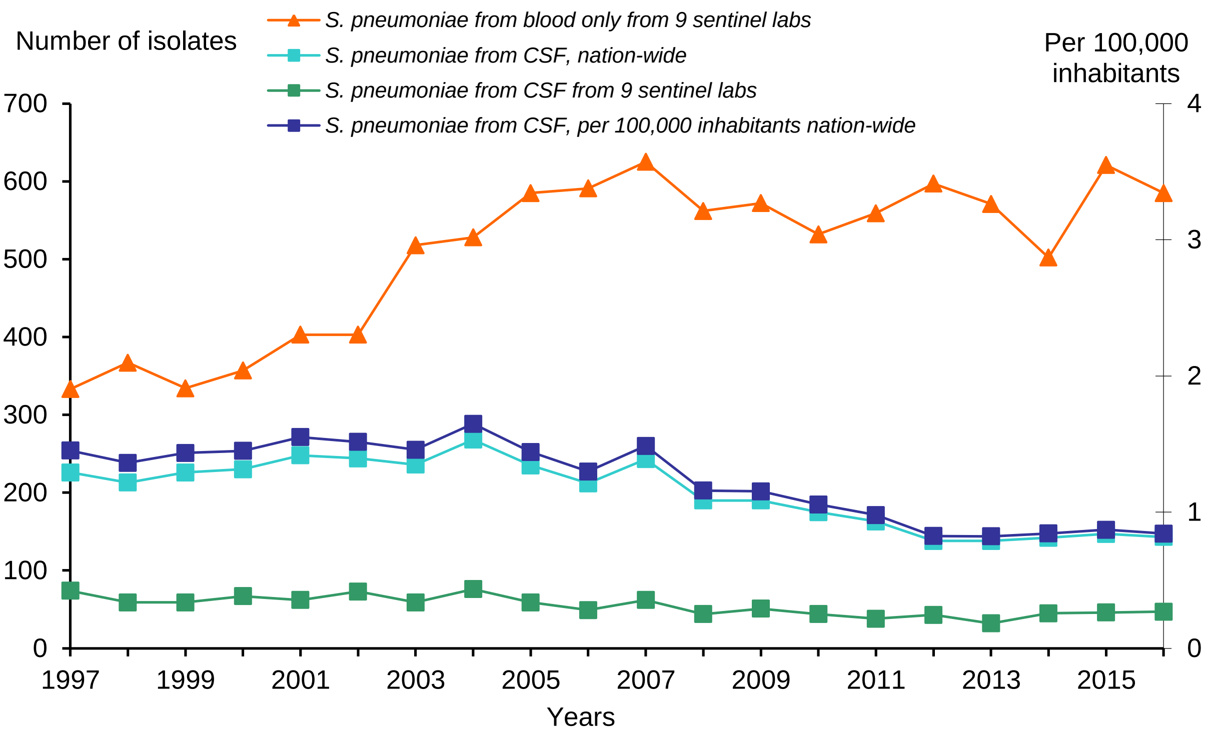

Figure 6.1 *Distribution of S. pneumoniae isolates, 1997-2016*

6.2 Antibiotic susceptibility

Among 143 isolates from CSF (or CSF and blood) and 762 isolates from the blood only, 22 isolates from the blood (2.4%) were intermediately susceptible to penicillin (0.06< MIC≤2.0 mg/L, table 6.1). Eight (5,6% from all CSF isolates or 1% off all isolates) strains isolated from CSF were resistant to penicillin (MIC > 0.06 mg/L).

Table 6.1 Susceptibility of *S. pneumoniae* isolates to penicillin, 2016

	Penicillin*				
	S	I	R	Total	%
MIC for CSF	MIC ≤ 0.06		MIC > 0.06		
CSF or CSF and blood	135	0	8	**143**	***15.8***
MIC for blood	MIC ≤ 0.06	0.06< MIC ≤ 2.0	MIC >2.0		
Blood only	740	22	0	**762**	***84.2***
Total	**875**	**22**	**8**	**905**	***100.0***
%	***96.7***	***2.4***	***0.9***	***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 65 – 69 year (Table 6.4).

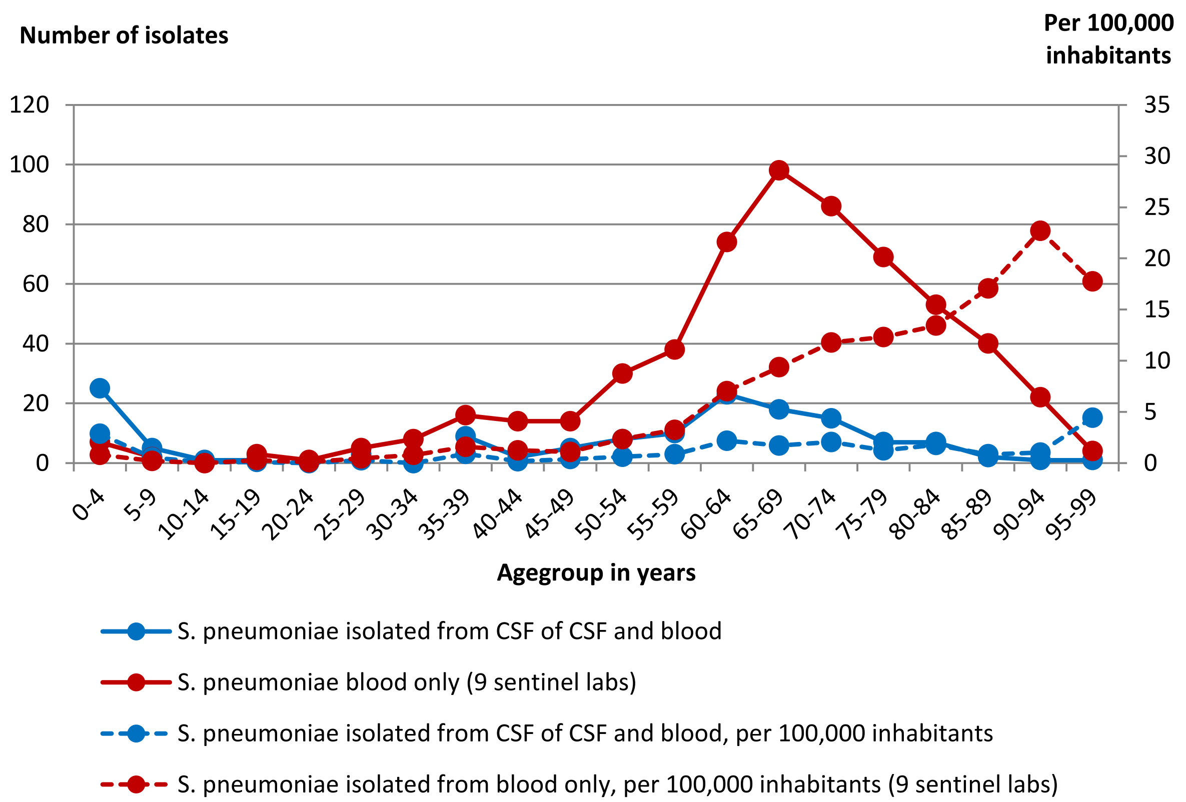

Figure 6.2 *Distribution of S. pneumoniae isolates received in 2016 according to age*

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 6.6 shows the distribution of CSF isolates according to serotype over the last 10 years. Table 6.7 shows the distribution of blood only isolates from the 9 selected laboratories according to serotype over the last 10 years. After the introduction of the 7-valent polysaccharide conjugate vaccine in the National Immunisation Programme the number of isolates with a vaccine type decreased dramatically. However, the effect was abrogated by an increase of the number of isolates with non-vaccine types (Table 6.6 and 6.7).

Table 6.2 *S. pneumoniae* isolates from CSF and/or blood (from the 9 sentinel laboratories), by serotype and age of patients, 2016

	AGE (MONTHS)			AGE (YEARS)										TOTAL	
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**	**T**	**%**
1	0	0	1	1	1	0	0	0	4	2	9	6	0	**23**	***3.7***
3	1	0	0	1	0	0	0	0	2	4	11	26	8	**52**	***8.2***
4	0	0	0	0	0	0	0	0	0	1	1	2	2	**6**	***1.0***
6	0	0	0	0	0	0	0	1	0	1	5	8	7	**22**	***3.5***
7	0	0	0	0	0	0	1	1	3	4	7	18	4	**38**	***6.0***
8	0	1	0	1	0	0	2	3	9	8	42	73	18	**156**	***24.7***
9	0	0	0	0	0	0	0	0	0	1	7	20	9	**37**	***5.9***
10	0	2	0	2	0	0	0	0	1	1	2	7	1	**14**	***2.2***
12	0	0	0	0	0	0	0	0	0	1	4	12	3	**20**	***3.2***
14	0	0	0	0	0	0	0	0	0	0	0	4	4	**8**	***1.3***
18	0	0	0	0	0	0	0	0	0	0	0	2	0	**2**	***0.3***
19	1	1	1	3	0	0	0	1	2	3	23	32	24	**88**	***13.9***
22	0	0	0	0	1	0	1	0	2	3	8	11	6	**32**	***5.1***
23	0	0	2	2	0	0	0	1	1	1	6	10	7	**28**	***4.4***
Others	0	1	1	2	1	0	0	1	3	2	28	40	28	**105**	***16.6***
Total	**2**	**5**	**5**	**12**	**3**	**0**	**4**	**8**	**27**	**32**	**153**	**271**	**121**	**631**	***100.0***
%	***0.3***	***0.8***	***0.8***	***1.9***	***0.5***	***0.0***	***0.6***	***1.3***	***4.3***	***5.1***	***24.2***	***42.9***	***19.2***	***100.0***	

Table 6.3 *S. pneumoniae* isolates from CSF (or CSF and blood)* nation-wide, by serotype and age of patients, 2016

	AGE (MONTHS)			AGE (YEARS)										TOTAL	
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**	**T**	**%**
1	0	1	0	1	0	0	0	0	1	0	0	0	0	**2**	***1.4***
3	0	2	2	4	3	0	0	1	0	1	9	7	0	**25**	***17.5***
4	0	0	0	0	0	0	0	0	1	0	0	0	0	**1**	***0.7***
6	0	1	0	1	0	0	0	0	0	0	1	2	2	**6**	***4.2***
7	0	0	0	0	0	0	0	1	1	0	0	2	0	**4**	***2.8***
8	0	4	0	4	0	0	0	0	0	0	5	7	2	**18**	***12.6***
9	0	0	0	0	0	0	0	0	0	1	1	2	1	**5**	***3.5***
10	0	2	0	2	1	0	0	0	1	0	1	1	1	**7**	***4.9***
12	0	1	1	2	0	0	0	0	0	0	3	6	1	**12**	***8.4***
14	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
18	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
19	0	3	1	4	0	0	0	0	1	1	4	1	2	**13**	***9.1***
22	0	0	0	0	0	0	1	0	0	2	7	1	1	**12**	***8.4***
23	0	1	0	1	0	0	0	1	1	1	5	2	0	**11**	***7.7***
Others	1	4	1	6	1	1	0	0	3	1	5	9	1	**27**	***18.8***
Total	**1**	**19**	**5**	**25**	**5**	**1**	**1**	**3**	**9**	**7**	**41**	**40**	**11**	**143**	***100.0***
%	***0.7***	***13.3***	***3.5***	***17.5***	***3.5***	***0.7***	***0.7***	***2.1***	***6.3***	***4.9***	***28.6***	***28.0***	***7.7***	***100.0***	

* From 10 patients with a pneumococcus isolated from blood, CSF was culture-negative but PCR was positive for pneumococcal DNA. Cases were in age groups 5-9 years (1), 20-29 years (1), 50-64 years (3), 65-79 years (3) and ≥80 (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, 2016

	AGE (YEARS)											TOTAL
TYPE	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-64**	**65-79**	**≥80**	
1	0	0.14	0	0	0	0	0.05	0	0	0	0	0.01
3	0	0.57	0.32	0	0	0.05	0	0.04	0.26	0.30	0	0.15
4	0	0	0	0	0	0	005	0	0	0	0	0.01
6	0	0.14	0	0	0	0	0	0	0.03	0.09	0.27	0.04
7	0	0	0	0	0	0.05	0.05	0	0	0.09	0	0.02
8	0	0.57	0	0	0	0	0	0	0.14	0.30	0.27	0.11
9	0	0	0	0	0	0	0	0.04	0.03	0.09	0.13	0.03
10	0	0.28	0.11	0	0	0	0.05	0	0.03	0.04	0.13	0.04
12	0	0.28	0	0	0	0	0	0	0.09	0.26	0.13	0.07
14	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0.57	0	0	0	0	0.05	0.04	0.11	0.04	0.27	0.08
22	0	0	0	0	0.10	0	0	0.08	0.20	0.04	0.13	0.07
23	0	0.14	0	0	0	0.05	0.05	0.04	0.14	0.09	0	0.06
Others	0.59	0.71	0.11	0.10	0	0	0.15	0.04	0.14	0.39	0.13	0.16
Total	**0.59**	**3.40**	**0.54**	**0.10**	**0.10**	**0.14**	**0.44**	**0.29**	**1.17**	**1.71**	**1.47**	**0.84**

Table 6.5 All *S. pneumoniae* isolates from blood only (from the 9 sentinel laboratories), by serotype of patients, 2016

	AGE (MONTHS)			AGE (YEARS)										TOTAL	
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**	**T**	**%**
1	0	0	1	1	1	0	0	0	3	2	9	6	0	**22**	***3.8***
3	1	0	0	1	0	0	0	0	2	4	7	23	8	**45**	***7.7***
4	0	0	0	0	0	0	0	0	0	1	1	2	2	**6**	***1.0***
6	0	0	0	0	0	0	0	1	0	1	5	7	7	**21**	***3.6***
7	0	0	0	0	0	0	1	0	3	4	7	17	4	**36**	***6.1***
8	0	0	0	0	0	0	2	3	9	8	42	69	18	**151**	***25.9***
9	0	0	0	0	0	0	0	0	0	0	6	18	9	**33**	***5.7***
10	0	1	0	1	0	0	0	0	0	1	1	7	1	**11**	***1.9***
12	0	0	0	0	0	0	0	0	0	1	4	10	3	**18**	***3.0***
14	0	0	0	0	0	0	0	0	0	0	0	4	4	**8**	***1.4***
18	0	0	0	0	0	0	0	0	0	0	0	2	0	**2**	***0.3***
19	1	0	0	1	0	0	0	1	2	3	21	31	22	**81**	***13.9***
22	0	0	0	0	1	0	0	0	2	1	7	11	6	**28**	***4.8***
23	0	0	2	2	0	0	0	0	1	0	5	9	7	**24**	***4.1***
Others	0	1	0	1	0	0	0	1	2	2	27	37	28	**98**	***16.8***
Total	**2**	**2**	**3**	**7**	**2**	**0**	**3**	**6**	**24**	**28**	**142**	**253**	**119**	**584**	***100.0***
%	***0.3***	***0.3***	***0.6***	***1.2***	***0.3***	***0.0***	***0.6***	***1.0***	***4.1***	***4.8***	***24.3***	***43.3***	***20.4***	***100.0***	

Table 6.6 Distribution of pneumococcal CSF isolates according to serotype nation-wide, 2007-2016

TYPE	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
	Year									
23-valent vaccine / 10-valent vaccine / 7-valent vaccine										
4	5	8	4	3	2	4	2	2	-	1
6B	12	11	3	-	2	-	-	-	1	-
9V	10	7	2	2	-	3	1	1	-	2
14	18	8	3	5	2	1	-	-	1	-
18C	17	8	6	5	5	2	2	-	1	-
19F	11	7	10	2	6	4	2	4	2	5
23F	22	17	5	4	2	1	-	-	1	-
Subtotal 7-valent vaccine	*95*	*66*	*33*	*21*	*19*	*15*	*7*	*7*	*6*	*8*
1	8	8	8	3	1	1	3	4	1	2
5	-	-	-	2	-	3	-	-	-	-
7F	36	25	25	20	28	16	15	8	7	4
Subtotal 10-valent vaccine	*139*	*99*	*66*	*46*	*48*	*35*	*25*	*19*	*14*	*14*
2	-	-	-	-	-	-	-	-	-	-
3	16	17	24	20	7	13	16	13	16	25
8	21	9	10	10	17	9	16	23	24	18
9N	4	1	3	6	7	4	2	6	6	3
10A	8	7	10	9	7	9	7	12	5	7
11A	4	2	8	1	5	1	1	3	2	3
12F	4	2	2	3	7	10	9	8	9	12
15B	1	4	8	2	3	1	-	-	-	5
17F	1	-	-	4	3	1	1	1	-	-
19A	9	8	6	20	16	6	9	7	10	8
20	-	1	-	1	-	-	1	1	1	-
22F	2	10	13	14	16	11	8	8	11	11
33F	5	6	6	7	5	6	3	2	4	4
Subtotal 23-valent vaccine	*214*	*166*	*156*	*143*	*141*	*106*	*98*	*103*	*102*	*110*
6A	5	4	6	5	1	1	1	3	-	1
6C	2	-	-	3	4	2	6	3	6	5
7B	-	-	-	-	-	-	1	-	-	-
10F	-	-	-	-	-	-	-	-	-	-
10B	-	-	-	-	-	1	-	1	1	-
12A	-	-	-	-	-	-	-	-	-	-
13	-	-	1	-	-	-	-	-	-	-
15A	1	1	-	1	1	1	4	6	7	2
15C	1	3	1	2	-	3	-	-	1	-
16F	2	2	-	5	4	-	5	2	1	3
17A	-	-	1	-	-	-	-	-	-	-
18F	-	-	-	-	-	-	-	-	-	-
18A	-	-	-	-	-	-	-	-	-	-
18B	1	1	-	-	-	1	-	-	-	-
21	1	-	-	-	1	-	-	-	-	-
22A	1	-	1	1	-	-	-	-	1	1
23A	3	1	3	3	2	4	4	4	5	5
23B	2	3	7	5	2	5	7	8	11	6
24F	3	2	6	1	1	4	4	7	7	1
24B	-	-	-	-	-	2	-	-	-	-
25	-	1	-	-	-	-	-	-	-	-
27	1	2	-	-	-	1	-	2	1	1
28F	-	-	-	-	-	-	1	-	-	-
28A	-	-	-	-	1	-	-	-	-	-
29	-	-	-	-	-	1	-	-	-	-
31	2	-	1	1	-	1	-	1	-	1
33A	-	-	-	-	-	-	-	-	-	-
34	1	1	1	-	1	-	-	-	1	1
35F	2	2	2	4	1	-	2	1	2	5
35B	1	-	-	1	-	1	3	1	1	1
37	-	-	1	-	1	2	1	-	-	-
38	-	1	3	1	-	2	1	-	-	-
Rough (n.t.)	-	-	-	-	-	-	-	-	-	-
Total	243	190	190	176	163	138	138	142	147	143

Table 6.7 Distribution of *S. pneumoniae* from blood only (from the 9 sentinel laboratories), according to serotype, 2007-2016

				Year									
			TYPE	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
23-valent vaccine	10-valent vaccine	7-valent	4	54	30	26	17	27	11	13	6	6	6
			6B	26	25	12	8	3	3	3	3	4	1
			9V	53	42	26	21	5	2	4	1	5	-
			14	84	54	34	22	19	12	8	2	7	8
			18C	13	15	15	7	8	4	8	2	2	2
			19F	11	9	10	5	9	3	5	7	8	6
			23F	39	13	12	13	5	3	1	2	1	1
			Subtotal 7-valent vaccine	*280*	*188*	*135*	*93*	*85*	*38*	*42*	*23*	*33*	*24*
			1	75	64	65	53	40	50	40	41	41	22
			5	3	2	6	7	11	8	9	2	1	-
			7F	55	65	86	72	91	92	75	53	56	36
			Subtotal 10-valent vaccine	*413*	*319*	*292*	*225*	*227*	*188*	*166*	*119*	*131*	*82*
			2	-	-	-	-	-	-	-	-	-	-
			3	30	31	34	30	36	45	40	31	35	45
			8	47	46	52	60	59	88	108	93	136	151
			9N	13	19	18	19	17	20	19	21	26	32
			10A	4	7	9	9	14	8	6	16	15	11
			11A	16	3	12	12	9	14	16	8	6	6
			12F	5	6	5	13	19	25	22	28	30	18
			15B	1	4	6	7	4	1	7	7	2	8
			17F	3	1	7	4	8	7	4	8	6	6
			19A	25	33	30	57	63	78	61	44	78	75
			20	3	3	3	3	4	-	1	4	2	3
			22F	18	24	24	29	37	41	45	34	43	28
			33F	6	10	11	10	15	22	12	12	19	18
			Subtotal 23-valent vaccine	*584*	*506*	*503*	*478*	*503*	*537*	*507*	*425*	529	483
			6A	10	18	11	9	2	6	2	-	2	-
			6C	2	1	7	9	7	10	10	7	21	20
			7C	1	-	-	-	-	-	-	-	-	-
			9A	-	-	-	-	-	1	-	1	-	1
			10F	-	1	-	-	-	-	-	1	-	-
			10B	-	-	-	-	-	-	1	-	-	-
			11B	1	-	-	-	-	-	-	-	-	-
			12A	-	-	-	-	-	-	-	-	-	-
			13	-	-	-	-	1	-	-	-	-	1
			15F	-	-	-	-	-	-	1	-	-	1
			15A	1	1	1	-	2	7	13	14	18	21
			15C	1	2	2	1	2	1	4	4	3	2
			16F	6	9	8	10	7	6	7	5	2	9
			17A	-	-	-	-	2	-	-	-	-	-
			18F	-	-	-	-	-	-	-	-	2	-
			18A	1	-	1	1	1	-	-	-	-	-
			18B	1	-	-	-	-	1	1	-	-	-
			21	-	-	-	-	-	-	2	1	-	-
			22A	2	1	-	1	1	-	1	-	1	-
			23A	6	3	9	7	2	6	6	7	7	12
			23B	1	3	6	3	9	3	6	15	5	11
			24F	1	7	-	2	3	2	4	4	7	1
			25F	-	1	-	-	-	-	-	-	1	-
			27	-	1	1	-	1	-	1	-	1	1
			28A	-	-	-	-	-	-	-	-	-	-
			29	-	-	-	-	-	1	-	-	-	-
			31	1	3	1	4	2	6	2	2	4	4
			33A	-	-	-	-	-	1	-	-	-	1
			34	1	-	1	1	-	1	2	1	-	1
			35F	1	2	4	5	6	5	6	7	7	6
			35A	-	-	-	-	-	1	-	-	-	-
			35B	-	-	4	-	3	1	7	6	8	8
			37	1	-	-	1	-	-	-	1	1	-
			38	2	3	5	-	3	-	1	2	2	1
			40	-	-	-	-	-	-	1	-	-	-
			Rough (n.t.)	-	-	-	-	2	-	-	-	-	-
			Total	624	562	564	532	559	596	585	503	621	584

Table 6.8 Distribution of *S. pneumoniae* isolates from CSF (or CSF and blood) nation-wide,

by serotype and age of patients, 2016.

Vaccine	TYPE	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total	%
		AGE (YEARS)											
23-valent vaccine / 10-valent vaccine / 7-valent vaccine	4	-	-	-	-	-	1	-	-	-	-	1	***0.7***
	6B	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	9V	-	-	-	-	-	-	1	1	-	-	2	***1.4***
	14	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	18C	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	19F	-	-	-	-	-	1	-	3	-	1	5	***3.5***
	23F	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	Subtotal 7-valent vaccine	*-*	*-*	*-*	*-*	*-*	*2*	*1*	*4*	*-*	*1*	*8*	
	1	1	-	-	-	-	1	-	-	-	-	2	***1.4***
	5	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	7F	-	-	-	-	1	1	-	-	2	-	4	***2.8***
	Subtotal 10-valent vaccine	*1*	*-*	*-*	*-*	*1*	*4*	*1*	*4*	*2*	*1*	*14*	
	2	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	3	4	3	-	-	1	-	1	9	7	-	25	***17.5***
	8	4	-	-	-	-	-	-	5	7	2	18	***12.6***
	9N	-	-	-	-	-	-	-	-	2	1	3	***2.1***
	10A	2	1	-	-	-	1	-	1	1	1	7	***4.9***
	11A	-	-	-	-	-	-	-	-	3	-	3	***2.1***
	12F	2	-	-	-	-	-	-	3	6	1	12	***8.4***
	15B	2	-	-	-	-	-	-	3	-	-	5	***3.5***
	17F	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	19A	4	-	-	-	-	-	1	1	1	1	8	***5.6***
	20	-	-	-	-	-	-	-	-	-	-	-	***0.0***
	22F	-	-	-	-	-	-	2	7	1	1	11	***7.7***
	33F	2	-	-	-	-	-	-	-	1	1	4	***2.8***
	Subtotal 23-valent vaccine	*21*	*4*	*-*	*-*	*2*	*5*	*5*	*33*	*31*	*9*	*110*	
	Other	4	1	1	1	1	4	2	8	9	2	33	***23.0***
	Total	25	5	1	1	3	9	7	41	40	11	143	***100.0***

7 *ESCHERICHIA COLI*

The Reference Laboratory received 50 *Escherichia coli* strains, 18 isolated from CSF (or CSF and blood) and 32 from blood only (table 7.1, 7.2 and 7.3). Eighty-two 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, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	20	-	-	20	-	-	1	5	**26**	***52***
K1	21	-	-	21	-	-	-	3	**24**	***48***
Total	**41**	**-**	**-**	**41**	**-**	**-**	**1**	**8**	**50**	***100***
%	***82***	***0***	***0***	***82***	***0***	***0***	***2***	***16***	***100***	

Table 7.2 Serotypes of *E. coli* isolates from CSF (or CSF and blood), by age of patients, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	4	-	-	4	-	-	1	5	**10**	***56***
K1	6	-	-	6	-	-	-	2	**8**	***44***
Total	**10**	**-**	**-**	**10**	**-**	**-**	**1**	**7**	**18**	***100***
%	***56***	***0***	***0***	***56***	***0***	***0***	***5***	***39***	***100***	

Table 7.3 Serotypes of *E. coli* isolates from blood only by age of patients, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	16	-	-	16	-	-	-	-	**16**	***50***
K1	15	-	-	15	-	-	-	1	**16**	***50***
Total	**31**	**-**	**-**	**31**	**-**	**-**	**-**	**1**	**32**	***100***
%	***97***	***0***	***0***	***97***	***0***	***0***	***0***	***3***	***100***	

Since 2012 all isolates were tested for the H – type. Fourty-six percent of all K1 isolates were of type H4 and H7, while 46% of the non-K1 isolates were H4 and H18 (table 7.4)

Table 7.4 H-type versus K-type of *E. coli* isolates from CSF and/or blood, 2016

TYPE	K1	Non K1	Total
H1	0	2	**2**
H4	6	7	**13**
H5	4	2	**6**
H6	4	1	**5**
H7	7	3	**10**
H9	0	2	**2**
H10	1	1	**2**
H16	0	1	**1**
H18	0	5	**5**
H21	0	1	**1**
H25	0	1	**1**
H33	1	0	**1**
H42	1	0	**1**
Total	**24**	**26**	**50**
%	***48***	***52***	***100***

Since 2016 the K1 is detected with Fage typing. The O and H typing was done with Whole Genome Sequencing. The types O non typable, O6, O8, O13, O15, O16, O17, O22, O25, O50, O75, O78, O82, O117 and O128 are prevalent among non-K1 isolates, while the types O non typable, O1, O2, O12, O13, O18, O36, O50, O75, O83 and O128 are 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, 2016

	O -	O1	O2	O6	O8	O12	O13	O15	O16	O17	O18	O22	O25	O32	O36	O50	O75	O78	O82	O83	O117	O128	
Non K1	4	0	0	1	1	0	1	4	1	1	0	1	3	1	0	2	1	2	1	0	1	1	***26***
K1	1	5	3	0	0	1	2	0	0	0	2	0	0	0	1	3	4	0	0	1	0	1	***24***
Total	**5**	**5**	**3**	**1**	**1**	**1**	**3**	**4**	**1**	**1**	**2**	**1**	**3**	**1**	**1**	**5**	**5**	**2**	**1**	**1**	**1**	**2**	***50***

At K1 isolates the O/H combination O75 H5 was found 4 times and O1 H7, 5 times.
At the non-K1 isolates O15 H18 and O25 H4 were dominant (4 respectivily 3 times)

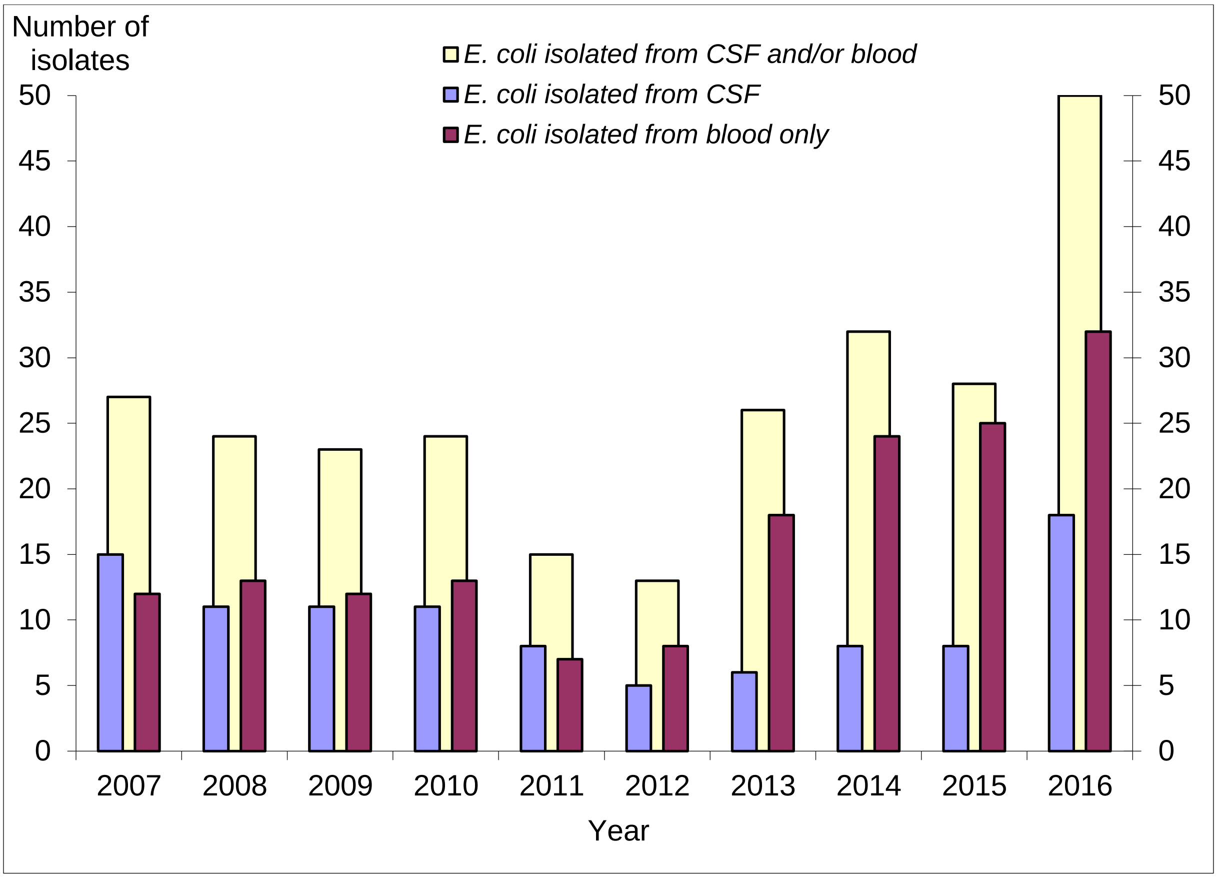

Figure 7.1 *Distribution of E. coli, 2007-2016*

8 *STREPTOCOCCUS AGALACTIAE* – (group B)

In 2016 the Reference Laboratory received 69 *Streptococcus agalactiae* isolates (2015: 65; 2014: 71; 2013: 72; 2012: 80, figure 8.1). Twenty *S. agalactiae* isolates were from CSF (or CSF and blood) and 49 from blood only (table 8.1, 8.2 and 8.3). Seventy-five 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 2016, Ia was decreased again to 7 (10%) cases.

Table 8.1 Serotypes of *S. agalactiae* isolates from CSF and/or blood, by age of patients, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	6	0	0	6	0	0	0	1	**7**	***10***
Ib	3	0	0	3	0	0	0	1	**4**	***6***
II	1	0	0	1	0	0	0	1	**2**	***3***
III	34	11	0	45	0	0	0	3	**48**	***70***
IV	3	0	0	3	0	0	0	0	**3**	***4***
V	5	0	0	5	0	0	0	0	**5**	***7***
Total	**52**	**11**	**0**	**63**	**0**	**0**	**0**	**6**	**69**	***100***
%	***75***	***16***	***0***	***91***	***0***	***0***	***0***	***9***	***100***	

Table 8.2 Serotypes of *S. agalactiae* isolates from CSF (or CSF and blood), by age of patients, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	0	0	0	0	0	0	0	0	**0**	***0***
Ib	0	0	0	0	0	0	0	1	**1**	***5***
II	0	0	0	0	0	0	0	1	**1**	***5***
III	10	5	0	15	0	0	0	3	**18**	***90***
IV	0	0	0	0	0	0	0	0	**0**	***0***
V	0	0	0	0	0	0	0	0	**0**	***0***
Total	**10**	**5**	**0**	**15**	**0**	**0**	**0**	**5**	**20**	***100***
%	***50***	***25***	***0***	***75***	***0***	***0***	***0***	***25***	***100***	

Table 8.3 Serotypes of *S. agalactiae* isolates from blood only, by age of patients, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	6	0	0	6	0	0	0	1	**7**	***14***
Ib	3	0	0	3	0	0	0	0	**3**	***6***
II	1	0	0	1	0	0	0	0	**1**	***2***
III	24	6	0	30	0	0	0	0	**30**	***62***
IV	3	0	0	3	0	0	0	0	**3**	***6***
V	5	0	0	5	0	0	0	0	**5**	***10***
Total	**42**	**6**	**0**	**48**	**0**	**0**	**0**	**1**	**49**	***100***
%	***86***	***12***	***0***	***98***	***0***	***0***	***0***	***2***	***100***	

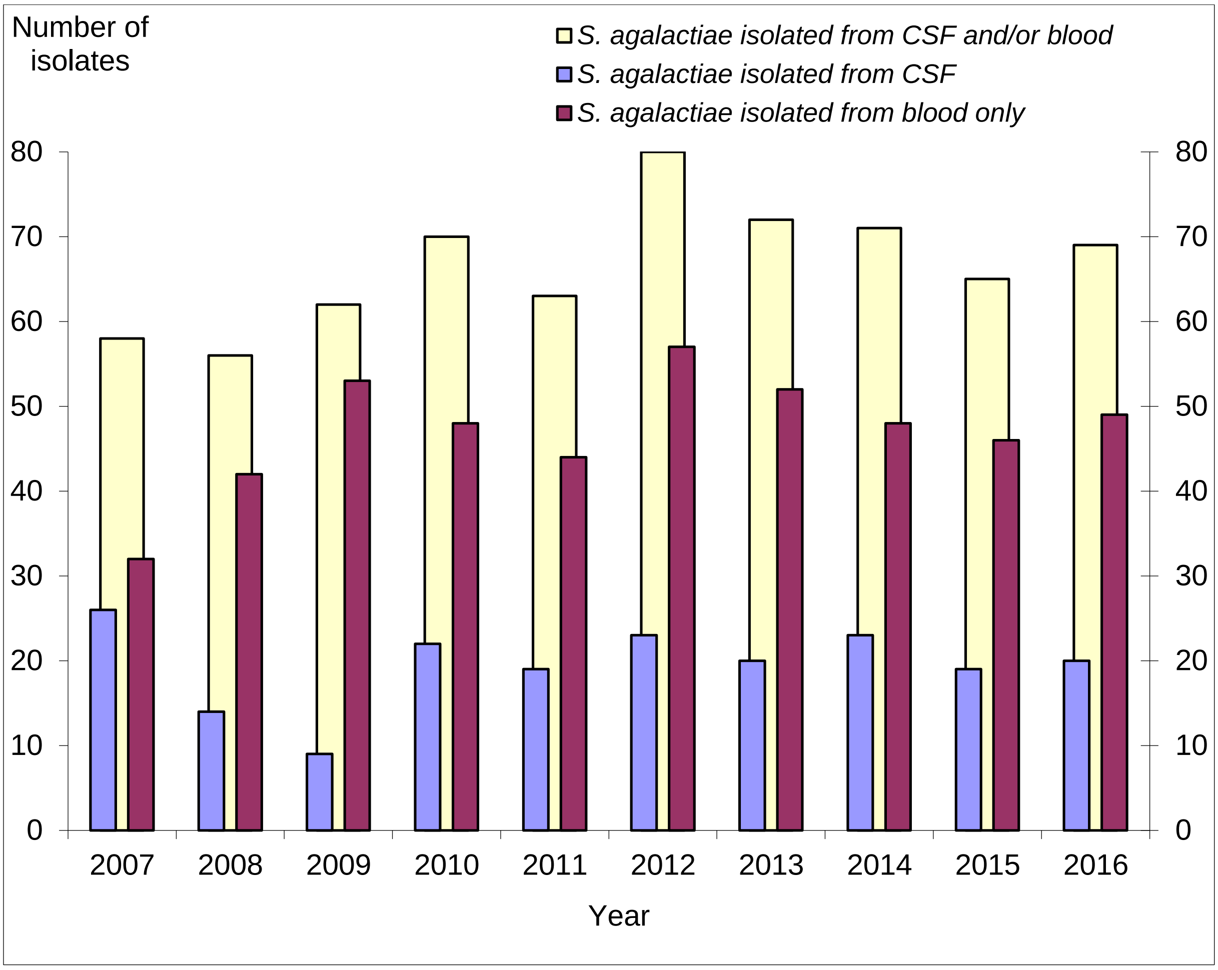

Figure 8.1 *Distribution of S. agalactiae, 2007-2016*

9 *LISTERIA MONOCYTOGENES*

Seventy strains of *Listeria monocytogenes* were submitted to the Reference Laboratory. Eleven isolates were from CSF (or CSF and blood) and 59 from blood only (figure 9.1). (2015: 8 CSF and 39 blood only; 2014: 19 CSF and 51 blood only; 2013: 6 CSF and 46 blood only). Most cases (85%) occurred among persons older than 50 years. In 2016 (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, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2a	1	0	0	1	0	0	3	30	**34**	***49***
1/2b	0	0	0	0	0	1	1	10	**12**	***17***
1/2c	0	0	0	0	0	0	1	0	**1**	***1***
3a	0	0	0	0	0	0	0	1	**1**	***1***
4b	2	0	0	2	0	0	2	18	**22**	***32***
Total	**3**	**0**	**0**	**3**	**0**	**1**	**7**	**59**	**70**	***100***
%	***4***	***0***	***0***	***4***	***0***	***1***	***10***	***85***	***100***	

Table 9.2 *L. monocytogenes* isolates from CSF (or CSF and blood), by type and age, 2016

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	3	3	***27***
1/2b	0	0	0	0	0	0	0	2	**2**	***18***
1/2c	0	0	0	0	0	0	0	0	**0**	***0***
3c	0	0	0	0	0	0	0	0	**0**	***0***
4b	0	0	0	0	0	0	0	6	**6**	***55***
Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**11**	***100***
%	***0***	***0***	***0***	***0***	***0***	***0***	***0***	***100***	***100***	

Table 9.3 *L. monocytogenes* isolates from blood only, by serotype and age, 2016

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2a	1	0	0	1	0	0	3	27	**31**	***52***
1/2b	0	0	0	0	0	1	1	8	**10**	***17***
1/2c	0	0	0	0	0	0	1	0	**1**	***2***
3c	0	0	0	0	0	0	0	1	**1**	***2***
4b	2	0	0	2	0	0	2	12	**16**	***27***
Total	3	0	0	3	0	1	7	48	59	***100***

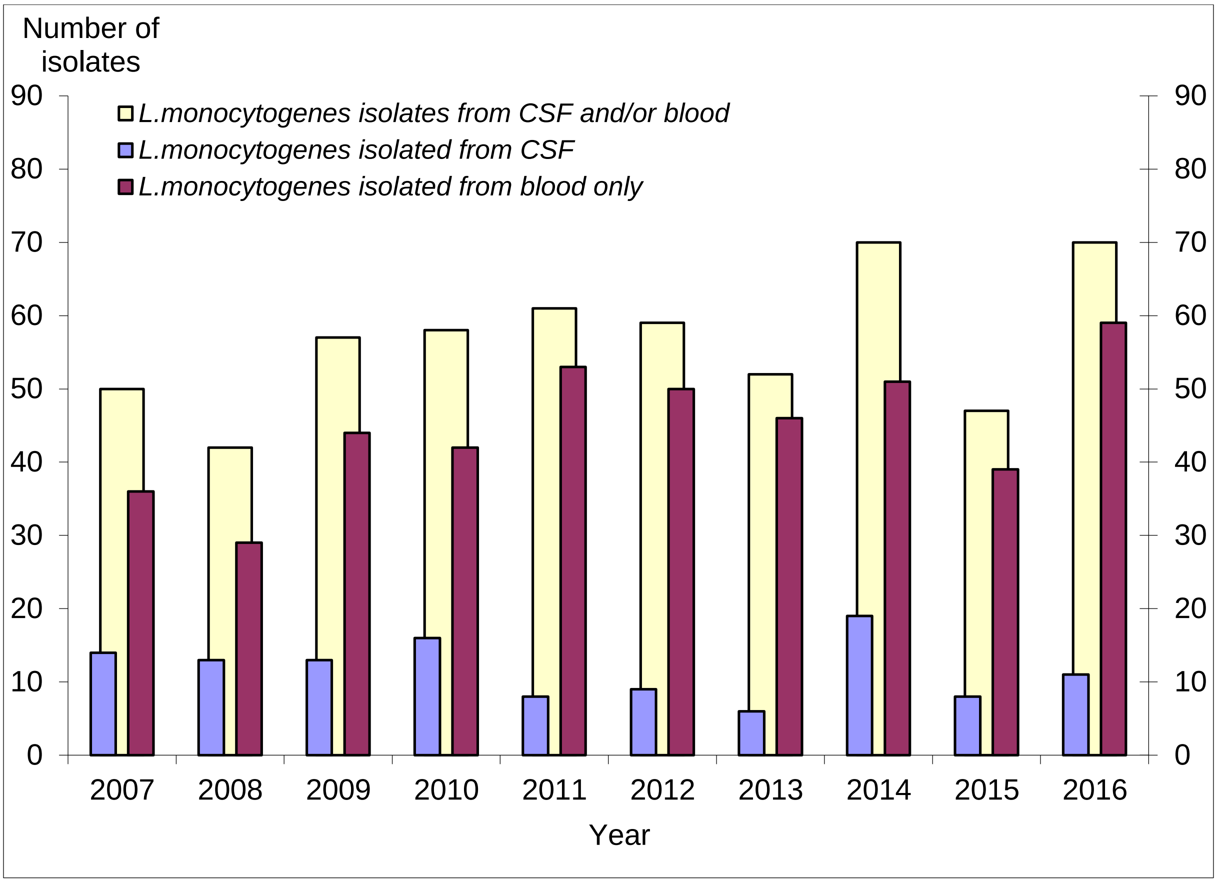

Figure 9.1 *Distribution of L. monocytogenes, 2007-2016*

10 *STREPTOCOCCUS PYOGENES*

Ten *Streptococcus pyogenes* isolates were submitted to the Reference Laboratory, 5 isolated from CSF (or CSF and blood) and 5 from blood only.

Table 10.1 *S. pyogenes* isolates from CSF and/or blood received in 2016 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	0	0	1	0	1	3	**5**	***50***
Blood	0	0	2	2	0	0	0	3	**5**	***50***
Total	**0**	**0**	**2**	**2**	**1**	**0**	**1**	**6**	**10**	***100***
%	***0***	***0***	***20***	***20***	***10***	***0***	***10***	***60***	***100***	

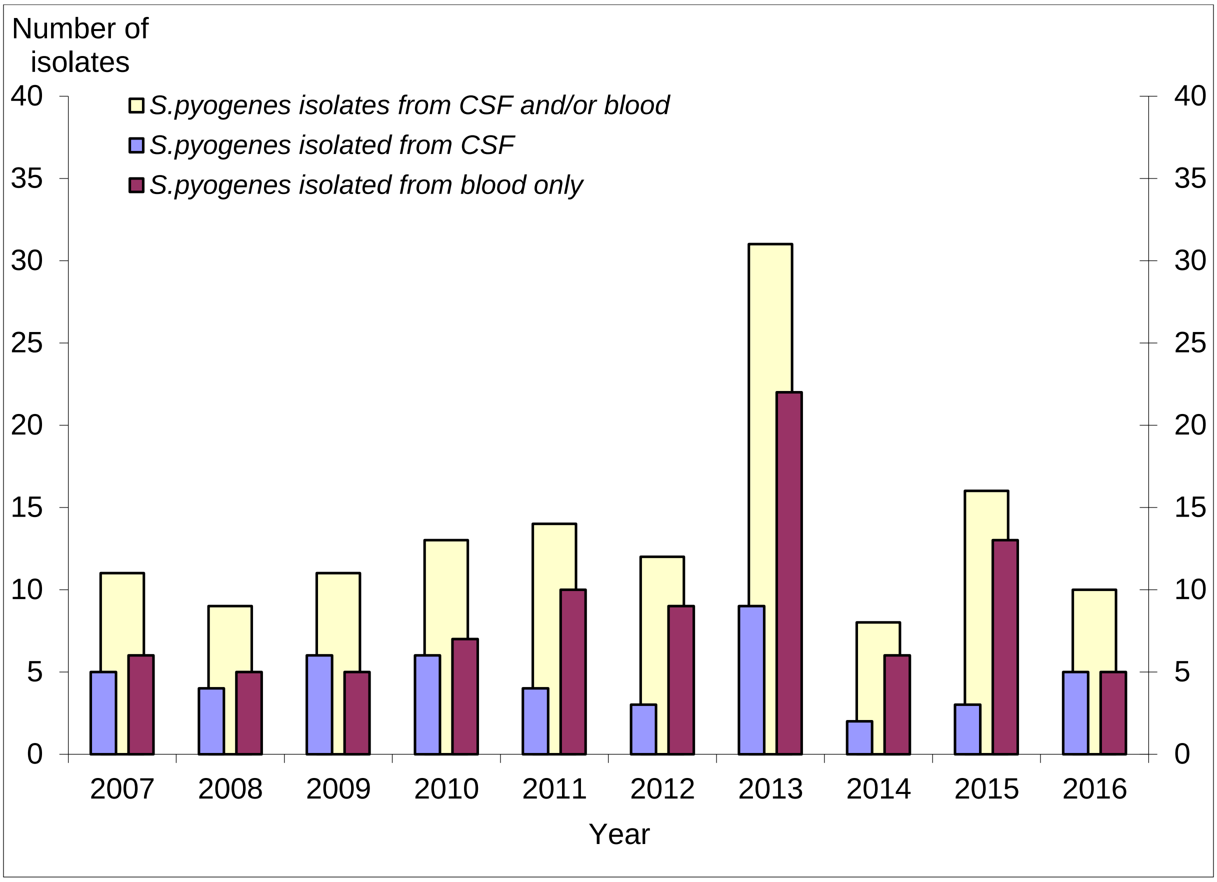

Figure 10.1 *Distribution of S. pyogenes, 2007-2016*

11 ANTIGEN AND DNA DETECTION

The Reference Laboratory received 109 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 109 specimens, 39 (36%) were positive by agglutination or PCR. Twenty (18%) (14 CSF, 4 sera and 2 DNA samples isolated from a skinbiopsy) were positive for *N. meningitidis* and 18 (16%) were positive for *S. pneumoniae*.

Thus, in 2016, PCR-positive, culture-negative CSF samples accounted for 28% (14/50) of cases of meningococcal meningitis registered in the database of the Reference Laboratory. For *S. pneumoniae*, this percentage was 11% (17/160).

Table 11.1 CSF and serum samples, tested for antigens or DNA, 2016

Antigen of	CSF (or DNA from CSF)	SERA	Other	TOTAL
C. neoformans	0	1	0	**1**
H. influenzae type b	0	0	0	**0**
DNA of				
***N. meningitidis* group B**	11	1	2	**14**
***N. meningitidis* group W**	2	3	0	**5**
***N. meningitidis* group Y**	1	0	0	**1**
S. pneumoniae	17*	1	0	18
Sub Total	**31**	**6**	**2**	**39**
Antigen and PCR negative	66	4	0	**70**
Total	**97**	**10**	**2**	**109**

* From 10 patients with a *S. pneumoniae* isolated from blood, the CSF was culture-negative but PCR-positive for pneumococcal 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 14 months to 19 years have been vaccinated. In 2016, 6 cases of meningococcal disease (4.4% of all cases, table 4.4) were due to serogroup C meningococci (2015: 8.3%; 2014: 4.1%; 2013: 5.4%; 2012: 2.5%). All six patients were not vaccinated because of age. This indicates that the vaccination programme is successful. (figure 12.1)

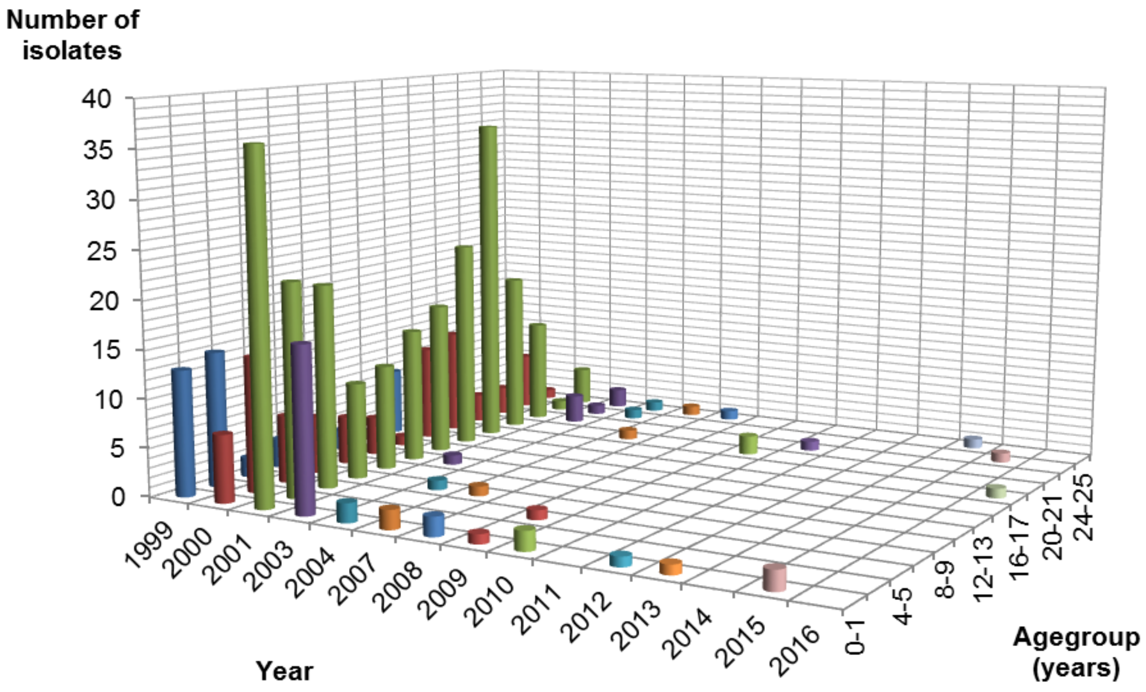

Figure 12.1 *Age distribution of N.meningitidis serogroup C invasive disease in the first 24 years of life, 1999-2016.*

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 57 cases (85%; table 4.9) of serogroup B meningococcal disease and 79 (58%) of all 136 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 a protein, tetanus toxoid. 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 2016, the number of invasive meningitis isolates of *H. influenzae* type b, received from patients that should have been vaccinated (<23 years of age) decreased from 14 to 11 (2015: 14; 2014: 12; 2013: 14; 2012: 11; 2011: 7) (figure 12.2 and 12.3). Of those 11 patients, Three had received all doses and one received three doses of the vaccine. Seven patients were not vaccinated. One because of age and one because of medical reasons.

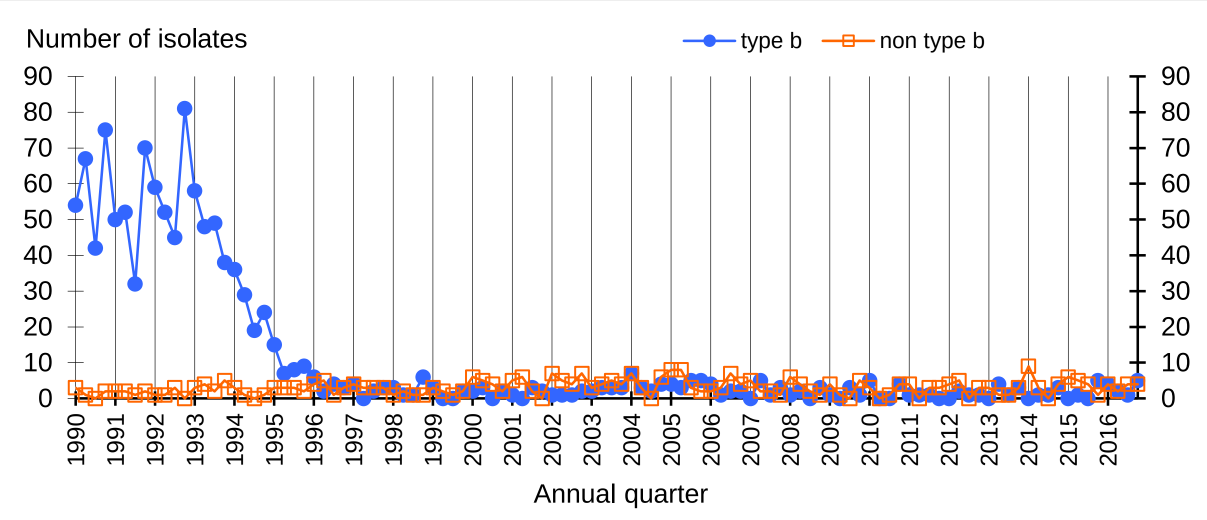

Figure 12.2 *The distribution of H. influenzae type b and non-type b meningitis cases according to annual quarter, 1990 –2016*

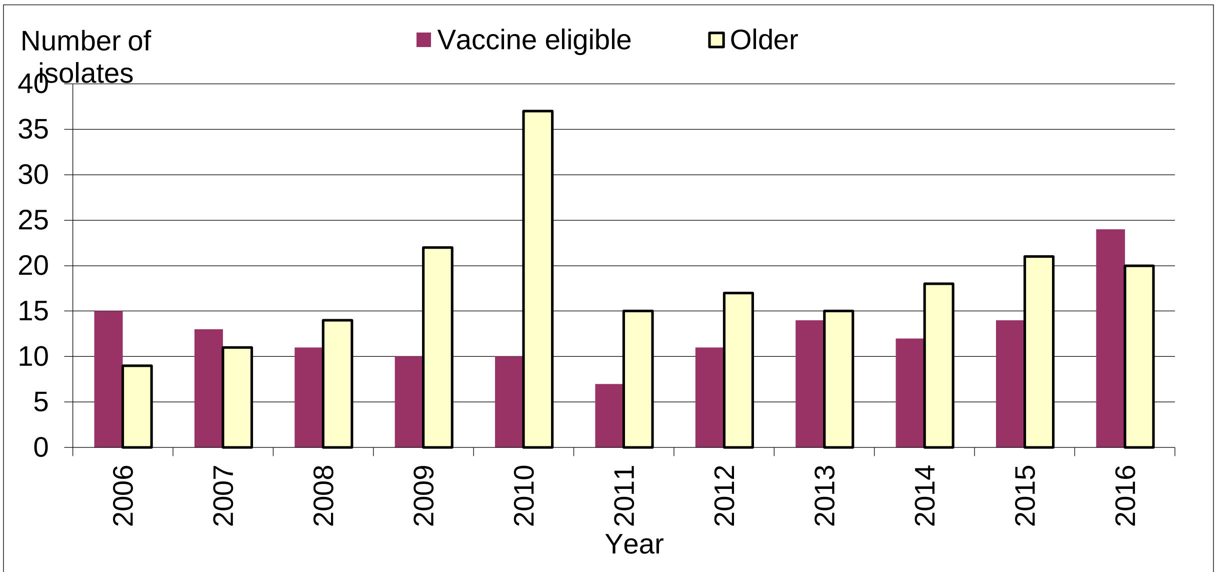

Figure 12.3 *The distribution of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 2006 –2016*

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 2016, 5.6 percent of the CSF isolates were of a serotype covered by this hepta-valent conjugate polysaccharide vaccine, while 9.8% of the isolates were covered by the 10-valent vaccine (table 6.6). In 2016 the proportion of CSF isolates with a PVC7 serotype was ten times lower than ten years ago (2016: 5.6%; 2006: 56%) as a result of the vaccination. There were 8 patients with invasive pneumococcal disease due to pneumonococci with a vaccine (PVC7) serotype (4, 9V and 19F) and 6 patients with invasive pneumococcal disease due to pneumonococci with a vaccine (PVC10 - 7) serotype (2 times type 1 and 4 times 7F). Those 14 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) (2015: 60%; 2014: 73%; 2013: 71%; 2012: 77%; 2011: 87%; 2010: 84%; 2009: 85%; 2008: 89%; 2007: 90%).

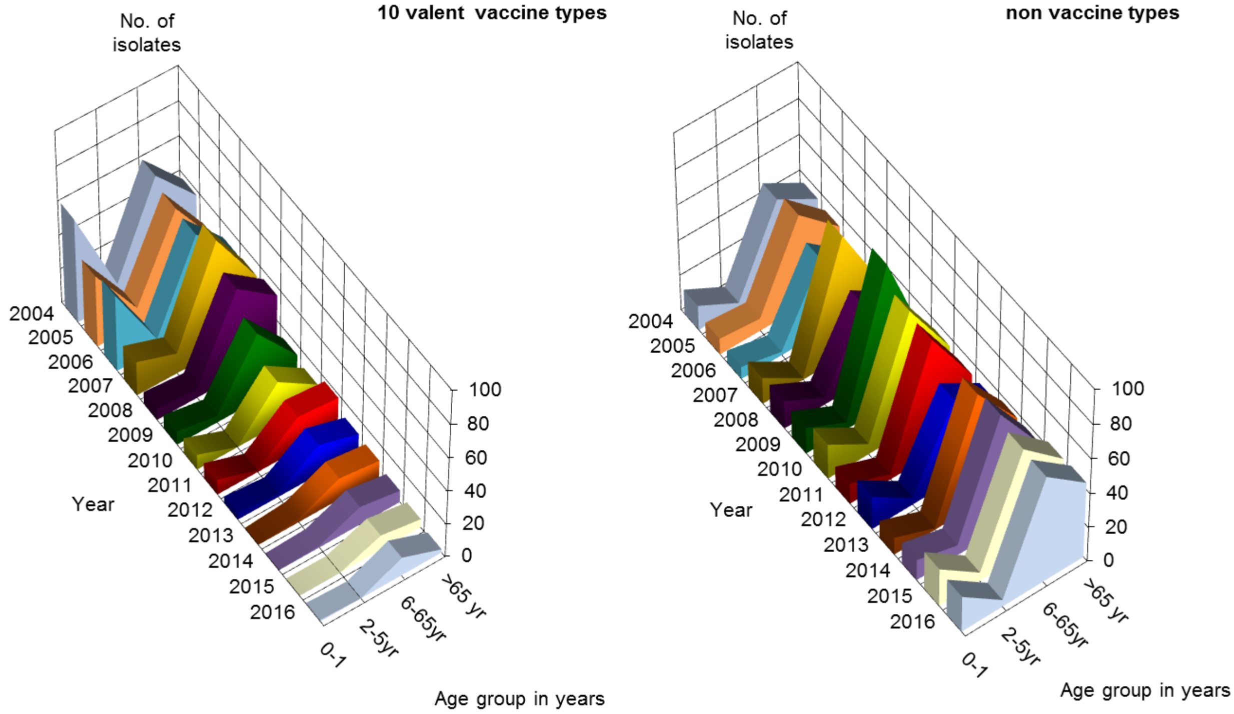

Figure 12.4 *The age distribution of S.pneumoniae invasive disease due to pneumococci of serotypes included in the hepta-valent conjugated polysaccharide vaccine, 2004-2016. Left: vaccine types. Right: types not included in this 10 valent vaccine*

13 PUBLICATIONS

1 Arenas J, Paganelli FL, Rodríguez-Castaño P, Cano-Crespo S, van der Ende A, van Putten JPM, Tommassen J, Expression of the Gene for Autotransporter AutB of *Neisseria meningitidis* Affects Biofilm Formation and Epithelial Transmigration. FRONT CELL INFECT MI 2016;6:162

2 Bijlsma MW. Bacterial meningitis: epidemiology, herd protection, clinical characteristics, and risk assessment. S.l.: s.n.; 2016. 173p. ISBN 978-94-6299-365- [UvA Dissertations Online] Proefschrift Universiteit van Amsterdam. (Promotor(s): van de Beek D; copromotor(s): van der Ende A, Brouwer MC)

3 Bijlsma MW, Brouwer MC, Bossuyt PM, Heymans MW, van der Ende A, Tanck MWT, van de Beek D, Risk scores for outcome in bacterial meningitis: Systematic review and external validation study. J INFECTION 2016;73 (5):393-401

4 Bijlsma MW, Brouwer MC, Kasanmoentalib ES, Kloek AT, Lucas MJ, Tanck MW, van der Ende A, van de Beek D, Community-acquired bacterial meningitis in adults in the Netherlands, 2006-14: a prospective cohort study. LANCET INFECT DIS 2016;16 (3):339-347

5 Costerus JM , Brouwer MC, Bijlsma MW, Tanck MW, van der Ende A, van de Beek D, Impact of an evidence-based guideline on the management of community-acquired bacterial meningitis: a prospective cohort study. CLIN MICROBIOL INFEC 2016;22 (11):928-933

6 Costerus JM , Brouwer MC, van der Ende A, van de Beek D, Repeat lumbar puncture in adults with bacterial meningitis. CLIN MICROBIOL INFEC 2016;22 (5):428-433

7 Costerus JM, Brouwer MC, van der Ende A, van de Beek D, Community-acquired bacterial meningitis in adults with cancer or a history of cancer. NEUROLOGY 2016;86 (9):860-866 [PubMed]

8 Dias SP, Brouwer MC, Bijlsma MW, van der Ende A, van de Beek D, Sex-based differences in pneumococcal serotype distribution in adults with pneumococcal meningitis. J INFECTION 2016;73 (6):616-619

9 Elberse KE, Wagenvoort GHJ, Pluister GN, de Melker HE, Sanders EAM, van der Ende A, Knol MJ, Pneumococcal population in the era of vaccination: changes in composition and the relation to clinical outcomes. FUTURE MICROBIOL 2016;11 (1):31-41

10 Ferwerda B, Valls Serón M, Jongejan A, Zwinderman AH, Geldhoff M, van der Ende A, Baas F, Brouwer MC, van de Beek D, Variation of 46 Innate Immune Genes Evaluated for their Contribution in Pneumococcal Meningitis Susceptibility and Outcome. EBIOMEDICINE 2016;10:77-84 [PubMed]

11 Jim KK, Engelen-Lee J, van der Sar AM, Bitter W, Brouwer MC, van der Ende A, Veening JW, van de Beek D, Vandenbroucke-Grauls CMJE, Infection of zebrafish embryos with live fluorescent *Streptococcus pneumoniae* as a real-time pneumococcal meningitis model. J NEUROINFLAMM 2016;13 (1):188

12 Kloek AT, van Setten J, van der Ende A, Bots ML, Asselbergs FW, Valls Serón M, Brouwer MC, van de Beek D, Ferwerda B, Exome Array Analysis of Susceptibility to Pneumococcal Meningitis. SCI REP-UK 2016;6:29351

13 Piet JR. Bacterial genetics in meningitis: Associating meningococcal and pneumococcal genes with clinical outcome. S.l.: s.n.; 2016. 206p. ISBN 978-94-6332-027-6 [UvA Dissertations Online] Proefschrift Universiteit van Amsterdam. (Promotor(s): van de Beek D; copromotor(s): van der Ende A)

14 Piet JR, van Ulsen P, Ur Rahman S, Bovenkerk S, Bentley SD, van de Beek D, van der Ende A, Meningococcal Two-Partner Secretion Systems and Their Association with Outcome in Patients with Meningitis. INFECT IMMUN 2016;84 (9):2534-2540

15 Savva A, Brouwer MC, Roger T, Valls Serón M, le Roy D, Ferwerda B, van der Ende A, Bochud PY, van de Beek D, Calandra T, Functional polymorphisms of macrophage

migration inhibitory factor as predictors of morbidity and mortality of pneumococcal meningitis. P NATL ACAD SCI USA 2016;113 (13):3597-3602
16 Valls Serón M, Ferwerda B, Engelen-Lee J, Geldhoff M, Jaspers V, Zwinderman AH, Tanck MW, Baas F, van der Ende A, Brouwer MC, van de Beek D, V-akt murine thymoma viral oncogene homolog 3 (AKT3) contributes to poor disease outcome in humans and mice with pneumococcal meningitis. ACTA NEUROPATHOL COM 2016;4 (1):50
17 van Samkar A. Zoonotic bacterial meningitis in adults: clinical characteristics, etiology, treatment and outcome. S.l.: s.n.; 2016. 151p. ISBN 978-94-6332-068-9 [UvA Dissertations Online] Proefschrift Universiteit van Amsterdam. (Promotor(s): van de Beek D; copromotor(s): Brouwer MC, van der Ende A)
18 van Samkar A, Brouwer MC, Schultsz C, van der Ende A, van de Beek D, *Capnocytophaga canimorsus* Meningitis: Three Cases and a Review of the Literature. ZOONOSES PUBLIC HLTH 2016;63 (6):442-448
19 van Samkar A, Brouwer MC, van der Ende A, van de Beek D, *Campylobacter Fetus* Meningitis in Adults: Report of 2 Cases and Review of the Literature. MEDICINE 2016;95 (8):e2858
20 van Samkar A, Brouwer MC, van der Ende A, van de Beek D, *Streptococcus equi* meningitis. CLIN MICROBIOL INFEC 2016;22 (1):e3-e4
21 van Samkar A, Brouwer MC, van der Ende A, van de Beek D, Zoonotic bacterial meningitis in human adults. NEUROLOGY 2016;87 (11):1171-1179
22 van Veen KEB, Brouwer MC, van der Ende A, van de Beek D, Bacterial meningitis in diabetes patients: a population-based prospective study. SCI REP-UK 2016;6:36996
23 van Veen KEB, Brouwer MC, van der Ende A, van de Beek D, Bacterial meningitis in hematopoietic stem cell transplant recipients: a population-based prospective study. BONE MARROW TRANSPL 2016;51 (11):1490-1495
24 van Veen KEB, Brouwer MC, van der Ende A, van de Beek D, Bacterial meningitis in patients with HIV: A population-based prospective study. J INFECTION 2016;72 (3):362-368
25 van Veen KEB, Brouwer MC, van der Ende A, van de Beek D, Bacterial meningitis in solid organ transplant recipients: a population-based prospective study. TRANSPL INFECT DIS 2016;18 (5):674-680
26 Wagenvoort GHJ, Knol MJ, de Melker HE, Vlaminckx BJ, van der Ende A, Rozenbaum MH, Sanders EAM, Risk and outcomes of invasive pneumococcal disease in adults with underlying conditions in the post-PCV7 era, The Netherlands. VACCINE 2016;34 (3):334-340
27 Wagenvoort GHJ, Sanders EAM, Vlaminckx BJ, Elberse KE, de Melker HE, van der Ende A, Knol MJ, Invasive pneumococcal disease: Clinical outcomes and patient characteristics 2-6 years after introduction of 7-valent pneumococcal conjugate vaccine compared to the pre-vaccine period, the Netherlands. VACCINE 2016;34 (8):1077-1085
28 Willemse N, Howell KJ, Weinert LA, Heuvelink A, Pannekoek Y, Wagenaar JA, Smith HE, van der Ende A, Schultsz C, An emerging zoonotic clone in the Netherlands provides clues to virulence and zoonotic potential of *Streptococcus suis*. SCI REP-UK 2016;6:28984

14 ACKNOWLEDGEMENTS

Many have contributed to the work of the Reference Laboratory 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 and dr. Karin Elberse) for ongoing financial support
- 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