BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2012

Amsterdam
The Netherlands

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

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1 INTRODUCTION

This is the **41th** 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 sending 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 65,000 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, 2013

dr. A. van der Ende PhD, biochemist
dr. L. Spanjaard MD PhD, medical microbiologist

2 ISOLATES, CSF SPECIMENS AND SERA RECEIVED

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 2012, the Reference Laboratory received isolates from CSF and / or blood from 1521 patients, and 36 specimens of CSF and/or serum wich were positive in PCR (or crypt. agglutination.) (table 2.1). Of these patients, 307 were confirmed cases of bacterial meningitis.

Table 2.1

	Number of specimens
Isolate (CSF and/or blood)	1304
CSF samples (without isolate)	205
Sera (and other fluid, without isolate)	12
Total	1521

In 2012, 54 clinical microbiology laboratories submitted isolates to the Reference Laboratory.

Table 2.2 shows the 1304 isolates according to species and to laboratory where cases were diagnosed.

Table 2.2 Number of isolates from CSF and/or blood received in 2012, according to laboratory

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Alkmaar	MCA lab. Med. Microbiologie	-	4	6	-	1	1	-	-	-	-	-	-	12
Amersfoort	Meander Medisch Centrum	-	3	2	-	1	-	-	-	-	-	-	-	6
Amsterdam	AMC Acad. Medisch Centrum	1	1	32	-	-	-	-	1	2	4	4	-	45
	VU Medisch centrum	3	-	1	-	1	-	-	-	-	1	-	-	6
	Onze Lieve Vrouwe Gasthuis	4	6	10	-	3	2	-	1	-	1	-	-	27
	Slotervaart Ziekenhuis	-	-	4	-	-	-	-	-	-	-	-	-	4
Apeldoorn	Gelre Ziekenhuizen	1	5	10	-	4	2	-	-	-	-	-	-	22
Breda	Amphia Ziekenhuis	2	6	11	5	5	1	-	-	-	-	1	-	31
Capelle ad IJssel	IJsselland Ziekenhuis	1	-	-	1	1	1	-	-	-	-	-	-	4
Delft	SSDZ (Reinier de Graaf groep)	3	-	5	-	-	1	-	-	-	-	-	-	9
Den Bosch	Regionaal laboratorium Den Bosch	4	3	4	-	3	-	1	-	-	-	-	-	15
Den Haag	Bronovo Ziekenhuis	1	1	1	-	-	-	-	-	-	-	-	-	3
	Leijenburg Ziekenhuis	4	3	9	-	1	2	-	1	-	-	-	-	20
Deventer	Deventer Ziekenhuis	-	1	3	-	3	3	-	-	-	-	-	-	10
Doetinchem	Slingeland Ziekenhuis	-	-	-	-	2	-	-	-	-	-	-	-	2
Dordrecht	RLM dordrecht	1	5	55	-	7	-	-	1	-	-	2	-	71
Ede	Gelderse Vallei	3	2	3	-	-	-	-	-	1	-	-	-	9
Enschede	RLM Enschede	5	8	87	1	11	3	2	-	-	-	-	-	117
Goes	Admiraal de Ruijterziekenhuis	-	-	-	-	-	1	-	-	-	-	-	-	1
Gouda	Groene Hart Ziekenhuis	1		2	1	-	-	-	-	-	-	-	-	4
Groningen	Lab. v. Infectieziekten	4	5	16	2	3	2	3	-	-	-	2	-	37
	UMC	-	2	6	-	-	-	-	-	-	-	-	-	8
Haarlem	St. Streeklab voor de Volksgezondheid	1	9	104	-	2	3	1	1	-	1	-	-	122
Harderwijk	St. Jansdal Ziekenhuis	-	-	3	-	-	-	-	-	-	-	-	-	3
Heerlen	Atrium Medisch Centrum	4	5	53	-	1	2	-	-	-	-	2	-	67
Hilversum	Centraal Bact. Ser. Lab.	1	-	7	-	-	1	-	1	-	-	2	-	12
Hoorn	Westfries gasthuis	-	-	1	-	-	1	-	-	-	-	-	-	2
Leeuwarden	Izore, centrum infectieziekten Friesland	3	5	86	-	7	2	-	-	-	-	1	-	104
Leiden	KML, Lab.voor Bacteriologie	2	-	2	-	2	1	-	-	-	-	5	-	12
	Diakonessen Ziekenhuis	2	-	1	-	-	-	-	-	-	-	-	-	3
Leiderdorp	Rijnland Ziekenhuis	-	-	1	-	2	-	-	-	-	-	-	-	3
Maastricht	Acad. Ziekenhuis Maastricht	-	1	1	-	-	-	-	-	-	-	-	-	2
Nieuwegein	St. Antonius Ziekenhuis	-	3	55	-	-	2	1	-	-	-	-	-	61
Nijmegen	Canisius Wilhelmina Ziekenhuis	3	6	2	-	2	-	1	-	-	-	-	-	14
	UMC St. Radboud	-	10	7	1	-	-	-	-	-	1	-	-	19
Roermond	St. Laurentius Ziekenhuis	1	-	-	-	-	-	-	-	-	-	-	-	1
Roosendaal	St. Fransiscus Ziekenhuis	5	1	2	-	-	1	-	-	-	-	-	-	9
Rotterdam	Erasmus MC Med. Microbiologie		7	6	-	1	-	-	-	-	-	-	-	14
Rotterdam	St.Franciscus Gasthuis	3	2	3	-	-	2	-	1	-	-	-	-	11
	Ikazia Ziekenhuis	-	2	-	-	1	-	-	-	-	-	-	-	3
	Maasstad Ziekenhuis	2	3	1	1	-	3	-	-	1	-	-	-	11
Schiedam	Vlietland locatie Schiedam	-	-	1	-	-	-	-	-	-	-	-	-	1
Sittard	Orbis Medisch Centrum	2	-	1	-	-	-	-	-	-	-	2	1	6
Terneuzen	ZorgSaam Zeeuws-Vlaanderen LVM	-	-	1	-	-	-	-	-	-	-	-	-	1

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Tilburg	Streeklab. Tilburg	-	5	67	-	1	4	1	-	1	-	1	-	80
Utrecht	Diakonessenhuis	1	1	4	-	-	2	-	-	-	-	-	-	8
	UMC Med. Microbiologie	2	3	11	-	2	2	-	-	-	-	1	-	21
Veldhoven	Lab. Med. Microbiologie	5	6	117	-	7	7	2	-	-	-	1	-	145
Velp	Ziekenhuis Velp	1	2	50	-	3	2	-	-	1	1	-	-	60
Venlo	Vie Curie medisch centrum	2	2	6	-	1	-	-	-	-	-	-	-	11
Weert	St. Jans gasthuis	1	-	1	-	-	-	-	-	-	1	-	-	3
Woerden	Zuwe Hofpoort Ziekenhuis	1	-	-	-	-	-	-	-	-	-	-	-	1
Zaandam	Zaans Medisch Centrum	-	7	4	-	1	2	-	-	-	-	-	-	14
Zwolle	Isala Klinieken	1	5	4	1	1	3	-	1	-	-	1	-	17
Absolute total*		**81**	**140**	**869**	**13**	**80**	**59**	**12**	**8**	**6**	**10**	**25**	**1**	**1304**

\# **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

The distribution of the isolates received in the 5 year period 2008 through 2012 is presented in table 2.3. The number of total isolates decreased from 1360 in 2008 to 1304 in 2012. The number of meningococcal disease cases reduced from 145 cases in 2008 to only 81 cases in 2012. 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 138 in 2012. The number of *Listeria monocytogenes* was high in 2005 (81), most likely due to an intensified surveillance performed by the RIVM. In 2012, the number of *L. monocytogenes* isolates was equal to that in 2010 and 2011. 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 2008 – 2012

Species	YEAR														
	2008			2009			2010			2011			2012		
	CSF	**Blood**	**Total**	**CSF**	**Blood**	**Total**	**CSF**	**Blood**	**Total**	**CSF**	**Blood**	**Total**	**CSF**	**Blood**	**Total**
N. meningitidis	61	84	145	52	87	139	53	79	132	37	53	90	41	40	81
H. influenzae	19	89	108	15	114	129	17	125	142	13	126	139	16	124	140
S. pneumoniae	190	726	916	190	735	925	176	658	834	163	753	916	138	731	869
E. coli	11	13	24	11	12	23	11	13	24	8	7	15	5	8	13
S. agalactiae	14	42	56	9	53	62	22	48	70	19	44	63	23	57	80
L. monocytogenes	13	29	42	13	44	57	16	42	58	8	53	61	9	50	59
S. pyogenes	4	5	9	6	5	11	6	7	13	4	10	14	3	9	12
S. aureus	9	-	9	10	-	10	8	-	8	4	-	4	7	1	8
Coag.neg.Staph.	6	-	6	6	-	6	6	-	6	-	-	-	6	-	6
C. neoformans	4	2	6	12	1	13	6	6	12	5	2	7	9	1	10
others	30	3	33	23	2	25	19	4	23	14	6	20	17	8	25
non viable	3	3	6	1	4	5	-	1	1	-	2	2	-	1	1
Total	364	996	1360	348	1057	1405	340	983	1323	275	1056	1331	274	1030	1304

CSF: CSF or CSF and blood
blood: blood only

The incidence of isolation of the different bacterial species from CSF and/or blood over the years 2008 to 2012 is shown in table 2.4. The incidence of *N. meningitidis* infection was 45% lower than in 2008. This is due to a decline in the number of cases of *N. meningitidis* group B. The incidence of *H. influenzae* infection was 60% lower than in the years before vaccination was introduced (2.1 in 1992; 0.84 in 2012). The incidence *of H. influenzae* infection increased from 1999 until 2010, mainly due to an increase in the number of cases of *H. influenzae* bacteraemia, but remained stable over the last three years.

Table 2.4 Number of isolates from CSF and/or blood per 100,000 inhabitants, 2008 - 2012

Species	YEAR				
	2008	**2009**	**2010**	**2011**	**2012**
N. meningitidis	0.88	0.84	0.80	0.54	0.48
H. influenzae	0.66	0.78	0.86	0.83	0.84
S. pneumoniae	5.58	5.61	5.03	5.50	5.19
E. coli	0.15	0.14	0.14	0.09	0.08
S. agalactiae	0.34	0.38	0.42	0.38	0.48
L. monocytogenes	0.26	0.35	0.35	0.37	0.35
S. pyogenes	0.05	0.07	0.08	0.08	0.07
S. aureus	0.05	0.06	0.05	0.02	0.05
Coag. neg. Staph.	0.04	0.04	0.04	-	0.04
C. neoformans	0.04	0.08	0.07	0.04	0.06
others	0.20	0.15	0.14	0.12	0.15
non viable	0.04	0.03	-	0.01	0.01
Total	8.29	8.52	7.98	7.99	7.79

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 was counted once for *S. pneumoniae* and once for *H. influenzae*.

Table 2.5 Total number of isolates from CSF and/or blood received in 2012, according to bacterial species and specimen source

Species		CSF or CSF and blood	Blood only	Total	%
Neisseria meningitidis		41	40	81	**6.2**
Haemophilus influenzae		16	124 [1]	140	**10.7**
Streptococcus pneumoniae		138	731 [2]	869	**66.6**
Escherichia coli		5	8	13	**1.0**
Streptococcus agalactiae		23	57	80	**6.1**
Listeria monocytogenes		9	50	59	**4.5**
Streptococcus pyogenes		3	9	12	**0.9**
Staphylococcus aureus		7	1	8	**0.6**
Coagulase-negative staphylococcus		6 [3]	-	6	**0.5**
Cryptococcus neoformans		9	1	10	**0.8**
Others total		17	8	25	**2.0**
Others	*Moraxella canis*	-	1	1	
	Peudomonas aeruginosa	1 [4]	-	1	
	Klebsiella oxytoca	1	-	1	
	Klebsiella pneumoniae	1	-	1	
	Haemophilus parainfluenzae	-	1	1	
	Streptococcus anginosus	1	-	1	
	Streptococcus dysgalactiae	2	-	2	
	Streptococcus infantis	1 [5]	-	1	
	Streptococcus intermedius	2	-	2	
	Streptococcus mitis	-	1	1	
	Streptococcus suis	1	-	1	
	Viridans streptococci	1	1	2	
	Enterococcus faecalis	2	-	2	
	Enterococcus faecium	1	-	1	
	Serratia marcescens	1	-	1	
	Fusobacterium necrophorum	1	-	1	
	Aggregatibacter aphrophilus	-	1	1	
	Actinomyces odontolyticus	1	-	1	
	Contamination	-	3	3	
Non viable		-	1 [6]	1	**0.1**
Total		274	1030	1304	**100.0**
%		**21.0**	**79.0**	**100.0**	

1 In three patient *S. pneumoniae* and *H. influenzae* were isolated from the blood
2 In two patients from whom *S. pneumoniae* was isolated from the blood, the CSF was culture-negative but positive in the PCR reaction
3 In one patient *Klebsiella pneumoniae* and *Staphylococcus epidermidis* were isolated from the CSF
4 In one patient *Pseudomonas aeruginosa* and *Streptococcus intermedius* were isolated from the CSF
5 In one patient *Streptococcus infantis* and *Actinomyces odontolyticus* were isolated from the CSF
6 Non viable, in one patient a meningococci was isolated from the blood. The isolate was non viable. The CSF was PCR positive for McB

3 BACTERIAL MENINGITIS - general data

In 2012, the Reference Laboratory received CSF isolates of 274 patients. Furthermore, 33 culture-negative CSF samples appeared to be positive by antigen detection or PCR (Table 11.1). Of these CSF samples, 16 were positive for *N. meningitidis,* 15 for *S. pneumoniae* and 2 for *C. neoformans*. Including these cases, the total number of patients with confirmed meningitis amounted to 307. The proportion of pneumococcal and meningococcal meningitis among these patients was 19% and 50%, respectively (Figure 3.1).

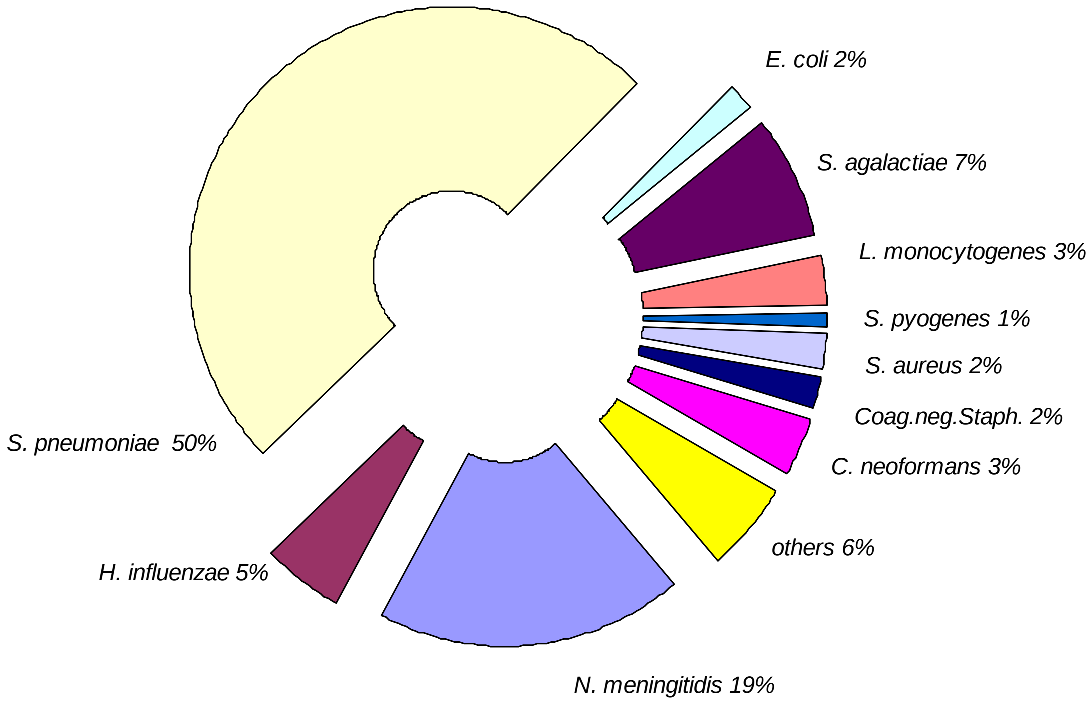

Figure 3.1 *Proportional distribution of CSF isolates and CSF positive samples, 2012*

Figure 3.2 shows the annual total number of bacterial isolates from CSF during the period 2003-2012. 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 3.5 and 1.6 during the period 2003-2012 (Figure 3.2).

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 0.10 per 100,000 and remained at this low level. The number of cases of meningococcal meningitis (with an isolate) decreased from 308 cases (incidence of 1.9) in 2002 to 37 cases (incidence of 0.3) in 2012, 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 0.8 in 2012 due to vaccination against pneumococci introduced in June 2006 in the National Immunisation Programme.

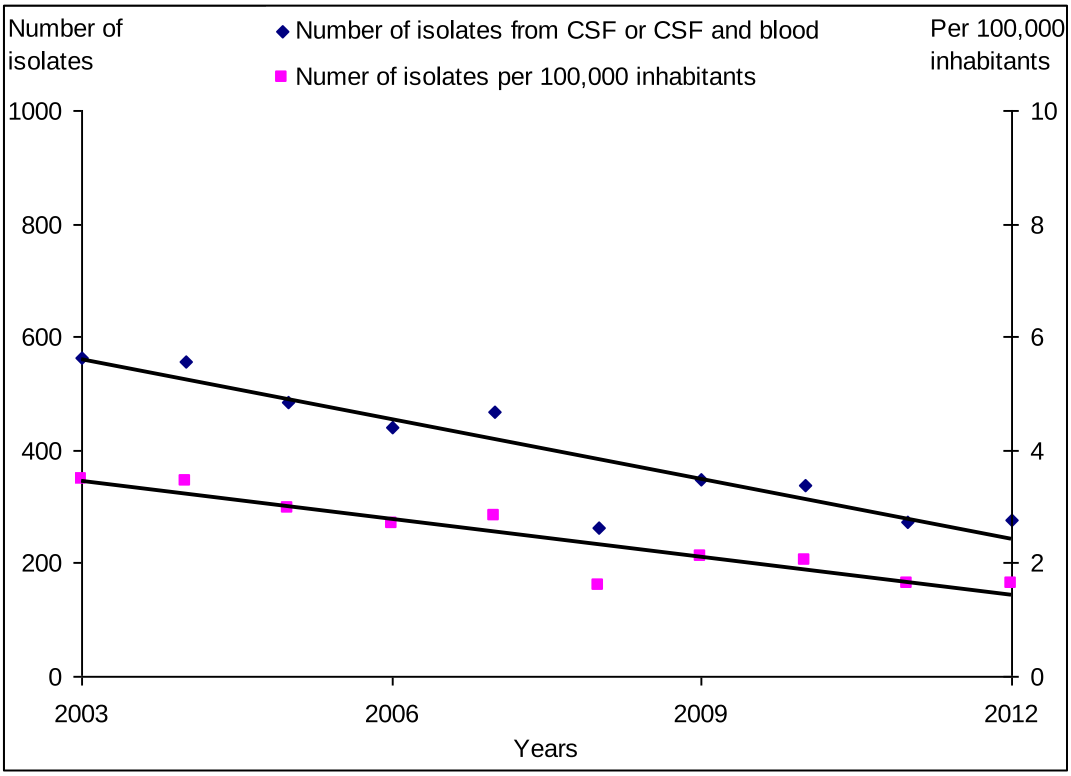

Figure 3.2 *Isolates from CSF, 2003-2012*

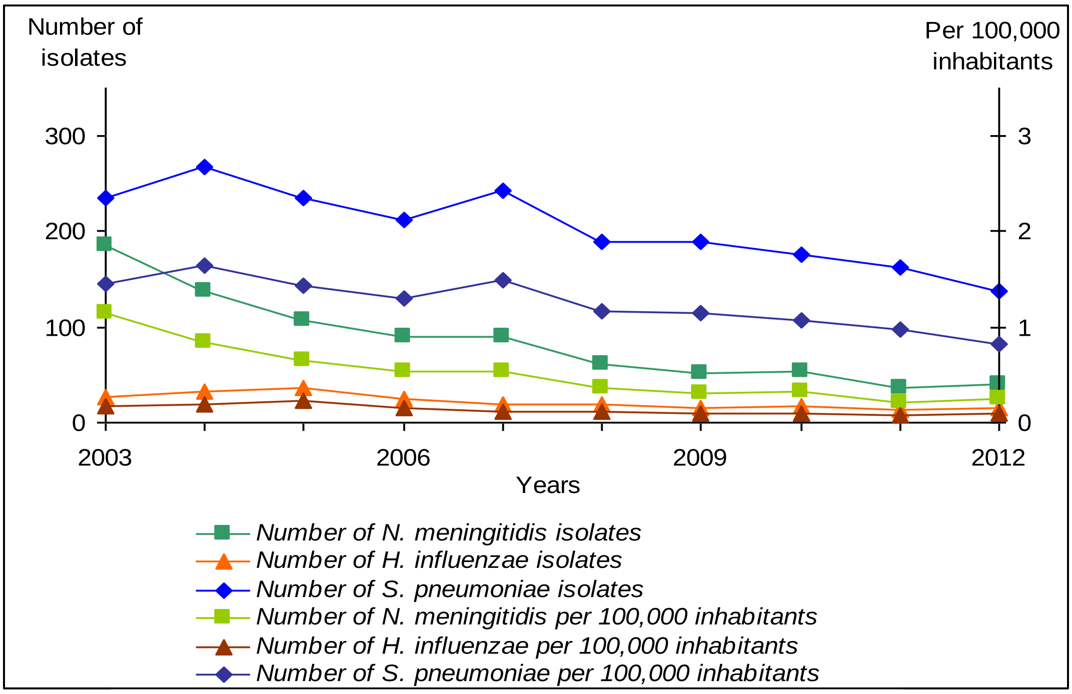

Figure 3.3 *Meningococcal, Haemophilus and pneumococcal meningitis, 2003-2012*

Table 3.1 shows the frequency of isolation of the different bacterial species from CSF by annual quarter. In previous years, most strains were received during the first quarter of the year. Last year (2012) we received most strains in the first and the last quarter.

Table 3.1 Isolates from CSF by annual quarter, 2012

	ANNUAL QUARTER					
SPECIES	**first**	**second**	**third**	**fourth**	**Total**	**%**
N. meningitidis	10	13	6	12	41	**15.0**
H. influenzae	4	7	1	4	16	**5.8**
S. pneumoniae	45	38	13	42	138	**50.4**
E. coli	1	2	1	1	5	**1.8**
S. agalactiae	3	5	10	5	23	**8.4**
L. monocytogenes	1	2	1	5	9	**3.3**
S. pyogenes	1	1	-	1	3	**1.1**
S. aureus	2	2	2	1	7	**2.5**
Coag.neg.Staph.	2	-	-	4	6	**2.2**
C. neoformans	4	1	3	1	9	**3.3**
Others	5	4	1	7	17	**6.2**
non viable	-	-	-	-	-	**-**
Total	78	75	38	83	274	**100.0**
%	**28.4**	**27.4**	**13.9**	**30.3**	**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 70% 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 74%). 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; 2004: 17 and 2012: 6).

Table 3.2 Isolates from CSF grouped according to patients' age, 2012

	AGE (MONTHS)			AGE (YEARS)											
SPECIES	**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	-	11	10	21	2	1	5	3	2	3	2	2	-	41	**15.0**
H. influenzae	-	3	3	6	1	-	-	-	-	3	1	4	1	16	**5.8**
S. pneumoniae	1	19	6	26	4	1	2	2	7	13	35	39	9	138	**50.4**
E. coli	1	2	-	3	-	-	-	-	-	1	-	1	-	5	**1.8**
S. agalactiae	14	5	-	19	-	-	-	-	-	-	2	1	1	23	**8.4**
L. monocytogenes	-	-	1	1	-	-	-	-	-	-	2	4	2	9	**3.3**
S. pyogenes	-	-	2	2	-	-	-	-	-	1	-	-	-	3	**1.1**
S. aureus	-	1	-	1	-	-	-	1	-	-	4	1	-	7	**2.5**
Coag.neg.Staph.	1	-	-	1	-	-	-	-	-	1	2	2	-	6	**2.2**
C. neoformans	-	-	-	-	-	-	-	1	2	3	1	2	-	9	**3.3**
Others	3	-	-	3	2	-	-	-	1	2	6	2	1	17	**6.2**
non viable	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total	20	41	22	83	9	2	7	7	12	27	55	58	14	274	**100.0**
%	**7.3**	**15.0**	**8.0**	**30.3**	**3.3**	**0.7**	**2.5**	**2.5**	**4.4**	**9.9**	**20.1**	**21.2**	**5.1**	**100.0**	

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, 2012

	AGE (YEARS)											
SPECIES	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-64**	**65-79**	**≥80**	**Total**
N. meningitidis	6.12	1.35	0.21	0.10	0.50	0.15	0.10	0.12	0.06	0.10	-	0.25
H. influenzae	1.67	0.41	0.10	-	-	-	-	0.12	0.03	0.20	0.15	0.10
S. pneumoniae	11.13	0.81	0.41	0.10	0.20	0.10	0.34	0.50	1.03	1.92	1.31	0.82
E. coli	1.67	-	-	-	-	-	-	0.04	-	0.05	-	0.03
S. agalactiae	10.58	-	-	-	-	-	-	-	0.06	0.05	0.15	0.14
L. monocytogenes	-	0.14	-	-	-	-	-	-	0.06	0.20	0.29	0.05
S. pyogenes	-	0.27	-	-	-	-	-	0.04	-	-	-	0.02
S. aureus	0.56	-	-	-	-	0.05	-	-	0.12	0.05	-	0.04
Coag.neg.Staph.	0.56	-	-	-	-	-	-	0.04	0.06	0.10	-	0.04
C. neoformans	-	-	-	-	-	0.05	0.10	0.12	0.03	0.10	-	0.05
Others	1.67	-	0.21	-	-	-	0.05	0.08	0.18	0.10	0.15	0.10
non viable	-	-	-	-	-	-	-	-	-	-	-	-
Total	33.95	2.98	0.93	0.20	0.70	0.34	0.58	1.04	1.62	2.86	2.04	1.64

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 0.8 and 1.5. The M/F ratio among patients infected with *C. neoformans* was 2.0. The overall M/F ratio was 1.1.

Table 3.4 Isolates from CSF according to patients' gender, 2012

SPECIES	**M**	**F**	**M/F-ratio**	**sex not known**	**Total**	**%**
N. meningitides	22	18	**1.2**	1	41	**15.0**
H. influenzae	7	9	**0.8**	-	16	**5.8**
S. pneumoniae	72	66	**1.1**	-	138	**50.4**
E. coli	3	2	**1.5**	-	5	**1.8**
S. agalactiae	9	13	**0.7**	1	23	**8.4**
L. monocytogenes	5	3	**1.7**	1	9	**3.3**
S. pyogenes	1	2	**0.5**	-	3	**1.1**
S. aureus	1	5	**0.2**	1	7	**2.5**
Coag.neg.Staph.	3	3	**1.0**	-	6	**2.2**
C. neoformans	6	3	**2.0**	-	9	**3.3**
Others	9	8	**1.1**	-	17	**6.2**
non viable	-	-	**-**	-	-	**-**
Total	138	132	**1.1**	4	274	**100.0**
%	**50.4**	**48.2**		**1.4**	**100.0**	

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2012, the Reference Laboratory received 81 *Neisseria meningitidis* isolates, of which 41 were isolated from CSF (or CSF and blood) (37 in 2011) and 40 from blood only (53 in 2011). This means that 50% of cases of 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 in previous years that the highest number of isolates was received in the first quarter of the year (figure 4.1). In 2012, this peak was not observed.

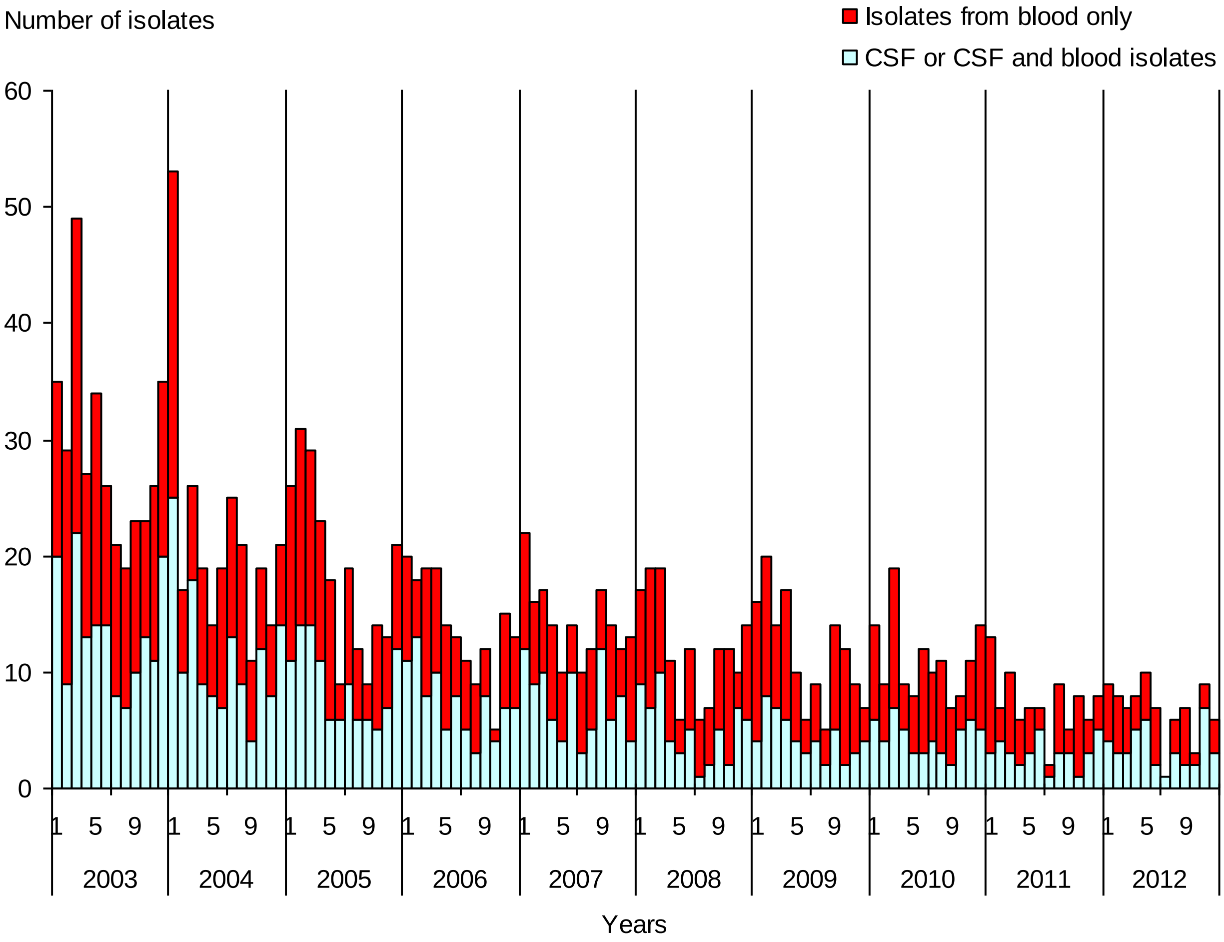

Figure 4.1 *Seasonal distribution of meningococcal disease, 2003-2012*

4.2 Antibiotic susceptibility

Sixtyfive percent of all isolates (53/81) (70% in 2011; 83% in 2010 and 92% in 2009) were susceptible to penicillin (MIC ≤ 0.064 µg/ml; CSF isolates 59%, isolates from blood only 68%). This reduced proportion of penicillin-susceptible isolates is mainly due to a doubling of the number of intermediate susceptible isolates (table 4.1, 4.2 and 4.3). 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 rifampicin.

Table 4.1 Susceptibility of *N. meningitidis* CSF and/or blood isolates to penicillin, 2012

	Penicillin*					
	MIC ≤ 0.064 sensitive	**0.064< MIC≤0.25**	**0.25< MIC≤1.0**	**MIC >1.0**	**Total**	**%**
CSF or CSF and blood	24	16	1	-	41	**51**
Blood only	27	13	**-**	-	40	**49**
Total number of isolates	51	29	1	-	81	**100**
%	**65**	**34**	**1**	**-**	**100**	

* MIC values in µg/ml

Table 4.2 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2008-2012

	Penicillin*								
	MIC ≤ 0.064 sensitive		**0.064< MIC≤0.25**		**0.25< MIC≤1.0**		**MIC >1.0**		**Total**
	n	%	n	%	n	%	n	%	
2008	58	**95.1**	3	**4.9**	**-**	**-**	-	-	61
2009	51	**98.1**	1	**1.9**	**-**	**-**	-	-	52
2010	43	**81.1**	10	**18.9**	**-**	**-**	-	-	53
2011	29	**78.4**	8	**21.6**	**-**	**-**	-	-	37
2012	24	**58.5**	16	**39.0**	1	**2.4**	-	-	41

* MIC values in µg/ml

Table 4.3 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2008-2012

	Penicillin*								
	MIC ≤ 0.064 sensitive		**0.064< MIC≤0.25**		**0.25< MIC≤1.0**		**MIC >1.0**		**Total**
	n	%	n	%	n	%	n	%	
2008	70	**83.3**	14	**16.7**	**-**	-	-	**-**	84
2009	77	**88.5**	10	**11.5**	**-**	-	-	**-**	87
2010	67	**84.8**	12	**15.2**	**-**	-	-	**-**	79
2011	34	**64.2**	19	**35.9**	**-**	-	-	**-**	53
2012	27	**67.5**	13	**32.5**	**-**	-	-	**-**	40

* MIC values in µg/ml

4.3 Serogroups

Serogroup B accounted for 80% (2011: 77%) of all isolates and group Y for about 12% (table 4.4). The proportion of serogroup Y isolates is gradually increasing since 2008 (2011: 17%; 2010: 8% and 2009: 5%), partly due to a decrease in the number of serogroup B isolates and to a small increase in the number of serogroup Y isolates. The remaining 8% of the isolates were of the rare serogroups C, W135, Y or Z. The serogroup distribution observed during the whole collection period 1959 - 2011 (figure 4.2) shows that in 2012 the number of group B isolates (65 cases) was the lowest since 1976. 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). 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 2012 only 2 serogroup C isolates were received (figure 4.3). However, it should be noted, that also the number of serogroup B has decreased, albeit that the decrease started earlier and was less steep.

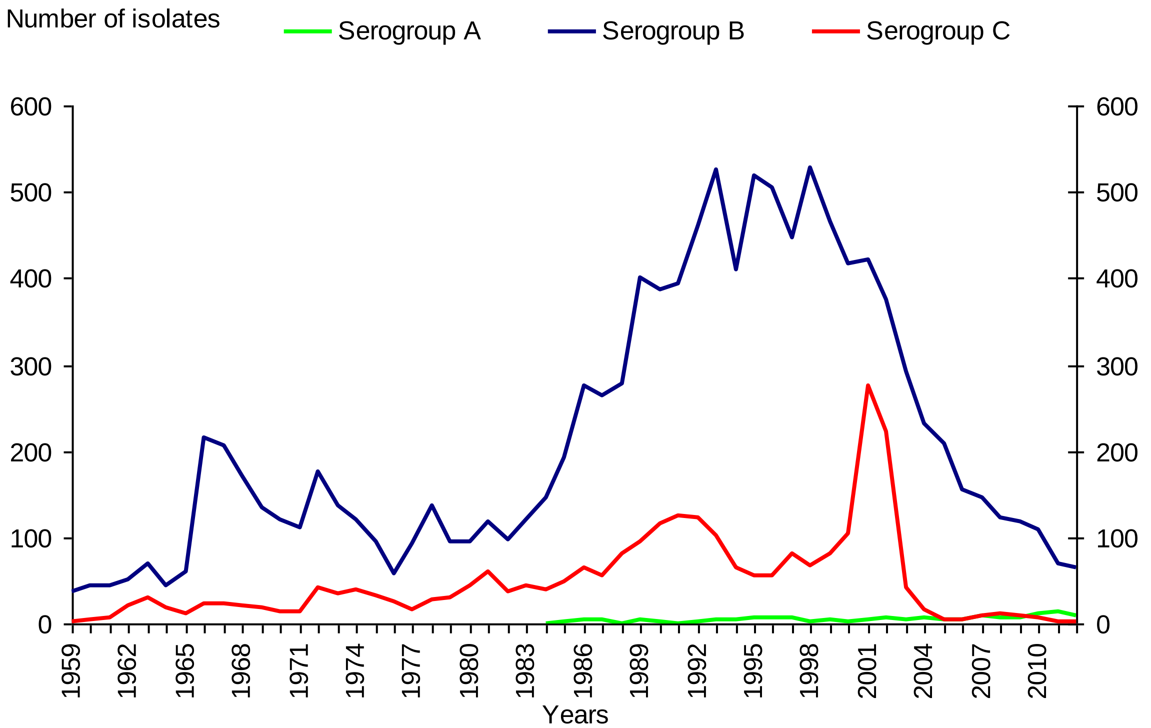

Figure 4.2. *Distribution of meningococcal serogroups, 1959-2012*

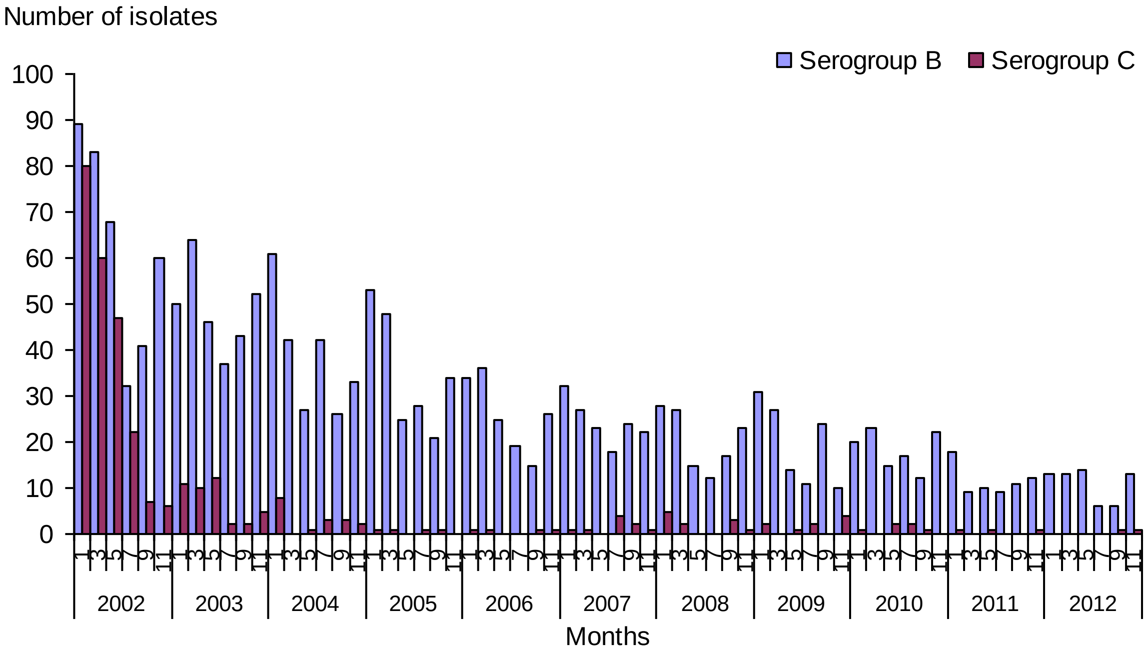

Figure 4.3 *Bimonthly distribution of meningococcal serogroups B and C, 2002-2012*

4.4 Serogroup and age

The age distribution of patients with meningitis and/or meningococcemia shows that 47% (38 of 81) of the patients was younger than 5 years (table 4.4, figure 4.4). Among patients from whom meningococci were isolated from blood only, 42.5% was younger than 5 years (table 4.7).

Table 4.4 Serogroups of *N. meningitidis* (all isolates: from CSF and /or blood, absolute numbers) by patient age, 2012

	AGE (MONTHS)			**AGE (YEARS)**										
SEROGROUP	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	**Total**	**%**
B	1	15	20	36	2	3	5	3	2	4	3	7	65	**80.3**
C	-	1	-	1	-	-	-	-	-	1	-	-	2	**2.5**
Y	-	-	1	1	3	-	-	-	-	2	2	2	10	**12.3**
W135	-	-	-	-	-	-	-	1	-	1	-	1	3	**3.7**
Z	-	-	-	-	1	-	-	-	-	-	-	-	1	**1.2**
Total	1	16	21	38	6	3	5	4	2	8	5	10	81	**100.0**
%	**1.2**	**19.8**	**25.9**	**46.9**	**7.4**	**3.7**	**6.2**	**4.9**	**2.5**	**9.9**	**6.2**	**12.3**	**100.0**	

Table 4.5 Serogroups of *N. meningitidis* (isolates from CSF, or CSF and blood; absolute numbers) by patient age, 2012

	AGE (MONTHS)			**AGE (YEARS)**										
SEROGROUP	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	**Total**	**%**
B	-	10	10	20	1	1	5	1	1	2	2	-	33	**80.5**
C	-	1	-	1	-	-	-	-	-	1	-	-	2	**4.9**
Y	-	-	-	-	1	-	-	-	-	2	-	2	5	**12.2**
W135	-	-	-	-	-	-	-	1	-	-	-	-	1	**2.4**
Z	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Total	-	11	10	21	2	1	5	2	1	5	2	2	41	**100.0**
%	**-**	**26.8**	**24.4**	**51.2**	**4.9**	**2.4**	**12.2**	**4.9**	**2.4**	**12.2**	**4.9**	**4.9**	**100.0**	

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), 2012

	AGE (YEARS)										
SEROGROUP	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	**Total**
B	5.57	1.35	0.10	0.10	0.50	0.10	0.10	0.10	0.06	-	0.20
C	0.56	-	-	-	-	-	-	0.05	-	-	0.01
Y	-	-	0.10	-	-	-	-	0.10	-	0.07	0.03
W135	-	-	-	-	-	0.10	-	-	-	-	0.01
Z	-	-	-	-	-	-	-	-	-	-	-
Total	6.12	1.35	0.21	0.10	0.50	0.19	0.10	0.24	0.06	0.07	0.25

Table 4.7 Serogroups of *N. meningitidis* (isolates from blood only, absolute numbers) by patient age, 2012

	AGE (MONTHS)			AGE (YEARS)										
SEROGROUP	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	**Total**	**%**
B	1	5	10	16	1	2	-	2	1	2	1	7	32	**80.0**
C	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**
Y	-	-	1	1	2	-	-	-	-	-	2	-	5	**12.5**
W135	-	-	-	-	-	-	-	-	-	1	-	1	2	**5.0**
Z	-	-	-	-	1	-	-	-	-	-	-	-	1	**2.5**
Total	1	5	11	17	4	2	-	2	1	3	3	8	40	**100.0**
%	**1.5**	**12.5**	**27.5**	**42.5**	**10.0**	**5.0**		**5.0**	**2.5**	**7.5**	**7.5**	**20.0**	**100.0**	

Table 4.8 Age distribution of meningococcemia (incidence per 100,000 inhabitants) by different serogroups of *N. meningitidis* (isolates from blood only), 2012

	AGE (YEARS)										
SEROGROUP	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-24**	**25-29**	**30-49**	**50-64**	**≥65**	**Total**
B	3.34	1.35	0.10	0.20	-	0.19	0.10	0.10	0.03	0.26	0.19
C	-	-	-	-	-	-	-	-	-	-	-
Y	-	0.14	0.21	-	-	-	-	-	0.06	-	0.03
W135	-	-	-	-	-	-	-	0.05	-	0.04	0.01
Z	-	-	0.01	-	-	-	-	-	-	-	0.01
Total	3.34	1.49	0.41	0.20	-	0.19	0.10	0.14	0.09	0.29	0.24

4.5 Group B meningococci

Figure 4.4 shows the age distribution of group B meningococcal disease. The age-specific incidences per 100,000 inhabitants in the age groups younger than 5 years, 15 - 19 years and 80 – 84 years were 3.9, 0.5 and 0.8 respectively. The age-specific incidences per 100,000 inhabitants in the age groups between 20 and 80 years and >85 years was less than 0.5.

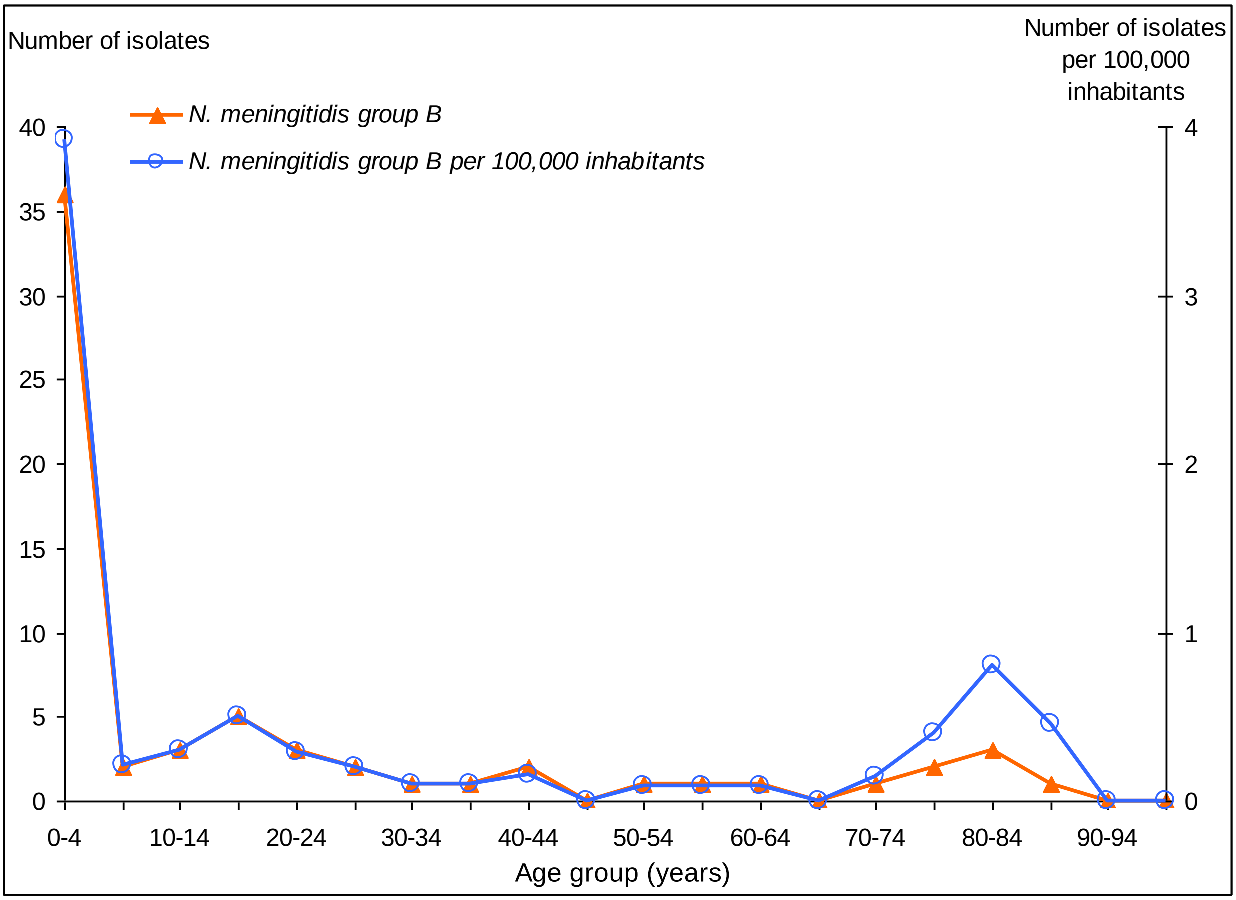

Figure 4.4 *Age distribution of serogroup B meningococcal disease in 2012*

4.6 *Distribution of PorA genosubtypes among serogroup B and C 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 2012, 31 different VR1/VR2 combinations were encountered among serogroup B meningococci (2006: 50; 2007: 39; 2008: 40; 2009: 43; 2010: 36; 2011: 32). The proportion of the dominant PorA genosubtype P1.7-2,4 decreased from 40% of all serogroup B isolates in 2000 to 9.2% in 2012 (figure 4.5, figure 4.7; table 4.9).

The two serogroup C isolates had the VR1/VR2 combination P1.5,2 and P1.12-1,13-2 respectively.

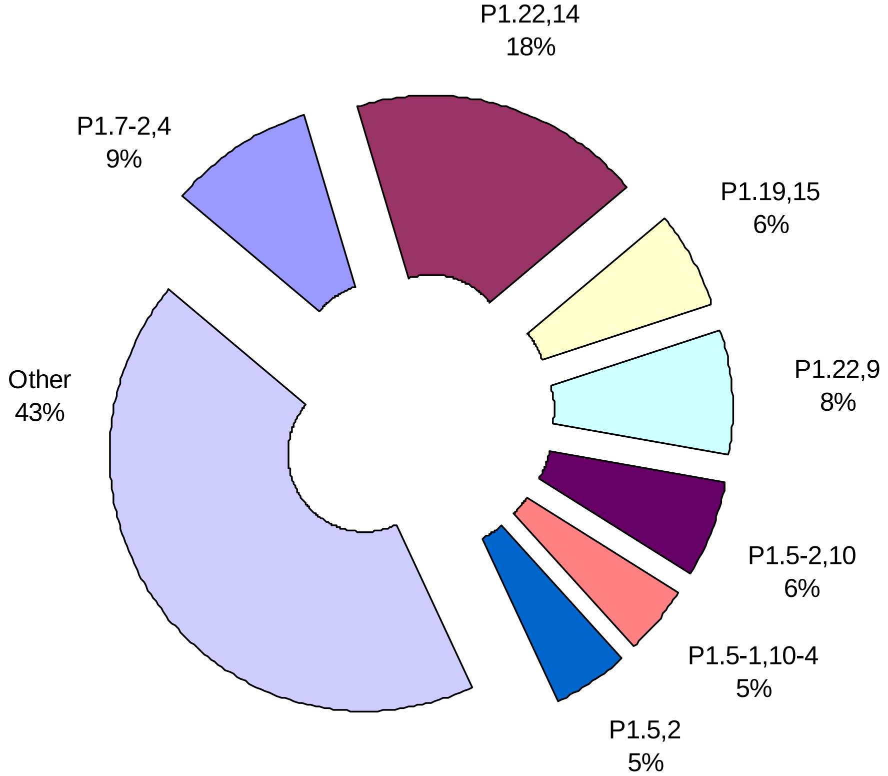

Figure 4.5 *Distribution of group B meningococcal PorA types, 2012*

Table 4.9 *N. meningitidis* serogroup B isolates according to PorA genosubtype, 2008-2012

	VR1,VR2 combination	YEAR 2008 No.	%	2009 No.	%	2010 No.	%	2011 No.	%	2012 No.	%
Vaccine types*	1.5-1, 2-2	1	**0.8**	-	**-**	1	**0.9**	-	**-**	-	**-**
	1.5-1, other	1	**0.8**	3	**2.6**	3	**2.8**	1	**1.4**	3	**4.6**
	1.5-2,10	10	**8.2**	11	**9.4**	11	**10.1**	2	**2.9**	4	**6.2**
	1.5-2, other	2	**1.6**	4	**3.4**	3	**2.8**	3	**4.4**	-	**-**
	1.7,16	-	**-**	4	**3.4**	2	**1.8**	1	**1.4**	-	**-**
	1.7, other	9	**7.4**	2	**1.7**	2	**1.8**	4	**5.8**	1	**1.5**
	1.7-1, 1	-	**-**	2	**1.7**	3	**2.8**	2	**2.9**	2	**3.1**
	1.7-1, other	1	**0.8**	-	**-**	-	**-**	-	**-**	-	**-**
	1.7-2,4	29	**23.8**	28	**23.9**	24	**22.0**	10	**14.5**	6	**9.2**
	1.7-2, other	15	**12.3**	15	**12.8**	7	**6.4**	4	**5.8**	8	**12.3**
	1.12-1, other	2	**1.6**	6	**5.1**	4	**3.7**	1	**1.4**	1	**1.5**
	1.18-1,3	1	**1.8**	1	**0.9**	2	**1.8**	2	**2.9**	1	**1.5**
	1.18-1, other	3	**1.5**	3	**2.6**	2	**1.8**	2	**2.9**	5	**7.7**
	1.19,15-1	-	**-**	-	**-**	-	**-**	1	**1.4**	-	**-**
	1.19, other	2	**1.6**	4	**3.4**	7	**6.4**	2	**2.9**	4	**6.2**
	1.22,14	14	**11.5**	13	**11.1**	20	**18.3**	14	**20.3**	12	**18.5**
	1.22,other	8	**6.6**	7	**6.0**	4	**3.7**	5	**7.3**	8	**12.3**
	Other, 14	2	**1.6**	2	**1.7**	3	**2.8**	2	**2.9**	-	**-**
	Other, 16	9	**7.4**	5	**4.3**	2	**1.8**	3	**4.4**	2	**3.1**
	Subtotal vaccine types	109	**89.3**	110	**94.0**	100	**91.7**	59	**85.5**	57	**87.7**
NVT**	Other	13	**10.7**	7	**6.0**	9	**8.3**	10	**14.5**	8	**12.3**
	Subtotal Non	13		7		9		10		8	
	Total	122	**100.0**	117	**100.0**	109	**100.0**	69	**100.0**	65	**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 and C 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 2012, 19 different FetA variants were observed among serogroup B meningococci. The dominant type is F1-5, accounting for 35% of group B meningococci (figure 4.6 and 4.7; table 4.10). In previous years this FetA type was strongly linked with PorA VR1/VR2 P1.7-2,4 and together to the MLST clonal complex ST41/44. In 2012, the diversity among the meningococcal isolates was much larger; 23 F1-5 types were linked with 17 different PorA types. FetA F1-5 was 4 times linked with PorA VR1/VR2 P1.7-2, 4 (4 in 2012; 8 in 2011; 20 in 2010).

The 3 serogroup C meningococci had the FetA types F1-14 and F3-3.

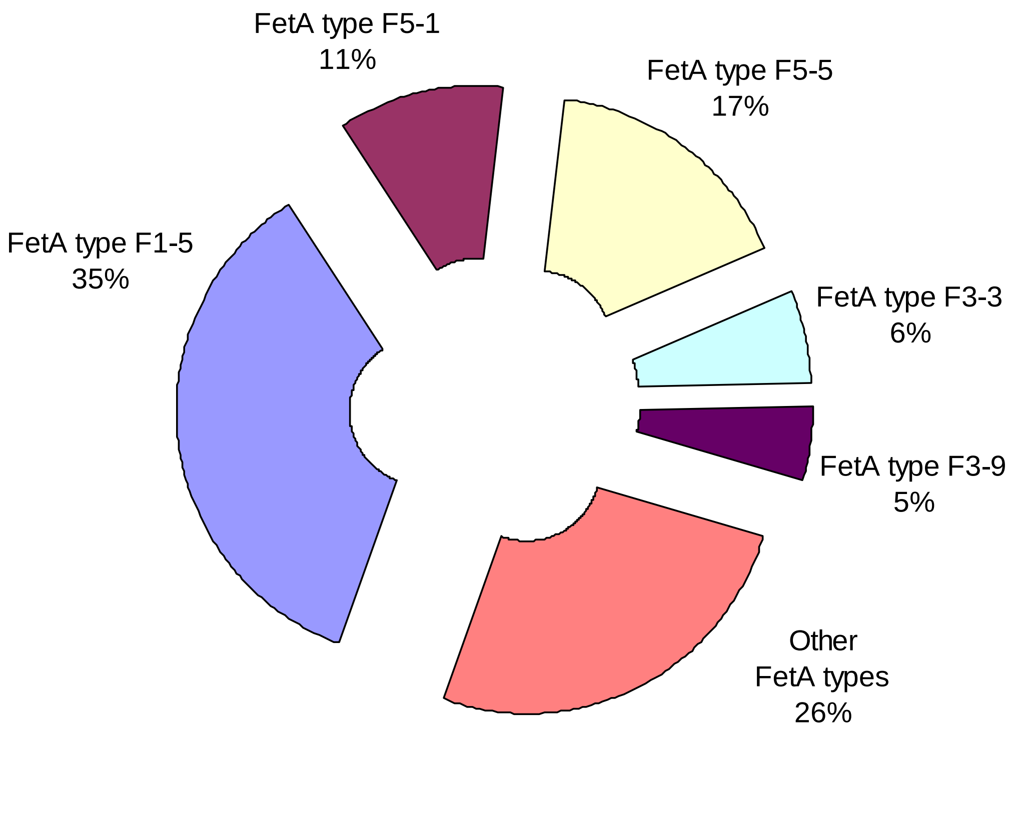

Figure 4.6 *Distribution of group B meningococcal FetA genosubtypes, 2012*

Table 4.10 *N. meningitidis* serogroup B isolates according to FetA genosubtype, 2008-2012

	YEAR									
	2008		**2009**		**2010**		**2011**		**2012**	
FetA type	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
F1-5	52	**42.6**	39	**33.3**	36	**33.0**	17	**24.6**	23	**35.4**
F1-7	10	**8.2**	5	**4.3**	12	**11.0**	4	**5.8**	2	**3.1**
F1-15	1	**0.8**	3	**2.6**	2	**1.8**	1	**1.5**	1	**1.5**
F3-3	9	**7.4**	10	**8.5**	4	**3.7**	6	**8.7**	4	**6.2**
F3-7	-	**-**	2	**1.7**	1	**0.9**	2	**2.9**	-	**-**
F3-9	2	**1.6**	2	**1.7**	1	**0.9**	3	**4.3**	3	**4.6**
F4-1	3	**2.5**	4	**3.4**	4	**3.7**	-	**-**	2	**3.1**
F5-1	17	**13.9**	19	**16.2**	20	**18.3**	8	**11.6**	7	**10.8**
F5-2	3	**2.5**	5	**4.3**	-	**-**	2	**2.9**	-	**-**
F5-5	10	**8.2**	12	**10.3**	13	**12.0**	10	**14.5**	11	**16.9**
F5-8	3	**2.5**	3	**2.6**	2	**1.8**	-	**-**	1	**1.5**
F5-12	2	**1.6**	3	**2.6**	1	**0.9**	2	**2.9**	2	**3.1**
Other	10	**8.2**	10	**8.5**	13	**12.0**	14	**20.3**	9	**13.8**
Total	122	**100.0**	117	**100.0**	109	**100.0**	69	**100.0**	65	**100.0**

In 2012, 31 different VR1/VR2 combinations and 19 different FetA variants were encountered among serogroup B meningococci. Among the dominant FetA type F1-5, accounting for 35% of group B meningococci, 4 were of P1.7-2,4:F1-5 (6% of group B meningococci).
Other frequently found combinations are P1.5-2,10:F5-1 (6%) and P1.22,14:F5-5 (15%) (Figure 4.7).

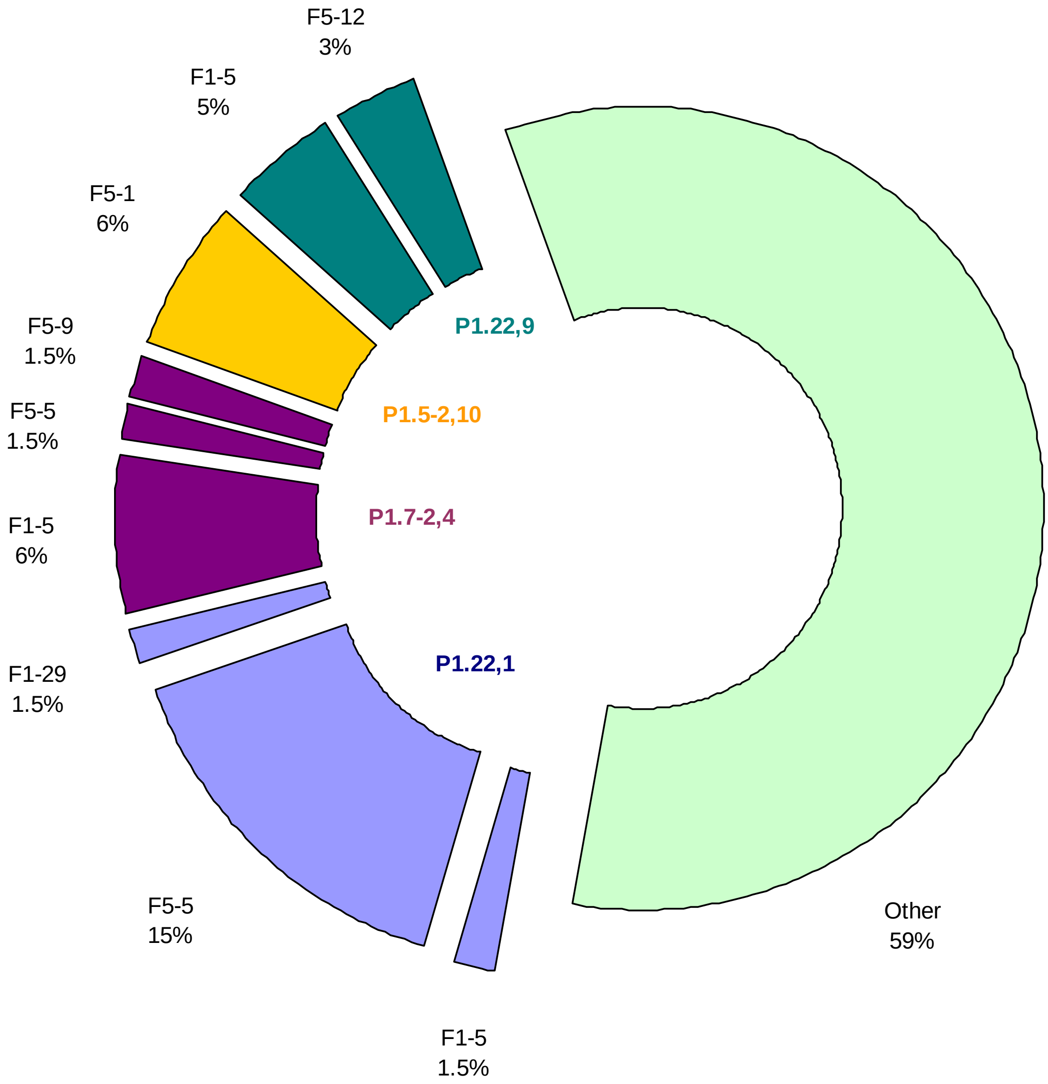

Figure 4.7 *Distribution of group B meningococcal PorA and FetA geno(sub)types, 2012*

5 *HAEMOPHILUS INFLUENZAE*

5.1 General features

In total, 140 *Haemophilus influenzae* isolates were submitted to the Reference Laboratory. This number is comparable to that of the last years (table 2.3, figure 3.3, figure 5.1). Sixteen strains were isolated from CSF (or CSF and blood) (2011: 13; 2010: 17; 2009: 15), and 124 from blood only. Twenty-eight (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 (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.

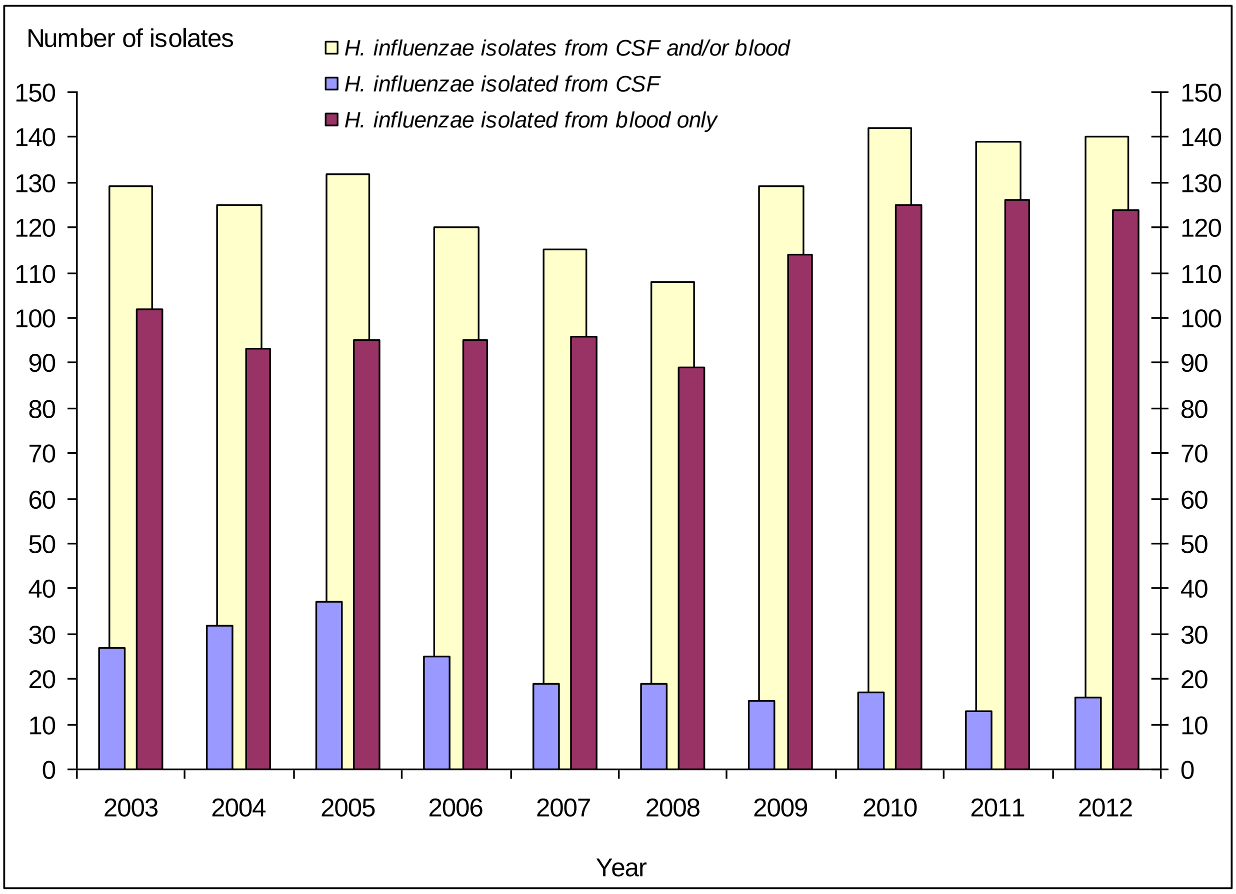

Figure 5.1 *Distribution of H. influenzae, 2003-2012*

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.

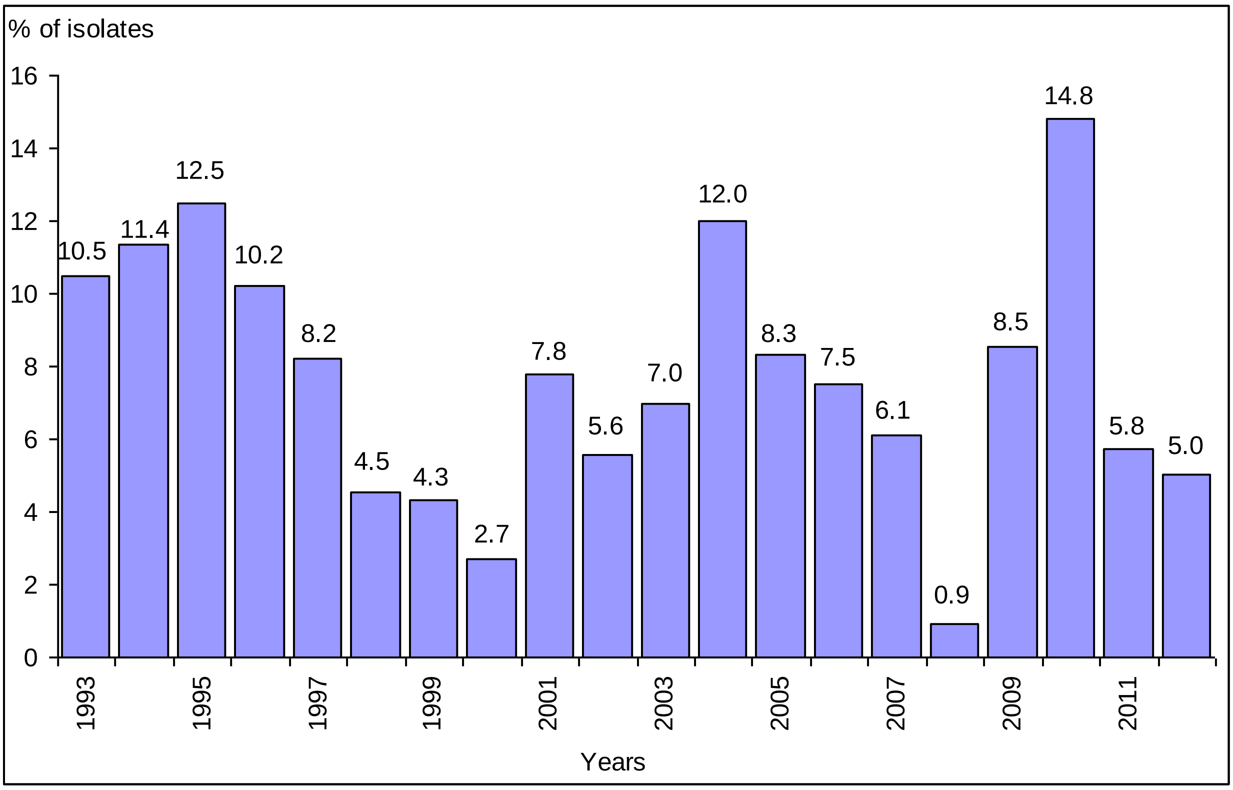

Figure 5.2 *Percentage β-lactamase producing H. influenzae, 1993-2012*

5.3 Serotype and age

Three cases of *H. influenzae* type b invasive disease were observed among children younger than 2 years of age (3 in 2011; 6 in 2010; 8 in 2009 and 4 in 2008) (figure 5.3). In total 101 non-typable *H.influenzae* were received; 12 isolated from CSF (or CSF and blood) and 89 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, 2012

	AGE (MONTH)				AGE (YEAR)					TOTAL	
TYPE	**0**	**1- 11**	**12-23**	**24-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
a	-	-	-	-	-	-	-	-	1	1	**0.7**
b	-	2	1	4	7	2	2	6	11	28	**20.0**
e	-	-	-	-	-	-	-	-	2	2	**1.4**
f	-	-	-	-	-	-	-	-	8	8	**5.7**
n.t.*	6	4	3	3	16	-	2	20	63	101	**72.2**
Total	6	6	4	7	23	2	4	26	85	140	**100.0**
%	**4.3**	**4.3**	**2.8**	**5.0**	**16.4**	**1.4**	**2.9**	**18.6**	**60.7**	**100.0**	

* non-typable

Table 5.2 *H.influenzae* isolates from CSF (or CSF and blood), according to serotype and age, 2012

	AGE (MONTH)				AGE (YEAR)					TOTAL	
TYPE	**0**	**1- 11**	**12-23**	**24-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
b	-	1	-	1	2	1	-	-	1	4	**25.0**
n.t.*	-	2	1	1	4	-	-	3	5	12	**75.0**
Total	-	3	1	2	6	1	-	3	6	16	**100.0**
%	**-**	**18.7**	**6.3**	**12.5**	**37.5**	**6.3**		**18.7**	**37.5**	**100.0**	

* non-typable

Table 5.3 *H. influenzae* isolates from blood only, according to serotype and age, 2012

TYPE	AGE (MONTH) 0	1-11	12-23	24-59	AGE (YEAR) 0-4	5-9	10-19	20-49	≥50	TOTAL Total	%
a	-	-	-	-	-	-	-	-	1	1	**0.8**
b	-	1	1	3	5	1	2	6	10	24	**19.4**
e	-	-	-	-	-	-	-	-	2	2	**1.6**
f	-	-	-	-	-	-	-	-	8	8	**6.4**
n.t.*	6	2	2	2	12	-	2	17	58	89	**71.8**
Total	6	3	3	5	17	1	4	23	79	124	**100**
%	**4.9**	**2.4**	**2.4**	**4.0**	**13.7**	**0.8**	**3.2**	**18.6**	**63.7**	**100.0**	

* non-typable

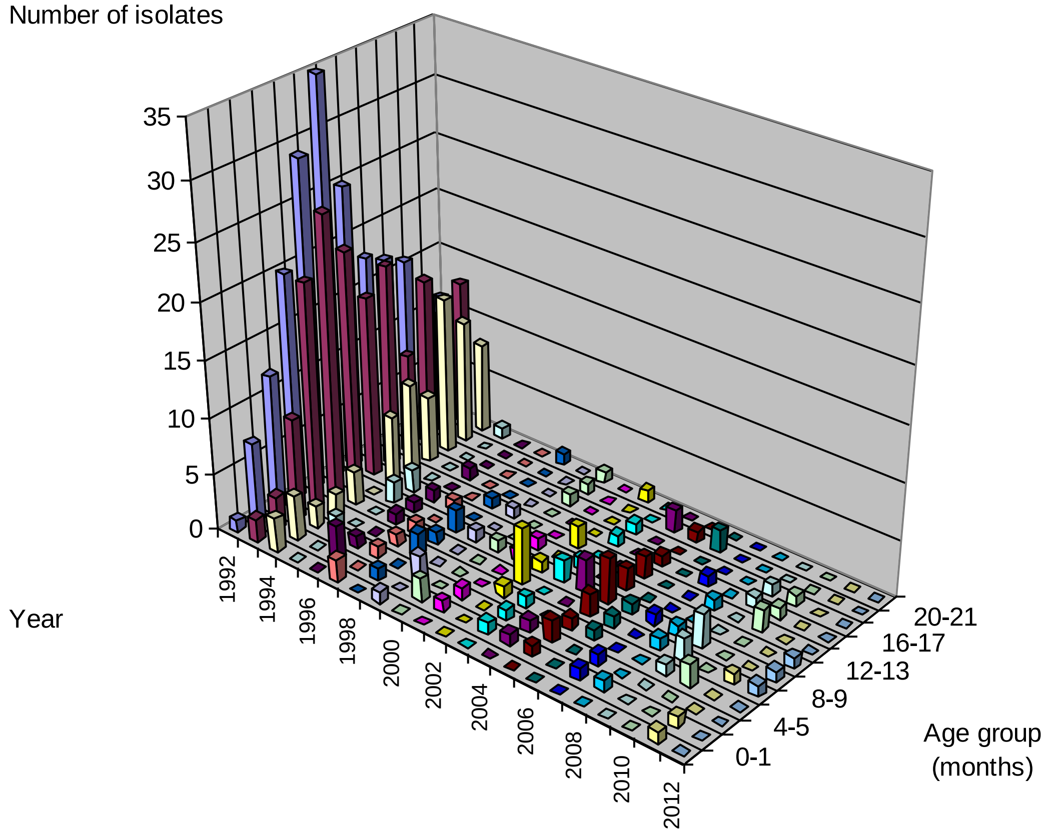

Figure 5.3 *Age distribution of H. influenzae type b invasive disease in the first two years of life, 1992-2012*

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 2012 the proportion of non-typable isolates was 72.7%.

Table 5.4 *H. influenzae* isolates from CSF and/or blood received from 1992 to 2012 according to year and serotype

YEAR	SEROTYPE a	b	d	e	f	n.t.*	TOTAL Total	% n.t.*	CSF (or CSF and blood)	Blood Only
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

* non-typable

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 2012 the number of non-typable isolates from CSF was twice the number of 2011, but similar to that of the years before 2011 (2008: 10; 2009: 9; 2010: 8; 2011: 6; 2012: 12). The number of non-typable *H. influenzae* isolates from blood increased during the period 1992 to 2012 from 15 to 89 (figure 5.4).

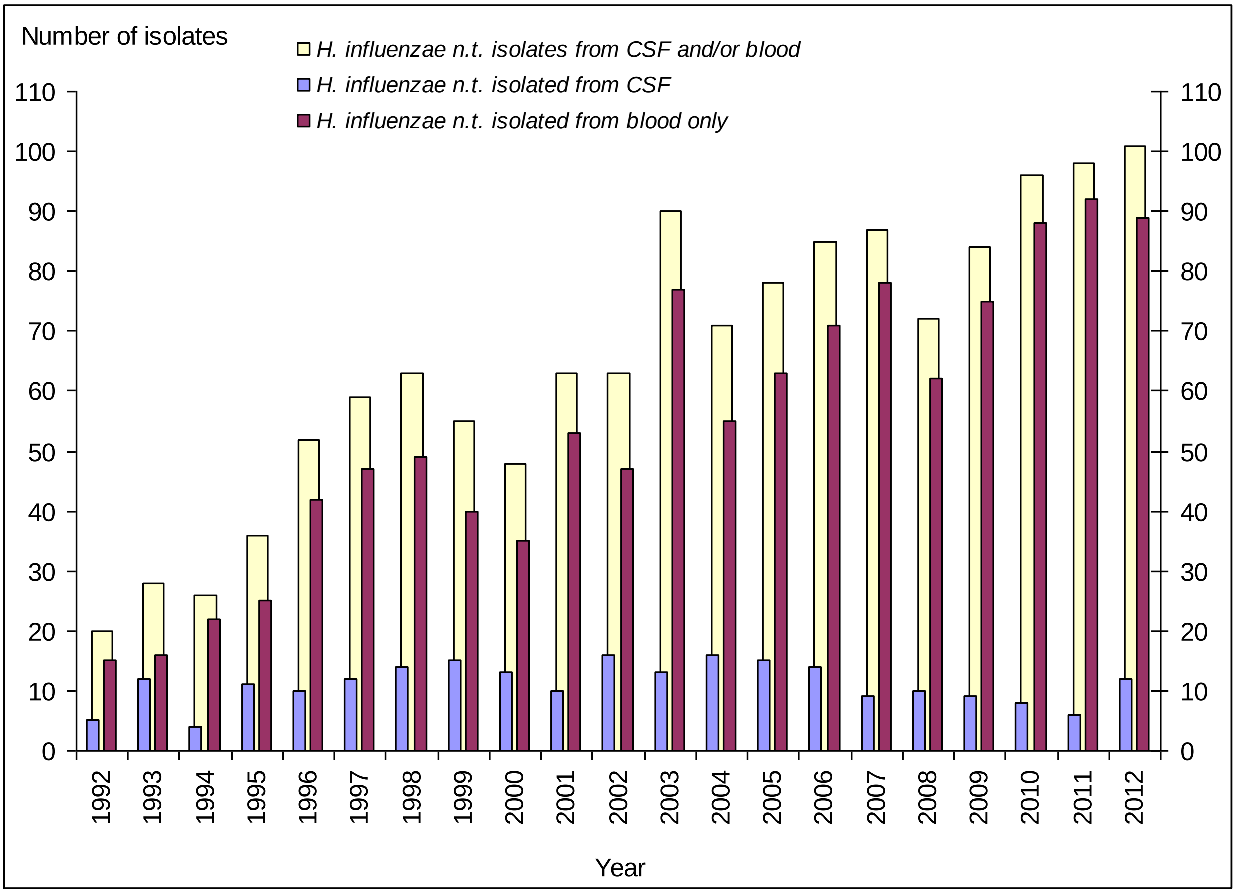

Figure 5.4 *Non-typable H. influenzae isolates from CSF and/or blood received from 1992-2012*

Table 5.5 Non-typable *H. influenzae* isolates from CSF and/or blood received from 2003 to 2012 according to year and biotype.

	Biotype							
YEAR	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**Total**
2003	21	32	27	5	4	1	-	90
2004	11	29	23	2	6	-	-	71
2005	7	48	16	1	5	1	-	78
2006	11	44	25	3	2	-	-	85
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

* non-typable

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

6 *STREPTOCOCCUS PNEUMONIAE*

6.1 General features

The Reference Laboratory received 869 *S. pneumoniae* isolates. Of these, 138 were isolated from CSF or from CSF and blood (table 2.3; figure 6.1). The incidence of pneumococcal meningitis slightly rose since 1990 from 1.0 to 1.6 in 2004; due to vaccination with the hepta-valent polyscaccharide conjugate vaccine it slightly decreased to 0.8 in 2012. 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 731 in 2012. This was due to a change in policy: from 2003 onwards, we asked only nine sentinel laboratories, evenly distributed over the country, to send 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.

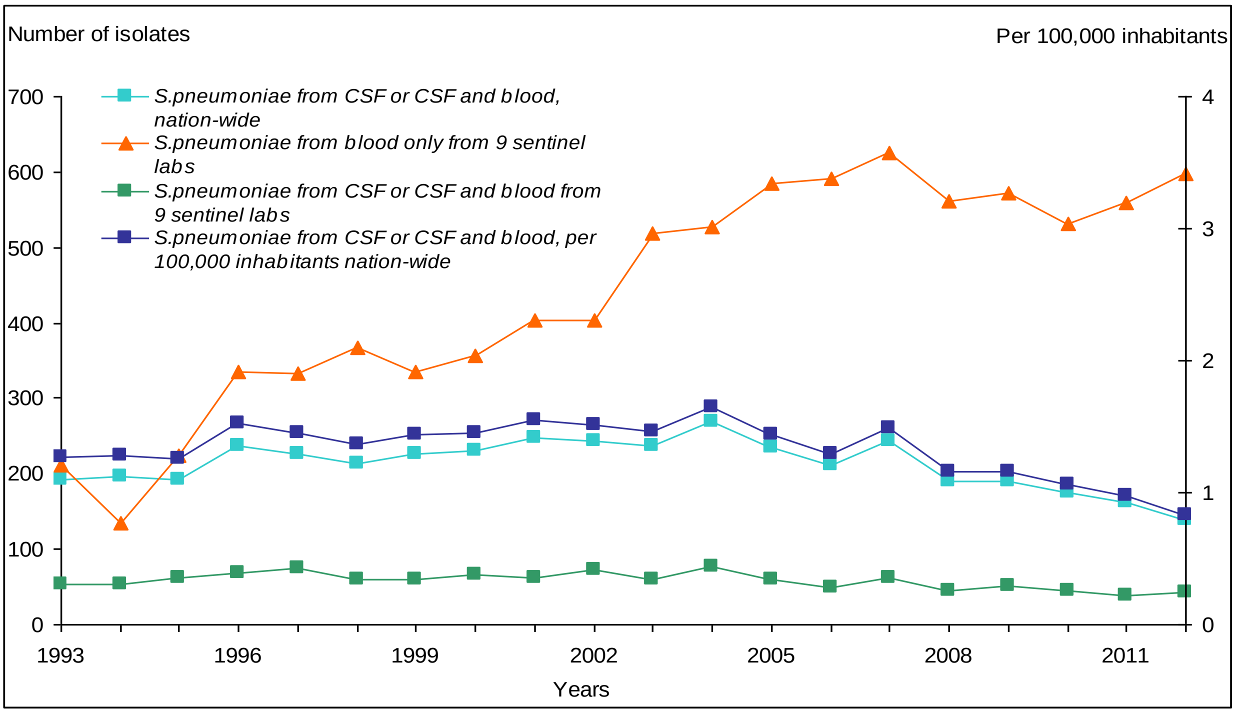

Figure 6.1 *Distribution of S. pneumoniae isolates, 1993-2012*

6.2 *Antibiotic susceptibility*

Among 138 isolates from CSF (or CSF and blood) and 731 isolates from the blood only, 2 (0.3%) and 13 (1.5%), respectively, were intermediately susceptible to penicillin (0.06< MIC≤1.0 mg/L, table 6.1). Two (0.3%) strains were resistant to penicillin (MIC > 1.0 mg/L).

Table 6.1 Susceptibility of *S. pneumoniae* isolates to penicillin, 2012

	Penicillin*				
	MIC ≤ 0.06	**0.06< MIC≤1.0**	**MIC >1.0**	**Total**	**%**
CSF or CSF and blood	135	2	1	138	**15.9**
Blood only	717	13	1	731	**84.1**
Total number of isolates	852	15	2	869	**100.0**
%	**98.0**	**1.7**	**0.3**	**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 children in their first year of life. A second peak was observed in the age group 65 – 75 year (Table 6.4).

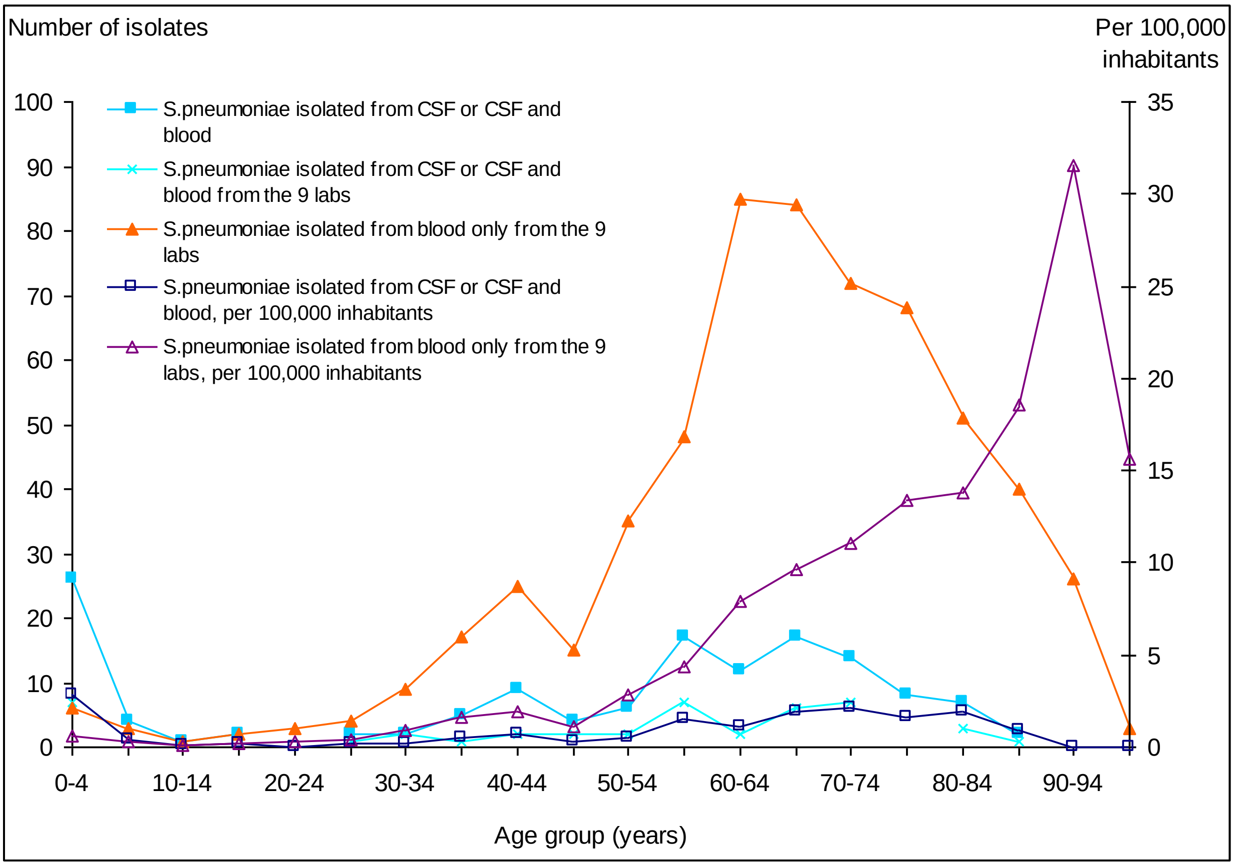

Figure 6.2 *Distribution of S. pneumoniae isolates received in 2012 according to age*

6.3 Distribution according to serotype

The relationship between age and major types of all isolates 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. In the age group 0-4 years the number of cases was lower than that of previous years, mainly because of the lower number of cases due to pneumococci with serotypes included in the hepta-valent conjugate vaccine (table 6.2, 6.3 and figure 12.4). Effect of the 7-valent vaccine can also been seen in table 6.6 and table 6.7. There was an overall reduction of the number of isolates from CSF, due to a reduction of the cases due to pneumococci with serotypes included in the vaccine.

The serotype distributions of CSF (or CSF and blood) and blood isolates only, are shown in table 6.5, 6.6 and 6.7. 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 6 years. From 2006 on those blood isolates were subtyped. 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, in particular 3, 8, 19A , 22F and 33F (Table 6.6 and 6.7).

Table 6.2 *S. pneumoniae* isolates from CSF and/or blood nation-wide, by serotype and age of patients, 2012

	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**	**%**
1	-	1	2	3	-	-	1	3	8	9	13	18	1	56	**6.4**
3	-	1	-	1	1	-	-	1	-	4	21	28	8	64	**7.4**
4	2	-	-	2	-	-	-	-	1	-	5	10	5	23	**2.6**
6	-	-	-	-	-	-	-	-	-	1	4	11	10	26	**3.0**
7	-	1	1	2	2	1	2	3	8	13	46	37	15	129	**14.8**
8	1	1	-	2	-	-	1	1	7	8	27	48	23	117	**13.5**
9	1	1	1	3	-	-	-	-	-	3	11	12	6	35	**4.0**
10	-	6	1	7	-	-	-	-	2	1	7	6	1	24	**2.8**
12	-	1	-	1	-	-	1	-	3	6	11	9	5	36	**4.1**
14	-	-	-	-	1	-	-	-	-	-	4	4	5	14	**1.6**
18	-	-	-	-	2	-	1	-	1	-	2	5	1	12	**1.4**
19	3	1	3	7	-	-	1	1	4	3	25	45	18	104	**12.0**
22	-	2	1	3	1	-	-	-	2	2	14	25	11	58	**6.7**
23	-	-	2	2	-	-	-	1	2	1	6	10	5	27	**3.1**
Others	1	10	6	17	1	1	1	3	4	10	31	44	31	144	**16.6**
Total	8	25	17	50	8	2	8	13	42	61	228	312	145	869	**100.0**
%	**0.9**	**2.9**	**2.0**	**5.8**	**0.9**	**0.2**	**0.9**	**1.5**	**4.9**	**7.0**	**26.2**	**35.9**	**16.7**	**100.0**	

Table 6.3 *S. pneumoniae* isolates from CSF (or CSF and blood) nation-wide, by serotype and age of patients, 2012

	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**	**%**
1	-	1	-	1	-	-	-	-	-	-	-	-	-	1	**0.7**
3	-	-	-	-	1	-	-	-	-	3	4	5	-	13	**9.4**
4	1	-	-	1	-	-	-	-	-	-	1	1	1	4	**2.9**
6	-	-	-	-	-	-	-	-	-	-	1	2	-	3	**2.2**
7	-	-	1	1	1	-	-	-	1	2	5	5	1	16	**11.6**
8	-	1	-	1	-	-	-	-	2	2	-	3	1	9	**6.5**
9	-	1	-	1	-	-	-	-	-	-	3	2	1	7	**5.1**
10	-	4	-	4	-	-	-	-	-	1	4	1	-	10	**7.3**
12	-	1	-	1	-	-	1	-	1	2	2	3	-	10	**7.3**
14	-	-	-	-	1	-	-	-	-	-	-	-	-	1	**0.7**
18	-	-	-	-	1	-	1	-	1	-	-	-	-	3	**2.2**
19	-	1	-	1	-	-	-	-	2	1	2	3	1	10	**7.2**
22	-	2	-	2	-	-	-	-	-	-	3	6	-	11	**8.0**
23	-	-	1	1	-	-	-	1	-	-	4	4	-	10	**7.2**
Others	-	8	4	12	-	1	-	1	-	2	6	4	4	30	**21.7**
Total	1	19	6	26	4	1	2	2	7	13	35	39	9	138	**100.0**
%	**0.7**	**13.8**	**4.3**	**18.8**	**2.9**	**0.7**	**1.5**	**1.5**	**5.1**	**9.4**	**25.4**	**28.3**	**6.4**	**100.0**	

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, 2012

	AGE (YEAR)											
TYPE	**0**	**1-4**	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-64**	**65-79**	**≥80**	**Total**
1	0.56	-	-	-	-	-	-	-	-	-	-	0.01
3	-	-	0.10	-	-	-	-	0.12	0.12	0.25	-	0.08
4	0.56	-	-	-	-	-	-	-	0.03	0.05	0.15	0.02
6	-	-	-	-	-	-	-	-	0.03	0.10	-	0.02
7	-	0.14	0.10	-	-	-	0.05	0.08	0.15	0.25	0.15	0.10
8	0.56	-	-	-	-	-	0.10	0.08	-	0.15	0.15	0.05
9	0.56	-	-	-	-	-	-	-	0.09	0.10	0.15	0.04
10	2.23	-	-	-	-	-	-	0.04	0.12	0.05	-	0.06
12	0.56	-	-	-	0.10	-	0.05	0.08	0.06	0.15	-	0.06
14	-	-	0.10	-	-	-	-	-	-	-	-	0.01
18	-	-	0.10	-	0.10	-	0.05	-	-	-	-	0.02
19	0.56	-	-	-	-	-	0.10	0.04	0.06	0.15	0.15	0.06
22	1.11	-	-	-	-	-	-	-	0.09	0.30	-	0.07
23	-	0.14	-	-	-	0.05	-	-	0.12	0.20	-	0.06
Others	4.45	0.54	-	0.10	-	0.05	-	0.08	0.18	0.20	0.58	0.18
Total	11.13	0.81	0.41	0.10	0.20	0.10	0.34	0.50	1.03	1.92	1.31	0.82

Table 6.5 All *S. pneumoniae* isolates from blood only nation-wide, by serotype and age of patients, 2012

	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**	**%**
1	-	-	2	2	-	-	1	3	8	9	13	18	1	55	**0.7**
3	-	1	-	1	-	-	-	1	-	1	17	23	8	51	**9.4**
4	1	-	-	1	-	-	-	-	1	-	4	9	4	19	**2.9**
6	-	-	-	-	-	-	-	-	-	1	3	9	10	23	**2.2**
7	-	1	-	1	1	1	2	3	7	11	41	32	14	113	**11.6**
8	1	-	-	1	-	-	1	1	5	6	27	45	22	108	**6.5**
9	1	-	1	2	-	-	-	-	-	3	8	10	5	28	**5.1**
10	-	2	1	3	-	-	-	-	2	-	3	5	1	14	**7.3**
12	-	-	-	-	-	-	-	-	2	4	9	6	5	26	**7.3**
14	-	-	-	-	-	-	-	-	-	-	4	4	5	13	**0.7**
18	-	-	-	-	1	-	-	-	-	-	2	5	1	9	**2.2**
19	3	-	3	6	-	-	1	1	2	2	23	42	17	94	**7.2**
22	-	-	1	1	1	-	-	-	2	2	11	19	11	47	**8.0**
23	-	-	1	1	-	-	-	-	2	1	2	6	5	17	**7.2**
Others	1	2	2	5	1	-	1	2	4	8	26	40	27	114	**21.7**
Total	7	6	11	24	4	1	6	11	35	48	193	273	136	731*	**100.0**
%	**1.0**	**0.8**	**1.5**	**3.3**	**0.6**	**0.1**	**0.8**	**1.5**	**4.8**	**6.6**	**26.4**	**37.4**	**18.6**	**100.0**	

* From 2 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) and 65-79 years (1).

Table 6.6 Distribution of pneumococcal CSF isolates according to serotype nation-wide, 2003-2012

Vaccine	TYPE	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
23-valent vaccine / 10-valent vaccine / 7-valent	4	12	15	8	12	5	8	4	3	2	4
	6B	20	28	17	21	12	11	3	-	2	-
	9V	16	10	10	6	10	7	2	2	-	3
	14	26	34	24	28	18	8	3	5	2	1
	18C	11	14	14	19	17	8	6	5	5	2
	19F	18	21	19	15	11	7	10	2	6	4
	23F	17	19	16	16	22	17	5	4	2	1
	Subtotal 7-valent vaccine	*120*	*141*	*108*	*117*	*95*	*66*	*33*	*21*	*19*	*15*
	1	3	7	6	1	8	8	8	3	1	1
	5	1	-	1	1	-	-	-	2	-	3
	7F	22	21	19	18	36	25	25	20	28	16
	Subtotal 10-valent vaccine	*146*	*168*	*134*	*137*	*139*	*99*	*66*	*46*	*48*	*35*
	2	-	-	-	-	-	-	-	-	-	-
	3	14	20	16	20	16	17	24	20	7	13
	8	14	11	15	10	21	9	10	10	17	9
	9N	2	5	10	2	4	1	3	6	7	4
	10A	5	7	6	7	8	7	10	9	7	9
	11A	2	-	5	3	4	2	8	1	5	1
	12F	3	2	1	1	4	2	2	3	7	10
	15B	4	3	3	-	1	4	8	2	3	1
	17F	3	1	-	-	1	-	-	4	3	1
	19A	5	8	7	2	9	8	6	20	16	6
	20	1	2	-	1	-	1	-	1	-	-
	22F	3	4	4	9	2	10	13	14	16	11
	33F	4	2	1	3	5	6	6	7	5	6
	Subtotal 23-valent vaccine	*206*	*234*	*202*	*195*	*214*	*166*	*156*	*143*	*141*	*106*
	6A	9	9	8	5	5	4	6	5	1	1
	6C	-	2	2	1	2	-	-	3	4	2
	10F	1	-	-	-	-	-	-	-	-	-
	10B	-	-	-	-	-	-	-	-	-	1
	12A	1	-	-	-	-	-	-	-	-	-
	13	1	-	1	-	-	-	1	-	-	-
	15A	2	-	-	-	1	1	-	1	1	1
	15C	3	1	2	1	1	3	1	2	-	3
	16F	-	2	4	2	2	2	-	5	4	-
	17A	-	-	-	-	-	-	1	-	-	-
	18F	-	1	1	-	-	-	-	-	-	-
	18A	-	1	-	-	-	-	-	-	-	-
	18B	2	1	1	1	1	1	-	-	-	1
	21	-	1	1	-	1	-	-	-	1	-
	22A	1	1	-	-	1	-	1	1	-	-
	23A	1	3	4	-	3	1	3	3	2	4
	23B	1	2	1	2	2	3	7	5	2	5
	24F	1	-	3	-	3	2	6	1	1	4
	24B	-	-	-	-	-	-	-	-	-	2
	25	-	-	-	-	-	1	-	-	-	-
	27	-	-	-	-	1	2	-	-	-	1
	28A	-	-	1	-	-	-	-	-	1	-
	29	-	1	-	-	-	-	-	-	-	1
	31	1	1	1	2	2	-	1	1	-	1
	33A	1	-	1	-	-	-	-	-	-	-
	34	2	-	-	-	1	1	1	-	1	-
	35F	-	4	-	1	2	2	2	4	1	-
	35B	-	2	1	1	1	-	-	1	-	1
	37	-	-	-	-	-	-	1	-	1	2
	38	1	1	1	1	-	1	3	1	-	2
	Rough (n.t.)	-	1	-	-	-	-	-	-	-	-
	Total	234	268	235	212	243	190	190	176	163	138

Table 6.7 Distribution of *S. pneumoniae* from blood only (from the 9 sentinel laboratories), according to serotype, 2006-2012

Vaccine	TYPE	2006	2007	2008	2009	2010	2011	2012
		Year						
23-valent vaccine / 10-valent vaccine / 7-valent	4	52	54	30	26	17	27	11
	6B	21	26	25	12	8	3	3
	9V	65	53	42	26	21	5	2
	14	86	84	54	34	22	19	12
	18C	12	13	15	15	7	8	4
	19F	19	11	9	10	5	9	3
	23F	29	39	13	12	13	5	3
	Subtotal 7-valent vaccine	*284*	*280*	*188*	*135*	*93*	*85*	*38*
23-valent vaccine / 10-valent vaccine	1	25	75	64	65	53	40	50
	5	-	3	2	6	7	11	8
	7F	75	55	65	86	72	91	92
	Subtotal 10-valent vaccine	*384*	*413*	*319*	*292*	*225*	*227*	*188*
23-valent vaccine	2	-	-	-	-	-	-	-
	3	32	30	31	34	30	36	45
	8	42	47	46	52	60	59	88
	9N	19	13	19	18	19	17	20
	10A	6	4	7	9	9	14	8
	11A	6	16	3	12	12	9	14
	12F	9	5	6	5	13	19	25
	15B	5	1	4	6	7	4	1
	17F	1	3	1	7	4	8	7
	19A	21	25	33	30	57	63	78
	20	2	3	3	3	3	4	-
	22F	19	18	24	24	29	37	41
	33F	10	6	10	11	10	15	22
	Subtotal 23-valent vaccine	*556*	*584*	*506*	*503*	*478*	*503*	*537*
	6A	7	10	18	11	9	2	6
	6C	-	2	1	7	9	7	10
	7C	2	1	-	-	-	-	-
	9A	-	-	-	-	-	-	1
	10F	-	-	1	-	-	-	-
	11B	-	1	-	-	-	-	-
	12A	-	-	-	-	-	-	-
	13	-	-	-	-	-	1	-
	15A	-	1	1	1	-	2	7
	15C	-	1	2	2	1	2	1
	16F	6	6	9	8	10	7	6
	17A	-	-	-	-	-	2	-
	18F	-	-	-	-	-	-	-
	18A	-	1	-	1	1	1	-
	18B	-	1	-	-	-	-	1
	21	-	-	-	-	-	-	-
	22A	3	2	1	-	1	1	-
	23A	2	6	3	9	7	2	6
	23B	1	1	3	6	3	9	3
	24F	1	1	7	-	2	3	2
	25F	1	-	1	-	-	-	-
	27	-	-	1	1	-	1	-
	28A	-	-	-	-	-	-	-
	29	-	-	-	-	-	-	1
	31	1	1	3	1	4	2	6
	33A	-	-	-	-	-	-	1
	34	1	1	-	1	1	-	1
	35F	2	1	2	4	5	6	5
	35A	-	-	-	-	-	-	1
	35B	3	-	-	4	-	3	1
	37	-	1	-	-	1	-	-
	38	3	2	3	5	-	3	-
	Rough (n.t.)	2	-	-	-	-	2	-
	Total	591	624	562	564	532	559	596

Table 6.8 Distribution of *S. pneumoniae* isolates from CSF (or CSF and blood) nation-wide, by serotype and age of patients, 2012.

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	1	1	4	**2.9**
	6B	-	-	-	-	-	-	-	-	-	-	-	**-**
	9V	-	-	-	-	-	-	-	1	2	-	3	**2.2**
	14	-	1	-	-	-	-	-	-	-	-	1	**0.7**
	18C	-	1	-	1	-	-	-	-	-	-	2	**1.5**
	19F	-	-	-	-	-	1	-	1	1	1	4	**2.9**
	23F	-	-	-	-	-	-	-	-	1	-	1	**0.7**
	Subtotal 7-valent vaccine	1	2	-	1	-	1	-	3	5	2	15	**10.9**
23-valent vaccine / 10-valent vaccine	1	1	-	-	-	-	-	-	-	-	-	1	**0.7**
	5	1	-	1	-	-	-	-	-	1	-	3	**2.2**
	7F	1	1	-	-	-	1	2	5	5	1	16	**11.6**
	Subtotal 10-valent vaccine	4	3	1	1	-	2	2	8	11	3	35	**25.4**
23-valent vaccine	2	-	-	-	-	-	-	-	-	-	-	-	**-**
	3	-	1	-	-	-	-	3	4	5	-	13	**9.4**
	8	1	-	-	-	-	2	2	-	3	1	9	**6.5**
	9N	1	-	-	-	-	-	-	2	-	1	4	**2.9**
	10A	4	-	-	-	-	-	-	4	1	-	9	**6.5**
	11A	-	-	-	-	-	-	-	-	-	1	1	**0.7**
	12F	1	-	-	1	-	1	2	2	3	-	10	**7.2**
	15B	-	-	-	-	-	-	-	1	-	-	1	**0.7**
	17F	-	-	-	-	-	-	-	-	1	-	1	**0.7**
	19A	1	-	-	-	-	1	1	1	2	-	6	**4.4**
	20	-	-	-	-	-	-	-	-	-	-	-	**-**
	22F	2	-	-	-	-	-	-	3	6	-	11	**8.0**
	33F	5	-	-	-	-	-	-	-	-	1	6	**4.4**
	Subtotal 23-valent vaccine	19	4	1	2	-	6	10	25	32	7	106	**76.8**
	Other	7	-	-	-	2	1	3	10	7	2	32	**23.2**
	Total	26	4	1	2	2	7	13	35	39	9	138	**100.0**

7 *ESCHERICHIA COLI*

The Reference Laboratory received 13 *Escherichia coli* strains, 5 isolated from CSF (or CSF and blood) and 8 from blood only (table 7.1, 7.2 and 7.3). The number of *E. coli* isolates from CSF was lower than that in the last years (figure 7.1). Seventy percent of the cases of *E. coli* meningitis occurred in the first month of life.

Interestingly, the types O4 and O55 are prevalent among non-K1 isolates, while the types O non typable, O1, O2, O6, O16, O18 and O45 are more often found among K1 isolates.

Since 2012 all isolates were tested for the H – type. Almost 55% of all K1 isolates were of type H7 (table 7.4)

Table 7.1 Serotypes of *E. coli* isolates from CSF and/or blood, by age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
Non K1	1	-	-	1	-	-	-	1	2	**15**
K1	8	2	-	10	-	-	1	-	11	**85**
Total	9	2	-	11	-	-	1	1	13	**100**
%	**70**	**15**	**-**	**85**	**-**	**-**	**7.5**	**7.5**	**100**	

Table 7.2 Serotypes of *E. coli* isolates from CSF (or CSF and blood), by age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
Non K1	-	-	-	-	-	-	-	1	1	**20**
K1	1	2	-	3	-	-	1	-	4	**80**
Total	1	2	-	3	-	-	1	1	5	**100**
%	**20**	**40**	**-**	**60**	**-**	**-**	**20**	**20**	**100**	

Table 7.3 Serotypes of *E. coli* isolates from blood only by age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
Non K1	1	-	-	1	-	-	-	-	1	**12**
K1	7	-	-	7	-	-	-	-	7	**88**
Total	8	-	-	8	-	-	-	-	8	**100**
%	**100**	**-**	**-**	**100**	**-**	**-**	**-**	**-**	**100**	

Table 7.4 H-type versus K-type of *E. coli* isolates from CSF and/or blood, 2012

TYPE	K1	Non K1	Total
H1	1	-	1
H5	1	-	1
H7	6	-	6
H19	-	1	1
H31	-	1	1
H34	1	1	1
H-rough	2	-	2
Total	11	2	13
%	**85**	**15**	**100**

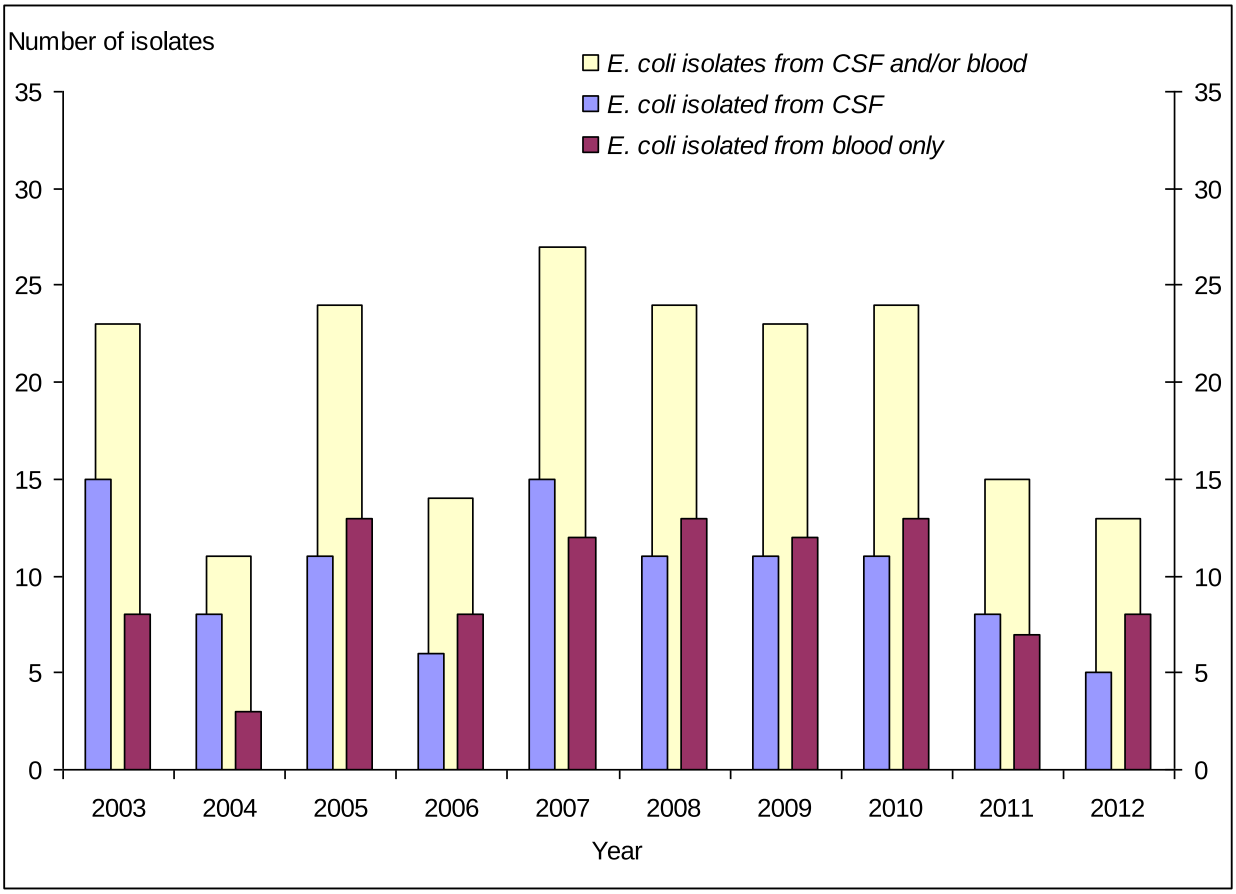

Figure 7.1 *Distribution of E. coli, 2003-2012*

8 *STREPTOCOCCUS AGALACTIAE* – (group B)

In 2012 the number of *Streptococcus agalactiae* isolates received by the Reference Laboratory increased to 80 (2011: 63; 2010: 70, figure 8.1). Twenty-three *S. agalactiae* isolates were from CSF (or CSF and blood) and 57 from blood only (table 8.1, 8.2 and 8.3). Seventy-three percent of the cases occurred in the first month of life. Serotype III was the most prevalent (table 8.1).

Table 8.1 Serotypes of *S. agalactiae* isolates from CSF and/or blood, by age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
Ia	18	6	-	24	-	-	-	1	25	**31**
Ib	4	-		4	-	-	-	-	4	**5**
II	5	-	-	5	-	-	-	-	5	**6**
III	27	10	-	37	-	-	1	3	41	**52**
IV	1	-	-	1	-	-	-	-	1	**1**
V	3	-	-	3	-	-	-	1	4	**5**
Total	58	16	-	74	-	-	1	5	80	**100**
%	**73**	**20**	**-**	**93**	**-**	**-**	**1**	**6**	**100**	

Table 8.2 Serotypes of *S. agalactiae* isolates from CSF (or CSF and blood), by age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
Ia	3	1	-	4	-	-	-	1	5	**22**
Ib	1	-	-	1	-	-	-	-	1	**4**
II	-	-	-	-	-	-	-	-	-	**-**
III	10	4	-	14	-	-	-	2	16	**70**
IV	-	-	-	-	-	-	-	-	-	**-**
V	-	-	-	-	-	-	-	1	1	**4**
Total	14	5	-	19	-	-	-	4	23	**100**
%	**61**	**22**	**-**	**83**	**-**	**-**	**-**	**17**	**100**	

Table 8.3 Serotypes of *S. agalactiae* isolates from blood only, by age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
Ia	15	5	-	20	-	-	-	-	20	**35**
Ib	3	-	-	3	-	-	-	-	3	**5**
II	5	-	-	5	-	-	-	-	5	**9**
III	17	6	-	23	-	-	1	1	25	**44**
IV	1	-	-	1	-	-	-	-	1	**2**
V	3	-	-	3	-	-	-	-	3	**5**
Total	38	11	-	55	-	-	1	1	57	**100**
%	**77**	**19**	**-**	**96**	**-**	**-**	**2**	**2**	**100**	

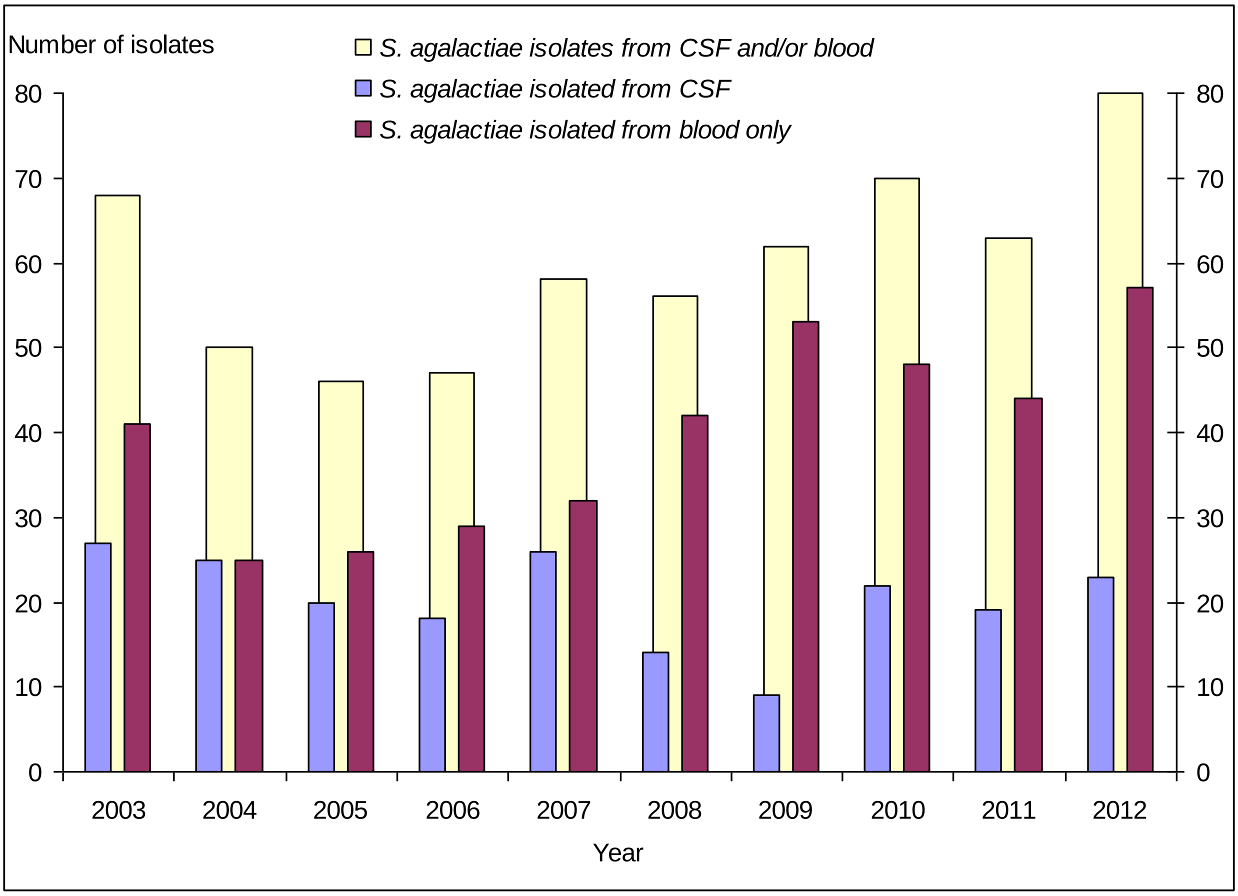

Figure 8.1 *Distribution of S. agalactiae, 2003-2012*

9 *LISTERIA MONOCYTOGENES*

Fifty-nine strains of *Listeria monocytogenes* were submitted to the Reference Laboratory. Nine isolates were from CSF (or CSF and blood) and 50 from blood only (figure 9.1). Most cases (88%) occurred among persons older than 50 years. In 2012 (as in previous years) serotypes 1/2a and 4b were most prevalent (table 9.1).

Table 9.1 *L. monocytogenes* isolates from CSF and/or blood, by type and age of patients, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
1/2a	-	-	-	-	-	-	-	17	17	**29**
1/2b	1	-	-	1	-	-	3	8	12	**20**
1/2c	-	-	-	-	-	-	1	4	5	**8**
3b	-	-	-	-	-	-	-	1	1	**2**
4b	-	-	1	1	-	-	1	22	24	**41**
Total	1	-	1	2	-	-	5	52	59	**100**
%	**2**	**-**	**2**	**4**	**-**	**-**	**8**	**88**	**100**	

Table 9.2 *L. monocytogenes* isolates from CSF (or CSF and blood), by type and age, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
1/2a	-	-	-	-	-	-	-	1	1	**11**
1/2b	-	-	-	-	-	-	-	1	1	**11**
1/2c	-	-	-	-	-	-	-	1	1	**11**
3b	-	-	-	-	-	-	-	-	-	**-**
4b	-	-	1	1	-	-	-	5	6	**67**
Total	-	-	1	1	-	-	-	8	9	**100**
%	**-**	**-**	**11**	**11**	**-**	**-**	**-**	**89**	**100**	

Table 9.3 *L. monocytogenes* isolates from blood only, by serotype and age, 2012

	AGE (MONTH)			AGE (YEAR)					TOTAL	
TYPE	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**	**%**
1/2a	-	-	-	-	-	-	-	16	16	**32**
1/2b	1	-	-	1	-	-	3	7	11	**22**
1/2c	-	-	-	-	-	-	1	3	4	**8**
3b	-	-	-	-	-	-	-	1	1	**2**
4b	-	-	-	-	-	-	1	17	18	**36**
Total	1	-	-	1	-	-	5	44	50	**100**
%	**2**	**-**	**-**	**2**	**-**	**-**	**10**	**88**	**100**	

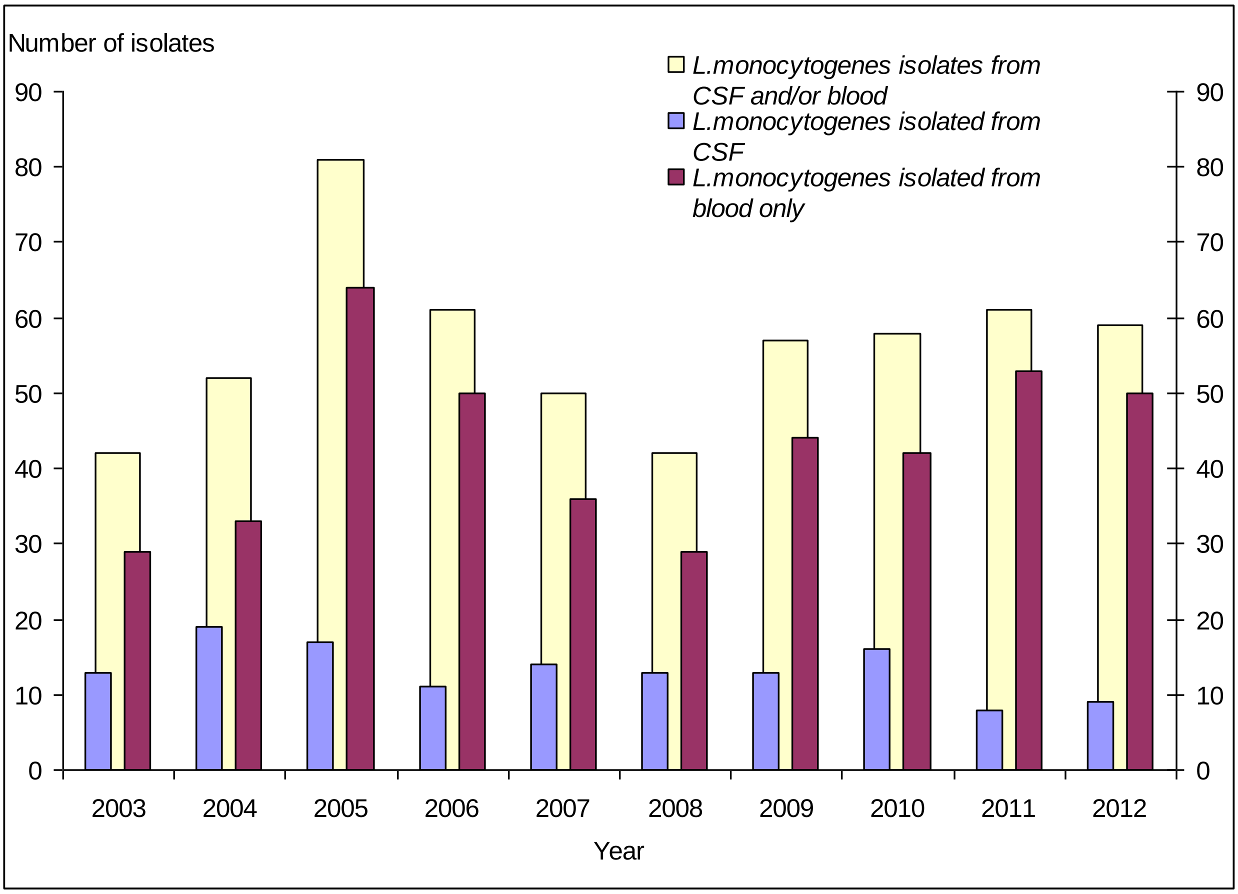

Figure 9.1 *Distribution of L. monocytogenes, 2003-2012*

10 *STREPTOCOCCUS PYOGENES*

Twelve *Streptococcus pyogenes* isolates were submitted to the Reference Laboratory, 3 isolated from CSF (or CSF and blood) and 9 from blood only.

The number of isolates from CSF decreased sinde 2010 (figure 10.1).

Table 10.1 *S. pyogenes* isolates from CSF and/or blood received in 2012 according to source of isolation and age

	AGE (MONTH)			AGE (YEAR)					TOTAL
Source	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-19**	**20-49**	**≥50**	**Total**
CSF	-	-	2	2	-	-	1	-	3
Blood	-	-	-	-	-	-	1	8	9
Total	-	-	2	2	-	-	2	8	12

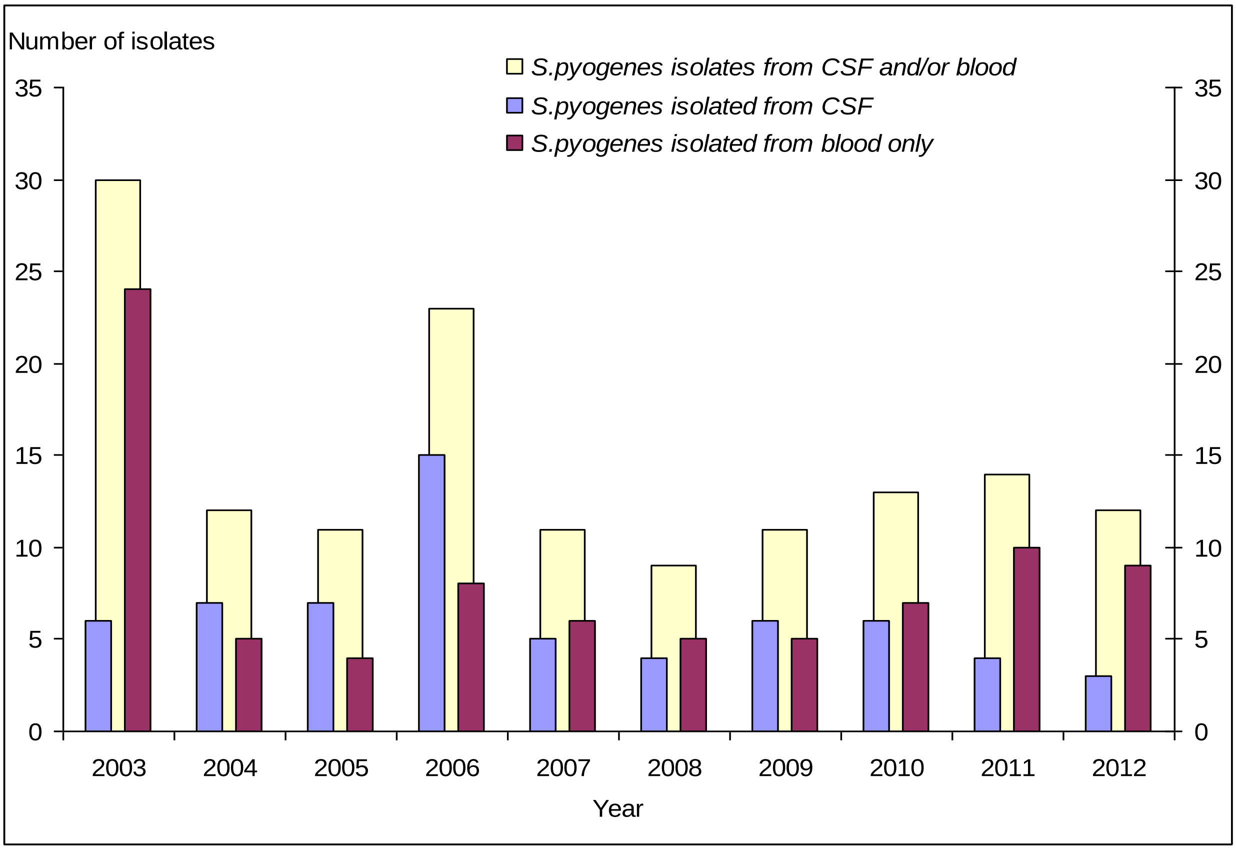

Figure 10.1 *Distribution of S. pyogenes, 2003-2012*

11 ANTIGEN AND DNA DETECTION IN CSF AND SERUM

The Reference Laboratory received 217 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 217 specimens, 36 (17 %) were positive by agglutination or PCR. Seventeen (16 CSF and 1 other) were positive for *N. meningitidis* and 16 (15 CSF and 1 other) were positive for *S. pneumoniae*.

Thus, in 2012, PCR-positive, culture-negative CSF samples accounted for 16 % of cases of meningococcal meningitis registered in the data base of the Reference Laboratory. For *S. pneumoniae*, this percentage was 10%.

Table 11.1 CSF and serum samples, tested for antigens or DNA, 2012

	NUMBER OF SPECIMENS			
	CSF (or CSF and serum)	**Sera**	**Other**	**Total**
Antigen of				
C. neoformans	2	1	-	3
H. influenzae type b	-	-	-	-
DNA of				
N. meningitidis	2	-	1	3
N. meningitidis group B	14	-	-	14
S. pneumoniae	15 *	-	1	16
Subtotal	**33**	**1**	**2**	**36**
Antigen and PCR target negative	**172**	**6**	**3**	**181**
Total	**205**	**7**	**5**	**217**

* From 2 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 2012, 2 cases of meningococcal disease (2.5% of all cases, table 4.4) were due to serogroup C meningococci (2011; 3.3%; 2010: 4.5%; 2009: 6.5%; 2002: 36%). Both patients were not vaccinated because of age. One was to young, the other to old for vaccination. (figure 12.1). This indicates that the vaccination programme is successful.

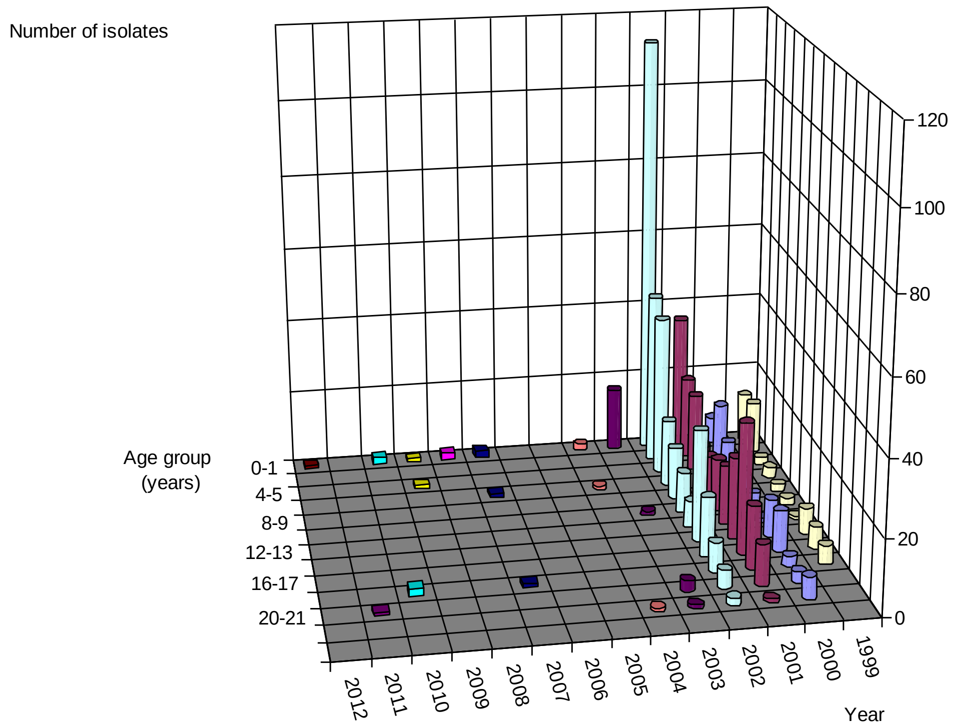

Figure 12.1 *Age distribution of N.meningitidis serogroup C invasive disease in the first 24 years of life, 1999-2012*

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 (88%; table 4.9) of serogroup B meningococcal disease and 70 (86%) of all cases of meningococcal disease.

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 2012, the number of invasive isolates of *H. influenzae* type b, received from patients that should have been vaccinated (<19 years of age) was similar those in previous years (2012: 11; 2011: 7; 2010: 10 and 2009: 10) (figure 12.2 and 12.3). Of those 11 patients, nine had received all doses of the vaccine. Two patients were not vaccinated.

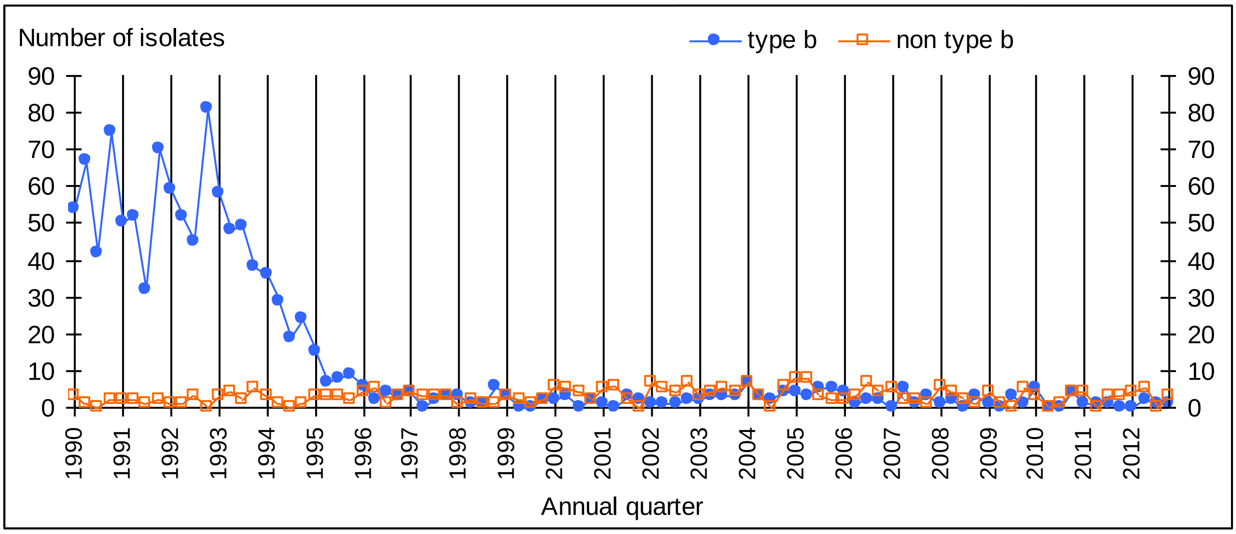

Figure 12.2 *The distribution of H. influenzae type b and non-type b meningitis cases according to annual quarter, 1990 –2012*

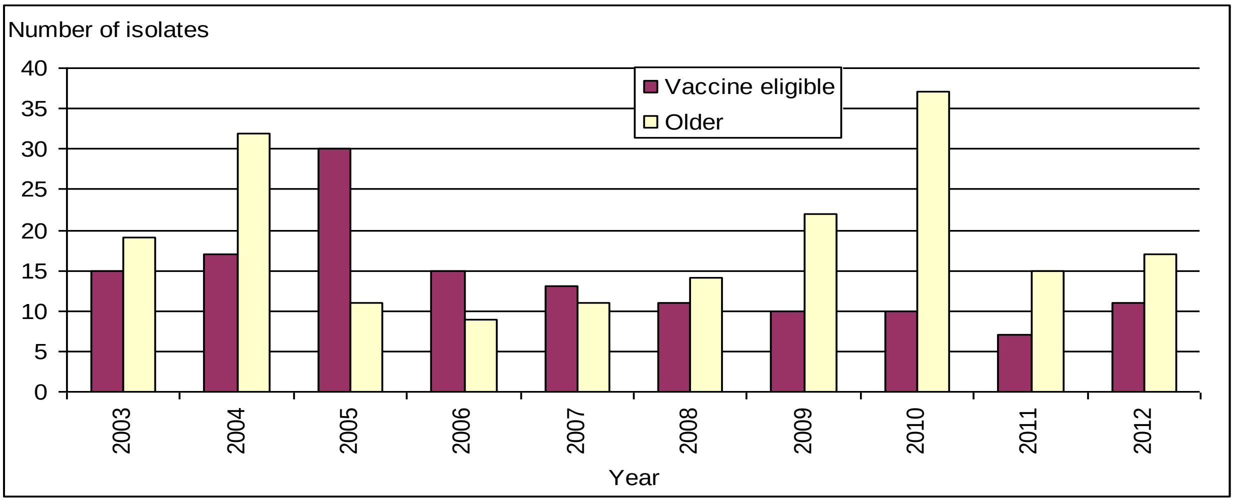

Figure 12.3 *The distribution of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 2003 –2012*

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. Eleven percent of the CSF isolates were of a serotype covered by this hepta-valent conjugate polysaccharide vaccine, while 25% of the isolates were covered by the 10-valent vaccine (table 6.6). The proportion of CSF isolates with a PVC7 serotype from te last year was lower than in previous years (2012:11% ; 2011:12%; 2010: 12%; 2009: 18%; 2008: 35%; 2007: 42%; 2006: 56%; 2005: 46%; 2004: 53%; 2003: 52%), most probably as a result of the vaccination. There was only one case of invasive disease due to *S. pneumoniae* with a vaccine serotype (18C) among a vaccinated (with PVC7) child. Three patiënts with an invasive disease due to *S. pneumoniae* with a vaccine serotype (1, 4 and 5) were not vaccinated because of age. There were two patients with an invasive disease due to *S. pneumoniae* with a (PVC10) vaccine serotype (7F). Those two children were vaccinated with the PVC7 vaccine. 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 (type 6A, which is not included in the vaccine but cross-reacts with 6B, accounted for another 0.7%) (table 6.6) (2011: 87%; 2010: 84%; 2009: 85%; 2008: 89%; 2007: 90%).

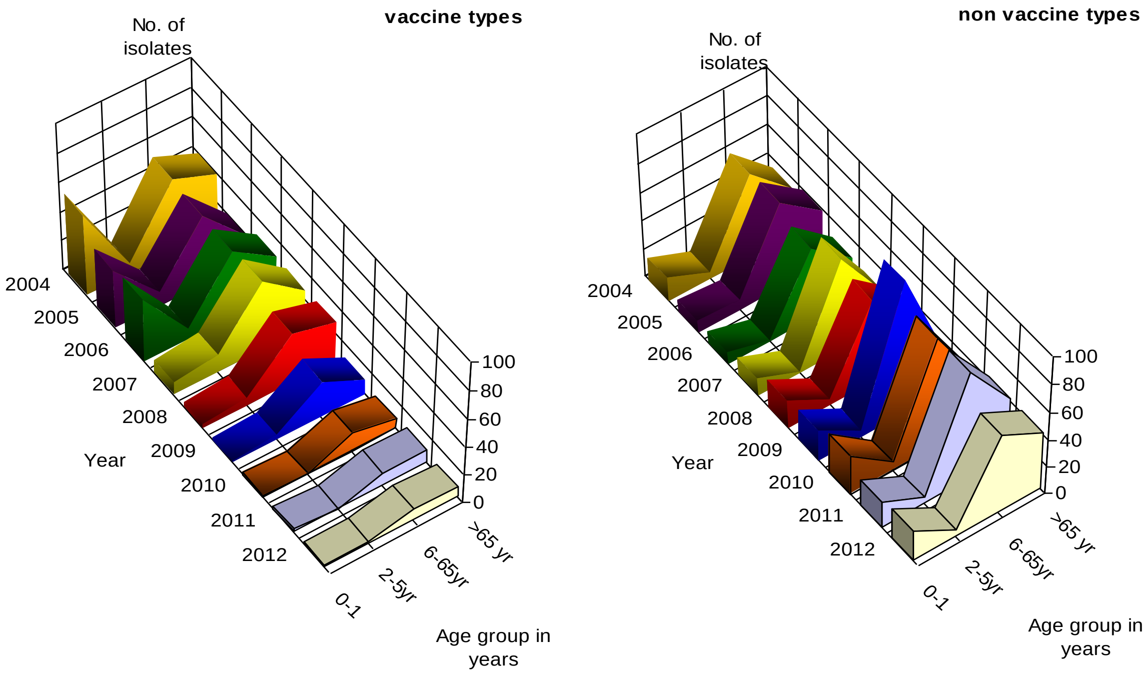

Figure 12.4 *The age distribution of S.pneumoniae invasive disease due to pneumococci of serotypes included in the hepta-valent conjugated polysaccharide vaccine, 2004-2012. Left: vaccine types. Right: types not included in this vaccine*

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