NETHERLANDS REFERENCE LABORATORY FOR BACTERIAL MENINGITIS

BACTERIAL MENINGITIS IN THE NETHERLANDS

ANNUAL REPORT 2014

AMC
Academic Medical Center
University of Amsterdam

RIVM
National Institute of Public Health
and
Environmental Protection

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Netherlands Reference Laboratory for Bacterial Meningitis (AMC/RIVM)
Bacterial meningitis in the Netherlands; annual report 2014
Amsterdam: University of Amsterdam, 2015

CONTENTS

1 INTRODUCTION

This is the **43th** 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 67,750 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, 2015

dr. A. van der Ende PhD, biochemist
dr. L. Spanjaard MD PhD, 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 2014, the Reference Laboratory received isolates from CSF and / or blood from 1243 patients, and 38 specimens of CSF and/or serum wich were positive in PCR (or crypt. agglutination.) (table 2.1/table 11.1). Of these patients, 318 were confirmed cases of bacterial meningitis.

Table 2.1

	Number of specimens
Isolate (CSF and/or blood)	1243
CSF samples (without isolate)	137
Sera (and other fluid, without isolate)	18
Total	**1398**

In 2013, 57 clinical microbiology laboratories submitted isolates to the Reference Laboratory.

Table 2.2 shows the 1243 isolates according to species and to laboratory where cases were diagnosed.

Table 2.2 Number of isolates from CSF and/or blood received in 2014, according to laboratory

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Alkmaar	MCA lab. Med. Microbiologie	5	6	6	2	-	-	-	1	-	-	-	-	**20**
Amersfoort	Meander Medisch Centrum	1	3	1	-	-	-	-	-	-	-	-	-	**5**
Amsterdam	Academisch Medisch Centrum AMC	1	2	23	6	6	-	1	2	1	1	13	-	**56**
	Academisch ziekenhuis VU	-	-	2	-	-	-	-	-	-	-	-	-	**2**
	Onze Lieve Vrouwe Gasthuis	3	4	17	1	3	1	1	1	-	1	-	-	**32**
Apeldoorn	Gelre Ziekenhuizen	3	2	3	-	-	1	-	-	-	-	-	-	**9**
Arnhem	Rijnstate	1	8	55	-	-	2	-	-	-	1	-	-	**67**
Breda	Amphia Ziekenhuis	2	3	6	3	1	3	-	-	-	1	1	-	**20**
Capelle ad IJssel	IJsselland Ziekenhuis	-	1	5	-	2	1	-	-	-	-	-	-	**9**
Delft	Reinier de Graaf groep	2	-	1	-	2	2	-	1	-	-	-	-	**8**
Den Bosch	Regionaal laboratorium Den Bosch	4	7	2	-	2	5	-	1	-	-	-	-	**21**
Den Haag	Haga Ziekenhuis, loc. Leyenburg	1	2	3	-	2	2	-	2	-	-	1	-	**13**
	MA Haaglanden, loc Westeinde	-	-		-	-	1							**1**
Deventer	Deventer Ziekenhuis	3	1	2	-	1	-	-	-	-	-	-	-	**7**
Doetinchem	Slingeland Ziekenhuis	-	2	3	-	1	-	-	1	-	-	-	-	**7**
Dordrecht	RLM Dordrecht / Gorinchem	1	2	56	2	12	-	1	-	-	-	1	-	**75**
Ede	Gelderse Vallei	1	5	3	-	-	-	-	2	-	-	1	-	**12**
Etten Leur	Stichting Huisartsen laboratorium	1	-		-	-	-	-	-	-	-	-	-	**1**
Gouda	Groene Hart Ziekenhuis	-	1	6	-	1	4	-	1	-	-	-	-	**13**
Groningen	Certe, Lab. v. Infectieziekten	5	16	7	1	3	7	-	-	-	-	-	-	**39**
	UMCG	-	2	4	2	-	-	-	-	-	1	-	-	**9**
Haarlem	St. Streeklab voor de Volksgezondheid	4	6	72	-	1	2	-	-	-	-	-	-	**85**
Harderwijk	St. Jansdal Ziekenhuis	2	1	1	-	-	-	-	-	-	-	-	-	**4**
Heerlen	Atrium Medisch Centrum	1	2	32	-	2	-	-	-	-	-	-	-	**37**
Hengelo	LabMicTa	3	5	84	-	4	1	-	1	-	-	1	1	**100**
Hilversum	Centraal Bact. Ser. Lab.	3	1	3	-	1	2	-	-	-	-	-	-	**10**
Hoorn	Westfries gasthuis	-	1	2	-	-	-	-	-	-	-	-	-	**3**
Leeuwarden	Izore, centrum infectieziekten Friesland	-	7	99	1	4	2	-	2	-	-	1	-	**116**
Leiden	Diakonessen Ziekenhuis	-	2	2	-	-	-		-	-	-	-	-	**4**
	LUMC, KML, Lab.voor Bacteriologie	2	4	6	1	-	3	-	2	-	1	4	-	**23**
Leiderdorp	Rijnland Ziekenhuis	-	-	2	-	-	-	1	-	-	-	-	-	**3**
Maastricht	Acad. Ziekenhuis Maastricht	2	-	2	-	-	-	-	-	-	-	-	-	**4**
Nieuwegein	St. Antonius Ziekenhuis	2	14	58	-	2	4	-	-	-	-	-	-	**80**
Nijmegen	Canisius Wilhelmina Ziekenhuis	2	3	2	-	-	1	-	-	-	-	-	-	**8**
	UMC St. Radboud	5	2	3	4	2	1	-	-	-	-	-	-	**17**

Location	Laboratory	Nm	Hi	Sp	Ec	Sag	Lm	Spy	Sau	Cns	Cn	Ot	nv	Total
Roermond	St. Laurentius Ziekenhuis	1	1		-	-	-	-	-	-	-	-	-	**2**
Roosendaal	St. Fransiscus Ziekenhuis	-	3	2	-	-	1	-	-	-	-	-	-	**6**
Rotterdam	Erasmus MC Med. Microbiologie	1	10	15	-	-	1	2	4	-	-	-	-	**33**
	Ikazia Ziekenhuis	1	1	3	-	-	-	-	-	-	-	-	-	**5**
	Maasstad Ziekenhuis	-	3	3	-	2	1	-	-	1	-	-	-	**10**
	St.Franciscus Gasthuis	1	1	1	-	2	1	-	1	-	-	-	-	**7**
Sittard	Orbis Medisch Centrum	-	1	1	-	-	-	-	-	-	-	-	-	**2**
Tilburg	Streeklab. Tilburg	1	9	70	-	1	2	-	-	-	-	2	-	**85**
Utrecht	Diakonessenhuis	-	1		-	2	7	-	-	-	-	-	-	**10**
	UMC Med. Microbiologie	-	10	16	4	3	3	-	-	-	1	-	-	**37**
Veldhoven	PAMM, Lab. Med. Microbiologie	5	4	67	4	5	4	2	*	-	-	-	-	**91**
Venlo	Vie Curie medisch centrum	-	-	4	-	-	1	-	1	-	-	-	-	**6**
Vlissingen	Lab. Voor Med. Microbiologie & Imm.	-	-	3	-	-	-	-	-	-	-	-	-	**3**
Weert	St. Jans gasthuis	-	1	1	-	-	-	-	-	-	-	-	-	**2**
Woerden	Zuwe Hofpoort Ziekenhuis	-	-	3	-	1	2	-	-	-	-	-	-	**6**
Zaandam	CoMicro ZMC	-	-	3	1	2	1	-	-	-	-	-	-	**7**
Zwolle	Isala Klinieken LMMI	3	1	4	-	1	1	-	-	-	-	1	-	**11**
Total		73	161	769	32	71	70	8	23	2	7	26	1	**1243**

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 2010 through 2014 is presented in table 2.3. The number of total isolates decreased from 1304 in 2012 to 1243 in 2014. The number of cases of meningococcal disease was lower compared to the number of cases in previous years (2014: 73; 2013: 111; 2012: 81). 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 142 in 2014. The number of *Listeria monocytogenes* was high in 2005 (81), most likely due to an intensified surveillance performed by the RIVM. In 2014, 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 2010 – 2014

Species	2010			2011			2012			2013			2014		
	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total	CSF	Blood	Total
N. meningitidis	53	79	132	37	53	90	41	40	81	39	72	111	31	42	73
H. influenzae	17	125	142	13	126	139	16	124	140	16	144	160	21	140	161
S. pneumoniae	176	658	834	163	753	916	138	731	869	138	768	906	142	627	769
E. coli	11	13	24	8	7	15	5	8	13	8	18	26	8	24	32
S. agalactiae	22	48	70	19	44	63	23	57	80	20	52	72	23	48	71
L. monocytogenes	16	42	58	8	53	61	9	50	59	6	46	52	19	51	70
S. pyogenes	6	7	13	4	10	14	3	9	12	9	22	31	2	6	8
S. aureus	8	0	8	4	0	4	7	1	8	5	18	23	13	10	23
Coag.neg.Staph.	6	0	6	0	0	0	6	0	6	6	0	6	2	0	2
C. neoformans	6	6	12	5	2	7	9	1	10	6	2	8	4	3	7
others	19	4	23	14	6	20	17	8	25	14	6	20	22	4	26
non viable	0	1	1	0	2	2	0	1	1	0	1	1	0	1	1
Total	**340**	**983**	**1323**	**275**	**1056**	**1331**	**274**	**1030**	**1304**	**267**	**1149**	**1416**	**287**	**956**	**1243**

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 2010 to 2014 is shown in table 2.4. The incidence of *H. influenzae* infection was 55% lower than in the years before vaccination was introduced (2.1 in 1992; 0.96 in 2014). 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, 2010 - 2014

Species	2010	2011	2012	2013	2014
N. meningitidis	0.80	0.54	0.48	0.66	0.43
H. influenzae	0.86	0.83	0.84	0.95	0.96
S. pneumoniae	5.03	5.50	5.19	5.40	4.57
E. coli	0.14	0.09	0.08	0.15	0.19
S. agalactiae	0.42	0.38	0.48	0.43	0.42
L. monocytogenes	0.35	0.37	0.35	0.31	0.42
S. pyogenes	0.08	0.08	0.07	0.18	0.05
S. aureus	0.05	0.02	0.05	0.14	0.14
Coag. neg. Staph.	0.04	0.00	0.04	0.04	0.01
C. neoformans	0.07	0.04	0.06	0.05	0.04
others	0.14	0.12	0.15	0.12	0.15
non viable	0.00	0.01	0.01	0.01	0.01
Total	**7.98**	**7.99**	**7.79**	**8.44**	**7.39**

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 2014, according to bacterial species and specimen source

Species		CSF or CSF and blood	Blood only	Total	%
Neisseria meningitidis		31	42	73	**5.9**
Haemophilus influenzae		21	140 [1]	161	**13.0**
Streptococcus pneumoniae		142 [2]	627	769	**61.9**
Escherichia coli		8	24	32	**2.6**
Streptococcus agalactiae		23	48 [3]	71	**5.7**
Listeria monocytogenes		19	51	70	**5.6**
Streptococcus pyogenes		2	6	8	**0.6**
Staphylococcus aureus		13	10 [4]	23	**1.8**
Coagulase-negative staphylococcus		2 [5]	-	2	**0.2**
Cryptococcus neoformans		4	3	7	**0.6**
Others total		22	4	26	**2.0**
Others	*Pseudomonas aeruginosa*	*3*	*0*	*3*	
	Klebsiella pneumonia	*2*	*0*	*2*	
	Enterobacter cloacae	*1*	*0*	*1*	
	Enterobacter ludwigii	*1*	*0*	*1*	
	Streptococcus anginosus	*1*	*0*	*1*	
	Streptococcus cristatus	*1*	*0*	*1*	
	Streptococcus pseudopneumoniae	*1*	*2*	*3*	
	Streptococcus dysgalactiae ssp equisimilis	*1*	*0*	*1*	
	Streptococcus gallolyticus ssp pasteurianus	*0*	*1*	*1*	
	Streptococcus mitis	*2*	*0*	*2*	
	Streptococcus oralis	*2*	*0*	*2*	
	Streptococcus sanguinis	*1*	*0*	*1*	
	Haemophilus parainfluenzae	*0*	*1*	*1*	
	Enterococcus faecium	*2*	*0*	*2*	
	Corynebacterium striatum	*1*	*0*	*1*	
	Serratia marcescens	*1*	*0*	*1*	
	Propionibacterium acnes	*1*	*0*	*1*	
	Rothia mucilaginosa	*1*	*0*	*1*	
Non viable		0	1 [6]	1	**0.1**
Total **%**		**287** **23.1**	**956** **76.9**	**1243**	**100.0**

1 In three patient *Streptococcus pneumoniae* and *Haemophilus influenzae* were isolated from the blood

2 In one patient in Januaryi a *Streptococcus pneumoniae* (35F) was iolated from CSF and blood and a *Haemophilus influenzae* from CSF, in April a *Streptococcus pneumonia (19F) was isolated from CSF* and blood. In September a *Streptococcus pneumonia (1-) was isolated from CSF.*

3 In one patient *Streptococcus mitis was isolated from CSF while Streptococcus agalactiae was isolated from the blood*

4 In one patient *Staphylococcus warneri* was isolated from CSF while *Staphylococcus aureus* was isolated from the blood

5 From 2 Coagulase-negative staphylococcus one was *Staphylococcus epidermidis* and the other a *Staphylococcus warneri*

6 Non viable, in one patient a *Streptococcus pneumoniae* was isolated from the blood. The isolate was non viable.

3 BACTERIAL MENINGITIS - general data

In 2014, the Reference Laboratory received CSF isolates of 287 patients. Furthermore, 31 culture-negative CSF samples appeared to be positive by antigen detection or PCR (Table 11.1). Of these CSF samples, 12 were positive for *N. meningitidis,* 18 for *S. pneumoniae* and 1 for *C. neoformans*. Including these cases, the total number of patients with confirmed meningitis amounted to 318. The proportion of meningococcal and pneumococcal meningitis among these patients was 14% and 50%, respectively (Figure 3.1). Of 12 meningococcal and 18 pneumococcal meningitis cases identified by a PCR positive CSF, 2 had a meningococciae isolate from the blood and 4 a pneumococciae isolate from the blood, respectively.

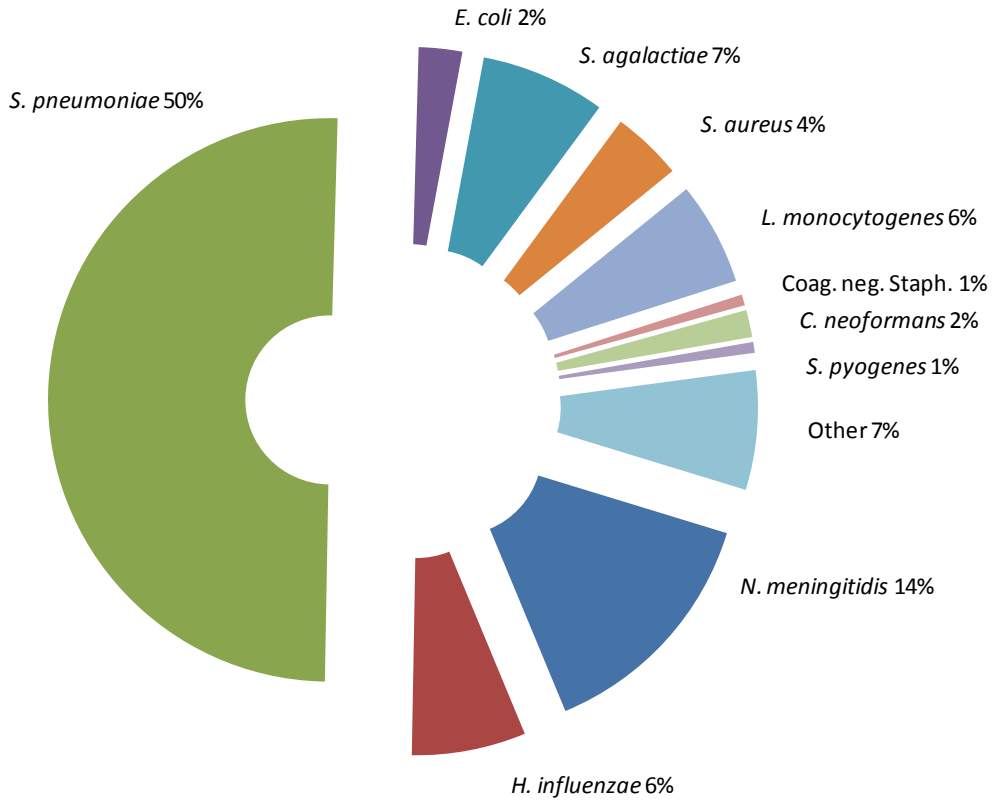

Figure 3.1 *Proportional distribution of CSF isolates and CSF positive samples, 2014*

Figure 3.2 shows the annual total number of bacterial isolates from CSF during the period 2004-2013. 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 3.0 and 1.6 during the period 2005-2014 (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 0.12 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 31 cases (incidence of 0.18) in 2014, 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 2014 due to vaccination against pneumococci introduced in June 2006 in the National Immunisation Programme.

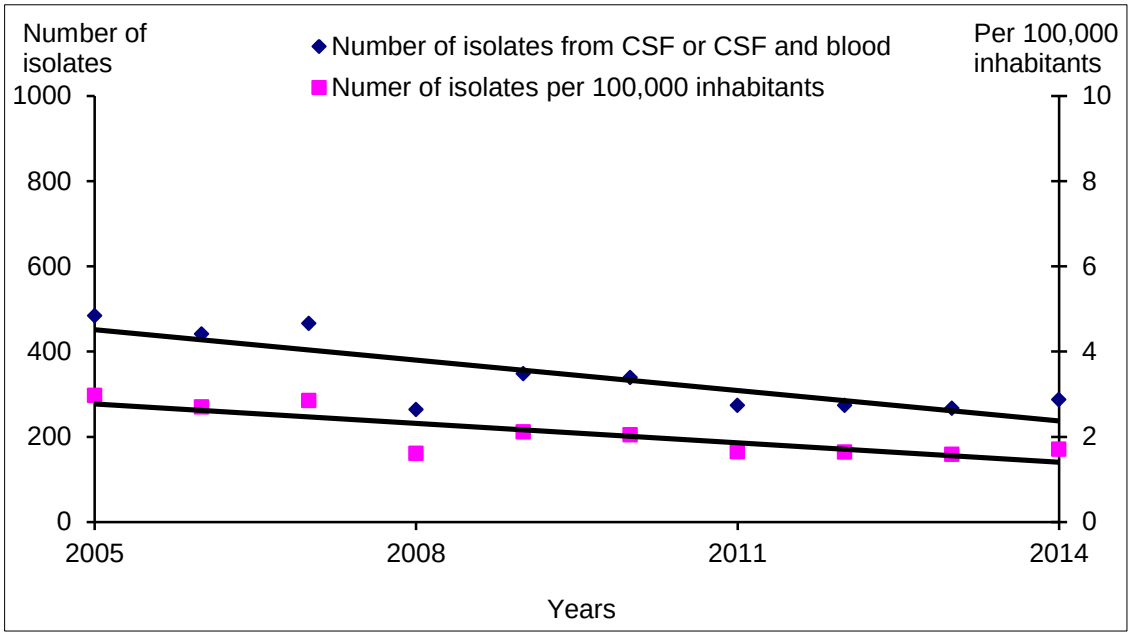

Figure 3.2 *Isolates from CSF, 2005-2014*

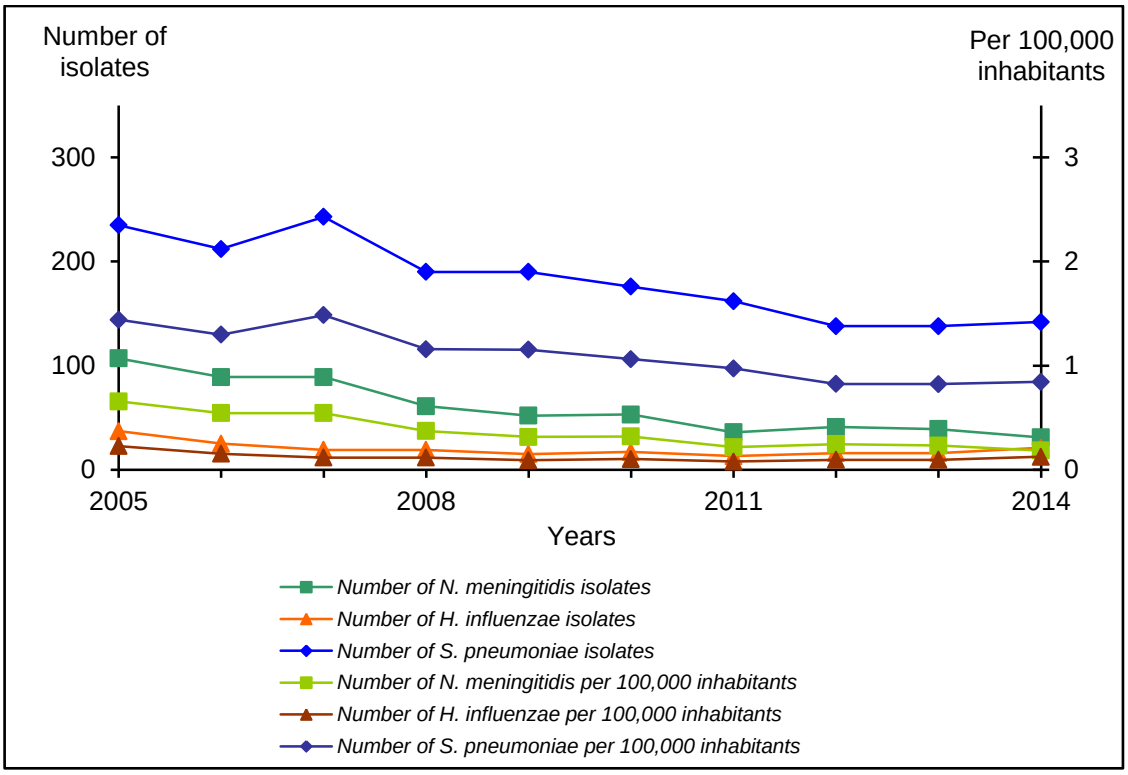

Figure 3.3 *Meningococcal, Haemophilus and pneumococcal meningitis, 2005-2014*

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, 2014

	ANNUAL QUARTER					
SPECIES	**First**	**Second**	**Third**	**Fourth**	**Total**	**%**
N. meningitidis	10	4	8	9	**31**	**10.8**
H. influenzae	9	4	1	7	**21**	**7.3**
S. pneumoniae	51	34	25	32	**142**	**49.5**
E. coli	2	2	3	1	**8**	**2.8**
S. agalactiae	2	7	3	11	**23**	**8.0**
L. monocytogenes	4	2	6	7	**19**	**6.6**
S. pyogenes	1	1	0	0	**2**	**0.7**
S. aureus	3	1	4	5	**13**	**4.5**
Coag.neg.Staph.	1	1	0	0	**2**	**0.7**
C. neoformans	2	2	0	0	**4**	**1.4**
Others	6	8	2	6	**22**	**7.7**
non viable	0	0	0	0	**0**	**-**
Total	**91**	**66**	**52**	**78**	**287**	**100.0**
%	***31.7***	***23.0***	***18.1***	***27.2***	***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 68% 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 50%). 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 2014: 8).

Table 3.2 Isolates from CSF grouped according to patients' age, 2014

	AGE (MONTHS)			AGE (YEARS)										TOTAL	
Group	**0**	**1-11**	**12-59**	**0-4**	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-64**	**65-79**	**≥80**	**Total**	**%**
N. meningitidis	0	7	9	16	2	0	7	1	1	0	3	1	0	**31**	**10.8**
H. influenzae	0	6	2	8	1	0	0	1	1	3	3	4	0	**21**	**7.3**
S. pneumoniae	0	10	6	16	7	2	0	2	5	14	49	40	7	**142**	**49.5**
E. coli	4	4	0	8	0	0	0	0	0	0	0	0	0	**8**	**2.8**
S. agalactiae	17	4	0	21	0	0	0	0	0	1	1	0	0	**23**	**8.0**
L. monocytogenes	0	0	0	0	0	1	0	0	0	0	4	12	2	**19**	**6.6**
S. pyogenes	0	0	1	1	0	0	0	0	0	0	1	0	0	**2**	**0.7**
S. aureus	1	1	1	3	0	0	0	1	3	0	1	3	2	**13**	**4.5**
Coag.neg.Staph.	0	1	0	1	0	0	0	0	0	0	1	0	0	**2**	**0.7**
C. neoformans	0	0	0	0	0	0	0	0	1	1	2	0	0	**4**	**1.4**
Others	3	1	2	6	0	0	2	4	1	2	5	2	0	**22**	**7.7**
non viable	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**-**
Total	**25**	**34**	**21**	**80**	**10**	**3**	**9**	**9**	**12**	**21**	**70**	**62**	**11**	**287**	***100***
%	***8.7***	***11.9***	***7.3***	***27.9***	***3.5***	***1.1***	***3.1***	***3.1***	***4.2***	***7.3***	***24.4***	***21.6***	***3.8***	***100***	

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, 2014

SPECIES	AGE (YEARS)											Total
	0	1-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	
N. meningitidis	4.09	1.24	0.21	0.00	0.70	0.05	0.05	0.00	0.09	0.05	0.00	**0.18**
H. influenzae	3.51	0.28	0.11	0.00	0.00	0.05	0.05	0.12	0.09	0.18	0.00	**0.12**
S. pneumoniae	5.85	0.83	0.75	0.20	0.00	0.10	0.25	0.56	1.43	1.82	0.98	**0.84**
E. coli	4.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.05**
S. agalactiae	12.28	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.03	0.00	0.00	**0.14**
L. monocytogenes	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.12	0.54	0.28	**0.11**
S. pyogenes	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	**0.01**
S. aureus	1.17	0.14	0.00	0.00	0.00	0.05	0.15	0.00	0.03	0.14	0.28	**0.08**
Coag.neg.Staph.	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	**0.01**
C. neoformans	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.04	0.06	0.00	0.00	**0.02**
Others	2.34	0.28	0.00	0.00	0.20	0.19	0.05	0.08	0.15	0.09	0.00	**0.13**
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	**34.51**	**2.89**	**1.07**	**0.30**	**0.90**	**0.43**	**0.59**	**0.83**	**2.04**	**2.82**	**1.53**	**1.71**

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 1.0 and 1.7. The M/F ratio among patients infected with *C. neoformans* or *S. aureus* were 3.0 and 5.0 respectivily. The overall M/F ratio was 1.3.

Table 3.4 Isolates from CSF according to patients' gender, 2014

SPECIES	M	F	M/F – ratio	Sex not known	Total	%
N. meningitidis	16	15	**1.1**	0	**31**	***10.8***
H. influenzae	15	6	**2.5**	0	**21**	***7.3***
S. pneumoniae	77	65	**1.2**	0	**142**	***49.5***
E. coli	5	3	**1.7**	0	**8**	***2.8***
S. agalactiae	14	9	**1.6**	0	**23**	***8.0***
L. monocytogenes	10	9	**1.1**	0	**19**	***6.6***
S. pyogenes	1	1	**1.0**	0	**2**	***0.7***
S. aureus	10	2	**5.0**	1	**13**	***4.5***
Coag.neg.Staph.	-	2	**0.0**	0	**2**	***0.7***
C. neoformans	3	1	**3.0**	0	**4**	***1.4***
Others	8	13	**0.6**	1	**22**	***7.7***
non viable	0	0	**0.0**	0	**0**	**-**
Total	**34.51**	**2.89**		**0.30**	**287**	***100.0***
%	***55.4***	***43.9***		***0.7***	***100.0***	

4 NEISSERIA MENINGITIDIS

4.1 General features

In 2014, the Reference Laboratory received 73 *Neisseria meningitidis* isolates, of which 32 were isolated from CSF (or CSF and blood) (39 in 2013) and 42 from blood only (72 in 2013). This means that 58% 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).

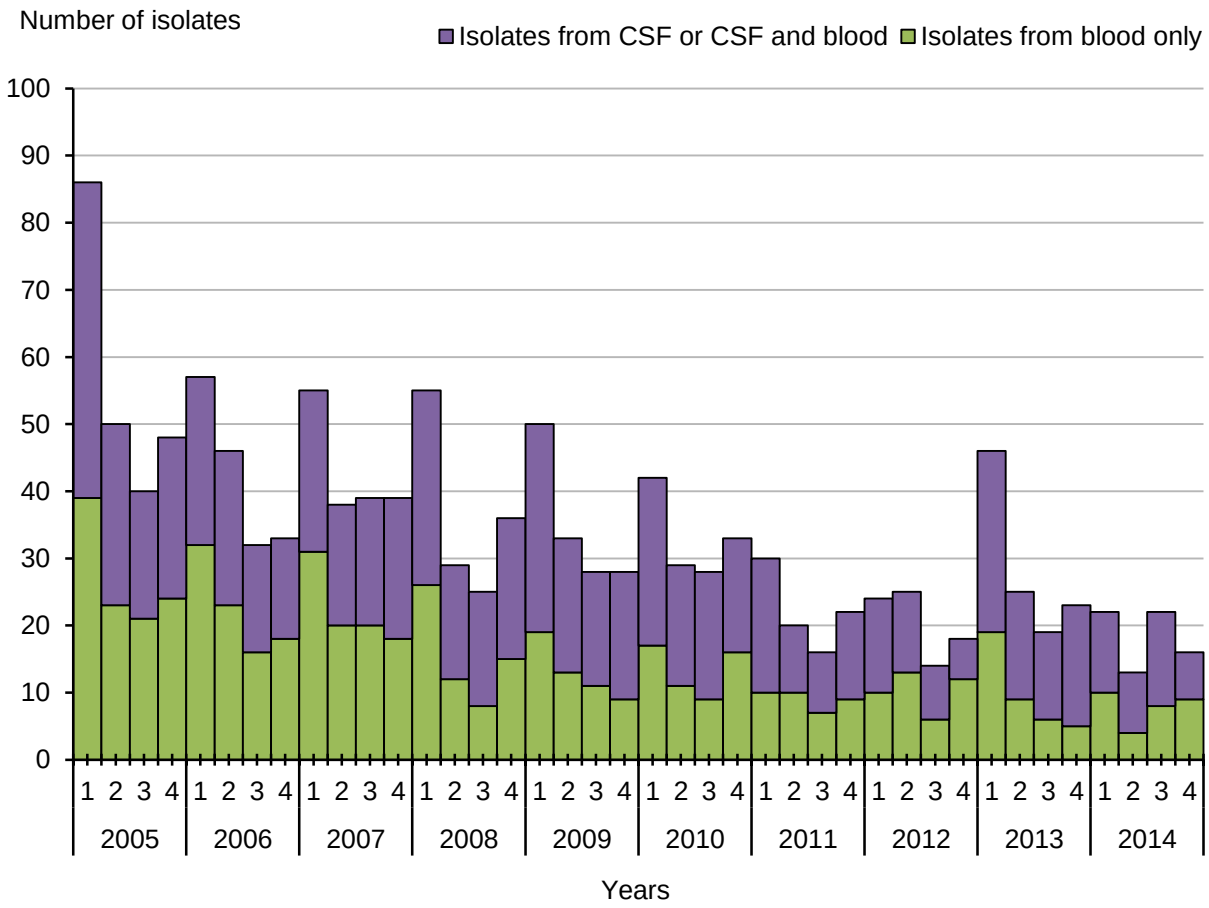

Figure 4.1 *Seasonal distribution of meningococcal disease, 2005-2014*

4.2 Antibiotic susceptibility

Eighty-six percent of all isolates (63/73) were susceptible to penicillin (MIC ≤ 0.064 µg/ml; CSF isolates 84%, isolates from blood only 88%). This is higher than in previous years. (79% in 2013; 65% in 2012; 70% in 2011; 83% in 2010 and 92% in 2009). This increased proportion of penicillin-susceptible isolates is mainly due to a 50% reduction 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 decrease 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, 2014

	Penicillin*					
	MIC ≤ 0.064 sensitive	0.064< MIC≤0.25	0.25< MIC≤1.0	MIC >1.0	Total	%
CSF or CSF and blood	26	5	0	0	**31**	**42**
Blood only	37	5	0	0	**42**	**58**
Total	**63**	**10**	**0**	**0**	**73**	**100**
%	**86**	**14**	**0**	**0**	**100**	

* MIC values in µg/ml

Table 4.2 Susceptibility of *N. meningitidis* isolated from CSF or CSF and blood to penicillin, 2009-2014

	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**

* MIC values in µg/ml

Table 4.3 Susceptibility of *N. meningitidis* isolated from blood only to penicillin, 2009-2014

	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

* MIC values in µg/ml

4.3 Serogroups

Serogroup B accounted for 73% (2013: 75%) of all isolates and group Y for about 17% (table 4.4). The proportion of serogroup Y isolates is gradually increasing since 2008 (2013: 14%; 2012: 12%; 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 11% of the isolates were of the rare serogroups C, W, E, X or Non Groupable. The serogroup distribution observed during the whole collection period 1959 - 2014 (figure 4.2) shows that in 2014 the number of group B isolates (53 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 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 2014 only 3 serogroup C isolates were received (figure 4.3).

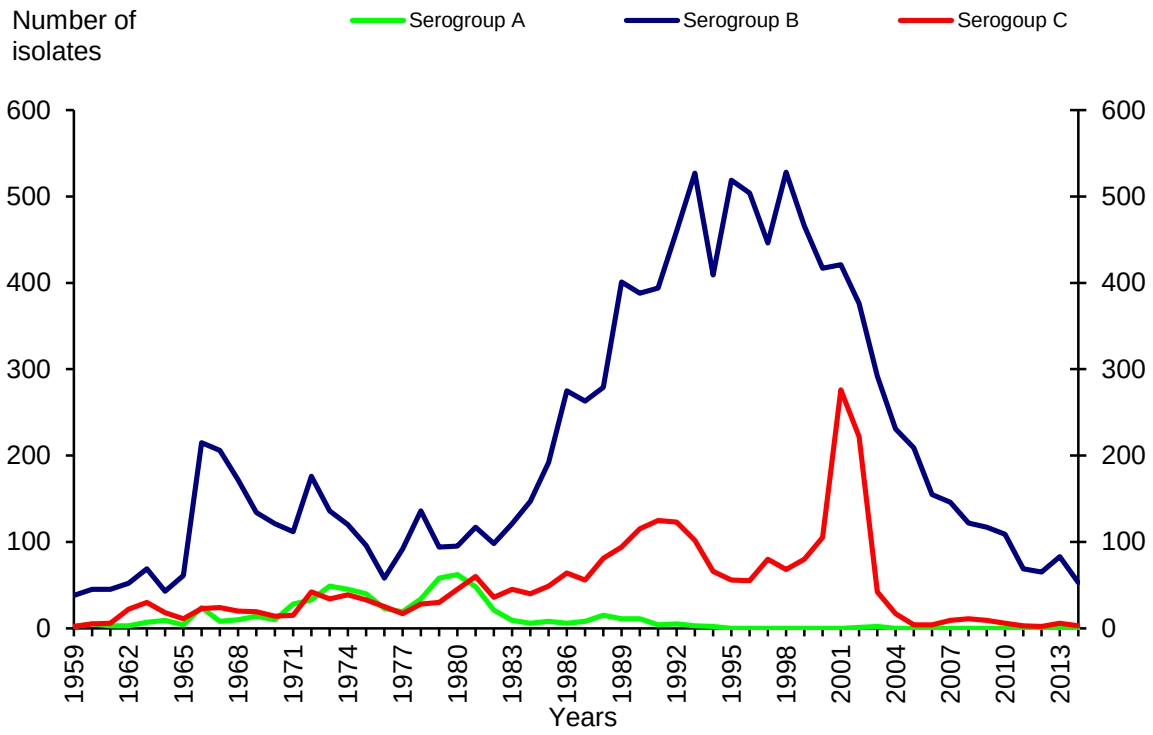

Figure 4.2. *Distribution of meningococcal serogroups A, B and C, 1959-2014*

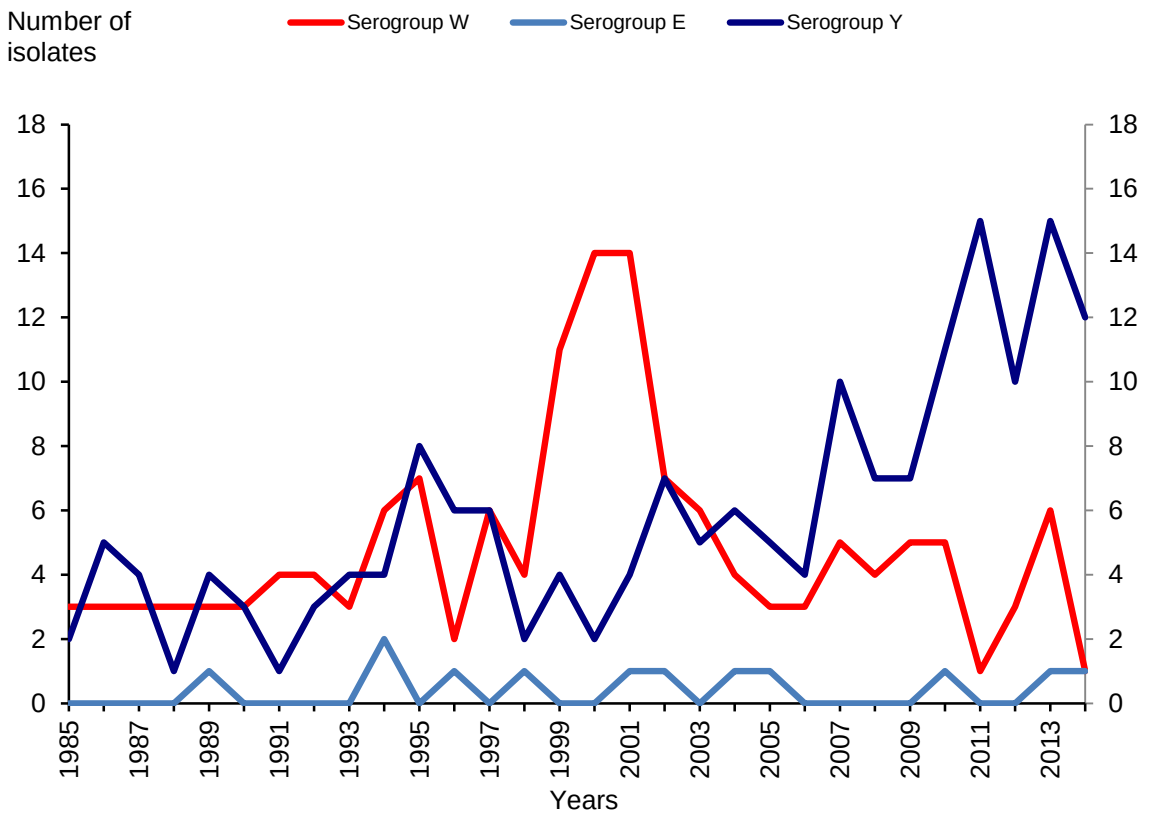

Figure 4.2.1. *Distribution of meningococcal serogroups Y, W and E, 1985-2014*

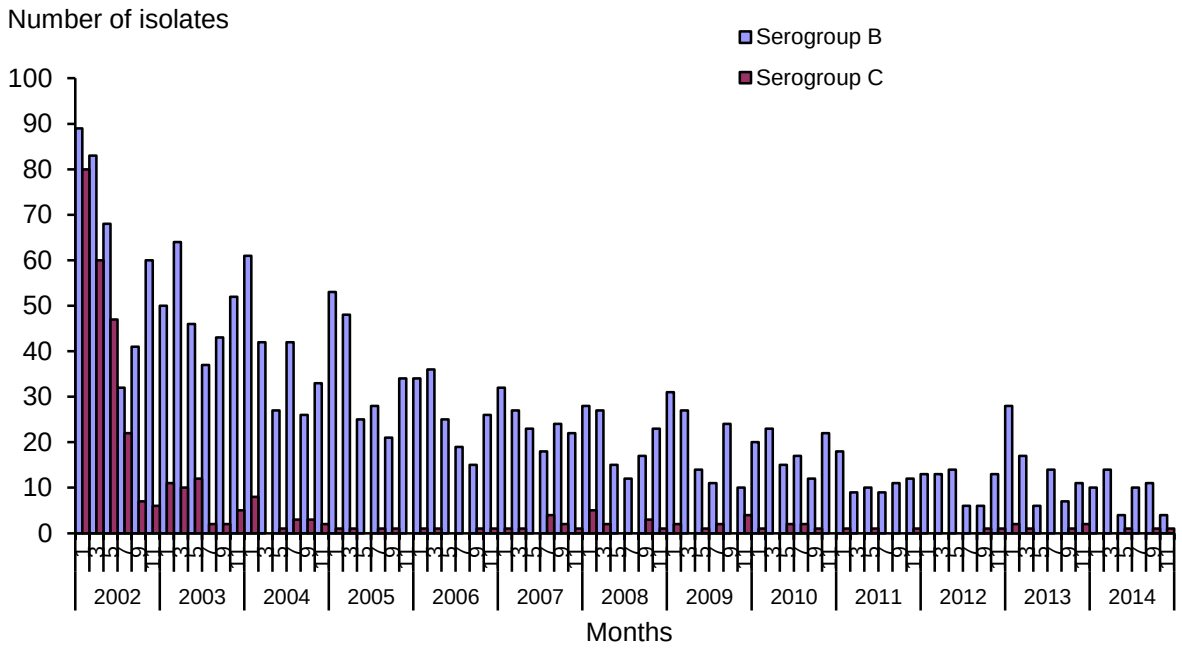

Figure 4.3 *Bimonthly distribution of meningococcal serogroups B and C, 2002-2014*

4.4 Serogroup and age

The age distribution of patients with meningitis and/or meningococcemia shows that 33% (24 of 73) of the patients was younger than 5 years (table 4.4, figure 4.4). Among patients from whom meningococci were isolated from blood only, 19% 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, 2014

	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	8	15	23	7	1	9	1	1	4	4	3	**53**	***72.6***
C	0	0	0	0	0	0	0	1	0	0	1	1	**3**	***4.1***
X	0	0	0	0	0	0	0	0	0	0	1	0	**1**	***1.4***
Y	0	1	0	1	1	0	2	0	2	0	1	5	**12**	***16.4***
W	0	0	0	0	0	0	0	0	0	0	1	0	**1**	***1.4***
E	0	0	0	0	0	0	1	0	0	0	0	0	**1**	***1.4***
n.g.	0	0	0	0	1	0	0	0	0	0	1	0	**2**	***2.7***
Total	**0**	**9**	**15**	**24**	**9**	**1**	**12**	**2**	**3**	**4**	**9**	**9**	**73**	***100.0***
%	***0.0***	***12.3***	***20.6***	***32.9***	***12.3***	***1.4***	***16.5***	***2.7***	***4.1***	***5.5***	***12.3***	***12.3***	***100.0***	

Table 4.5 Serogroups of *N. meningitidis* (isolates from CSF, or CSF and blood; absolute numbers) by patient age, 2014

	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	9	15	2	0	6	0	0	1	2	0	**26**	***83.9***
C	0	0	0	0	0	0	0	0	0	0	0	1	**1**	***3.2***
X	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
Y	0	1	0	1	0	0	1	0	1	0	0	0	**3**	***9.7***
W	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
E	0	0	0	0	0	0	0	0	0	0	0	0	**0**	***0.0***
n.g.	0	0	0	0	0	0	0	0	0	0	1	0	**1**	***3.2***
Total	**0**	**7**	**9**	**16**	**2**	**0**	**7**	**0**	**1**	**1**	**3**	**1**	**31**	***100.0***
%	***0.0***	***22.6***	***29.0***	***51.6***	***6.5***	***0.0***	***22.6***	***0.0***	***3.2***	***3.2***	***9.7***	***3.2***	***100***	

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), 2014

	AGE (YEARS)										TOTAL
Group	0	1-4	5-9	10-14	15-19	20-24	25-29	30-49	50-64	≥65	T
B	3.51	1.24	0.21	0.00	0.60	0.00	0.00	0.02	0.06	0.00	**0.15**
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	**0.01**
X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
Y	0.58	0.00	0.00	0.00	0.10	0.00	0.10	0.00	0.00	0.00	**0.02**
W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
E	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**
n.g.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	**0.01**
Total	**4.09**	**1.24**	**0.21**	**0.00**	**0.70**	**0.00**	**0.10**	**0.02**	**0.09**	**0.03**	**0.18**

Table 4.7 Serogroups of *N. meningitidis* (isolates from blood only*, absolute numbers) by patient age, 2014

	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	2	6	8	5	1	3	1	1	3	2	3	**27**	***64.2***
C	0	0	0	0	0	0	0	1	0	0	1	0	**2**	***4.8***
X	0	0	0	0	0	0	0	0	0	0	1	0	**1**	***2.4***
Y	0	0	0	0	1	0	1	0	1	0	1	5	**9**	***21.4***
W	0	0	0	0	0	0	0	0	0	0	1	0	**1**	***2.4***
E	0	0	0	0	0	0	1	0	0	0	0	0	**1**	***2.4***
n.g.	0	0	0	0	1	0	0	0	0	0	0	0	**1**	***2.4***
Total	**0**	**2**	**6**	**8**	**7**	**1**	**5**	**2**	**2**	**3**	**6**	**8**	**42**	***100.0***
%	***0.0***	***4.8***	***14.2***	***19.0***	***16.7***	***2.4***	***11.9***	***4.8***	***4.8***	***7.2***	***14.2***	***19.0***	***100.0***	

* From 2 patients with a meningococci isolated from blood, CSF was culture-negative but PCR was positive for meningococcal group B DNA. Cases were in age groups 1-11 months and 15-19 years

Table 4.8 Age distribution of meningococcemia (incidence per 100,000 inhabitants) by different serogroups of *N. meningitidis* (isolates from blood only), 2014

	AGE (YEARS)										TOTAL
Group	0	1-4	5-9	10-14	15-19	20-24	25-29	30-49	50-64	≥65	T
B	1.17	0.83	0.53	0.10	0.30	0.09	0.10	0.07	0.06	0.10	**0.16**
C	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.03	0.00	**0.01**
X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	**0.01**
Y	0.00	0.00	0.11	0.00	0.10	0.00	0.10	0.00	0.03	0.17	**0.05**
W	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	**0.01**
E	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	**0.01**
n.g.	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.01**
Total	**1.17**	**0.83**	**0.75**	**0.10**	**0.50**	**0.19**	**0.19**	**0.07**	**0.17**	**0.27**	**0.25**

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 and 15 - 19 years were 2.6 and 0.9 respectively. The age-specific incidences per 100,000 inhabitants in the age groups >19 years was less than 0.2 except for the age group 35-39 years (incidence of 0.3).

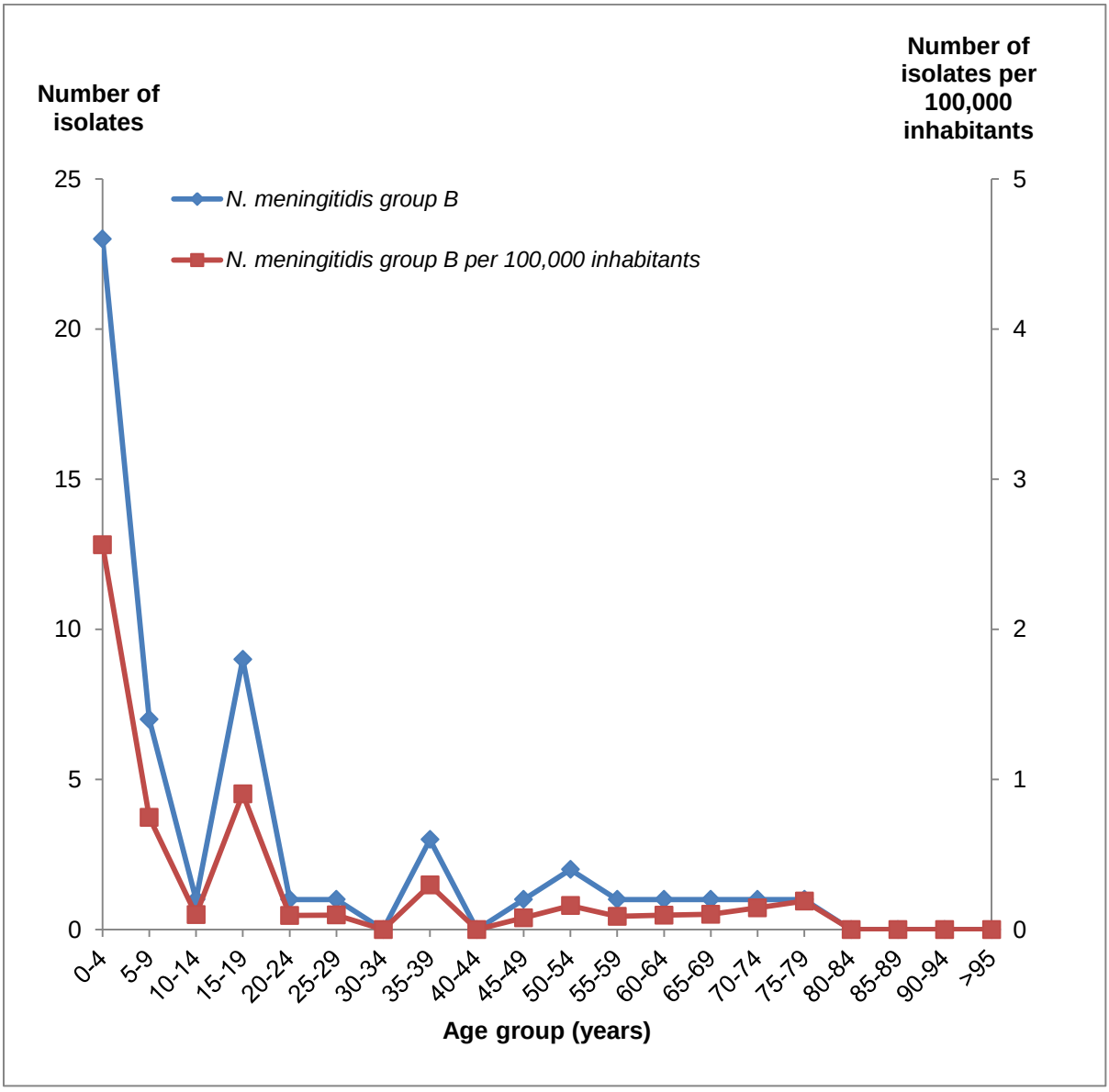

Figure 4.4 *Age distribution of serogroup B meningococcal disease in 2014*

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 2014, 28 different VR1/VR2 combinations were encountered among serogroup B meningococci (2011: 32; 2012: 31; 2013: 39). The proportion of the dominant PorA genosubtype P1.7-2,4 decreased from 40% of all serogroup B isolates in 2000 to 15% in 2014 (figure 4.5, figure 4.7; table 4.9).

The three serogroup C isolates had the 3 differtent VR1/VR2 combinations. P1.17,16-4, P1.21-2,28 and P1.5,2 respectively.

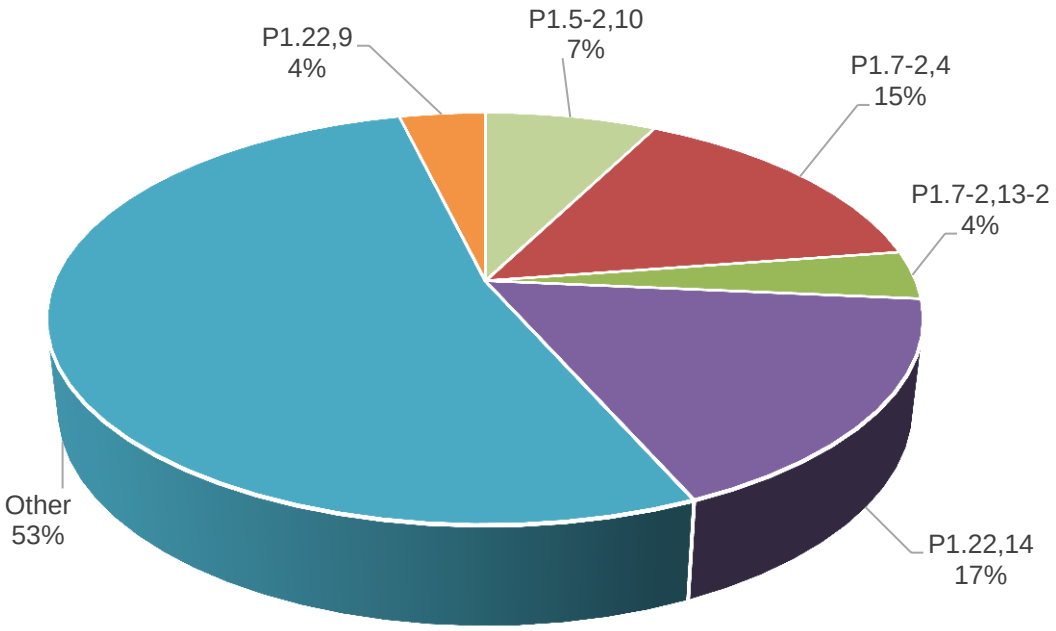

Figure 4.5 *Distribution of group B meningococcal PorA types, 2014*

Table 4.9 *N. meningitidis* serogroup B isolates according to PorA genosubtype, 2010-2014

	VR1,VR2 combination	YEAR									
		2010		2011		2012		2013		2014	
		No.	%	No.	%	No.	%	No.	%		%
Vaccine types*	1.5-1, 2-2	1	**0.9**	-	**-**	-	**-**	-	**-**	-	**-**
	1.5-1, other	3	**2.8**	1	**1.4**	3	**4.6**	1	**1.2**	1	**1.9**
	1.5-2,10	11	**10.1**	2	**2.9**	4	**6.2**	7	**8.4**	4	**7.5**
	1.5-2, other	3	**2.8**	3	**4.4**	-	**-**	-	**-**	1	**1.9**
	1.7,16	2	**1.8**	1	**1.4**	-	**-**	1	**1.2**	-	**-**
	1.7, other	2	**1.8**	4	**5.8**	1	**1.5**	5	**6.0**	1	**1.9**
	1.7-1, 1	3	**2.8**	2	**2.9**	2	**3.1**	-	**-**	1	**1.9**
	1.7-1, other	-	**-**	-	**-**	-	**-**	-	**-**	1	**1.9**
	1.7-2,4	24	**22.0**	10	**14.5**	6	**9.2**	7	**8.4**	8	**15.0**
	1.7-2, other	7	**6.4**	4	**5.8**	8	**12.3**	13	**15.7**	3	**5.7**
	1.12-1, other	4	**3.7**	1	**1.4**	1	**1.5**	1	**1.2**	1	**1.9**
	1.18-1,3	2	**1.8**	2	**2.9**	1	**1.5**	3	**3.6**	-	**-**
	1.18-1, other	2	**1.8**	2	**2.9**	5	**7.7**	3	**3.6**	9	**17.0**
	1.19,15-1	-	**-**	1	**1.4**	-	**-**	3	**3.6**	2	**3.7**
	1.19, other	7	**6.4**	2	**2.9**	4	**6.2**	3	**3.6**	3	**5.7**
	1.22,14	20	**18.3**	14	**20.3**	12	**18.5**	9	**10.9**	9	**17.0**
	1.22,other	4	**3.7**	5	**7.3**	8	**12.3**	6	**7.3**	3	**5.7**
	Other, 14	3	**2.8**	2	**2.9**	-	**-**	2	**2.4**	1	**1.9**
	Other, 16	2	**1.8**	3	**4.4**	2	**3.1**	3	**3.6**	1	**1.9**
	Subtotal vaccine types	100	**91.7**	59	**85.5**	57	**87.7**	67	**80.7**	49	**92.5**
NVT**	Other	9		10		8		16		4	
	Subtotal Non	9	**8.3**	10	**14.5**	8	**12.3**	16	**19.3**	4	**7.5**
	Total	109	**100.0**	69	**100.0**	65	**100.0**	83	**100.0**	53	**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 2014, 16 different FetA variants were observed among serogroup B meningococci. The dominant type is F5-1, accounting for 19% 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 with PorA VR1/VR2 P1.7-2,4 and together to the MLST clonal complex ST41/44. In 2014, the diversity among the meningococcal isolates was much smaller; 8 F1-5 types were linked with 6 different PorA types. FetA type F1-5 was 7 times linked with PorA VR1/VR2 P1.7-2 (5 in 2013; 4 in 2012; 8 in 2011; 20 in 2010).

The three serogroup C meningococci had the FetA types F3-3 (once) and twice Fet A type F3-9.

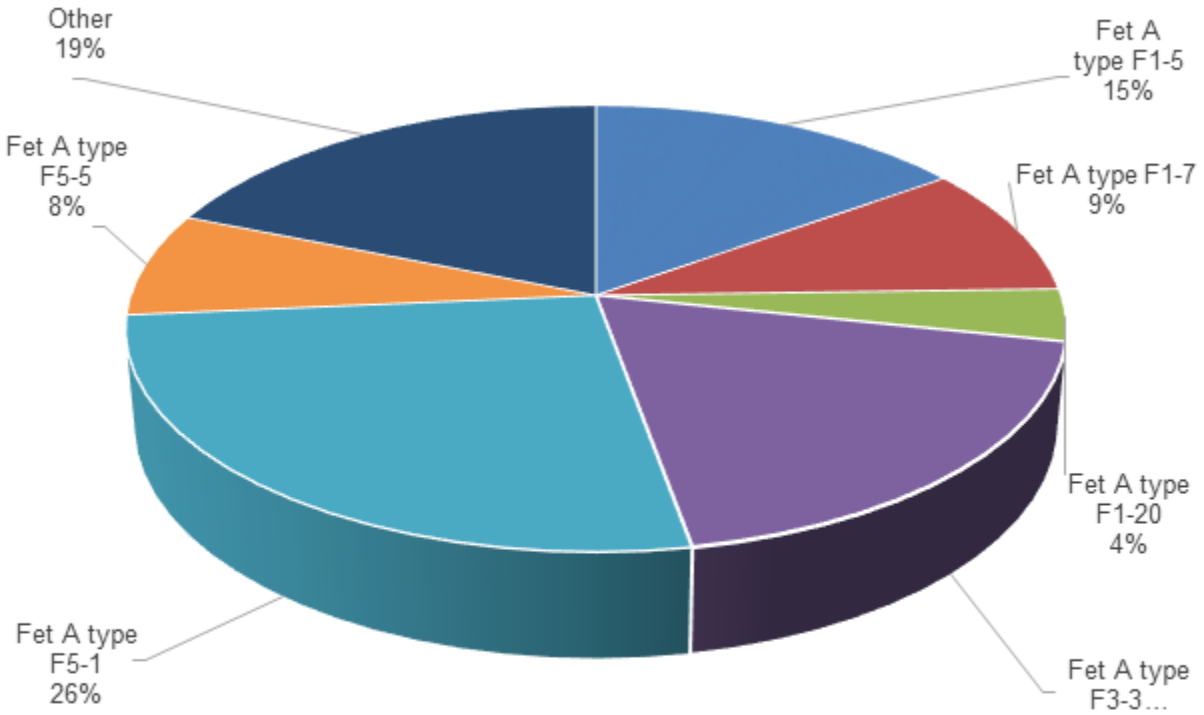

Figure 4.6 *Distribution of group B meningococcal FetA genosubtypes, 2014*

Table 4.10 *N. meningitidis* serogroup B isolates according to FetA genosubtype, 2010-2014

	YEARS									
FetA type	**2010**		**2011**		**2012**		**2013**		**2014**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
F1-5	36	33.0	17	24.6	23	35.4	17	20.5	8	15.1
F1-7	12	11.0	4	5.8	2	3.1	6	7.2	5	9.4
F1-15	2	1.8	1	1.5	1	1.5	1	1.2	1	1.9
F3-3	4	3.7	6	8.7	4	6.2	6	7.2	10	18.9
F3-7	1	0.9	2	2.9	-	-	-	-	-	-
F3-9	1	0.9	3	4.3	3	4.6	2	2.4	1	1.9
F4-1	4	3.7	-	-	2	3.1	2	2.4	1	1.9
F5-1	20	18.3	8	11.6	7	10.8	14	16.9	14	26.4
F5-2	-	-	2	2.9	-	-	2	2.4	-	-
F5-5	13	12.0	10	14.5	11	16.9	8	9.7	4	7.5
F5-8	2	1.8	-	-	1	1.5	-	-	1	1.9
F5-12	1	0.9	2	2.9	2	3.1	-	-	1	1.9
Other	13	12.0	14	20.3	9	13.8	25	30.1	7	13.2
Total	**109**	**100.0**	**69**	**100.0**	**65**	**100.0**	**83**	**100.0**	**53**	**100.0**

In 2014, 28 different VR1/VR2 combinations and 16 different FetA variants were encountered among serogroup B meningococci. Among the dominant FetA type F5-1, accounting for 26% of group B meningococci, 4 were of P1.5-2,10:F5-1 (7.5% of group B meningococci).
Other frequently found combinations are P1.7-2,4:F1-5 (11%) and P1.22,14:F5-5 (8%) (Figure 4.7).

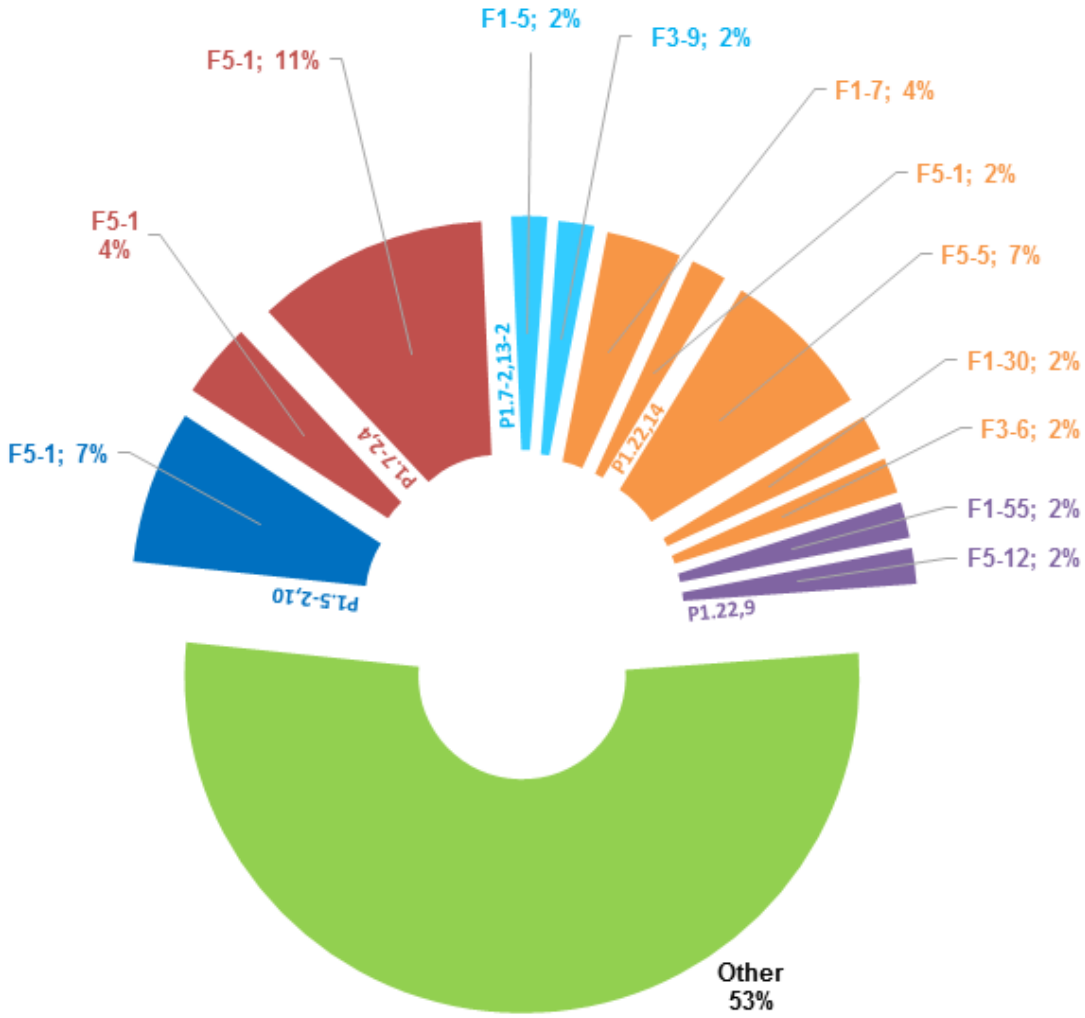

Figure 4.7 *Distribution of group B meningococcal PorA and FetA geno(sub)types, 2014*

5 *HAEMOPHILUS INFLUENZAE*

5.1 General features

In total, 161 *Haemophilus influenzae* isolates were submitted to the Reference Laboratory. This number is higher than that of the last years (table 2.3, figure 3.3, figure 5.1). Twenty-one strains were isolated from CSF (or CSF and blood) (2013: 16; 2012: 16; 2011: 13), and 140 from blood only. Thirty (19%) 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.

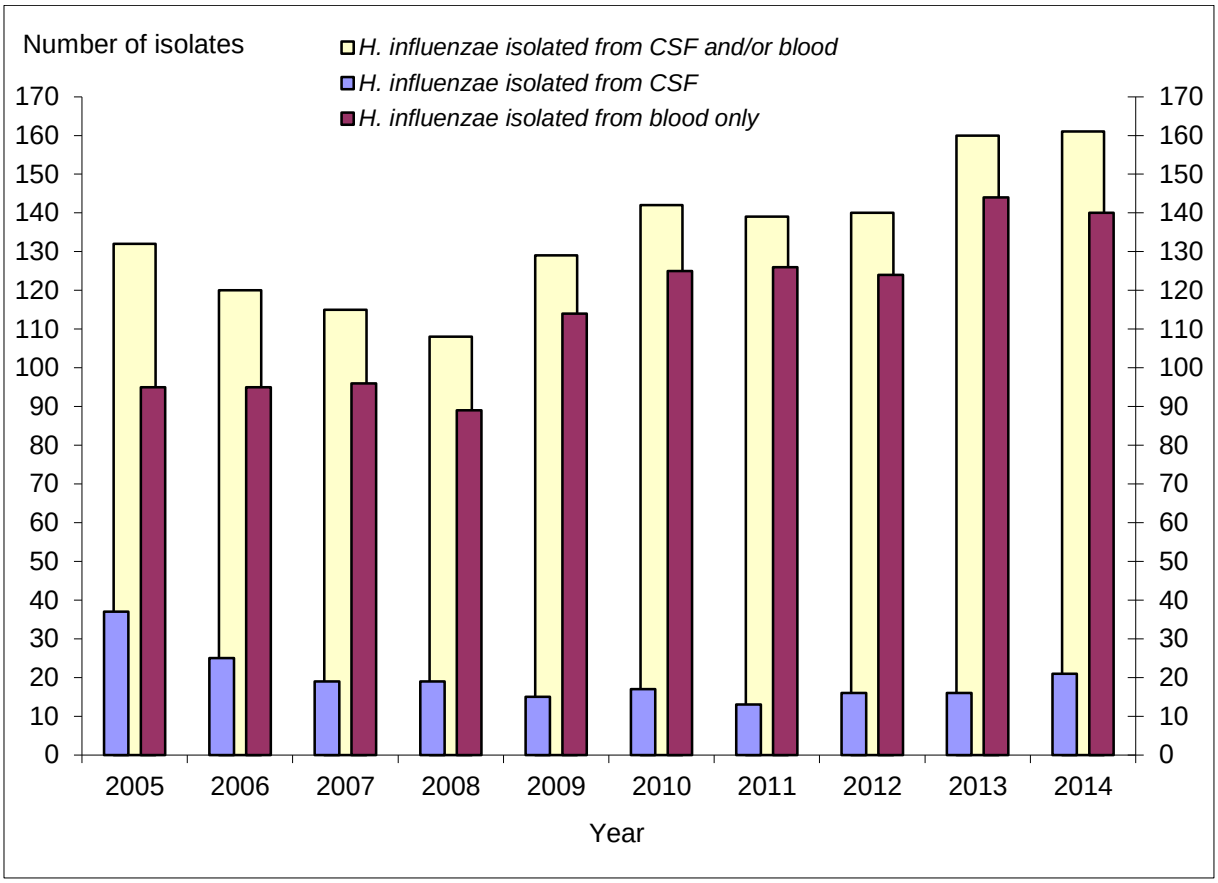

Figure 5.1 *Distribution of H. influenzae, 2005-2014*

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.

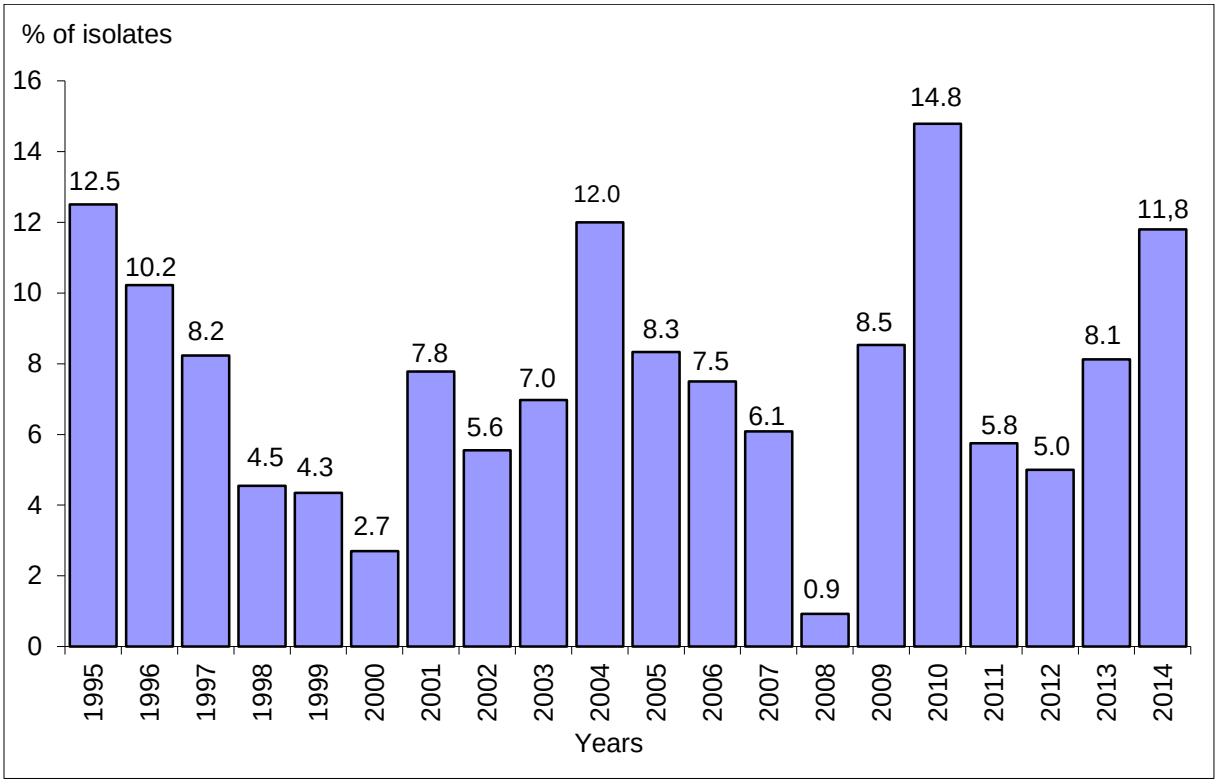

Figure 5.2 *Percentage β-lactamase producing H. influenzae, 1995-2014*

5.3 Serotype and age

Six cases of *H. influenzae* type b invasive disease were observed among children younger than 2 years of age (9 in 2013; 3 in 2012; 3 in 2011; 6 in 2010) (figure 5.3). In total 117 non-typable *H.influenzae* were received; 15 isolated from CSF (or CSF and blood) and 102 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, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
a	1	1	-	2	-	-	-	-	**2**	***1.2***
b	1	5	3	9	2	1	6	12	**30**	***18.6***
d	-	-	-	-	-	-	-	1	**1**	***0.6***
e	-	-	-	-	-	-	-	3	**3**	***1.9***
f	-	-	-	-	1	-	-	7	**8**	***5.0***
n.t.*	5	3	3	11	2	-	16	88	**117**	***72.7***
Total	**7**	**9**	**6**	**22**	**5**	**1**	**22**	**111**	**161**	***100***
%	***4.4***	***5.6***	***3.7***	***13.7***	***3.1***	***0.6***	***13.7***	***68.9***	***100***	

* non-typable

Table 5.2 *H.influenzae* isolates from CSF (or CSF and blood), according to serotype and age, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
a	-	1	-	1	-	-	-	-	1	***4.8***
b	-	5	-	5	-	-	-	-	5	***23.8***
d	-	-	-	-	-	-	-	-	-	***0.0***
e	-	-	-	-	-	-	-	-	-	***0.0***
f	-	-	-	-	-	-	-	-	-	***0.0***
n.t.*	-	-	2	2	1	-	5	7	15	***71.4***
Total	**-**	**6**	**2**	**8**	**1**	**-**	**5**	**7**	**21**	***100.0***
%	***0.0***	***28.6***	***9.5***	***38.1***	***4.8***	***0.0***	***23.8***	***3.3***	***100.0***	

* non-typable

Table 5.3 *H. influenzae* isolates from blood only, according to serotype and age, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
a	1	-	-	1	-	-	-	-	**1**	***0.7***
b	1	-	3	4	2	1	6	12	**25**	***17.9***
d	-	-	-	-	-	-	-	1	**1**	***0.7***
e	-	-	-	-	-	-	-	3	**3**	***2.1***
f	-	-	-	-	1	-	-	7	**8**	***5.7***
n.t.*	5	3	1	9	1	-	11	81	**102**	***72.9***
Total	**7**	**3**	**4**	**14**	**4**	**1**	**17**	**104**	**140**	***100.0***
%	***5.0***	***2.1***	***2.9***	***10.0***	***2.9***	***0.7***	***12.1***	***74.3***	***100.0***	

* non-typable

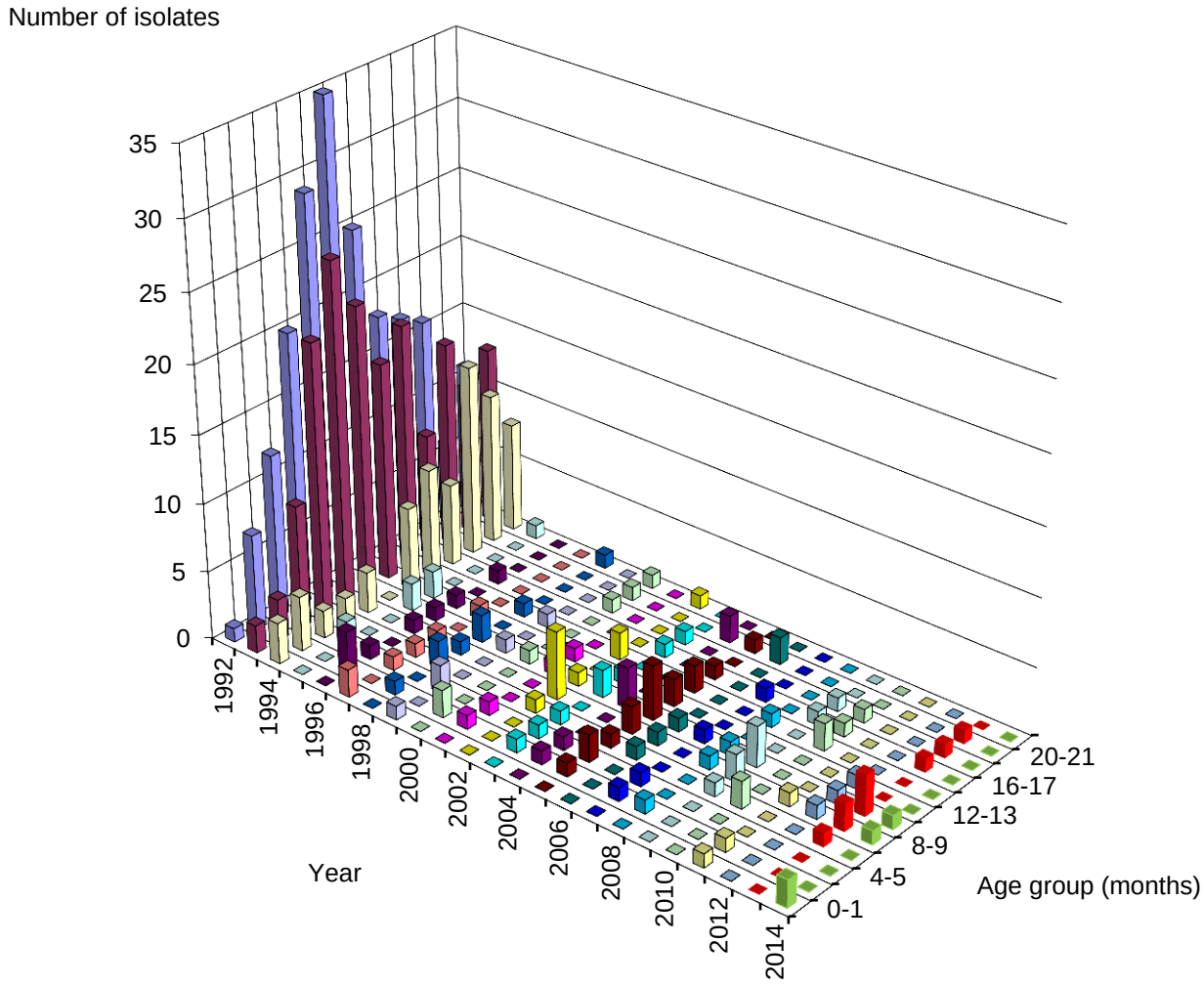

Figure 5.3 *Age distribution of H. influenzae type b invasive disease in the first two years of life, 1992-2014*

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 2014 the proportion of non-typable isolates was 73%.

Table 5.4 *H. influenzae* isolates from CSF and/or blood received from 1992 to 2014 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**

* 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 2014 15 non-typable isolates from CSF were received; 2.5 times more than in 2013. The number of non-typable *H. influenzae* isolates from blood increased during the period 1992 to 2014 from 15 to 102 (figure 5.4).

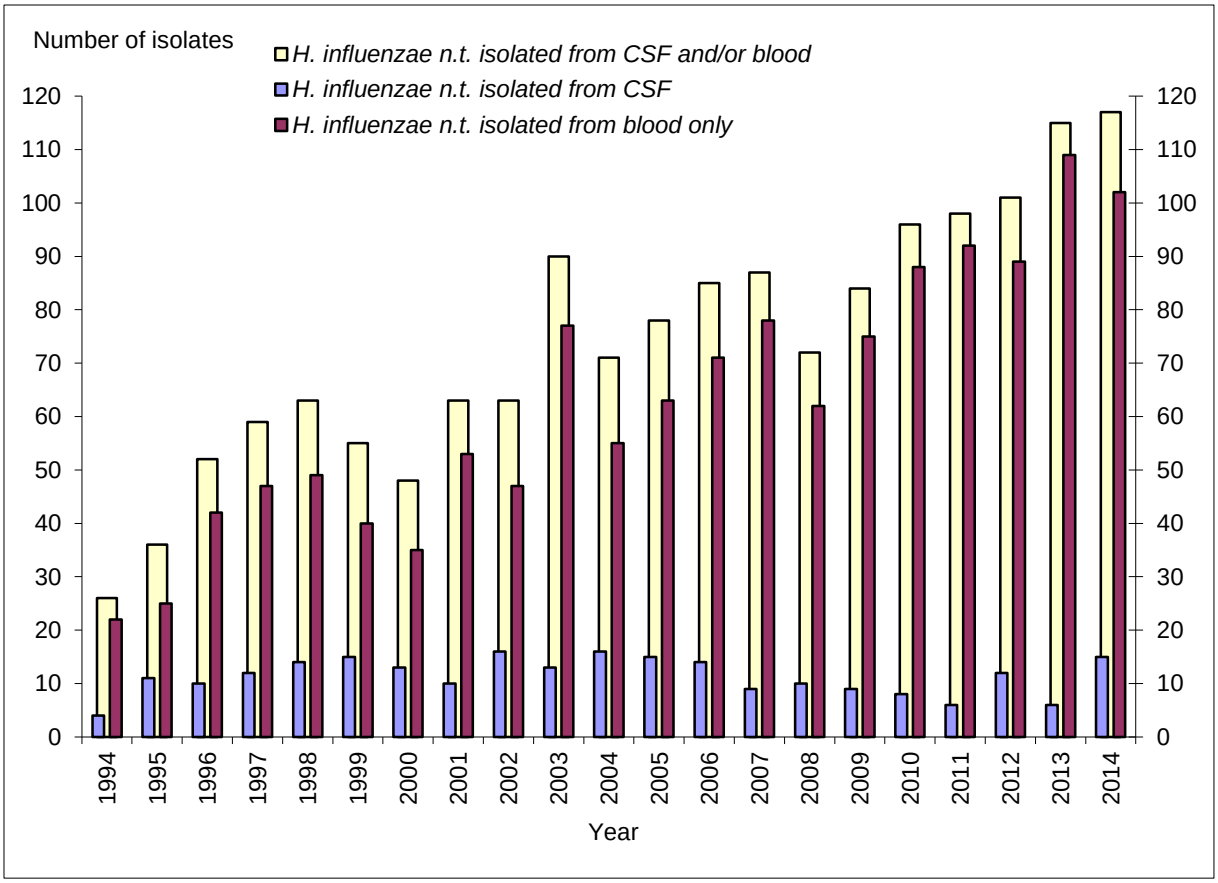

Figure 5.4 *Non-typable H. influenzae isolates from CSF and/or blood received from 1994-2014*

Table 5.5 Non-typable *H. influenzae* isolates from CSF and/or blood received from 2005 to 2014 according to year and biotype.

	Biotype							Total
YEAR	I	II	III	IV	V	VI	VII	
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**
2013	25	44	30	7	7	2	-	**115**
2014	16	56	32	1	9	3	-	**117**

*non-typable

Among non-serotypable *H. influenzae* isolates biotype II was the predominant biotype during the last ten years. The number of Biotype II isolates was higher than that in 2013 (Table 5.5)

6 STREPTOCOCCUS PNEUMONIAE

6.1 General features

The Reference Laboratory received 769 *S. pneumoniae* isolates. Of these, 142 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 2014. 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 627 in 2014. 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.

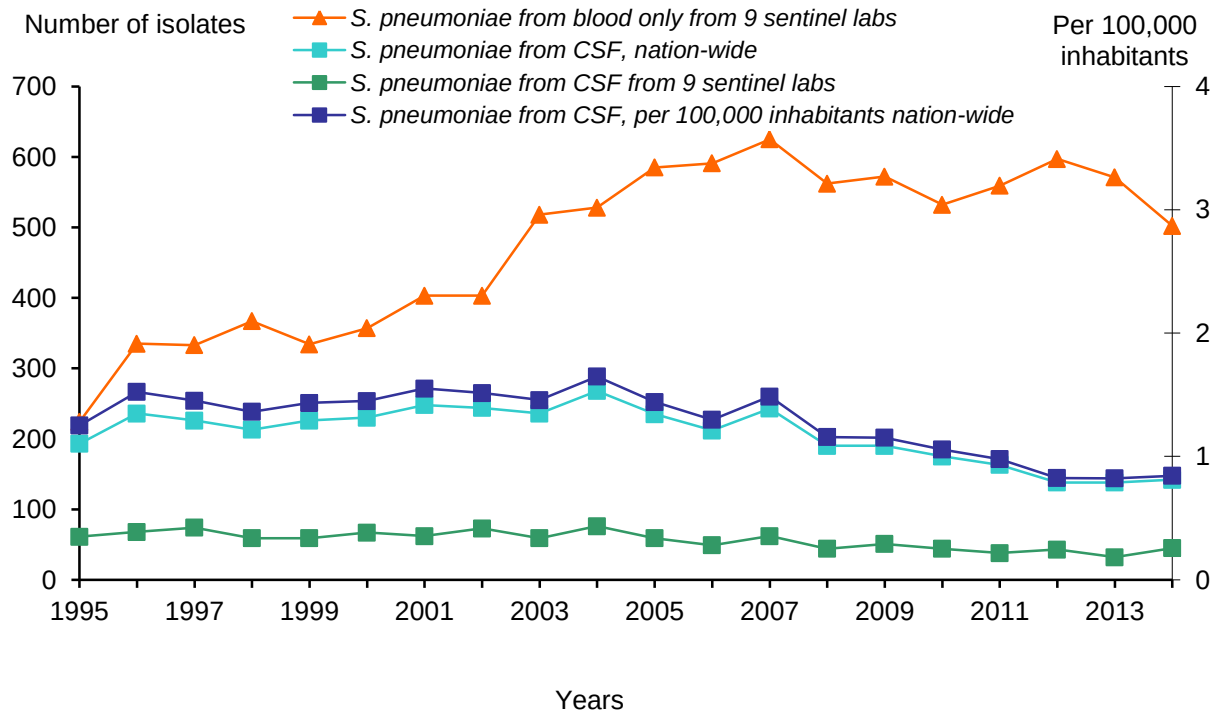

Figure 6.1 *Distribution of S. pneumoniae isolates, 1995-2014*

6.2 Antibiotic susceptibility

Among 142 isolates from CSF (or CSF and blood) and 627 isolates from the blood only, 16 isolates from the blood (2.1%) were intermediately susceptible to penicillin (0.06< MIC≤2.0 mg/L, table 6.1). Seven (0.9%) strains isolated from CSF were resistant to penicillin (MIC > 0.06 mg/L).

Table 6.1 Susceptibility of *S. pneumoniae* isolates to penicillin, 2014

	Penicillin*				
	S	I	R		
MIC for CSF	MIC ≤ 0.06		MIC > 0.06		
MIC for blood	MIC ≤ 0.06	0.06< MIC ≤ 2.0	MIC >2.0	**Total**	**%**
CSF or CSF and blood	135	0	7	**142**	***18.5***
Blood only	611	16	0	**627**	***81.5***
Total	**746**	**16**	**7**	**769**	***100.0***
%	***97.0***	***2.1***	***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 60 – 64 year (Table 6.4).

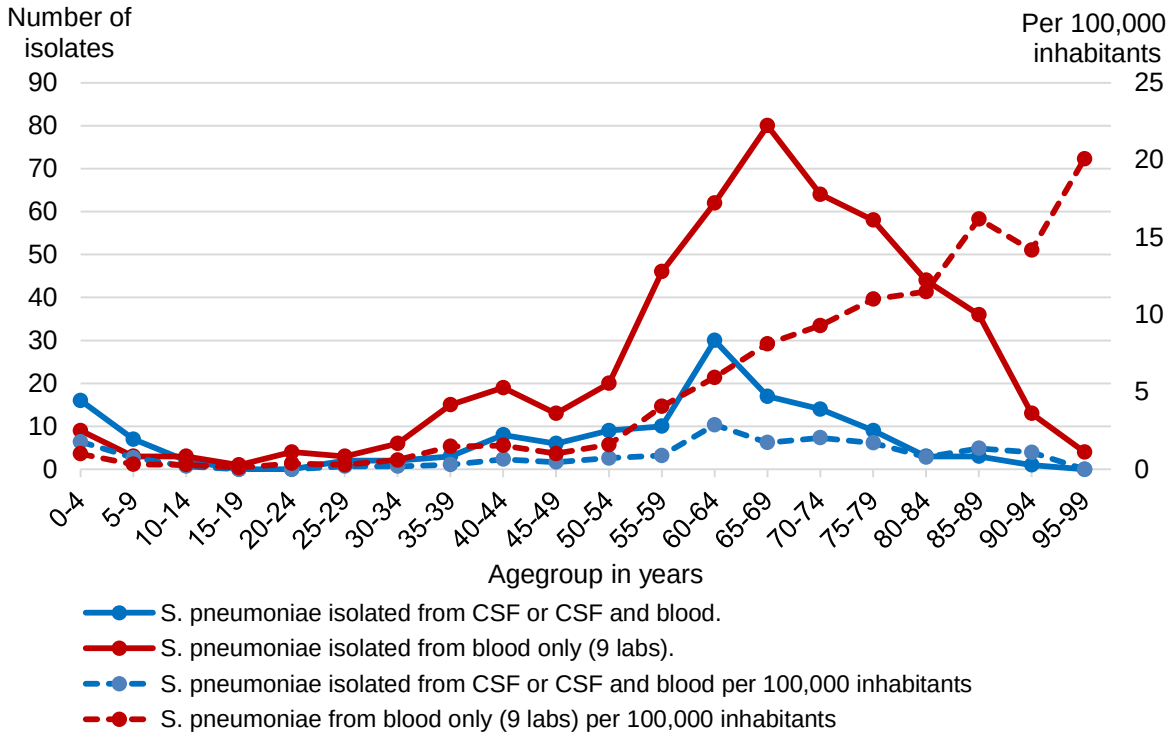

Figure 6.2 *Distribution of S. pneumoniae isolates received in 2014 according to age*

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. Effect of the 10-valent vaccine can been seen in table 6.6 and table 6.7. In the age group 0-4 years the number of cases was higher than in 2013 (35 cases), due to higher number of cases due to pneumococci with serotypes not included in the deca-valent conjugate vaccine (table 6.2, 6.3 and figure 12.4). 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 7 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 nation-wide, by serotype and age of patients, 2014

	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	3	3	4	0	0	1	6	8	10	18	5	55	***7.2***
3	1	0	0	1	2	0	0	0	2	3	14	20	8	50	***6.5***
4	0	0	0	0	0	0	0	0	1	0	2	6	2	11	***1.4***
6	0	0	1	1	0	1	0	0	1	1	4	8	5	21	***2.7***
7	0	0	2	2	1	1	0	1	6	7	17	22	10	67	***8.7***
8	1	1	0	2	1	1	1	3	1	12	32	72	14	139	***18.2***
9	0	0	0	0	0	0	0	0	0	2	9	14	9	34	***4.4***
10	0	5	1	6	1	1	0	0	1	1	11	9	4	34	***4.4***
12	0	1	0	1	1	0	0	1	2	3	15	18	1	42	***5.5***
14	0	0	0	0	0	0	0	0	0	0	2	0	2	4	***0.5***
18	0	0	0	0	0	0	0	0	0	0	1	2	0	3	***0.4***
19	0	2	4	6	3	0	0	0	1	2	22	29	14	77	***10.0***
22	0	0	0	1	0	0	0	1	1	6	19	14	9	51	***6.6***
23	1	1	2	4	0	0	0	0	1	0	20	18	4	47	***6.1***
Others	1	7	11	19	2	1	1	3	6	5	26	39	32	134	***17.4***
Total	**4**	**17**	**25**	**46**	**15**	**5**	**2**	**10**	**29**	**50**	**204**	**289**	**119**	**769**	***100.0***
%	***0.5***	***2.2***	***3.3***	***6.0***	***2.0***	***0.6***	***0.3***	***1.3***	***3.8***	***6.5***	***26.5***	***37.6***	***15.4***	***100.0***	

Table 6.3 *S. pneumoniae* isolates from CSF (or CSF and blood) nation-wide, by serotype and age of patients, 2014

	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	0	0	2	0	0	0	0	1	0	1	0	**4**	***2.8***
3	0	0	0	0	1	0	0	0	0	3	3	4	2	**13**	***9.2***
4	0	0	0	0	0	0	0	0	0	0	1	1	0	**2**	***1.4***
6	0	0	0	0	0	1	0	0	1	1	0	2	1	**6**	***4.2***
7	0	0	0	0	1	0	0	0	0	1	2	2	2	**8**	***5.6***
8	0	1	0	1	0	1	0	1	0	2	11	7	0	**23**	***16.2***
9	0	0	0	0	0	0	0	0	0	1	2	3	1	**7**	***4.9***
10	0	4	1	5	0	0	0	0	0	1	5	2	0	**13**	***9.2***
12	0	0	0	0	0	0	0	0	1	2	3	2	0	**8**	***5.6***
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	0	2	2	2	0	0	0	0	1	4	2	0	**11**	***7.8***
22	0	0	0	0	0	0	0	1	0	1	3	3	0	**8**	***5.6***
23	0	0	0	0	0	0	0	0	0	0	7	5	0	**12**	***8.5***
Others	0	5	3	8	1	0	0	0	3	0	8	6	1	**27**	***19.0***
Total	**0**	**10**	**6**	**16**	**7**	**2**	**0**	**2**	**5**	**14**	**49**	**40**	**7**	**142**	***100.0***
%	***0.0***	***7.1***	***4.2***	***11.3***	***4.9***	***1.4***	***0.0***	***1.4***	***3.5***	***9.9***	***34.5***	***28.2***	***4.9***	***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, 2014

	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	0.21	0	0	0	0	0.04	0	0.05	0	**0.02**
3	0	0	0.11	0	0	0	0	0.12	0.09	0.18	0.28	**0.08**
4	0	0	0	0	0	0	0	0	0.03	0.05	0	**0.01**
6	0	0	0	0.10	0	0	0.05	0.04	0	0.09	0.14	**0.04**
7	0	0	0.11	0	0	0	0	0.04	0.06	0.09	0.28	**0.05**
8	0.58	0	0	0.10	0	0.05	0	0.08	0.32	0.32	0	**0.14**
9	0	0	0	0	0	0	0	0.04	0.06	0.14	0.14	**0.04**
10	2.33	0.14	0	0	0	0	0	0.04	0.15	0.09	0	**0.08**
12	0	0	0	0	0	0	0.05	0.08	0.09	0.09	0	**0.05**
14	0	0	0	0	0	0	0	0	0	0	0	**0.00**
18	0	0	0	0	0	0	0	0	0	0	0	**0.00**
19	0	0.28	0.21	0	0	0	0	0.04	0.12	0.09	0	**0.07**
22	0	0	0	0	0	0.05	0	0.04	0.09	0.14	0	**0.05**
23	0	0	0	0	0	0	0	0	0.20	0.23	0	**0.07**
Others	2.92	0.41	0.11	0	0	0	0.15	0	0.23	0.27	0.14	**0.16**
Total	**5.85**	**0.83**	**0.75**	**0.20**	**0.00**	**0.10**	**0.25**	**0.56**	**1.43**	**1.82**	**0.98**	**0.84**

Table 6.5 All *S. pneumoniae* isolates from blood only* nation-wide, by serotype and age of patients, 2014

	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	Total	%
1	0	0	2	2	1	0	0	1	6	7	8	12	4	**41**	***8.1***
3	1	0	0	1	0	0	0	0	2	0	9	13	6	**31**	***6.1***
4	0	0	0	0	0	0	0	0	1	0	0	4	1	**6**	***1.2***
6	0	0	1	1	0	0	0	0	0	0	3	5	1	**10**	***2.0***
7	0	0	1	1	0	1	0	1	5	6	14	18	7	**53**	***10.5***
8	0	0	0	0	0	0	0	2	0	7	20	52	12	**93**	***18.5***
9	0	0	0	0	0	0	0	0	0	1	7	8	7	**23**	***4.6***
10	0	0	0	0	0	1	0	0	1	0	5	6	4	**17**	***3.4***
12	0	0	0	0	0	0	0	1	1	1	11	13	1	**28**	***5.6***
14	0	0	0	0	0	0	0	0	0	0	1	0	1	**2**	***0.4***
18	0	0	0	0	0	0	0	0	0	0	0	2	0	**2**	***0.4***
19	0	0	0	0	1	0	0	0	1	1	15	20	13	**51**	***10.1***
22	0	0	0	0	0	0	0	0	1	4	10	11	8	**34**	***6.8***
23	0	0	2	2	0	0	0	0	1	0	9	9	3	**24**	***4.8***
Others	0	0	2	2	1	1	1	2	2	5	16	29	29	**88**	***17.5***
Total	**1**	**0**	**8**	**9**	**3**	**3**	**1**	**7**	**21**	**32**	**128**	**202**	**97**	**503**	***100.0***
%	***0.2***	***0.0***	***1.6***	***1.8***	***0.6***	***0.6***	***0.2***	***1.4***	***4.2***	***6.4***	***25.4***	***40.1***	***19.3***	***100.0***	

* From 8 patients with a pneumococcus isolated from blood, CSF was culture-negative but PCR was positive for pneumococcal DNA. Cases were in age groups 30-39 (1), 50-64 years (4) and 65-79 years (3)

Table 6.6 Distribution of pneumococcal CSF isolates according to serotype nation-wide, 2005-2014

			TYPE	Year 2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
23-valent vaccine	10-valent vaccine	7-valent	4	8	12	5	8	4	3	2	4	2	2
			6B	17	21	12	11	3	-	2	-	-	-
			9V	10	6	10	7	2	2	-	3	1	1
			14	24	28	18	8	3	5	2	1	-	-
			18C	14	19	17	8	6	5	5	2	2	-
			19F	19	15	11	7	10	2	6	4	2	4
			23F	16	16	22	17	5	4	2	1	-	-
			Subtotal 7-valent vaccine	*108*	*117*	*95*	*66*	*33*	*21*	*19*	*15*	*7*	*7*
			1	6	1	8	8	8	3	1	1	3	4
			5	1	1	-	-	-	2	-	3	-	-
			7F	19	18	36	25	25	20	28	16	15	8
			Subtotal 10-valent vaccine	*134*	*137*	*139*	*99*	*66*	*46*	*48*	*35*	*25*	*19*
			2	-	-	-	-	-	-	-	-	-	-
			3	16	20	16	17	24	20	7	13	16	13
			8	15	10	21	9	10	10	17	9	16	23
			9N	10	2	4	1	3	6	7	4	2	6
			10A	6	7	8	7	10	9	7	9	7	12
			11A	5	3	4	2	8	1	5	1	1	3
			12F	1	1	4	2	2	3	7	10	9	8
			15B	3	-	1	4	8	2	3	1	-	-
			17F	-	-	1	-	-	4	3	1	1	1
			19A	7	2	9	8	6	20	16	6	9	7
			20	-	1	-	1	-	1	-	-	1	1
			22F	4	9	2	10	13	14	16	11	8	8
			33F	1	3	5	6	6	7	5	6	3	2
			Subtotal 23-valent vaccine	*202*	*195*	*214*	*166*	*156*	*143*	*141*	*106*	*98*	*103*
			6A	8	5	5	4	6	5	1	1	1	3
			6C	2	1	2	-	-	3	4	2	6	3
			7B	-	-	-	-	-	-	-	-	1	-
			10F	-	-	-	-	-	-	-	-	-	-
			10B	-	-	-	-	-	-	-	1	-	1
			12A	-	-	-	-	-	-	-	-	-	-
			13	1	-	-	-	1	-	-	-	-	-
			15A	-	-	1	1	-	1	1	1	4	6
			15C	2	1	1	3	1	2	-	3	-	-
			16F	4	2	2	2	-	5	4	-	5	2
			17A	-	-	-	-	1	-	-	-	-	-
			18F	1	-	-	-	-	-	-	-	-	-
			18A	-	-	-	-	-	-	-	-	-	-
			18B	1	1	1	1	-	-	-	1	-	-
			21	1	-	1	-	-	-	1	-	-	-
			22A	-	-	1	-	1	1	-	-	-	-
			23A	4	-	3	1	3	3	2	4	4	4
			23B	1	2	2	3	7	5	2	5	7	8
			24F	3	-	3	2	6	1	1	4	4	7
			24B	-	-	-	-	-	-	-	2	-	-
			25	-	-	-	1	-	-	-	-	-	-
			27	-	-	1	2	-	-	-	1	-	2
			28F	-	-	-	-	-	-	-	-	1	-
			28A	1	-	-	-	-	-	1	-	-	-
			29	-	-	-	-	-	-	-	1	-	-
			31	1	2	2	-	1	1	-	1	-	1
			33A	1	-	-	-	-	-	-	-	-	-
			34	-	-	1	1	1	-	1	-	-	-
			35F	-	1	2	2	2	4	1	-	2	1
			35B	1	1	1	-	-	1	-	1	3	1
			37	-	-	-	-	1	-	1	2	1	-
			38	1	1	-	1	3	1	-	2	1	-
			Rough (n.t.)	-	-	-	-	-	-	-	-	-	-
			Total	235	212	243	190	190	176	163	138	138	142

Table 6.7 Distribution of *S. pneumoniae* from blood only (from the 9 sentinel laboratories), according to serotype, 2006-2014

		TYPE	2006	2007	2008	2009	2010	2011	2012	2013	2014
			Year								
23-valent vaccine	10-valent vaccine	7-valent	4	52	54	30	26	17	27	11	13	6
			6B	21	26	25	12	8	3	3	3	3
			9V	65	53	42	26	21	5	2	4	1
			14	86	84	54	34	22	19	12	8	2
			18C	12	13	15	15	7	8	4	8	2
			19F	19	11	9	10	5	9	3	5	7
			23F	29	39	13	12	13	5	3	1	2
			Subtotal 7-valent vaccine	*284*	*280*	*188*	*135*	*93*	*85*	*38*	*42*	*23*
			1	25	75	64	65	53	40	50	40	41
			5	-	3	2	6	7	11	8	9	2
			7F	75	55	65	86	72	91	92	75	53
			Subtotal 10-valent vaccine	*384*	*413*	*319*	*292*	*225*	*227*	*188*	*166*	*119*
			2	-	-	-	-	-	-	-	-	-
			3	32	30	31	34	30	36	45	40	31
			8	42	47	46	52	60	59	88	108	93
			9N	19	13	19	18	19	17	20	19	21
			10A	6	4	7	9	9	14	8	6	16
			11A	6	16	3	12	12	9	14	16	8
			12F	9	5	6	5	13	19	25	22	28
			15B	5	1	4	6	7	4	1	7	7
			17F	1	3	1	7	4	8	7	4	8
			19A	21	25	33	30	57	63	78	61	44
			20	2	3	3	3	3	4	-	1	4
			22F	19	18	24	24	29	37	41	45	34
			33F	10	6	10	11	10	15	22	12	12
			Subtotal 23-valent vaccine	*556*	*584*	*506*	*503*	*478*	*503*	*537*	*507*	*425*
			6A	7	10	18	11	9	2	6	2	-
			6C	-	2	1	7	9	7	10	10	7
			7C	2	1	-	-	-	-	-	-	-
			9A	-	-	-	-	-	-	1	-	1
			10F	-	-	1	-	-	-	-	-	1
			10B	-	-	-	-	-	-	-	1	-
			11B	-	1	-	-	-	-	-	-	-
			12A	-	-	-	-	-	-	-	-	-
			13	-	-	-	-	-	1	-	-	-
			15F	-	-	-	-	-	-	-	1	-
			15A	-	1	1	1	-	2	7	13	14
			15C	-	1	2	2	1	2	1	4	4
			16F	6	6	9	8	10	7	6	7	5
			17A	-	-	-	-	-	2	-	-	-
			18F	-	-	-	-	-	-	-	-	-
			18A	-	1	-	1	1	1	-	-	-
			18B	-	1	-	-	-	-	1	1	-
			21	-	-	-	-	-	-	-	2	1
			22A	3	2	1	-	1	1	-	1	-
			23A	2	6	3	9	7	2	6	6	7
			23B	1	1	3	6	3	9	3	6	15
			24F	1	1	7	-	2	3	2	4	4
			25F	1	-	1	-	-	-	-	-	-
			27	-	-	1	1	-	1	-	1	-
			28A	-	-	-	-	-	-	-	-	-
			29	-	-	-	-	-	-	1	-	-
			31	1	1	3	1	4	2	6	2	2
			33A	-	-	-	-	-	-	1	-	-
			34	1	1	-	1	1	-	1	2	1
			35F	2	1	2	4	5	6	5	6	7
			35A	-	-	-	-	-	-	1	-	-
			35B	3	-	-	4	-	3	1	7	6
			37	-	1	-	-	1	-	-	-	1
			38	3	2	3	5	-	3	-	1	2
			40	-	-	-	-	-	-	-	1	-
			Rough (n.t.)	2	-	-	-	-	2	-	-	-
			Total	591	624	562	564	532	559	596	585	503

Table 6.8 Distribution of *S. pneumoniae* isolates from CSF (or CSF and blood) nation-wide, by serotype and age of patients, 2014.

		TYPE	AGE (YEARS) 0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-64	65-79	≥80	Total	%
23-valent vaccine	10-valent vaccine	7-valent vaccine 4	-	-	-	-	-	-	-	1	1	-	2	**1.4**
		6B	-	-	-	-	-	-	-	-	-	-	-	**-**
		9V	-	-	-	-	-	-	-	-	1	-	1	**0.7**
		14	-	-	-	-	-	-	-	-	-	-	-	**-**
		18C	-	-	-	-	-	-	-	-	-	-	-	**-**
		19F	-	2	-	-	-	-	-	2	-	-	4	**2.8**
		23F	-	-	-	-	-	-	-	-	-	-	-	**-**
		Subtotal 7-valent vaccine	-	*2*	-	-	-	-	-	*3*	*2*	-	*7*	**4.9**
		1	-	2	-	-	-	-	1	-	1	-	4	**2.8**
		5	-	-	-	-	-	-	-	-	-	-	-	**-**
		7F	-	1	-	-	-	-	1	2	2	2	8	**5.6**
		Subtotal 10-valent vaccine	-	*5*	-	-	-	-	*2*	*5*	*5*	*2*	*19*	**13.4**
		2	-	-	-	-	-	-	-	-	-	-	-	**-**
		3	-	1	-	-	-	-	3	3	4	2	13	**9.2**
		8	1	-	1	-	1	-	2	11	7	-	23	**16.2**
		9N	-	-	-	-	-	-	1	2	2	1	6	**4.2**
		10A	5	-	-	-	-	-	1	4	2	-	12	**8.5**
		11A	-	-	-	-	-	-	-	2	1	-	3	**2.1**
		12F	-	-	-	-	-	1	2	3	2	-	8	**5.6**
		15B	-	-	-	-	-	-	-	-	-	-	-	**-**
		17F	-	-	-	-	-	-	-	-	1	-	1	**0.7**
		19A	2	-	-	-	-	-	1	2	2	-	7	**4.9**
		20	-	-	-	-	-	-	-	-	1	-	1	**0.7**
		22F	-	-	-	-	1	-	1	3	3	-	8	**5.6**
		33F	1	-	-	-	-	-	-	1	-	-	2	**1.4**
		Subtotal 23-valent vaccine	*9*	*6*	*1*	*-*	*2*	*1*	*13*	*36*	*30*	*5*	*103*	**72.5**
		Other	7	1	1	-	-	4	1	13	10	2	39	**27.5**
		Total	16	7	2	-	2	5	14	49	40	7	142	**100.0**

7 *ESCHERICHIA COLI*

The Reference Laboratory received 32 *Escherichia coli* strains, 8 isolated from CSF (or CSF and blood) and 24 from blood only (table 7.1, 7.2 and 7.3). The number of *E. coli* isolates from CSF was dubbled since 2012 (figure 7.1). Seventy-five percent of the cases of *E. coli* meningitis occurred in the first month of life.

Interestingly, the types O non typable, O8, O15, O21, O73, O87, O88 and O107 are prevalent among non-K1 isolates, while the types O non typable, O1, O2, O18, O23, O25, O68, O70, O113 and O117 are more often found among K1 isolates.

Table 7.1 Serotypes of *E. coli* isolates from CSF and/or blood, by age of patients, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	11	1	-	12	-	-	-	-	**12**	***38***
K1	13	7	-	20	-	-	-	-	**20**	***62***
Total	**24**	**8**	**-**	**32**	**-**	**-**	**-**	**-**	**32**	***100***
%	***75***	***25***	***0***	***100***	***0***	***0***	***0***	***0***	***100***	

Table 7.2 Serotypes of *E. coli* isolates from CSF (or CSF and blood), by age of patients, 2014

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

Table 7.3 Serotypes of *E. coli* isolates from blood only by age of patients, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
Non K1	11	1	-	12	-	-	-	-	**12**	***50***
K1	9	3	-	12	-	-	-	-	**12**	***50***
Total	**20**	**4**	**-**	**24**	**-**	**-**	**-**	**-**	**24**	***100***
%	***83***	***17***	***0***	***100***	***0***	***0***	***0***	***0***	***100***	

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

Table 7.4 H-type versus K-type of *E. coli* isolates from CSF and/or blood, 2014

TYPE	K1	Non K1	Total
H1	1	0	**1**
H4	5	0	**5**
H6	0	3	**3**
H7	11	0	**11**
H10	0	3	**3**
H18	1	1	**2**
H19	0	2	**2**
H31	1	0	**1**
H38	0	1	**1**
H-rough	0	1	**1**
H-	1	0	**1**
Total	**20**	**12**	**32**
%	***63***	***37***	***100***

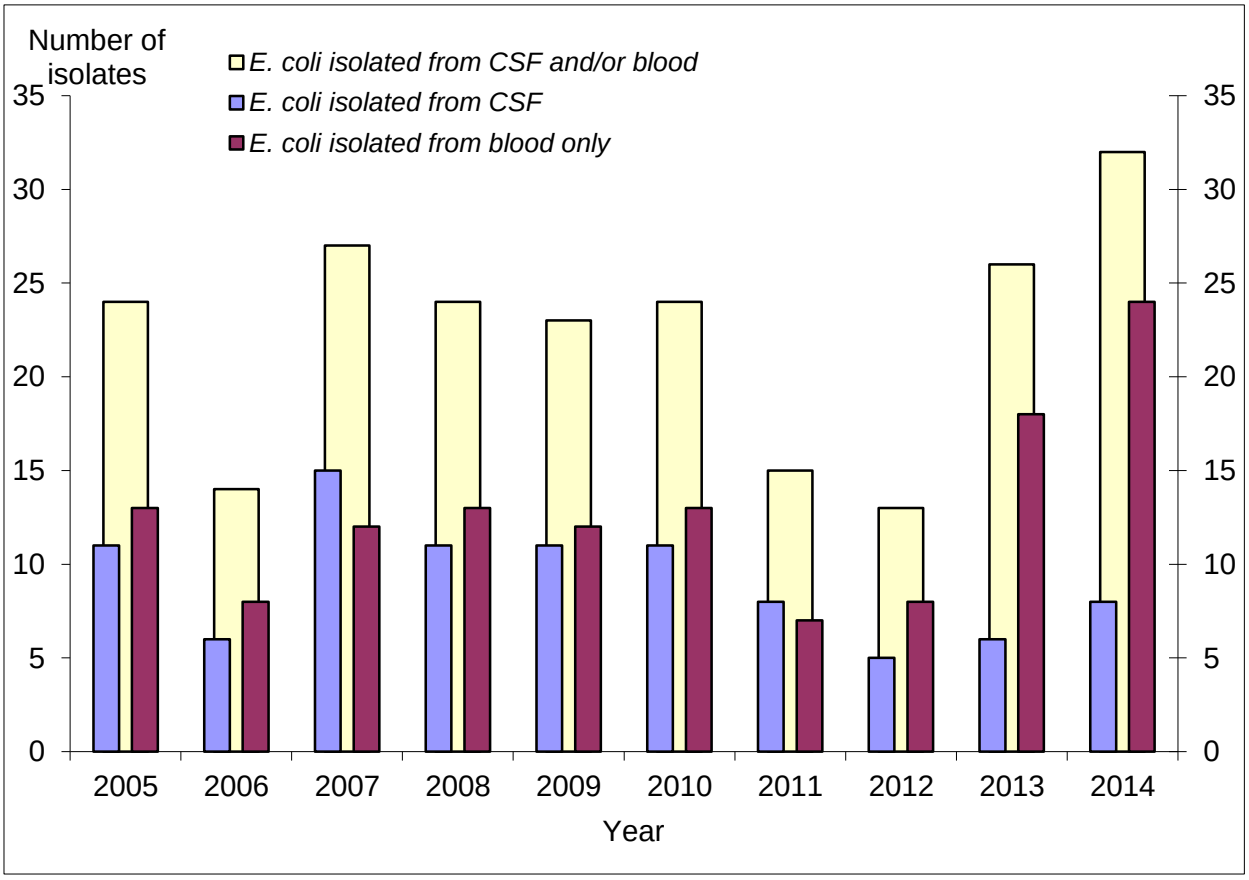

Figure 7.1 *Distribution of E. coli, 2005-2014*

8 *STREPTOCOCCUS AGALACTIAE* – (group B)

In 2014 the Reference Laboratory received 71 *Streptococcus agalactiae* isolates, similar to that in the previous year (2014: 71; 2013: 72; 2012: 80; 2010: 63, figure 8.1). Twenty-three *S. agalactiae* isolates were from CSF (or CSF and blood) and 48 from blood only (table 8.1, 8.2 and 8.3). Eighty-seven 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, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	5	1	-	6	-	-	-	-	**6**	***8.5***
Ib	3	-	-	3	-	-	-	-	**3**	***4***
II	5	-	-	5	-	-	-	-	**5**	***7***
III	44	5	-	49	-	-	1	-	**50**	***70***
IV	1	1	-	2	-	-	-	-	**2**	***3***
n.t.	-	-	-	-	-	-	-	1	**1**	***1.5***
V	2	-	-	2	-	-	-	-	**2**	***3***
VI (NT6)	2	-	-	2	-	-	-	-	**2**	***3***
Total	**62**	**7**	**0**	**69**	**0**	**0**	**1**	**1**	**71**	***100***
%	***87***	***10***	***0***	***97***	***0***	***0***	***1.5***	***1.5***	***100***	

Table 8.2 Serotypes of *S. agalactiae* isolates from CSF (or CSF and blood), by age of patients, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	-	1	-	1	-	-	-	-	**1**	***4***
Ib	-	-	-	-	-	-	-	-	**-**	***0***
II	-	-	-	-	-	-	-	-	**-**	***0***
III	15	3	-	18	-	-	1	-	**19**	***84***
IV	-	-	-	-	-	-	-	-	**-**	***0***
n.t.	-	-	-	-	-	-	-	1	**1**	***4***
V	1	-	-	1	-	-	-	-	**1**	***4***
VI (NT6)	1	-	-	1	-	-	-	-	**1**	***4***
Total	**17**	**4**	**-**	**21**	**-**	**-**	**1**	**1**	**23**	***100***
%	***87***	***10***	***0***	***97***	***0***	***0***	***1.5***	***1.5***	***100***	

Table 8.3 Serotypes of *S. agalactiae* isolates from blood only, by age of patients, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	Total	%
Ia	5	-	-	5	-	-	-	-	**5**	***10***
Ib	3	-	-	3	-	-	-	-	**3**	***6***
II	5	-	-	5	-	-	-	-	**5**	***10***
III	29	2	-	31	-	-	-	-	**31**	***66***
IV	1	1	-	2	-	-	-	-	**2**	***4***
n.t.	-	-	-	-	-	-	-	-	**-**	**-**
V	1	-	-	1	-	-	-	-	**1**	***2***
VI (NT6)	1	-	-	1	-	-	-	-	**1**	***2***
Total	**45**	**3**	**-**	**48**	**-**	**-**	**-**	**-**	**48**	***100***
%	***94***	***6***	**-**	***100***	**-**	**-**	**-**	**-**	***100***	

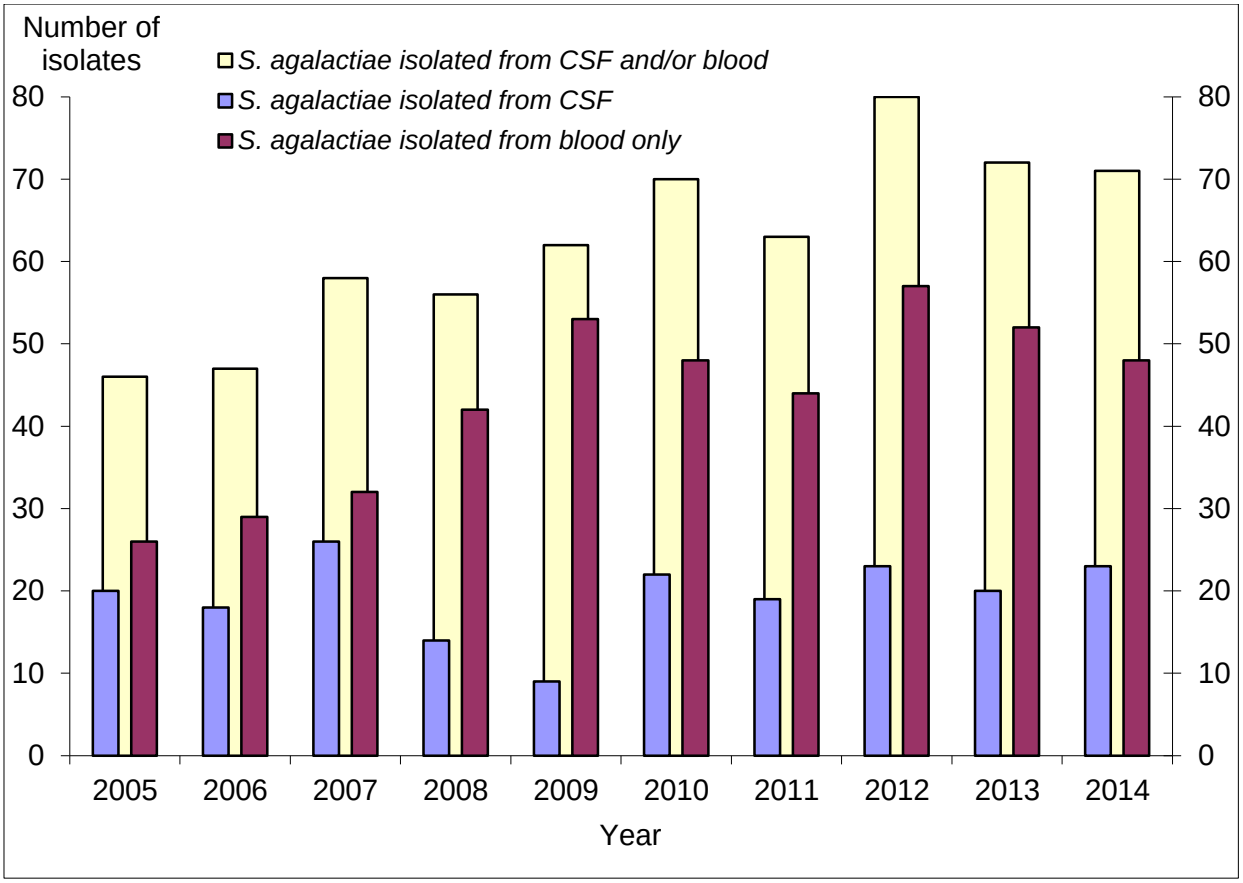

Figure 8.1 *Distribution of S. agalactiae, 2005-2014*

9 *LISTERIA MONOCYTOGENES*

Seventy strains of *Listeria monocytogenes* were submitted to the Reference Laboratory. Nineteen isolates were from CSF (or CSF and blood) and 51 from blood only (figure 9.1). (2013: 6 CSF and 46 blood only). Most cases (92%) occurred among persons older than 50 years. In 2014 (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, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2	-	-	-	-	-	-	-	1	**1**	***1***
1/2a	-	-	-	-	-	-	1	24	**25**	***36***
1/2b	-	-	-	-	-	-	1	10	**11**	***16***
1/2c	-	-	-	-	-	-	-	3	**3**	***4***
4b	2	-	-	2	-	1	1	26	**30**	***43***
Total	**2**	**-**	**-**	**2**	**-**	**1**	**3**	**64**	**70**	***100***
%	***3***	**-**	**-**	***3***	**-**	***1***	***4***	***92***	***100***	

Table 9.2 *L. monocytogenes* isolates from CSF (or CSF and blood), by type and age, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2	-	-	-	-	-	-	-	-	-	**-**
1/2a	-	-	-	-	-	-	-	6	**6**	***32***
1/2b	-	-	-	-	-	-	-	3	**3**	***16***
1/2c	-	-	-	-	-	-	-	-	**-**	**-**
4b	-	-	-	-	-	1	-	9	**10**	***52***
Total	**-**	**-**	**-**	**-**	**-**	**1**	**-**	**18**	**19**	***100***
%	**-**	**-**	**-**	**-**	**-**	***5***	**-**	***95***	***100***	

Table 9.3 *L. monocytogenes* isolates from blood only, by serotype and age, 2014

TYPE	AGE (MONTHS)			AGE (YEARS)					TOTAL	
	0	1-11	12-59	0-4	5-9	10-19	20-49	≥50	T	%
1/2	-	-	-	-	-	-	-	1	**1**	***2***
1/2a	-	-	-	-	-	-	1	18	**19**	***37***
1/2b	-	-	-	-	-	-	1	7	**8**	***16***
1/2c	-	-	-	-	-	-	-	3	**3**	***6***
4b	2	-	-	2	-	-	1	17	**20**	***39***
Total	**2**	**-**	**-**	**2**	**-**	**-**	**3**	**46**	**51**	***100***
%	***4***	**-**	**-**	***4***	**-**	**-**	***6***	***90***	***100***	

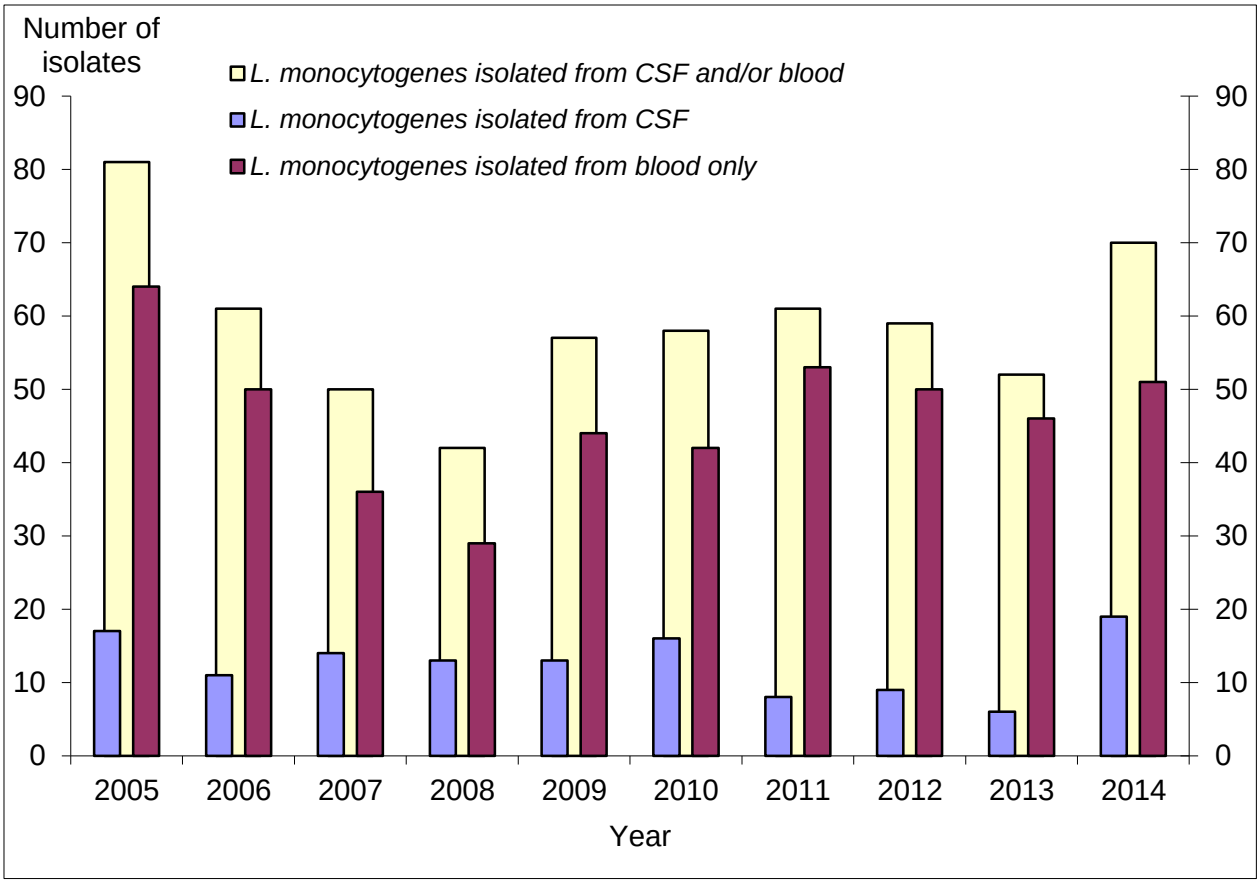

Figure 9.1 *Distribution of L. monocytogenes, 2005-2014*

10 *STREPTOCOCCUS PYOGENES*

Eight *Streptococcus pyogenes* isolates were submitted to the Reference Laboratory, 2 isolated from CSF (or CSF and blood) and 6 from blood only. These numbers are lower than those in the previous year, but similar to those in the years before 2013.

Table 10.1 *S. pyogenes* isolates from CSF and/or blood received in 2014 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	-	-	1	1	-	-	-	1	**2**	***25***
Blood	-	1	1	2	1	-	1	2	**6**	***75***
Total	**-**	**1**	**2**	**3**	**1**	**-**	**1**	**3**	**8**	***100***
%	**-**	***12.5***	***25***	***37.5***	***12.5***	**-**	***12.5***	***37.5***	***100***	

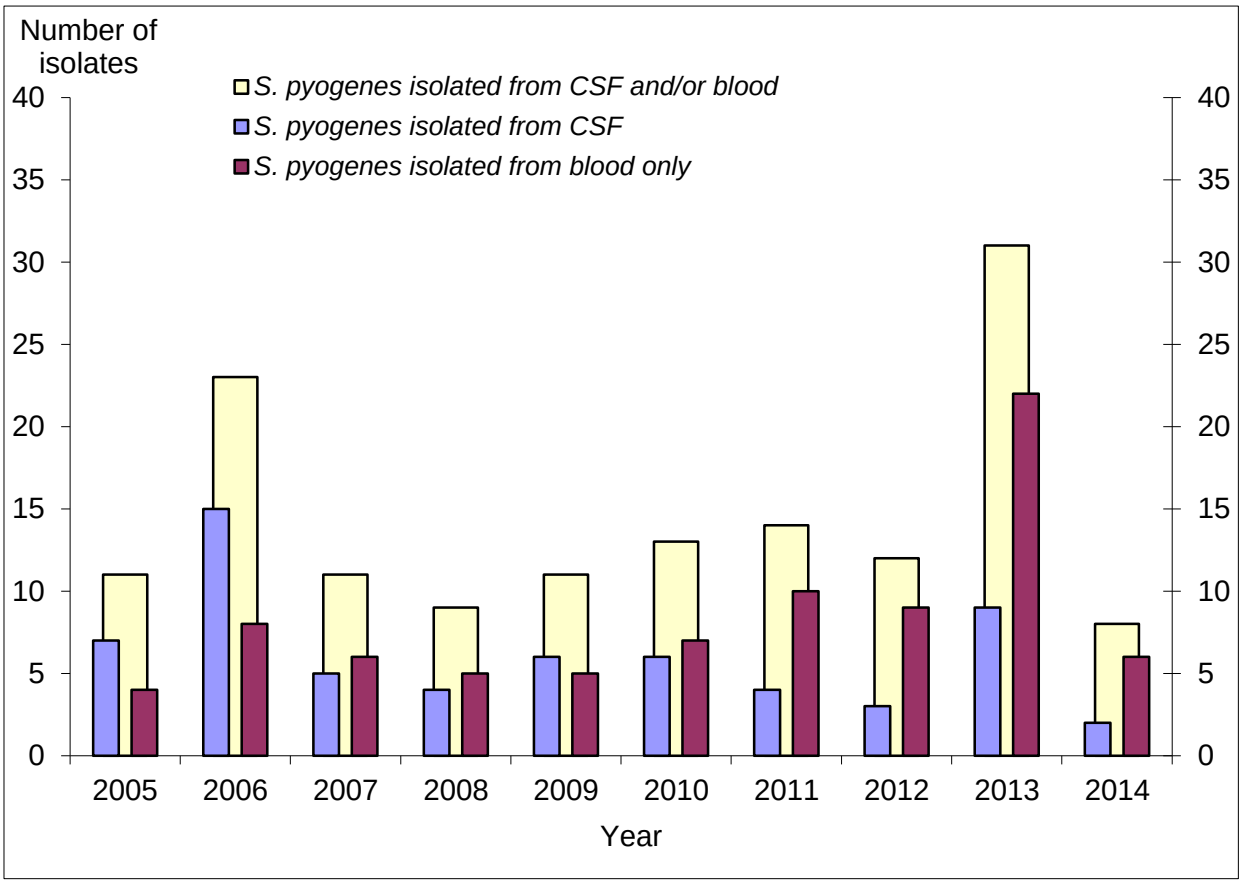

Figure 10.1 *Distribution of S. pyogenes, 2005-2014*

11 ANTIGEN AND DNA DETECTION

The Reference Laboratory received 155 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 155 specimens, 38 (25 %) were positive by agglutination or PCR. Thirteen (12 CSF and 1 DNA sample isolated from a skinbiopsy) were positive for *N. meningitidis* and 21 (18 CSF, 2 serum and 1 other) were positive for *S. pneumoniae*.

Thus, in 2014, PCR-positive, culture-negative CSF samples accounted for 22 % of cases of meningococcal meningitis registered in the database of the Reference Laboratory. For *S. pneumoniae*, this percentage was 9%.

Table 11.1 CSF and serum samples, tested for antigens or DNA, 2014

Antigen of	CSF (or CSF and SERUM)	SERA	OTHER	TOTAL
C. neoformans	1	3	0	**4**
H. influenza type b	0	0	0	**0**
DNA of				
N. meningitidis	1	0	0	1
N. meningitidis group B	10	0	1	11
N. meningitidis group W	1	0	0	1
S. pneumoniae	18	2	1	21
Sub Total	**31***	**5**	**2**	**38**
Antigen and PCR negative	106	8	3	**117**
Total	**137**	**13**	**5**	**155**

* From 6 patients with a *S. pneumoniae* isolated from blood, the CSF was culture-negative but PCR-positive for pneumococcal DNA.
From 2 patients with a *N. meningitidis* isolated from blood, the CSF was culture-negative but PCR-positive for meningococcal DNA.

12 VACCINATION PROSPECTS

12.1 N. meningitidis

In the Netherlands, vaccination against serogroup C meningococcal disease has been introduced in June, 2002. All children born on or after June 1st, 2001 are vaccinated at the age of 14 months as part of the regular National Immunisation Programme. In addition, between June, 2002 and October, 2002 children and adolescents from 14 months to 19 years have been vaccinated. In 2014, 3 cases of meningococcal disease (4.1% of all cases, table 4.4) were due to serogroup C meningococci (2013: 5.4%; 2012: 2.5%; 2011: 3.3%; 2010: 4.5%; 2009: 6.5%; 2002: 36%). All three patients were not vaccinated, two because of age (50 and 70 years of age) and one because of nationality (Poland). This indicates that the vaccination programme is successful. (figure 12.1)

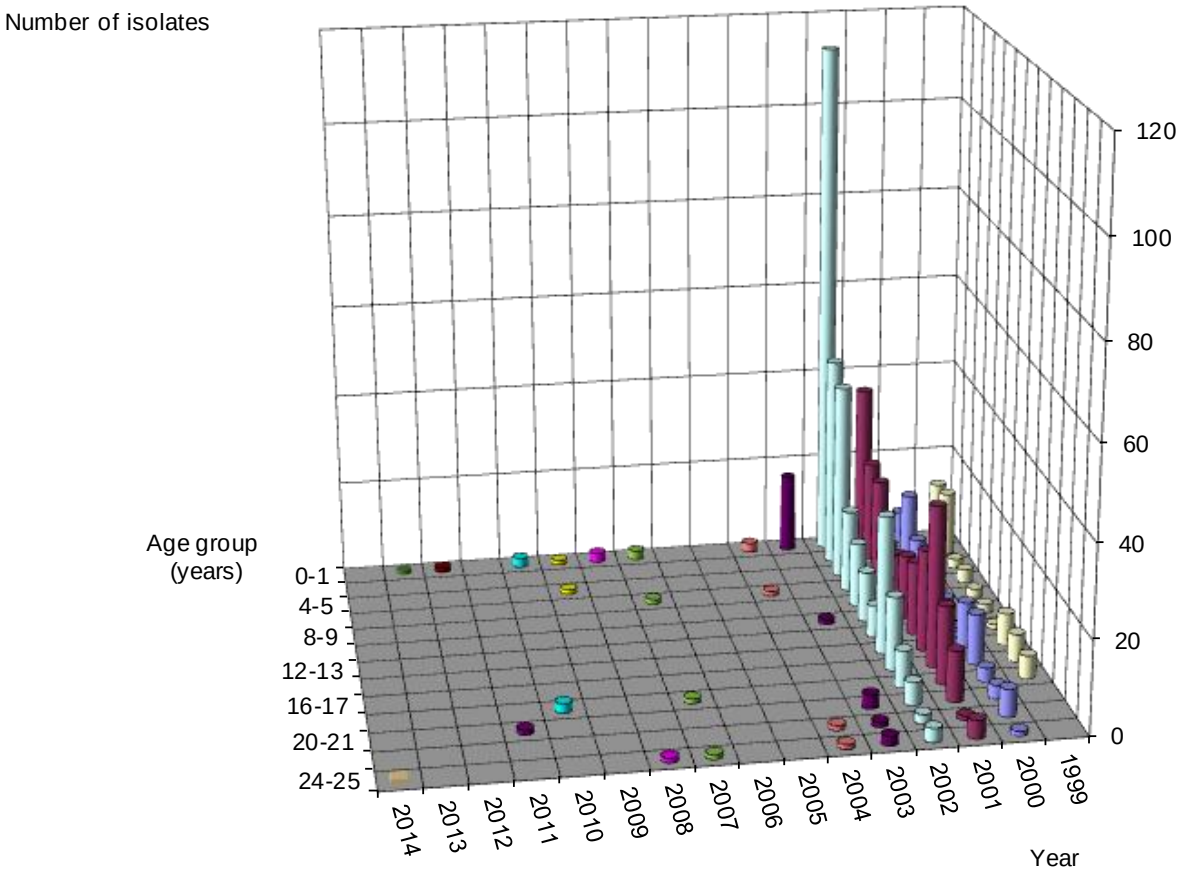

Figure 12.1 *Age distribution of N.meningitidis serogroup C invasive disease in the first 24 years of life, 1999-2014.*

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 49 cases (93%; table 4.9) of serogroup B meningococcal disease and 61 (84%) of all 73 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 2014, the number of invasive isolates of *H. influenzae* type b, received from patients that should have been vaccinated (<21 years of age) decreased from 14 to 12 in 2014 (2013: 14; 2012: 11; 2011: 7; 2010: 10 and 2009: 10) (figure 12.2 and 12.3). Of those 12 patients, six had received all doses and one received only three doses of the vaccine. Four patients (5 cases) were not vaccinated, two of them because of age.

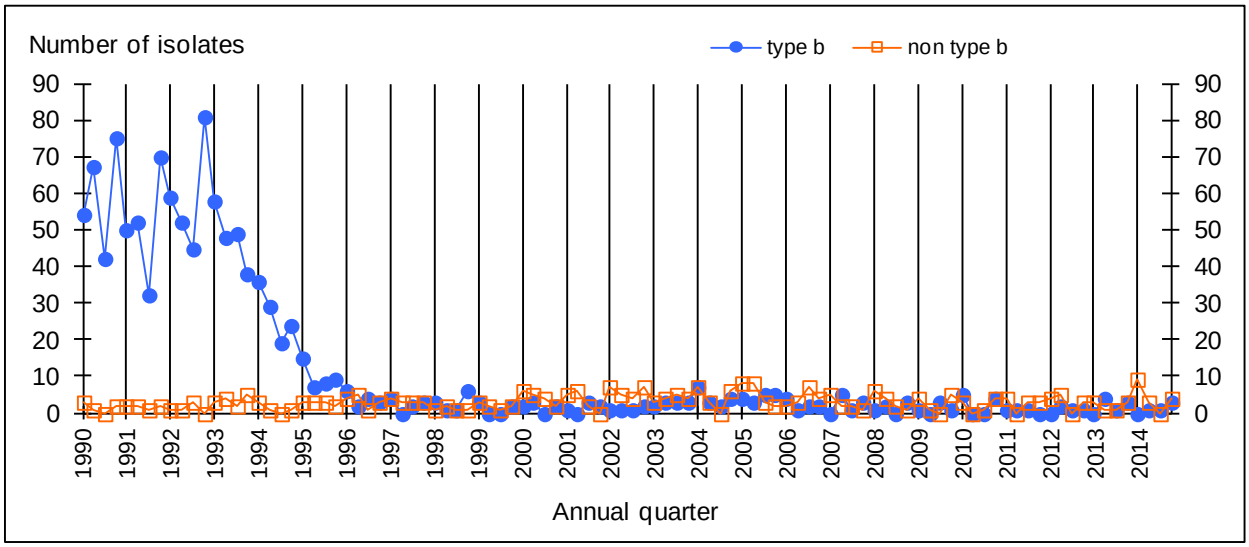

Figure 12.2 *The distribution of H. influenzae type b and non-type b meningitis cases according to annual quarter, 1990 –2014*

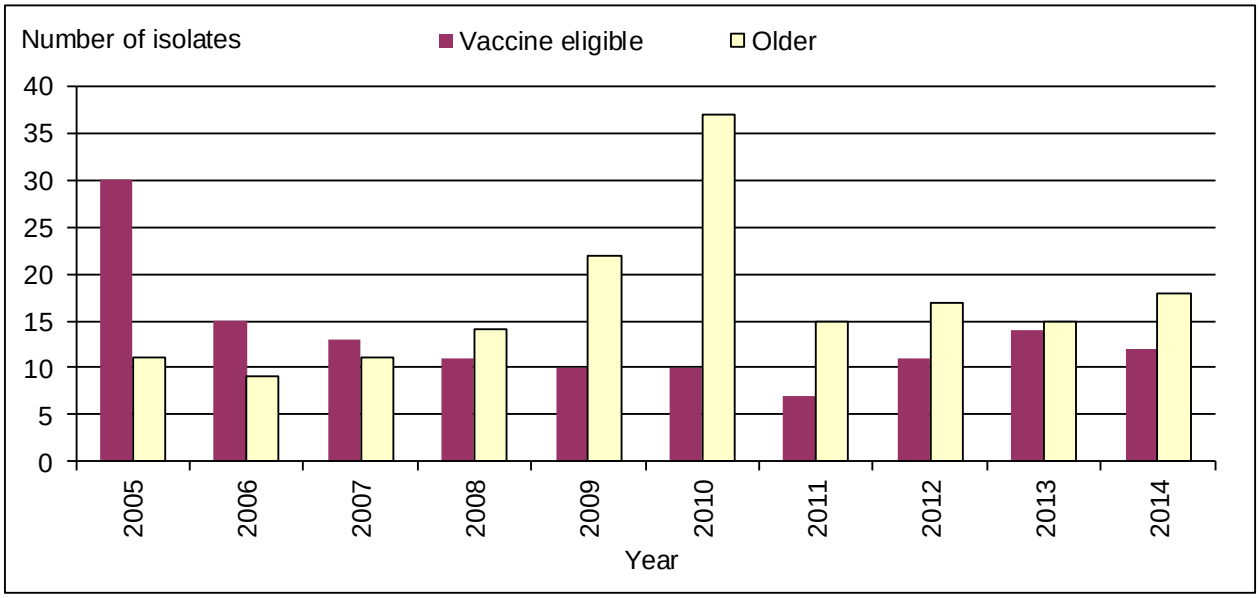

Figure 12.3 *The distribution of H. influenzae type b cases (CSF or blood) among patients eligible for vaccination and among older patients, 2005 –2014*

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 2014, five percent of the CSF isolates were of a serotype covered by this hepta-valent conjugate polysaccharide vaccine, while 13% of the isolates were covered by the 10-valent vaccine (table 6.6). In 2014 the proportion of CSF isolates with a PVC7 serotype was lower than that in previous years (2014: 5%; 2013: 5%; 2012:11% ; 2011:12%; 2010: 12%; 2009: 18%; 2008: 35%; 2007: 42%; 2006: 56%; 2005: 46%; 2004: 53%; 2003: 52%), as a result of the vaccination. There were 7 patients with invasive pneumococcal disease due to pneumonococci with a vaccine (PVC7) serotype (4, 9V and 19F). One case of serotype 19F invasive pneumococcal disease, was 5 years of age, who received four doses of PVC7. The remaining 6 cases were not vaccinated because of age (9, 55, 62, 63, 72 and 76 yr). There was one child with an invasive disease due to *S. pneumoniae* with a (PVC10) vaccine serotype (7F). This child received four doses of PVC10. 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-three 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 2.1%) (table 6.6) (2013: 71%; 2012: 77%; 2011: 87%; 2010: 84%; 2009: 85%; 2008: 89%; 2007: 90%).

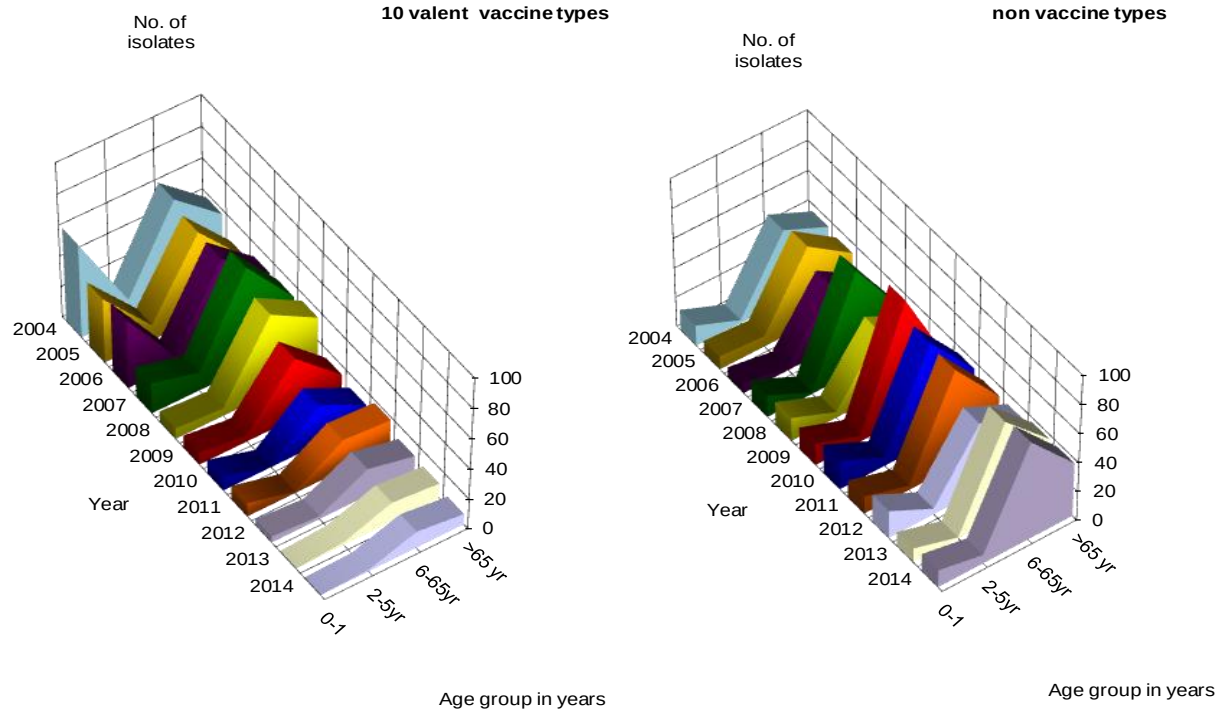

Figure 12.4 *The age distribution of S.pneumoniae invasive disease due to pneumococci of serotypes included in the hepta-valent conjugated polysaccharide vaccine, 2004-2014. Left: vaccine types. Right: types not included in this vaccine*

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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
- Dr. D. Notermans (Laboratory for Infectious Diseases and Perinatal Screening (LIS), RIVM) for typing of *E. coli* and *S. pyogenes*
- 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