

Invasive group B streptococcal disease in infants: a 19-year nationwide study. Serotype distribution, incidence and recurrent infection

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SUMMARY

During the period 1984–2002, 472 cases of invasive group B streptococcal (GBS) disease in infants aged 0–90 days in Denmark were registered. The overall incidence was 0·4/1000 live births. Most infants (73%) had early-onset GBS infection with 53% registered within the first day. Serotype III predominated (59%) with other serotypes as follows: Ia (16%), Ib (8%), NT (7%), II (6%), other serotypes (5%). Recurrence of GBS infection was registered in six infants, and the interval with no antibiotic therapy varied from 2 to 39 days. The serotypes of the isolates obtained from first and second episodes were identical (serotype III in five, and serotype Ia in one infant). Paired isolates were indistinguishable by PFGE and antibiotic susceptibility testing. Invasive GBS infections in infants are still a problem in Denmark, and recurrent infections are registered in 1% of these infants.

INTRODUCTION

Group B streptococcus (GBS) (*Streptococcus agalactiae*) has been one of the most frequent causes of infectious mortality and morbidity in infants younger than 90 days since the 1970s. The majority of the infections are early-onset disease (EOD) (age 0–6 days), where the infant acquires GBS from the mother via transmission *in utero* or in the birth canal [1, 2]. The most common clinical manifestations of EOD are pneumonia and septicaemia. Meningitis is more common in late-onset disease (LOD) (age 7–90 days), when GBS can be transferred from nosocomial or community sources [2], although mothers of infants with LOD often carry the same GBS serotype as that causing infection in their infants [2].

Recurrence of invasive GBS disease in infants is uncommon but has been described in other case series

[3–16]. In this study we estimated the frequency of recurrent GBS infections in infants (age 0–90 days) in Denmark in a 19-year period from 1984 to 2002 and we describe the clinical presentations of these infections. In the near future introduction of GBS serotype-specific vaccines directed towards fertile women may be a possible prophylactic strategy against invasive GBS infections in infants through transmission of protective antibodies to the foetus. We present the serotype distribution and incidences of the early- and late-onset GBS infections in infants registered from 1984 to 2002 in Denmark.

METHODS

Bacterial isolates

The Streptococcus Unit, Statens Serum Institut, Denmark, serves as the National Streptococcus Reference Centre and receives the vast majority of invasive GBS isolates as pure cultures from local clinical microbiology departments for the purposes

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of national surveillance and serotyping. Information concerning the infants' age and gender were received together with clinical isolates. Included in this study were GBS isolates from blood and cerebrospinal fluid (CSF) collected from infants younger than 90 days admitted to Danish hospitals from 1 January 1984 to 31 December 2002. Local clinical microbiology departments submit blood and CSF isolates to the Streptococcus Unit as part of routine surveillance, but other sterile site isolates are not submitted as part of this routine. Not included in this paper were 11 GBS isolates from lung, synovial fluid and tissue from a hip received by the Streptococcus Unit during the study period.

After confirmatory identification of the group B antigen, all isolates were serotyped by precipitin test as described by Lancefield [17] with specific anti-capsular-polysaccharide rabbit antibodies (Ia, Ib and II–VIII; Statens Serum Institut, Copenhagen, Denmark). Non-serotypable isolates were designated NT. Capsular antigens were extracted with hydrochloric acid (HCl), and during the period 1984–1998 an extract concentration of 0.2 N HCl was used for serotyping. Since January 1999 all GBS isolates received were serotyped using both 0.1 and 0.2 N HCl because of observation of false-negative results by the standard Lancefield extraction method [18]; isolates were then stored at -80°C .

Recurrent episodes

A recurrent episode was defined as a case where two isolates from the same infant were registered at the Streptococcus Unit and it was possible to identify an interval without antibiotic therapy between the first and second episode and a clinical recovery after the first episode. The infants' hospital files relating to the recurrent episode were read. Ten infants had two separate isolates registered at the Streptococcus Unit from two different dates. Based on the hospital files, four infants were excluded because there was no clinical recovery after the first treatment or because there was not an antibiotic-free interval.

Pulsed field gel electrophoresis (PFGE)

The six isolates from the three patients with recurrent infections after January 1999 were analysed by PFGE as described elsewhere [19], supplementing the lysis buffer with 22 $\mu\text{l/ml}$ mutanolysin (Sigma, St. Louis, MO, USA) [20]. PFGE patterns were

considered to be identical if there was no band difference.

Antibiotic susceptibility tests

Minimal inhibitory concentrations (MICs) were determined on the same six isolates using E-test strips (AB Biodisk, Solna, Sweden) on Mueller–Hinton agar with 5% lysed horse blood (Becton Dickinson, Microbiology Systems, Cockeysville, MD, USA) towards penicillin G, ampicillin and gentamicin. Break-points were defined according to National Committee for Clinical Laboratory Standards (NCCLS) [21] and the Swedish Reference Group for Antibiotics (SRGA) [22]. Time-kill curves were performed towards penicillin G and ampicillin with concentrations 4 and 16 times the MIC and towards gentamicin with a concentration twice the MIC. Each isolate was inoculated with no antibiotic as a growth control. By spectrophotometry the absorbance at 620 nm was determined every 15 min and growth and kill of the bacteria were detected [23].

Statistical analysis

Fisher's exact test was used for statistical analysis. A *P* value of <0.05 was considered significant. The SAS System, release 8.02 (SAS Institute Inc., Cary, NC, USA) was used for all analysis. The annual number of inhabitants and the gender distribution in the period 1984–2002 were obtained from Statistics Denmark.

RESULTS

Invasive GBS infections

During the period 1984–2002 Statens Serum Institut registered 472 infants (0–90 days) with invasive GBS infections; 209 infections (44%) were in girls and in 2% the gender was not recorded. The average incidence was 0.4/1000 live births over the review period, increasing from 0.1/1000 live births in 1984 to 0.8/1000 in 1995. In 2002 the incidence decreased to 0.3/1000 live births (Table 1). The age of affected infants on the date of the sample ranged from 0–87 days (median 1 day). Most (71%) of the neonatal invasive GBS infections were EOD and 53% of these infants had an infection with GBS on the first day of life. The incidences of EOD and LOD were 0.3 and 0.1/1000 live births respectively. The median

Table 1. *Invasive GBS disease in infants (0–90 days) in Denmark 1984–2002 (numbers and incidences)*

Year	Live births	EOD (0–6 days)		LOD (7–90 days)		Total	
		<i>n</i>	Incidence*	<i>n</i>	Incidence*	<i>n</i>	Incidence*
1984	51 800	4	0.1	1	0.0	5	0.1
1985	53 749	18	0.3	7	0.1	25	0.5
1986	55 312	9	0.2	3	0.1	12	0.2
1987	56 221	14	0.2	6	0.1	20	0.4
1988	58 844	11	0.2	3	0.1	14	0.2
1989	61 351	13	0.2	4	0.1	17	0.3
1990	63 433	12	0.2	6	0.1	18	0.3
1991	64 358	9	0.1	1	0.0	10	0.2
1992	67 726	19	0.3	11	0.2	30	0.4
1993	67 369	34	0.5	13	0.2	47	0.7
1994	69 666	30	0.4	10	0.1	40	0.6
1995	69 771	44	0.6	9	0.1	53	0.8
1996	67 638	29	0.4	5	0.1	34	0.5
1997	67 640	15	0.2	13	0.2	28	0.4
1998	66 170	22	0.3	5	0.1	27	0.4
1999	66 232	9	0.1	13	0.2	22	0.3
2000	67 081	18	0.3	10	0.1	28	0.4
2001	65 485	13	0.2	7	0.1	20	0.3
2002	64 153	15	0.2	7	0.1	22	0.3
Total	12 03 999	338	0.3	134	0.1	472	0.4

EOD, Early-onset disease; LOD, late-onset disease.

* Incidence is calculated as *n* per 1000 live births.

age of the infants in the EOD group was 0 days (mean 1 day), while the infants with LOD had a median age of 22 days (mean 29 days). Meningitis was documented in 54 infants (16%) with EOD and in 53 (40%) with LOD ($P < 0.0001$). The outcome of the GBS infections was registered from 1999 to 2002. Eight infants died, resulting in a mortality rate of 9% (range 5–14%) in the 4-year period. Half of the infants with a fatal outcome were female, and half had EOD. Type III was identified in 63% of infants. The age distribution in the infants with fatal outcome paralleled that of the entire group of affected infants.

Invasive GBS isolates

The Streptococcus Unit received 512 isolates from blood and CSF from the 472 infants. The annual number of GBS isolates sent to the Streptococcus Unit varied from six in 1984 to 55 in 1995. In 2002, the Statens Serum Institute received 22 isolates from infants with invasive GBS disease. Over the whole study period, isolates from blood and CSF comprised 71% and 13% respectively, of referred strains. Forty isolates (8%) were from both blood and CSF.

The most frequent serotypes regardless of age were serotype III (57%, range 41–83%), Ia (17%,

range 0–32%), Ib (7%, range 0–20%), NT (6%, range 0–33%) and II (6%, range 0–15%) (Table 2). Other serotypes [IV, V and VI accounted for 5% (range 0–11%)]. Serotypes VII and VIII were not identified from blood or CSF (Fig.). There was no difference between the sexes in the serotype distribution. Blood and CSF isolates from cases of EOD were equally distributed regarding the serotypes Ia, IV and V. Serotypes II and NT appeared only among isolates from blood whereas serotype III was isolated relatively more frequently from CSF than from blood ($P < 0.01$). In three cases with a fatal outcome GBS was identified in both blood and CSF. The remaining isolates were from blood. In 1999 all the isolates received were retested (using both 0.1 and 0.2 N HCl to extract the capsular antigens); as a result, three isolates initially determined as NT were re-assigned to serotype III. During 2000–2002 two, none and a single isolate respectively were designated NT despite the use of a higher concentration of HCl.

Recurrent invasive GBS disease in infants

Recurrence of GBS disease in infants was documented in 6 of the 472 infants from four different hospitals in Denmark in the study period. Three of

Table 2. Invasive GBS isolates from infants in Denmark 1984–2002

Specimen ...	Early onset						Late onset						Total	Per cent of total
	Blood			CSF*			Blood			CSF*				
	F	M	U	F	M	U	F	M	U	F	M	U		
Ia	29	31	0	4	5	1	9	5	1	6	3	1	95	19
Ib	16	11	0	0	2	0	0	4	1	0	3	0	37	7
II	9	17	0	0	0	0	2	1	0	0	0	0	29	6
III	64	79	3	21	15	2	27	44	0	20	19	0	294	57
IV	0	2	0	0	1	0	0	1	0	0	1	0	5	1
V	9	7	0	2	1	0	1	0	1	0	0	0	21	4
NT‡	8	19	1	0	0	0	1	2	0	0	0	0	31	6
Total	135	166	4	27	24	3	40	57	3	26	26	1	512	100

Distribution of serotypes by onset, specimen and sex.

* CSF, cerebrospinal fluid.

† F, female; M, male; U, unknown.

‡ NT, non-typable.

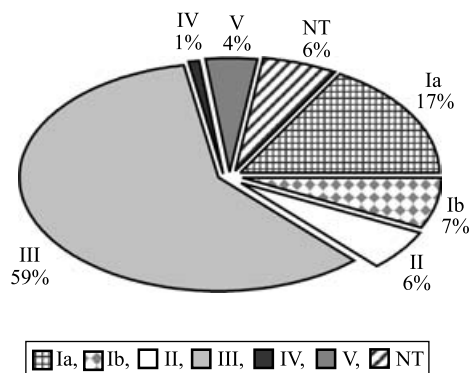


Fig. Average serotype distribution of invasive GBS isolates in infants in Denmark 1984–2002.

the six were boys, two had a twin sibling and one was triplet C. The median ages of the infants at the first and second episode of the invasive GBS infections were 5 days (range 0–54 days) and 30 days (range 25–99 days) respectively. The interval with no antibiotic therapy varied from 2 to 39 days (median 5 days). The length of intravenous antibiotic treatment in the first episode varied from 6 to 45 days (median 22 days) and in the second episode from 7 to 60 days (median 24 days). Three infants had septicaemia in both episodes. One infant had GBS isolated from CSF in the first episode, but in the recurrence GBS could be identified in blood only, and for one patient the opposite was the case. Finally one infant had GBS identified in both blood and CSF. Serotype III was identified both at first and second episode in five patients. The remaining two isolates were

serotype Ia. No foci were identified in any of the patients. No underlying diseases were found in the six infants and none of the six infants died.

PFGE analysis did not differentiate between the isolates from the first and second episode in the three recurrent cases identified after January 1999 and there were no differences between the results of the antibiotic susceptibility testing of the three pairs of GBS isolates. The results of the MIC and the time-kill curves were identical for the isolates from the first and second episodes when tested against penicillin, ampicillin and gentamicin; also, the four-fold divergence in the concentration of penicillin and ampicillin showed no differences in the killing kinetics. The tested antibiotic induced no kill of the GBS isolates but a significant inhibition of the growth compared with the control containing no antibiotic.

DISCUSSION

Here we have presented surveillance data of serotype distribution, incidences and details of recurrent infections from a 19-year nationwide study of invasive GBS disease in infants in Denmark.

The EOD increased from 0.1/1000 live births in 1984 to 0.6/1000 live births in 1995 followed by a decline to 0.3/1000 live births in Denmark in 2002. The incidence of LOD varied between 0.1 and 0.2/1000 live births during 1984–2002. These incidences reflect the minimum estimates of invasive GBS disease since only blood and CSF isolates were included in this study, whereas isolates from other sterile sites

(i.e. lung, bone and joint) were excluded. National streptococcal surveillance in Denmark is based on voluntary submission of invasive isolates and a retrospective investigation of the number of streptococcal blood isolates obtained in and submitted from each microbiological department during 1999–2002 showed that we received a consistent ratio. We were not able to determine an exact value of the completeness of the surveillance of invasive GBS infections in infants, but we received the vast majority (approximately 70%) of all β -haemolytic blood isolates disregarding serogroups and age of patients (data not shown). The consequence is an underestimation of the disease burden of invasive GBS infections in infants, although we still consider the incidence of the disease to be the best possible estimate.

In the literature the incidence of invasive GBS infection in infants varies between 0.4–5.5/1000 live births in different countries and compared to that our average incidence of 0.4/1000 live births is relatively low [24, 25]. From 1984 to 1995 we observed an increase in the incidence of GBS infections in infants, which was parallel to that observed in other haemolytic streptococcal infections, indicating a potential increased awareness among the microbiologists consequent on the prominence of the topic of haemolytic streptococcal infections in the literature. However, the incidence of the other streptococcal infections continued to increase after 1995 (data not shown) and only the incidence of GBS infections in infants declined.

In three American active surveillance areas the incidence of EOD declined from 1.7/1000 live births in 1993 to 0.6/1000 live births in 1998 after the implementation of guidelines concerning antibiotic prophylaxis, while the incidence of the LOD was constant at 0.2/1000 live births [26]. The Centers for Disease Control and Prevention (CDC), the American Academy of Pediatrics, and the American College of Obstetricians and Gynecologists published common guidelines in 1996 for risk- or screening-based strategies aimed at reducing the incidence of early-onset invasive GBS diseases in infants [27]. The guidelines were revised in 2002 and the risk-based strategy was abandoned [28].

In Denmark the American guidelines have never been implemented as a preventive routine, although risk-based prophylaxis has generally been recommended [29]. After 1995 the incidence of GBS infections in infants declined in contrast to the incidences of other haemolytic streptococcal infections.

The decline in the incidences of EOD since 1995 might reflect an increased awareness of GBS infections in infants and of the use of antibiotic prophylaxis to women pre- or intra-partum. Another explanation would be a biological serotype variation or a variation in the frequency or referral of isolates from local microbiology departments. There was no difference in the serotype distribution before and after 1995, and it seems unlikely that the ratios of submitted invasive GBS isolates from local microbiology departments have changed without affecting the submission of other haemolytic streptococci.

The most common serotype over the period of 19 years was serotype III (57%). The serotypes VII and VIII were not identified in blood or CSF, although we identified one GBS isolate with serotype VIII from the umbilical veins from a boy with EOD in 2002, but despite repeated cultures no GBS was identified in blood or CSF [30].

The overall serotype distribution during 1984–2002 is similar to that described elsewhere [24, 31, 32]. Serotype Ia was found in 40% and serotype III in 27% of the cases with EOD collected from six study sites in the United States [33]. In Japan the serotypes VII and VIII were identified in 5% and 2% of the cases with EOD and LOD respectively [32].

In future, it may become possible to vaccinate women of fertile age with GBS-conjugated vaccines including the most prevalent serotypes in order to eliminate colonization by GBS and thereby prevent EOD [34, 35]. A conjugated tetravalent vaccine (Ia, Ib, III and V) would cover 88% of the invasive GBS infections in infants in Denmark. Coverage would increase to 93% if serotype II was added in a pentavalent vaccine.

In adults recurrent infections have been registered in approximately 5% of the invasive GBS infections [36]. Recurrence of invasive GBS infection in infants is uncommon and has been described in a number of case series, but the frequency of recurrent invasive GBS infections in infants has rarely previously been estimated [3–16]. In Denmark recurrence constituted 1% of the registered invasive GBS infections in infants. Yagupsky [37] identified 4% with recurrent GBS disease among survivors of EOD. The recurrence rates should be considered as minimum estimates since not all infants with recurrent infections would be identified by the reference centre. Obviously it is a challenge to distinguish between a recurrent infection and a re-infection. In our cases the intervals without antibiotic treatment between the first and

second episodes were 2 to 39 days, and the serotypes were identical in the first and recurrent episode, in agreement with other case series.

Asymptomatic carriage of GBS or mastitis in the nursing mother has been identified as a cause of invasive GBS infection and could be a possible explanation of the recurrent episode in our first and second cases [13, 38]. In two cases, GBS carriage in the parents was sought, but the bacteria were not found. No information on exogenous sources of GBS carriage or infection was available in the other hospital files. Due to the limited antibiotic-free interval in three of the cases it seems unlikely that these recurrent episodes were re-infections with new GBS isolates. The underlying mechanism of the recurrence could be explained by persistent mucosal carriage and penicillin tolerance, defined as MIC/MBC (minimum bactericidal concentration) ≥ 32 [7–9, 15, 39]. Combination therapy has been suggested in all cases of neonatal GBS infection because GBS strains can be penicillin tolerant [39]. The six infants in our study received combination therapy, initially ampicillin and aminoglycoside (gentamicin or netilmicin). In 7 of the 12 episodes treatment was changed to penicillin when GBS was identified, but only in one episode was treatment with an aminoglycoside discontinued. Finally one infant was treated twice intravenously with penicillin for 6 weeks. While treatment with the combination of ampicillin/penicillin and aminoglycoside is no guarantee against recurrent GBS infection it may still be the best alternative to eradicate persistent mucosal carriage of GBS [39].

Siegel has suggested that the MBC of ampicillin or penicillin should be determined for GBS isolates from blood or CSF prior to discontinuing the aminoglycoside [7]. However, laborious time-kill curves are considered to be the most reliable method for detecting tolerance, while the lack of standardization of a method for MBC determination has raised questions about the use of the MBC [40]. Our three GBS isolates from the recurrent episodes identified after 1999 were not killed by penicillin or ampicillin but the growth of the bacteria was inhibited in the time-kill curves. We did not test for the penicillin–gentamicin synergy, because the aim of the antibiotic susceptibility testing was to differentiate between the isolates from the first and second episode only, and not to evaluate the antibiotic treatment of the infants. The time-kill curves for the isolates from first and second episode from each patient were identical; implicating that the recurrent episode was presumably

caused by the same strain as the first episode. The use of rifampicin to eradicate GBS colonization has been discussed [10, 11, 41] and has recently been proposed for both mother and infant in cases of recurrence of invasive GBS infection [42]. The efficacy of the antibiotic treatment was controlled in four of the cases by lumbar punctures during or immediately after the antibiotic treatment, and no GBS were detected in CSF suggesting eradication of the GBS.

PFGE can discriminate between different GBS isolates from the same patient and between patients (between cases and exogenous sources) [4, 5]. In all our cases the GBS isolates from the first and the recurrent episodes were identical, and in addition the isolates from each of the three cases differed from isolates with the same serotype (data not shown). Together with the antibiotic susceptibility-testing information, this supports, but does not prove, that the second episodes were recurrences rather than re-infections.

Our 19-year national surveillance of invasive GBS infections in infants shows that these infections are still a problem in Denmark and introducing enhanced surveillance as described by Heath et al. [43] will raise further the total number of cases ascertained.

In Denmark, risk-based strategies for prevention of EOD have been recommended, and the incidence of EOD has declined since 1995. During the period 1994–2002 recurrent infections occurred only in 1% of the infections. The serotypes III and Ia constitute the vast majority of all invasive infections. So far no invasive GBS serotype VIII isolates have been detected in infants. National and international surveillance of the incidence and serotype distribution will continue to be required to inform and evaluate potential preventive strategies.

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