Bacterial meningitis: epidemiology, herd protection, clinical characteristics, and risk assessment
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Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study

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Abstract

Group B streptococcus is the most common cause of neonatal infections. We studied the clinical and molecular epidemiology of invasive group B streptococcus infection in children younger than 3 months in the Netherlands over 25 years. We assessed the effect of the Dutch guidelines, introduced in 1999, for prevention of group B streptococcus, consisting of intravenous antibiotic prophylaxis during labour in cases of premature labour, prolonged rupture of membranes, or fever during delivery. We did this nationwide surveillance study with data from 1987 to 2011, from the Netherlands Reference Laboratory for Bacterial Meningitis. We included data for patients aged 3 months or younger with positive blood culture or cerebrospinal fluid culture for group B streptococcus and Escherichia coli infection. Early onset was defined as less than 7 days after birth and late onset was defined as 7 or more days after birth. We did multilocus sequence typing of a random subset of group B streptococcus samples to assess changes in sequence type (Mann-Kendall trend test) and the distribution of clonal complexes ($\chi^2$ and Fisher exact test) before the introduction of prevention guidelines (1987-99) and afterwards (2000-11). We compared incidences and the distribution of clonal complexes before and after the introduction of guidelines. Most cases of group B streptococcus had early onset (696/1075; 65%). The incidence of invasive group B streptococcus infection increased from 0.20 per 1000 livebirths in 1987, to 0.32 per 1000 livebirths in 2011 ($p<0.0001$). The incidence of early-onset disease increased from 0.11 per 1000 livebirths to 0.19 per 1000 livebirths ($p<0.0001$). The incidence of invasive Escherichia coli infection was 0.05 in 1987, and 0.16 in 2011 ($p=0.17$). Early-onset group B streptococcus infection caused by isolates belonging to clonal complex 17 was more common in the post-implementation period than in the pre-implementation period ($p=0.002$). The introduction of prevention guidelines for invasive group B streptococcus disease in 1999 did not reduce the incidence of disease in neonates. The guidelines should be reassessed and alternative approaches to prevent infant invasive group B streptococcus disease should be sought.
Introduction

Group B streptococcus (*Streptococcus agalactiae*) is the most common cause of neonatal infections.¹ Perinatal group B streptococcus infections are usually classified as early-onset disease, occurring in the first week of life, or late-onset disease, occurring between 1 week and 3 months of age.² Early-onset disease is thought to develop in the foetus after aspiration of amniotic fluid infected with bacteria that have ascended from the colonised genital tract of the mother.³ The pathogenesis of late-onset infections is less well understood.³ Pathogens can be acquired during passage through the birth canal, but nosocomial and community sources are also probably involved.³

The incidence of group B streptococcus in high-income countries varies geographically and over time.² Karen Edmond and colleagues⁴ did a systematic review to define the present worldwide incidence of group B streptococcus disease in infants younger than 3 months. Limited to data for 2000-11, the estimated overall incidence was 0.53 per 1000 livebirths (95% CI 0.44–0.62) in Europe, 0.67 per 1000 livebirths (0.54–0.80) in North America, and 0.00 per 1000 livebirths (0.00–0.44) in Australia. The incidence in low-income countries, as systematically reviewed by Alemnew Dagnew and coworkers,⁵ ranged from 0.00 to 3.06 per 1000 livebirths with variation within and between geographic regions. Risk factors associated with neonatal group B streptococcus disease include maternal colonisation, male sex, black ethnic origin, low concentrations of maternal antibodies against group B streptococcus, prematurity, prolonged rupture of membranes, and intrapartum fever.²

In 1986, a randomised controlled trial⁶ involving 160 women with group B streptococcus colonisation and various perinatal risk factors (premature labour, prolonged rupture of membranes, or intrapartum fever) showed that intravenous ampicillin prophylaxis during labour significantly reduced neonatal group B streptococcus disease. On the basis of these results, various prevention programmes have been initiated. Two major strategies have been adopted in high-income countries. The first approach is based on risk stratification of women at the time of delivery. Prophylaxis with antibiotics is recommended for women in labour with clinical risk factors for disease transmission, such as intrapartum fever, heavy colonisation with group B streptococcus (ie, bacteriuria), having previously had a child with group B streptococcus disease, preterm delivery, or an interval between rupture of membranes and time of delivery of 18 h or more. The second approach is based on universal screening of pregnant women by vaginal and rectal swabs for group B streptococcus. Intrapartum antibiotic prophylaxis is offered to carriers. In those who were not tested before 35 weeks of gestation, antibiotic prophylaxis is offered to women with any of the clinical risk factors. In the Netherlands, narrow-spectrum penicillin or amoxicillin are preferentially used (intravenously, starting 4 h before delivery if possible). In case of penicillin allergy, macrolides or clindamycin are recommended. Investigators cautioned that, although intrapartum antibiotics are effective for prevention of group B streptococcus disease, they might
select for other virulent and more drug-resistant pathogens, such as *Escherichia coli*. In 1999, the National Society of Obstetrics and Gynaecology and the National Society of Paediatrics in the Netherlands introduced an approach based on risk factors to reduce the occurrence of invasive group B streptococcus disease.

Multilocus sequence typing enables the identification of genetically related group B streptococcus isolates on the basis of similarities in seven conserved housekeeping genes. A combination of unique sequences of the seven loci defines a sequence type. Genetically similar isolates can be grouped into clonal complexes. Five major clonal complexes have been identified in neonates and adults: cc1, cc12, cc19, cc17, and cc23. Sequence types 17 and 19 cause more neonatal group B streptococcus disease than would be expected on the basis of the proportion of pregnant women with asymptomatic carriage. However, changes in the incidence of different genotypes over more than 10 years have not been studied. We assessed the clinical and molecular epidemiology of invasive group B streptococcus infection over 25 years and assess the effect of the introduction of the prevention programme in Netherlands on the incidence of neonatal group B streptococcus disease.

**Methods**

**Study design and patients**

We used data from a nationwide surveillance study of bacterial meningitis and infant bacteraemia that is done by the Netherlands Reference Laboratory for Bacterial Meningitis. From 1975 onwards, medical microbiology laboratories throughout the country have submitted to the laboratory samples of cerebrospinal fluid and blood from all children with invasive group B streptococcus or *E coli* infection. Information about the probable underlying disease and the immunological status of patients is not included. The study covers 84% of patients with meningitis in the Netherlands. We included all patients aged 3 months or younger for whom an isolate was received between Jan 1, 1987, and Dec 31, 2011. Patient age was calculated as the number of days between the date of birth and earliest known date of the illness, which mostly was the date of culture from the first positive sample culture. If the culture date was missing, we used the date that the material was sent to or received by the laboratory. If no date of birth or early date of illness or age was recorded, the patient was excluded.

We did this study with anonymous patient data and in accordance with Dutch privacy legislation. Additional institutional review board approval is not required for the assessment of anonymised laboratory surveillance data.
**Procedures**

We did serogrouping and serotyping as previously described,\(^\text{15}\) using monospecific antiserum samples against group B streptococcus serotypes IA, IB, II, III, IV, V, VI, VII, and VIII.

Invasive infection was defined as a positive culture from cerebrospinal fluid or blood. Early-onset disease was defined as invasive infection within 7 days after birth. Late-onset disease was defined as invasive infection between 7 days and 3 months after birth. On the basis of implementation of the guidelines for the prevention of neonatal group B streptococcus infections in the Netherlands, the study period was divided into a pre-implementation period (1987-99) and post-implementation period (2000-11).

For multilocus sequence typing, we selected a random sample of all available strains, stratified by calendar year, with the function “sample” from the R-base package. We did multilocus sequence typing as previously described.\(^\text{9}\) Briefly, we used PCR to amplify fragments from seven housekeeping genes (\(\text{adhP}\), \(\text{pheS}\), \(\text{atr}\), \(\text{glnA}\), \(\text{sdhA}\), \(\text{glicK}\), and \(\text{tkt}\)). The seven PCR products were purified and sequenced. We assigned an allele number to each fragment on the basis of its sequence. We then assigned each isolate a sequence type on the basis of the allelic profile of the seven amplicons. We assigned allele and sequence types with the group B streptococcus multilocus sequence typing database. We combined sequence profiles of the Dutch isolates with those of all group B streptococcus isolates in the database with the compare option in eBURST (version 3.0). We assigned isolates to the same group when they shared identical alleles at six of the seven loci with at least one other member of the group. We assigned isolates to a clonal complexes as previously described.\(^\text{10,16}\)

**Statistical analysis**

We calculated incidences with population data obtained from Statistics Netherlands with the use of StatLine. We used R (version 2.15.0) for the statistical analyses. We used Pearson’s \(\chi^2\) test to assess goodness of fit. We estimated the significance of trends with the Mann-Kendall trend test. For this test, neither normal distribution nor linearity of the trend are needed. This test assesses the increase or decrease of the data elements in the time series. To compare the change in incidence before and after the introduction of prevention guidelines, we calculated the slope of the incidence-time regression line with least-square mean linear regression. We compared the slopes with Student’s \(t\)-test. For the difference in distribution of clonal complexes before and after the introduction of prevention guidelines, we dichotomised values for clonal complexes and analysed them by Pearson’s or Fisher exact \(\chi^2\) test, as appropriate. We considered a \(p\) value of less than 0.05 as statistically significant.
Results

From 1987 to 2011, 1075 cases of invasive group B streptococcus infection (median age 3 days, IQR 1-14) and 474 cases of invasive E. coli infection (median age 11 days, IQR 6-22) were identified in children aged 3 months or younger. Infection was more common in boys than in girls for both group B streptococcus (p=0.004) and E. coli infection (p<0.0001, table 1). The incidence of invasive group B streptococcus infection increased from 0.20 per 1000 livebirths in 1987, to 0.32 per 1000 livebirths in 2011 (p<0.0001), while that of invasive E. coli infection was 0.05 cases per 1000 livebirths in 1987 and 0.16 cases per 1000 livebirths in 2011 (p=0.17, figure 1).

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Group B streptococcus (n=1075)</th>
<th>Escherichia coli (n=474)</th>
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<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>546 (51%)</td>
<td>267 (56%)</td>
</tr>
<tr>
<td>Girls</td>
<td>456 (42%)</td>
<td>170 (36%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>73 (7%)</td>
<td>37 (8%)</td>
</tr>
<tr>
<td><strong>Median age</strong></td>
<td>3 days (1-14)</td>
<td>11 days (6-22)</td>
</tr>
</tbody>
</table>

Figure 1. Incidence of group B streptococcus and Escherichia coli invasive disease in the Netherlands among patients aged 3 months or younger. Vertical dashed line represents the introduction of prevention guidelines in Netherlands in 1999.

Early-onset invasive group B streptococcus infection occurred in 696 neonates (65%) and late-onset invasive group B streptococcus infection occurred in 379 (35%, figure 2). The incidence of early-onset group B streptococcus disease increased from 0.11 to 0.19 per 1000 livebirths (p<0.00001, figure 2). The incidence of late-onset disease increased from 0.03 to 0.13 per 1000 livebirths (p=0.004). The increase in infection in the pre-implementation and post-implementation period were not significantly different for early-onset disease (0.00087 vs 0.0057, p=0.20) or late-onset
late-onset disease increased from 0.03 to 0.13 per 1000 livebirths (p<0.0001, figure 2). Early-onset invasive E. coli occurred in 163 neonates (34%) and late-onset disease in 311 (66%, figure 2).

**Figure 2.** Incidence of group B streptococcus (A) and Escherichia coli (B) by age. Vertical dashed line represents the introduction of prevention guidelines in Netherlands in 1999.

We did a subgroup analysis of group B streptococcus confirmed by cerebrospinal fluid samples compared with blood culture. Of 1075 confirmed group B streptococcus infections before the age of 3 months, 424 (39%) were cerebrospinal fluid culture positive and 644 (61%) were solely blood culture positive. During the study period, the incidence of blood culture only group B streptococcus increased significantly for both early-onset disease (p<0.0001) and late-onset disease (p<0.0001), but the incidence of cases confirmed from cerebrospinal fluid did not change significantly (appendix).

Of 1075 isolates, 645 (60%) were of serotype III, and 171 (16%) were of serotype IA. The remaining isolates were distributed over eight other serotypes or could not be typed. The distribution of serotypes was not different in the post-implementation period compared with in the pre-implementation period (appendix). Of 167 isolates, we identified 27 different sequence types. Three
sequence types accounted for 66% of the isolates: 44 (26%) isolates were ST17, 41 (25%) were ST19, and 25 (15%) were ST23. The Dutch isolates were mainly distributed in two groups. One group with ST17 as the founder contained 132 (79%) of the Dutch isolates and a second group with ST23 as the founder contained 35 (21%) of the Dutch isolates (figure 3). 51 isolates (31%) of the first group were assigned to clonal complex 19, 49 (29%) to clonal complex 17, 20 (12%) to clonal complex 12, nine (5%) to clonal complex 1, one (1%) to clonal complex 7, and two (1%) to clonal complex 4; the 35 (21%) of the second group to clonal complex 23. Most of the isolates of clonal complex 17 and clonal complex 19 were serotype III (appendix).

The proportion of isolates belonging to clonal complex 17 was higher in the post-implementation period than in the pre-implementation period (37/99 [37%] vs 12/68 [18%], p=0.006), whereas the isolates belonging to clonal complex 19 were less common in the post-implementation period (21/99 [21%] vs 30/68 [44%], p=0.002, figure 4). The proportion of clonal complex 17 increased among cases of early-onset group B streptococcus disease after implementation (21/63 [33%] post-implementation vs 2/34 [6%] pre-implementation; p=0.002), whereas the proportion of clonal complex 19 decreased among cases of late-onset disease (12/63 [19%] vs 12/34 [35%], p=0.02; figure 4).

Figure 3. Lineage of group B streptococcus isolates.
Green isolates are unique to the Dutch population. Magenta isolates were found in our Dutch sample and in the database. The size of the dot is proportional to the number of isolates. Sequence types connected by black line are single locus variants of each other. The labelled sequence types were common, central to clonal complexes, or numerous single locus variants. ST17 and ST23, the founders of group 1 and 2, respectively. Yellow dots are secondary founders.
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Figure 4. Distribution of clonal complexes.
Overall (A), and stratified by early and late onset (B).

See Online for appendix
Discussion

We showed a 60% increase in the incidence of infant invasive group B streptococcus infection in the Netherlands over the past 25 years despite the introduction in 1999 of guidelines for prevention of neonatal group B streptococcus disease. Results of a previous study of a shorter period suggested that the introduction of the guidelines stabilised the incidence of neonatal group B streptococcal disease. However, we present evidence that, by contrast with what was anticipated, the incidence of invasive group B streptococcus infection has further increased with the same trend as before the introduction of the guidelines. This increase was mainly a result of a rise in the number of cases caused by group B streptococcus belonging to clonal complex 17.

After the guidelines for the use of intrapartum antibiotic prophylaxis were issued in the USA, a large decrease of both group B streptococcal sepsis and meningitis before the age of 7 days occurred, from 1.7 per 1000 livebirths in 1990, to 0.6 per 1000 livebirths in 1998. After the introduction of universal screening in 2002 in the USA, the incidence of early-onset disease fell further, from 0.47 per 1000 livebirth in 1999-2000, to 0.34 per 1000 livebirths in 2003-05, which is still higher than the incidence of 0.2 per 1000 livebirths in Netherlands. Contrary to the USA and most European countries, the UK and Netherlands still use a risk factor-based approach for intrapartum use of antibiotics.

The implementation of universal screening has led to a substantial increase in the use of antibiotics during labour. Between 1998 and 2002, 35% of mothers who delivered term infants in Utah, USA, received intrapartum antibiotics. A surveillance study in ten US states showed that the proportion of mothers receiving intrapartum antibiotics rose from 27% to 32% from 1998 to 2004. However, preventive intrapartum antibiotics are only effective against early-onset disease.

Our findings offer no explanation for the increase in incidence over the past 25 years in Netherlands. Possible explanations include changes in the host, medical practice, increased submission of isolates to the National Laboratory, or the pathogen itself.

Population structure probably plays a part in differences in incidences between regions and countries. A meta-analysis showed large regional differences in the incidence of group B streptococcus disease, ranging from 0.15 cases per 1000 livebirths in the western Pacific, to 1.21 cases per 1000 livebirths in Africa. In the USA, the incidence of group B streptococcus disease is higher in African-American than in European-American neonates. This disparity could explain the differences in incidence between these reports and our study.

Additionally, increased recognition of symptoms and a lower threshold for diagnostic tests might have increased registration and so resulted in higher incidence, but this possibility cannot account for the stable incidence of E. coli infection. Another potential explanation is the change
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in survival over time of pre-term and immature neonates, who are more vulnerable to group B streptococcus infection. However, prematurely born neonates more often have *E. coli* infection than group B streptococcus infection.¹

Our study is based on historical data. We cannot rule out a reduction in under-reporting by improved submission of isolates to the national laboratory, resulting in an apparent increase in the incidence of group B streptococcus. The incidence of both early-onset and late-onset cerebrospinal fluid-based culture-confirmed infections did not change during the study period (39% of all cases). However, changes in diagnostic practice—eg, blood cultures are increasingly common when sepsis in neonates is suspected—might have increased the number of submitted isolates from blood. Additionally, the incidence of *E. coli* disease among children younger than 3 months did not change during our observation period. Furthermore, our findings are consistent with observations from elsewhere. In the UK, the incidence of disease in children up to 90 days of age also increased, from 0.3 cases per 1000 livebirths in 1977-78, to 0.72 cases per 1000 livebirths in 2000-01.²⁸ Results of an epidemiological study ²⁷ of England and Wales showed a steady increase for both early-onset and late-onset group B streptococcus infection of up to 5% per year during a 20-year surveillance period. In the USA, the incidence of early-onset of group B streptococcus disease decreased between 2000 and 2003, but increased significantly from 2003 to 2006.²⁹ By contrast, in Denmark, the incidence of early-onset disease among neonates decreased during 1992-2001.³⁰ In Denmark, a modified regimen of the risk-based approach for prevention of early-onset group B streptococcus disease was recommended in 1999-2000 and fully implemented during 1999-2001.

Finally, the emergence of new and more virulent group B streptococcus types could be another explanation.³¹ The increase of group B streptococcus was associated with a concomitant change in the distribution of clonal complexes. In the post-implementation period more cases were caused by clonal complex 17 and fewer by clonal complex 19, compared with the pre-implementation period. Isolates of clonal complex 17 have been associated with invasive disease in neonates.³² Isolates of clonal complex 17 express a unique serine-rich repeat protein (Ssr-2) and, in a mouse model of neonatal sepsis,³³ have a significantly lower lethal dose for 90% of mice than do group B streptococcus isolates that do not express Ssr-2.

Most strains in clonal complexes 1, 17, 19, and 23, cluster into a dominant capsular serotype (cps): cpsV for clonal complex 1, cpsIII for clonal complex 17, cpsIII for clonal complex 19, and cpsIA for clonal complex 23.³⁴ In our study, almost all isolates of clonal complex 17 and clonal complex 19 were of serotype III. Serotype III is the main cause of group B streptococcus disease in Netherlands, which is relevant for the development of vaccines based on capsular polysaccharides. Comparison of the serotype distribution before and after the introduction of the prevention guidelines showed a similar proportion of serotype III group B streptococcus in both periods, caused by an increase in clonal complex 17 and a decrease in clonal complex 19.
A limitation of our study is that patient age was measured as the difference between the date of birth and the earliest known date of the illness, mostly the date of cerebrospinal fluid or blood culture. This approach might have resulted in an underestimation of cases. Because incidence of group B streptococcus decreases with age, underestimation would be more likely for the group with early-onset disease. However, we calculated age in the same way for patients with *E. coli* and those with group B streptococcus. Moreover, the incidence of both early-onset and late-onset group B streptococcus disease increased. Therefore, this possible underestimation of the true incidence cannot explain the difference in trends between both pathogens.

Furthermore, our incidence estimates might be underestimated because we included only culture-confirmed cases. Also, although we describe the most extensive series published thus far, this study was limited by the fact that the surveillance system does not collect data for underlying diseases or for outcome of the disease.

This study, based on surveillance data, cannot establish the effectiveness of neonatal group B streptococcus prevention based on risk stratification of women at the time of delivery. The incidence of group B streptococcus would have probably been higher in the absence of prevention. However, even if current practice is (partly) effective, and rising incidence is (partly) caused by changes in diagnostic or reporting practices, the number of neonates with positive group B streptococcus cultures is not decreasing. We believe that our study should lead to a reassessment of current practices. Whether the Netherlands should move to a culture-based screening programme is beyond the scope of this study. Results from a cost-effectiveness study in 2006 in the Netherlands showed a much higher cost-effectiveness ratio per quality-adjusted life-year gained for the screening-based strategy compared with the risk-based strategy. The effect of an increasing incidence of neonatal group B streptococcus disease on the cost-effectiveness of both strategies remains to be assessed. Vaccination against group B streptococcus is a promising alternative to be seriously considered for prevention of neonatal invasive group B streptococcus infection, although vaccines are still at an early stage of development.
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References


Supplementary material

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Supplementary figure 1. Incidence rates of GBS invasive disease in the Netherlands among patients aged 3 months or less. (A) CSF culture confirmed; (B) Blood culture confirmed.

Supplementary figure 1A. Age less than 1 week (early-onset disease), diamonds with solid line and age between 1 week and 3 months (late-onset disease), dashed line.
Supplementary figure 1B.
Age less than 1 week (early-onset disease), diamonds with solid line and age between 1 week and 3 months (late-onset disease), dashed line.

Supplementary figure 2. GBS serotypes distribution. Serotypes of all GBS isolates stratified by pre- and post-implementation period.
White bars: guidelines’ pre-implementation period; black bars: guidelines’ post-implementation period. Numbers indicate absolute numbers of isolates.
Supplementary figure 3. Association between GBS serotypes and clonal complex. Serotype distribution among the 167 randomly selected samples for clonal complex analysis.