ORIGINAL ARTICLE

Carriage of group B streptococcus in pregnant women from Oxford, UK

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Objective: To investigate asymptomatic vagino-rectal carriage of group B streptococcus (GBS) in pregnant women.**Methods:** Women in the final trimester of pregnancy were recruited. A single vagino-rectal swab was taken, with consent, for culture of GBS. Two microbiological methods for isolation of GBS from vagino-rectal swabs were compared. The distribution of capsular serotypes of the GBS identified was determined. Epidemiological data for a subset (n = 167) of the pregnant women participating were examined.**Results:** 21.3% were colonised vagino-rectally with GBS. Risk factors for neonatal GBS disease (maternal fever, prolonged rupture of membranes, and preterm delivery) were present in 34 of 167 women (20.4%), and the presence of these factors correlated poorly with GBS carriage. Capsular serotypes III (26.4%), IA (25.8%), V (18.9%), and IB (15.7%) were prevalent in the GBS isolates. Selective broth culture of vagino-rectal swabs was superior to selective plate culture, but the combination of both methods was associated with increased detection of GBS (7.5%). An algorithm for the identification of GBS from vagino-rectal swabs was developed.**Conclusions:** GBS carriage is prevalent in pregnant women in Oxfordshire, UK. The poor correlation between risk factors and GBS carriage requires further investigation in larger groups, given that the identification of these surrogate markers is recommended to guide administration of intrapartum antibiotic prophylaxis by the Royal College of Obstetricians of the UK. A selective broth culture detected more GBS carriers than a selective plate culture.

Group B streptococcus (GBS), is the leading cause of neonatal sepsis in many parts of the world including the United Kingdom.¹ Administration of intrapartum antibiotic prophylaxis (IAP) to the GBS colonised woman is an effective strategy for the prevention of early onset neonatal sepsis.² The Royal College of Obstetricians has published guidelines (Guideline No 36, <http://www.rcog.org.uk>) for the prevention of neonatal GBS infection in the UK that recommend the identification of risk factors for early onset neonatal GBS disease in the mother to guide the administration of IAP. The Centers for Disease Control and Prevention (CDC) in the USA have recommended universal screening of pregnant women to identify GBS carriers, who will then be offered IAP.³

The development of national guidelines for prevention of neonatal GBS has been hampered in the UK by a lack of epidemiological data. The investigations that have been published have given different results. A study carried out in London in 1987 identified a carriage rate of GBS of 28% in pregnant women.⁴ Similarly, 26% of women studied in the Republic of Ireland had GBS identified on perineal and vaginal swabs.⁵ In contrast, only 4% of women in Wales were carriers of GBS.⁶

The aim of this study was to determine the carriage rate of GBS in pregnant women in Oxfordshire, UK. Further aims were to gather demographic and clinical information on a subset of women studied, and to examine different microbiological methods of GBS isolation from swabs.

METHODS

A prospective carriage study was carried out in Oxfordshire, UK over a two year period (2001–2003). Pregnant women attending antenatal care at hospital and community units attached to the John Radcliffe Hospital, Oxford, and at the Horton Hospital, Banbury were recruited. A single

vagino-rectal swab for culture of GBS was taken from participants who were in the final few weeks of pregnancy (34 weeks to full term). Questionnaires were used to gather demographic and epidemiological information about the pregnancy for women attending the Horton Hospital. Information was not collected from women attending the John Radcliffe Hospital other than GBS carriage status. Data were entered onto a Microsoft Access database for analysis. Local policy was followed to guide the administration of IAP. The Oxfordshire ethics committee granted ethical approval for the study (C00.035).

An algorithm for the identification of GBS in vagino-rectal swab cultures was developed (fig 1). Two selective methods for detection of GBS from vagino-rectal swabs were tested for all swabs. Todd-Hewitt (TH) broth with gentamicin (8 mg/l) and nalidixic acid (15 mg/l), as recommended by the CDC,⁷ was compared with a selective plate (NNA) containing 5% horse blood agar with neomycin (30 mg/l) and nalidixic acid (15 mg/l). Each vagino-rectal swab was first smeared over a section of the selective plate (and spread with a flamed platinum loop) and then cut off into the selective broth.

Incubation of plates and broths was carried out overnight in a humidified atmosphere enriched with 5% CO₂. A 10 µl loopful of the TH broth culture was then subcultured onto Columbia plates with 5% horse blood and incubated overnight. Colonies with a characteristic small zone of β-haemolysis on blood agar or NNA plates were further examined. Confirmatory tests were Gram staining, showing Gram positive cocci in pairs or short chains, which were catalase negative and grew on but did not hydrolyse aesculin in bile-aesculin agar.⁸ Identification of GBS was then

Abbreviations: GBS, group B streptococcus; IAP, intrapartum antibiotic prophylaxis

Figure 1 Algorithm for laboratory handling of vagino-rectal swabs for the isolation and identification of group B streptococcus.

confirmed by the detection of the Lancefield group B antigen, using the rapid latex agglutination test (Oxoid, Basingstoke, UK). Isolates of GBS for study were stored at -80°C in tryptone-soy broth with glycerol (10%).

The capsular serotypes of the GBS isolates were determined using latex agglutination on glass slides. The methods followed were based upon those of Slotved *et al.*⁹ Antisera to the nine GBS serotypes (IA, IB to VIII) were supplied by Statens Serum Institut, Denmark. Strains were cultured overnight on blood agar plates, then inoculated into Todd-Hewitt broths (1 ml) in sterile capped tubes. Tubes were incubated overnight at 37°C . On a glass slide, 1 μl of antisera was mixed with 5 μl of broth suspension of organism. The slide was rocked for a maximum of 30 seconds. Agglutination indicated a positive reaction.

RESULTS

We recruited 748 women over the two year study period (2001–2003), of whom 167 were from the Horton Hospital, Banbury, and the remainder from the John Radcliffe Hospital (JRH), Oxford. In all, 159 women (21.3%) were carriers of GBS. There was no significant difference between carriage rates at the JRH, a tertiary referral hospital, and at the Horton, which is a district general hospital.

Epidemiological and clinical information for a subset of women

For the 167 mothers recruited at the Horton Hospital, further epidemiological data were available and are shown in table 1. There were 170 infants born to 167 women, of whom 93 (54.7%) were female.

Table 1 Ethnicity, marital status, and number of pregnancies recorded for 167 pregnant women from Oxfordshire, UK

Epidemiological information	n (%)
<i>Ethnic origin</i>	
British-white	155 (92.8)
Other-white	7 (4.2)
Asian-other	2 (1.2)
African	1 (0.6)
Irish-white	1 (0.6)
Pakistani	1 (0.6)
Total	167
<i>Pregnancy</i>	
First	75 (44.9)
Second	54 (32.3)
Third	21 (12.6)
Fourth	8 (4.8)
Fifth	3 (1.8)
Sixth	3 (1.8)
Seventh	3 (1.8)
Total	167
<i>Marital status</i>	
Married	152 (91.0)
Single	7 (4.2)
Cohabiting	5 (3.0)
Divorced	2 (1.2)
Widowed	1 (0.6)
Total	167

For the 167 mothers recruited at the Horton Hospital (of whom 29 (17.4%) were GBS carriers), further data were available from questionnaires. In 34 mothers (20.4%) a risk factor for GBS colonisation was identified (fever $>37.5^{\circ}\text{C}$, prolonged rupture of membranes >18 hours, preterm delivery <37 weeks). The correlation between GBS carrier status and identification of a risk factor was not good (table 2). GBS carrier status was not significantly associated with any risk factor, and 24 of 29 women with GBS did not develop a risk factor.

Participating women at the Horton Hospital were asked to recall whether they had been given antibiotics at any time during their pregnancy. Twenty six women (15.6%) said that they had taken antibiotics (table 3). Approximately 50% of women could not recall why they were treated and what the antibiotic was called. Urinary sepsis was the most frequent reason given, and amoxicillin or cephalixin were commonly used. Only one of the 26 cases receiving oral antibiotics was a GBS carrier.

Twelve women (7.2%) were given intravenous antibiotics (coamoxiclav, amoxicillin, or cefuroxime) during labour. The indication for antibiotic treatment was emergency caesarean section in four cases, two each had fever or prolonged rupture of membranes, one had premature delivery, and for three cases the indication was not recorded. The rate of instrumental delivery was 34.1% overall, and was not significantly affected by GBS carrier status ($p = 0.3$).

Microbiological methods and capsular serotyping

GBS was detected in vagino-rectal swabs using the selective TH broth on 147 of 159 occasions (92.5%), as compared with 130 of 159 occasions (81.8%) using the selective NNA plate (table 4). Capsular serotypes III and IA were most prevalent among the GBS carried in this study. Approximately 2% of isolates were not typeable by capsular serotyping (fig 2).

DISCUSSION

The carriage rate of GBS in pregnant women in Oxfordshire is 21.3%. This is similar to that seen in London (1987)⁴ and in

Table 2 The occurrence of risk factors for GBS carriage during pregnancy, with results of vagino-rectal screening for GBS and level of significance, in 167 women from Oxfordshire, UK

Risk factor	GBS carrier (n = 29)	GBS negative (n = 138)	p Value*
Any risk factor	5	29	0.8
Fever $>37.5^{\circ}\text{C}$	2	9	0.6
PROM >18 hours	2	14	0.5
Preterm <37 weeks	0	3	0.6
Fever and PROM	1	3	0.5

*Yates corrected test used, except where values were <5 , then Fisher's exact test was used.

PROM, prolonged rupture of the membranes.

Table 3 Antibiotics given orally at some time during pregnancy to 26 pregnant women and indication for treatment (self-reported by participating women by questionnaire). Figures are Number (%)

Oral antibiotic therapy	n (%)
<i>Antibiotics given</i>	
Amoxicillin	6 (23.0%)
Cephalexin	6 (23.0%)
Erythromycin	2 (7.7%)
Penicillin	1 (3.9%)
Do not remember	11 (42.3%)
Total	26
<i>Indication for antibiotics</i>	
UTI	9 (34.6%)
ROM	1 (3.9%)
Chest infection	2 (7.7%)
Do not remember	14 (53.9%)
Total	26

ROM, rupture of membranes; UTI, urinary tract infection.

Table 4 Comparison of two selective microbiological methods of culturing GBS in vagino-rectal swabs

Method	NNA positive	NNA negative	Total
TH positive	118	29	147
TH negative	12	0	12
Totals	130	29	159

NNA, blood agar with nalidixic acid and neomycin; TH, Todd-Hewitt broth with gentamicin and nalidixic acid.

Ireland (1991),⁵ but is considerably higher than in North Wales (2002).⁶ This may represent different study methodology or may suggest geographical variation in GBS carriage rates in the United Kingdom.

The Horton Hospital, Banbury, is a small district hospital in a rural area of Oxfordshire. This site was chosen as the focus for a questionnaire based epidemiological study of GBS carriage in order to avoid a bias towards complicated and high risk pregnancies that may be seen at the tertiary referral hospital (JRH, Oxford).

The carriage rate of GBS (17.4%) in Banbury was not significantly different from that found at the JRH (22.4%, $p = 0.2$). Racially, the population studied was not diverse, and 93% of women questioned called themselves "white

Figure 2 Distribution of capsular serotypes in 159 asymptotically carried isolates of GBS in pregnant women in Oxfordshire. Values are n (%).

British". By analysis of risk factors and carriage rates it is estimated that a maximum of 25% of women in this study would have been treated with IAP according to a risk factor approach and a minimum of 21% under the screening approach of the CDC guidelines.³ The presence of risk factors did not correlate well with vagino-rectal carriage of GBS in this study. However, Oddie and Embleton¹⁰ in a UK based study, did find a correlation between the presence of risk factors in mothers and the occurrence of GBS disease in their neonates, suggesting that analysis of larger groups of pregnant women may be required. Current guidance from the Royal College of Obstetricians (Guideline No 36, <http://www.rcog.org.uk>) recommends that the presence of surrogate markers of GBS neonatal diseases be used to guide the administration of IAP.

Antibiotic use was recorded, as this could have an impact on GBS carriage during pregnancy. The results showed that 15.6% of women recalled recent oral antibiotic use. The finding that the majority of women could not recall why or with what drug they had been treated suggests that education of the pregnant woman or the wording of the questionnaire may need to be improved.

The selective TH broth method was superior to the selective NNA plate method, detecting 92.5% compared with 81.8% of GBS in vagino-rectal swabs. This is in keeping with the recommendations of the CDC that selective broth culture is the single best method of detecting GBS carriage.⁷ Dunn *et al*^{11, 12} found that the sensitivity of TH selective broth culture was 86–93% and of NNA culture was 78–85%. It is likely that a screening method based upon the use of one of these methods—the selective broth culture being preferred by the authors—would therefore not pick up all carriers.

The distribution of capsular serotypes seen in this collection of asymptotically carried isolates is similar to that recorded in the recent national UK surveillance study of invasive neonatal GBS.^{13, 14} The carriage study reported from Ireland⁹ had fewer serotype V isolates, but similar proportions of serotypes IA, IB, II, and III. Capsular serotype data were not available for two other UK carriage studies.

In conclusion, in Oxfordshire, UK, 21.3% of pregnant women were colonised vagino-rectally with GBS. Selective broth culture of vagino-rectal swabs was superior to selective plate culture, but the combination of both methods was associated with increased detection of GBS (7.5% extra). Capsular serotypes IA, III, and V were prevalent in the carried GBS isolates.

Potential future work in the UK should focus on large multicentre carriage studies of GBS in pregnant women, examination of culture versus rapid detection methods^{15–18} for identifying GBS carriage, and randomised controlled trials of strategies for prevention of neonatal GBS infection.

Take home message

- Overall, 21.3% of pregnant women were colonised vagino-rectally with group B streptococcus.
- There was a poor correlation between risk factors and GBS carriage.
- A selective broth culture detected more GBS carriers than a selective plate culture.

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