Colonization with group B streptococci during pregnancy and labor

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Mohammed Rohi El Cheikh Khalil,

Kolding, Denmark,

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Preface

This thesis was accomplished at the Faculty of Health Sciences, Institute of Regional Health Research, University of Southern Denmark, during the period 2014 – 2017. Both the principal supervisor, Clinical Professor, MD, PhD Jens Kjølseth Møller from the Institute of Regional Health Research, University of Southern Denmark and the co-supervisor clinical Professor, MD, Dr. Med Niels Uldbjerg from the Clinical Institute, Aarhus University, provided supervision.

The PhD thesis presents results from two different cohorts: 1) a prospective Danish cohort with unselected Danish women in labor at term (n=902), and 2) a retrospective population-based cohort consisting of all pregnant women (n=36,097) from the catchment area of Lillebaelt Hospital, Denmark, during the period January 2002 – December 2012.

The study has the overall aims to evaluate three screening methods for intrapartum group B streptococcus colonization in a Danish cohort and to investigate the possible association between preterm delivery and colonization of group B streptococci cultured in urine during pregnancy.

List of papers

This thesis is based on the following four studies, which will be referred to by their roman numerals in the text:

Paper I

Intrapartum PCR assay versus antepartum culture for assessment of vaginal carriage of group B streptococci in a Danish cohort at birth

Mohammed Rohi Khalil, Niels Uldbjerg, Poul Bak Thorsen, Jens Kjølseth Møller.

PLoS One 2017 Jul 5; 12(7):e0180262.

Paper II

Risk-based screening combined with a PCR-based test for group B streptococci diminishes the use of antibiotics in laboring women

Mohammed Rohi Khalil, Niels Uldbjerg, Poul Bak Thorsen, Birgitte Henriksen, Jens Kjølseth Møller.

European Journal of Obstetrics & Gynecology and Reproductive Biology 215 (2017) 188–192.

Paper III

Number of colony forming units in urine at 35–37 weeks' gestation as predictor of the vaginal load of Group B Streptococci at birth

Mohammed Rohi Khalil, Poul Bak Thorsen, Jens Kjølseth Møller, Niels Uldbjerg.

European Journal of Obstetrics & Gynecology and Reproductive Biology 223 (2018) 68–71.

Paper IV

Group B streptococci cultured in urine during pregnancy associated with preterm delivery: a selection problem?

Mohammed Rohi Khalil, Niels Uldbjerg, Jens Kjølseth Møller, Poul Bak Thorsen.

The Journal of Maternal-Fetal & Neonatal Medicine 2018 Mar 29:1-191.

Abbreviations

ACOG	American College of Obstetricians and Gynecologists
AAP	American Academy of Pediatrics
ASB	Asymptomatic bacteriuria
CDC	Centers for Disease Control and Prevention, USA
CI	Confidence interval
CFU/mL	Colony-forming units per mL
NICE	National Institute of Health and Care Excellence
EOGBS	Early-onset neonatal group B streptococcal disease
EONS	Early onset neonatal sepsis
GBS	Group B streptococcus
IAP	Intrapartum antibiotic prophylaxis
LOGBS	Late-onset neonatal group B streptococcal disease
LH+	Positive likelihood ratio
NPV	Negative predictive value
NAAT	Nucleic acid amplification test
PCR	Polymerase chain reaction
PPV	Positive predictive value
PROM	Pre-labor ruptures of membranes
PTD	Preterm delivery
RCOG	Royal College of Obstetricians and Gynecologists, Great Britain
ROM	Rupture of membranes

Thesis at a glance

Paper	Objectives	Study design	Methods	Conclusions
I	To compare the performance of an antepartum culture-based screening strategy and an intrapartum PCR assay for the prediction of intrapartum vaginal carriage of GBS.	A prospective observational study enrolling 902 unselected Danish pregnant women at term.	A culture-strategy based on rectovaginal cultures at 35-37 weeks of gestation and a PCR strategy based on PCR assay on intrapartum vaginal swab samples.	In a Danish population, the intrapartum PCR assay performs better than the antepartum culture for identification of intrapartum vaginal GBS.
II	To assess the performance of a polymerase chain reaction – group B streptococci test (PCR-GBS test) – in deciding antibiotic prophylaxis in women in labor at term.	A prospective observational study enrolling 902 unselected Danish pregnant women at term.	During labor, vaginal swabs were used for both GBS cultures (reference standard) and the PCR-GBS test. The presence of risk factors for EOGBS was recorded.	In programs aiming to treat only GBS-carriers who have risk factors for EOGBS, it may be possible to reduce penicillin usage by two-thirds, from 12% to 4%.
III	To evaluate how well GBS colony numbers in the antepartum urine at 35-37 weeks predict the level of GBS colonization of the vagina at birth.	A prospective observational study enrolling 902 unselected Danish pregnant women at term.	Exposure was GBS CFU/mL urine at 35-37 weeks of gestation. Outcome was vaginal GBS colonization at birth assessed by a semi-quantitative culture method.	Screening for urinary GBS colonization at 35-37 weeks of gestation does not perform satisfactorily as a stand-alone screening marker for risk of EOGBS.
IV	To investigate a possible association between GBS cultured in urine during pregnancy and preterm delivery (PTD) in a Danish cohort of pregnant women.	A retrospective population-based cohort consisting of 36,097 pregnant women, during the period January 2002 to December 2012.	The cohort of 34,285 singleton pregnancies used in this study was divided into three groups. The primary outcome was preterm delivery (before 37 weeks of gestation).	No association between asymptomatic GBS bacteriuria and PTD was found. Previous suggestions of such findings can be due to a selection problem.

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Introduction

Early onset neonatal sepsis (EONS) is still a leading cause of mortality and morbidity in term infants [1, 2]. Group B streptococcus (GBS, *Streptococcus agalactiae* or *Lancefield group B Streptococcus*) is a major cause of severe early-onset infection (EOGBS) in newborn infants, defined as GBS acquired before seven days of age [3, 4]. EOGBS is associated with manifestations of severe disease such as respiratory distress, pneumonia, sepsis, or meningitis within the first 24-48 hours of life [5]. The most important risk factor for EOGBS is vaginal colonization that causes vertical transmission of bacteria to the infant during labor and delivery [6]. Therefore, identification of pregnant women colonized with GBS is essential in the prevention of EOGBS. The incidence of EOGBS ranges from 0.5 to 3.0 per 1,000 live births, with 4-10% mortality [7, 8]. It has been estimated that in the absence of any intervention, approximately 50% of babies born by colonized mothers become colonized, and 1–2% of them progress to develop invasive disease [9, 10].

Intrapartum antibiotic prophylaxis (IAP) is the most effective available intervention against EOGBS. However, the widespread use of intrapartum antibiotics is a matter of general concern and caution because of the risk of antibiotic resistance and an unfavorable colonization pattern of the gut microbiota of the new born [11], which has been linked to later problems such as allergy, obesity, and diabetes [12-14]. The debate about the most effective strategy for identifying candidates for IAP therefore continues.

GBS colonization of the genital tract

GBS is an encapsulated gram-positive streptococcus that colonizes the gastrointestinal and genital tract among pregnant women. The gastrointestinal tract acts as natural reservoir and is likely the source for vaginal colonization [15, 16], where GBS tends to be present transiently or intermittently rather than permanently [17-21]. Women colonized with GBS prenatally have a 25-fold higher risk of delivering a baby with EOGBS compared with non-colonized women [6, 15]. The rate fluctuates

from 8% to 36% in Denmark, despite the reported obstetrical cohorts being remarkably homogeneous [22, 23], and from 6.5 to 30% in Europe [24]. The colonization rate of pregnant women may vary with characteristics such as age, parity, socio-economic status, geographic location, ethnicity, sexual behavior, body sites sampled, and microbiological procedures [25-29].

Maternal GBS colonization has also been associated with increased risk of urinary tract infection and adverse pregnancy outcome such as endometritis [30], chorioamnionitis [30, 31], preterm delivery, and intrauterine death [32].

GBS infections can present from day 7 to 89 as late-onset infection (LOGBS) [33], which can be acquired from the mother (approximately 50% of infants with LOGBS are colonized at birth with the same GBS serotype as the mother) [34] or from environmental sources [33].

International guidelines for prevention of EOGBS

International guidelines outline two main strategies for identification of women who should be offered intrapartum antibiotic prophylaxis (IAP), the *risk-based* and *the culture-based screening approaches*. Until 1996, both approaches were recommended in guidelines from the Centers for Disease Control (CDC) in the USA, as there was insufficient evidence to recommend one approach over the other. In 2002, the CDC guidelines were reviewed in response to further evidence demonstrating that the risk of EOGBS was significantly lower among the infants of universally screened women than among those in the risk-based group [24]. The culture-screening was over 50% more effective than the risk-based approach of preventing EOGBS [24]. Unfortunately, there had not been prospective and randomized studies for comparison of the efficacy of the two approaches [35].

CDC recommends screening at 35-37 weeks of gestation for GBS recto-vaginal colonization, and IAP will be offered to positive carriers. In USA, the introduction of culture-based screening strategy was followed by a decrease in the EOGBS rate from 1.7 to 0.4 cases per 1,000 births [36].

Despite the widespread implementation of IAP, most cases of EOGBS occur among premature infants or among term infants born to mothers screened GBS-negative [3, 37-39]. In the latest updated CDC guidelines, it is recommended that women with unknown GBS colonization status at the time of birth should have IAP administered in the presence of intrapartum risk factors [5].

The Royal College of Obstetricians and Gynecologists, UK, (RCOG) recommends the risk-based approach and has defined five risk factors: 1) previous infant with EOGBS, 2) GBS bacteriuria during the current pregnancy, 3) temperature >38°C, 4) rupture of membranes (ROM) ≥18 hours, or 5) delivery at <37 weeks of gestation. They claim that 66% of EOGBS neonates are born to mothers with one or more of these risk factors [40-42]. However, it is of concern that a substantial fraction of the women in labor, who are at risk according to the RCOG definition are GBS-negative, and up to 50% of EOGBS cases develop in neonates born to mothers colonized with GBS without any of these risk factors [43-45]. The rates of EOGBS in the UK and Netherlands have risen considerably over the past 20 years. A recent (2014–2015) enhanced surveillance has reported rates of 0.54 and 0.36 per 1,000 live births for EOGBS and LOGBS infections, respectively. These increments have occurred despite the introduction of national guidelines in 2003 recommending a risk-based prevention approach for offering IAP [46]. Further, in 2012 RCOG recommended IAP to women with GBS vaginal carriage detected during the current pregnancy [47], and in 2013 recommended offering IAP to all women, who go into labor before 37 weeks of pregnancy with or without rupture of membranes [48]. According to RCOG, IAP for prevention of EOGBS is now recommended in the following scenarios:

- women in confirmed preterm labor (before 37 weeks of gestation);
- women with a previous baby with early- or late-onset GBS infection;
- women who have had GBS in their urine during the current pregnancy;

- women tested positive for GBS during the current pregnancy (includes incidental or intentional testing);
- women who are carriers of GBS and have term rupture of membranes;
- women who are GBS carriers and have preterm rupture of membranes, along with induction of labor as soon as reasonably possible; and
- women with fever (≥38°C) and in labor regardless of their GBS carrier status.

Although RCOG still recommends against universal GBS screening for all pregnant women, if performed, testing should occur at 35 to 37 weeks of gestation.

The decrease in the EOGBS rate in USA must be categorized as a success. However, one might wonder why the EOGBS rate in some other countries like Denmark is 0.1-0.4/1,000 live births [49], Sweden is 0.4/1,000 live births [50], Norway is 0.5/1,000 live births [51], and Finland is 0.6-0.7/1,000 live births [52], even though they have not implemented this antepartum culture-based screening program.

Regardless of the strategy used, methods with more rapid and accurate identification of GBS-carriers at labor may optimize the use of IAP and thereby reduce the incidence of EOGBS. Recently, several real-time polymerase chain reaction (PCR) tests for the rapid detection of GBS have become commercially available and have been shown to be tests of high sensitivity and specificity [19, 26, 53-55]. This advanced technology may help to overcome the limitations associated with antepartum culture screening methods and may offer point-of-care tests for intrapartum screening.

To facilitate a consensus towards European guidelines for the management of pregnant women in labor and during pregnancy for the prevention of GBS perinatal disease, a conference was organized in 2013 with group of European experts in neonatology, gynecology-obstetrics, and

clinical microbiology. The consensus conference recommended IAP based on a universal intrapartum GBS screening strategy using a rapid real-time testing [56].

Neither the performance of the universal antepartum screening culture strategy, nor that of an intrapartum PCR test has been evaluated in a Danish population of pregnant women, where the risk-based approach is still recommended, and the risk of babies acquiring EOGBS is very low.

GBS bacteriuria and vaginal GBS colonization

GBS bacteriuria during pregnancy is considered a marker of heavy maternal colonization and has been associated with an increased risk of EOGBS [5, 18, 57-62]. However, little is known about the association between the antepartum GBS-urinary colony count and the intrapartum load of GBS in the vagina. If this association is strong, quantification of GBS in the urine may constitute an effective tool for assessing the risk of EOGBS and minimizing the number of women who should be screened for vaginal GBS colonization intrapartum by a PCR test. Antepartum GBS screening of urine might even be able to replace the intrapartum vaginal PCR-GBS test, which is usually not quantitative [63, 64] and is perhaps too sensitive by finding insignificant numbers of GBS in the vagina.

GBS vaginal colonization and preterm delivery

Group B streptococci are a component of the normal vaginal bacterial microflora. During pregnancy, specific changes in bacterial flora can lead to bacterial overgrowth that may increase the risk of ascending infection through the cervix, resulting in bacterial infection of fetal membranes and decidua [65]. This ascending infection causes secretion of proteases that degrade the extracellular matrix within the fetal membranes and/or a host inflammatory response with cytokine production, stimulation of prostaglandin, and protease synthesis. This increases uterine contractility and may result in preterm delivery [66-68].

GBS bacteriuria and preterm delivery (PTD)

Vaginal carriage of GBS during pregnancy is common, and the colonization rate ranges from 6.5% to 36% [22-24]. In pregnancy, GBS colonization causes asymptomatic bacteriuria (ASB) or urinary tract infection [69]. Maternal GBS bacteriuria is considered a surrogate for heavy genital tract colonization and is a recognized risk factor for EOGBS [5, 18, 57-62]. Due partly to dilation of the renal pelvis and ureters already in the eighth week of pregnancy [70], pregnant women are at increased risk of bacteria ascending to the kidneys causing pyelonephritis [71]. Pyelonephritis seems to be an important independent risk factor for PTD [72-74]. Untreated pyelonephritis is associated with low birth weight, prematurity, premature labor, hypertension, preeclampsia, maternal anemia, and amnionitis [75]. Bladder infection during pregnancy is also associated with increased risk of maternal hypertension, anemia, and amnionitis [61]. However, GBS is a poor immunogenic pathogen, and in the absence of systemic inflammation it is highly unlikely to lead to PTD [76].

Pregnant women with GBS in the urine are treated with antibiotics to reduce adverse maternal and neonatal complications like the risk of pyelonephritis, PTD, and low birth weight [77]. GBS bacteriuria is associated with an increased risk of chorioamnionitis [77], which is itself a risk factor for PTD, however a broader role for GBS in triggering PTD remains uncertain [78, 79]. Due to the risk of neonatal and maternal outcomes, it is recommended to screen patients for ASB in early gestation [80]. Some authors recommend repeating the test every trimester to increase the detection rate of ASB [81].

Treatment and follow-up to prevent recolonization in pregnant women with GBS in the urine reduce the incidence of PTD [60]. However, this presumed reduction could not be rediscovered in a subsequent Cochrane review and other studies [82-84]. Treatment does not eliminate GBS colonization, and subsequent re-colonization is common [85] contributing to recurrent presence of

GBS in urine [84]. Several authors have even postulated that antibiotic administration may alter vaginal flora to allow heavy growth of other potentially pathogenic organisms in the upper genital tract, which may subsequently lead to PTD [77, 83]. However, the association between GBS bacteriuria during pregnancy and PTD remains controversial [83, 85-87].

Aim and objectives of the thesis

General aim

The aims of this study were to evaluate three screening methods for intrapartum GBS colonization in a Danish cohort and to investigate the possible association between preterm delivery and colonization of group B streptococci cultured in urine during pregnancy.

Objectives

1. To compare the performance of an antepartum culture-based screening strategy and an intrapartum polymerase chain reaction assay for the prediction of intrapartum vaginal carriage of group B streptococci in a Danish cohort. (Paper I)
2. To assess the performance of a polymerase chain reaction – group B streptococci test in deciding antibiotic prophylaxis in women in labor at term. (Paper II)
3. To evaluate how well group B streptococci colony numbers in the urine at 35-37 weeks of gestation predict the load of group B streptococci colonization of the vagina at birth. (Paper III)
4. To investigate the possible association between preterm delivery and group B streptococci detected in urine culture during pregnancy. (Paper IV)

Materials and Methods

Study Design and population

Studies I, II, and III are three clinical studies based on the same study population, where 2,343 pregnant women attending the prenatal clinic at Lillebaelt Hospital, Kolding, Denmark (with an average of 3,200 deliveries per year) over a 15-month period between April 2013 and June 2014 were invited to participate in this prospective observational study. As 1,364 women declined to participate, 979 participants were in the final cohort (Figure 1). Studies I and II are based on antepartum vaginal and rectal cultures sampled by the pregnant women themselves at 35-37 weeks of gestation, and an intrapartum vaginal swab sample collected by midwives. Study III supplements Study I and II with midstream clean catch urine samples submitted at 35-37 weeks of gestation. All participants provided written informed consent.

Study IV is a retrospective population-based cohort consisting of all pregnant women (n=36,097) from the catchment area of Lillebaelt Hospital, Denmark, during the period January 2002 – December 2012, of whom 17.5% singleton pregnant women submitted urine samples (one or more times) for culturing at the Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark, and gave birth to 6,014 babies.

Figure 1

Flowchart of a prospective Danish cohort of unselected women in labor at term.

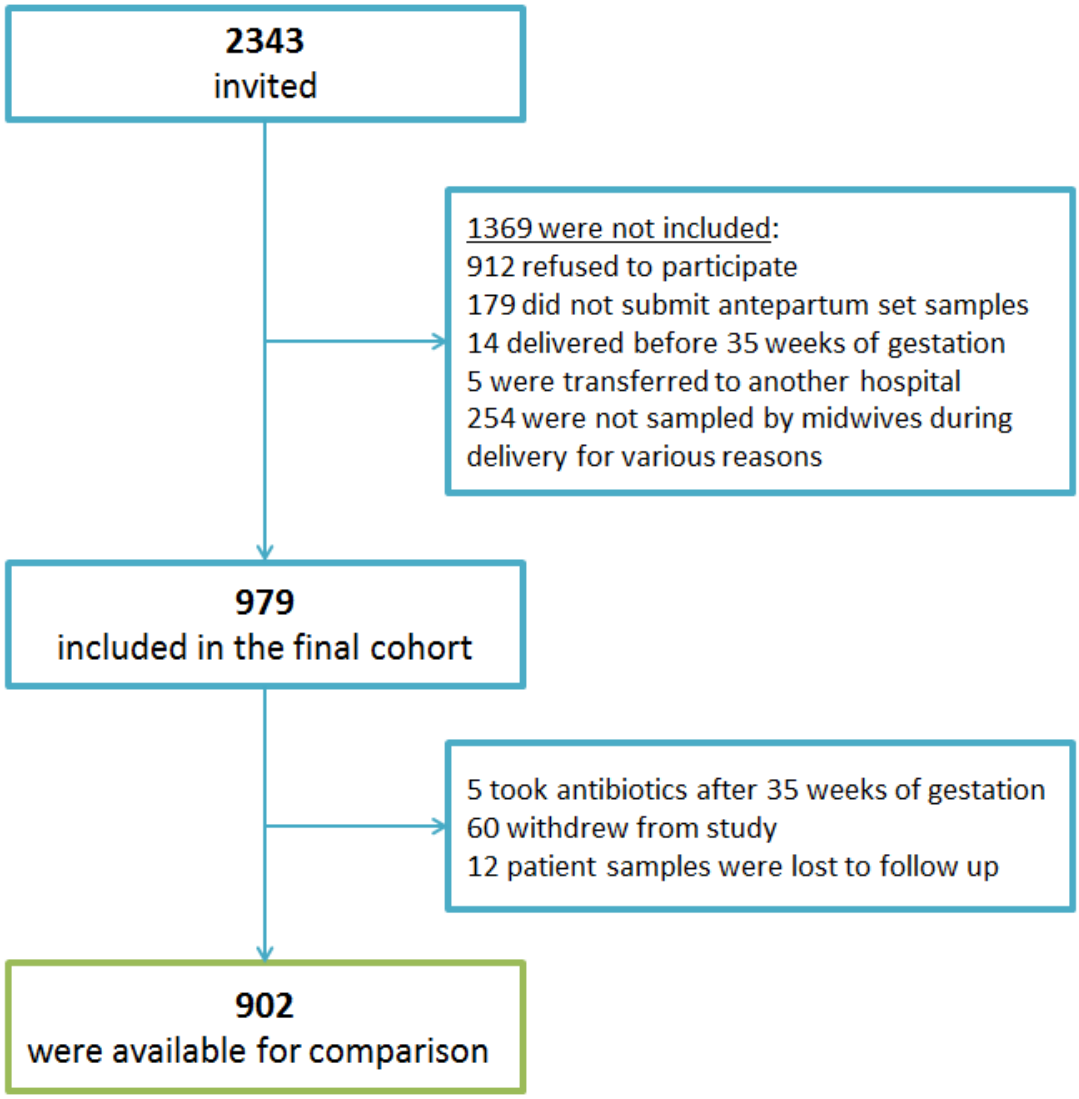
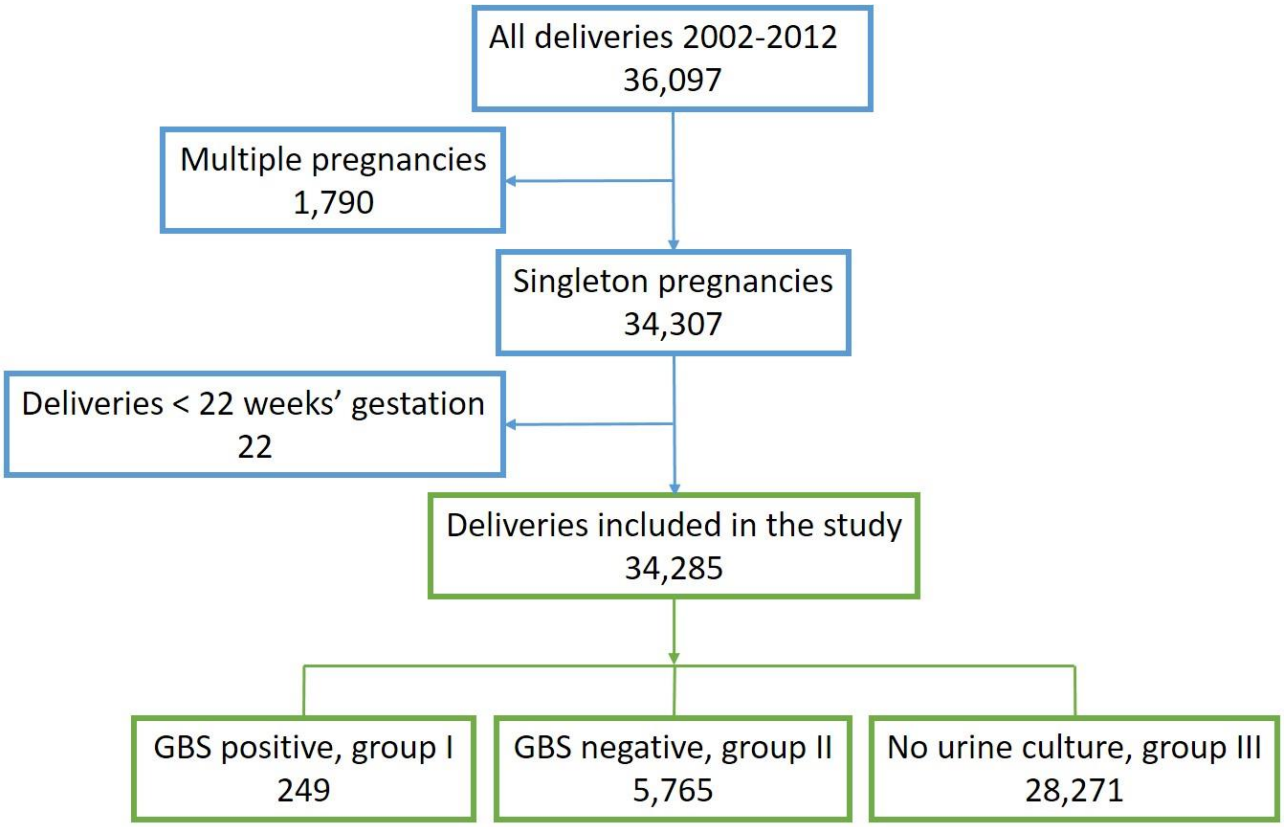


Figure 2

Flowchart describing the cohort, a retrospective population-based cohort



Collection and culture of specimens (for Study I, II, and III)

At 35-37 weeks of gestation, each participant obtained self-administered vaginal and rectal swab samples for culture during a planned visit to the outpatient clinic. During labor, the midwife collected a vaginal swab sample for both culture (reference standard) and a PCR assay for GBS. All samples were collected using nylon flocked swabs submerged separately into 1 ml of E-Swab transport medium (E-Swab, Copan Diagnostics, Brescia, Italy).

In addition to the written information with text and drawings on how women should obtain a self-administered vaginal and rectal swab sample for culture, two instructional videos were available to all participants on the project website.

Inclusion criteria

- Pregnant women attending the prenatal clinic at Lillebaelt Hospital, Kolding, Denmark.

All pregnant women in the catchment area of Lillebaelt Hospital give birth at this clinic, as there are no private or other public alternatives. Only very complicated pregnancies like extreme preterm deliveries are referred to the regional University Hospital.

Exclusion criteria

- Women treated with antibiotics after 35 weeks of gestation
- Preterm labor (before 37+0 weeks of gestation)
- Age under 18 years
- Women with a communication barrier as language or mental health conditions

Culture of GBS

The sampling was carried out by inserting and rotating one E-Swab 1.5-2 cm inside the vagina and another one in the rectum by inserting the swab 1.5-2 cm beyond the anal sphincter. All samples were sent to the Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark.

Samples were cultured at the time of arrival to the laboratory; if received after 8 PM, they were kept at 4^0C until the next morning. Direct plating without a prior enrichment of the specimen in a culture broth was carried out by streaking the E-Swab specimen on a selective Granada agar plate. The vaginal and rectal swabs from the same patient were seeded on different sides of the same Granada agar plate (BioMérieux®, Spain) (Figure 3). The Granada agar plates were incubated immediately after seeding in the CO_2-containing atmosphere at 35 °C. The specimen tubes containing the vaginal intrapartum E-Swab sample medium were subsequently frozen at minus 80°C for later PCR analysis. The Granada agar plates were read after one and two days of incubation.

Urine samples were seeded on a 5% blood agar plate and read after incubation for 24 or 48 hours depending on the initial growth of bacteria. GBS bacteriuria was classified according to the number of colony-forming units per mL (CFU/mL).

- For Study I, growth of intrapartum vaginal culture was classified semi-quantitatively as plates having only growth of few GBS colonies (1+), some (2+) or many (3+).

- For Study II, the presences of risk factors for EOGBS (Early Onset Group B Streptococcal disease) were recorded: 1) Bacteriuria during current pregnancy, 2) Prior infant with EOGBS 3) Temperature above 38.0^{o}C during labor, and 4) Rupture of membranes ≥18 hours.

- For Study III, GBS bacteriuria was classified according to the number of colony-forming units per mL (CFU/mL). Low colony counts refer to < 10^4 CFU/mL, and high colony counts refer to ≥ 10^4 CFU/mL.

Figure 3
The inoculation of the swabs on Granada agar plate from the same patient, indicated by R for the rectum and V for the vagina

MALDI TOF

All GBS-like colonies (identified by their orange color on Granada agar plates) were routinely confirmed as *Streptococcus agalactiae* by identification using the Microflex LT ™ MALDI-TOF system (Bruker Daltonik, Germany).

MALDI TOF stands for Matrix assisted laser desorption ionization (MALDI) and Time of flight (TOF). It is a technique that uses a laser energy absorbing matrix to create ions from large molecules. It has become a standard method in clinical microbiology for identification of micro-organisms such as bacteria or fungi. The method is based on a pure culture of the microbe in question. A portion of a colony is placed onto a metal plate and overlaid with matrix. After laser irradiation of the matrix and sample material, the charged molecules (ions) are accelerated into the mass spectrometer. The mass spectra generated are analyzed by dedicated software and compared with stored profiles of reference strains. Species diagnosis by use of mass spectra is much faster, more accurate, and cheaper than previous laboratory methods based on biochemical tests [88].

The specimens were analyzed without prior enrichment to make the culture findings comparable with the results of the PCR assay.

Real-time polymerase chain reaction (PCR)

The analysis of 902 vaginal samples was performed by real-time PCR on BDMAX™ without enrichment of the specimen in a culture broth prior to analysis (Figure 4). The BDMAX™ System automatically extracts the target nucleic acid and amplifies a section of the cfb gene sequence of the GBS chromosome (Becton, Dickinson), if present. The BDMAX™ assay includes an Internal Process Control to monitor for the presence of potential inhibitory substances as well as system or reagent failures that may be encountered during the entire process. The results are interpreted and produced by the BDMAX™ software as a qualitative answer, either positive or negative for GBS. In a small number of cases, the specimens were initially undetermined because of inhibition,

reagent failure, or system errors. This led to additional testing by taking a new aliquot of the sample and repeating the DNA extraction and PCR assay. The PCR analyses were performed retrospectively on frozen samples for practical reasons [89].

Figure 4

Real-time PCR on BDMAX™

The results of the GBS culture and PCR tests were read by independent laboratory technicians and recorded separately.

The amplified targets are detected in real time using Scorpions® chemistry-based fluorogenic oligonucleotide probe molecules specific to the amplicons for the respective targets. Scorpions chemistry features a bi-functional molecule which includes a PCR primer covalently attached to a probe. Figure 5 is a diagrammatic representation of Scorpions functionality. In Steps 1 and 2, the Scorpions primer is extended on the target DNA. In Step 3, the extended primer is heat-denatured, along with the stem loop of the probe, thereby causing the fluorophore to disassociate. In Step 4, the extended Scorpions primer is rearranged and binds to the newly extended DNA strand as it cools and begins to fluoresce in a target-specific manner, while the un-extended primer is quenched.

Figure 5

Mechanism of action of Scorpions chemistry

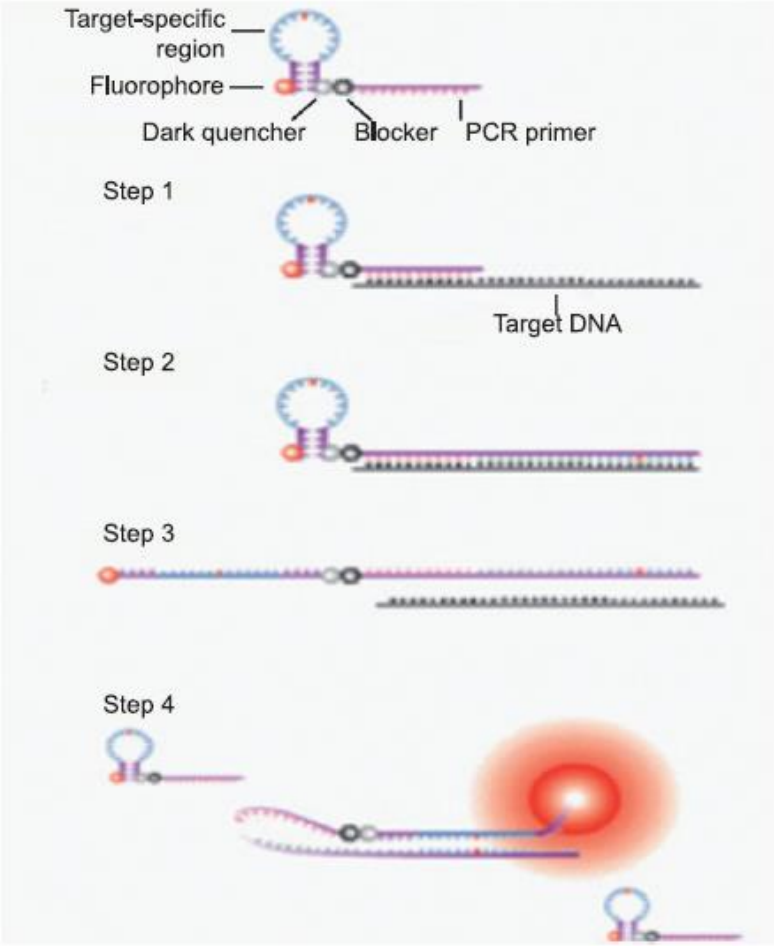
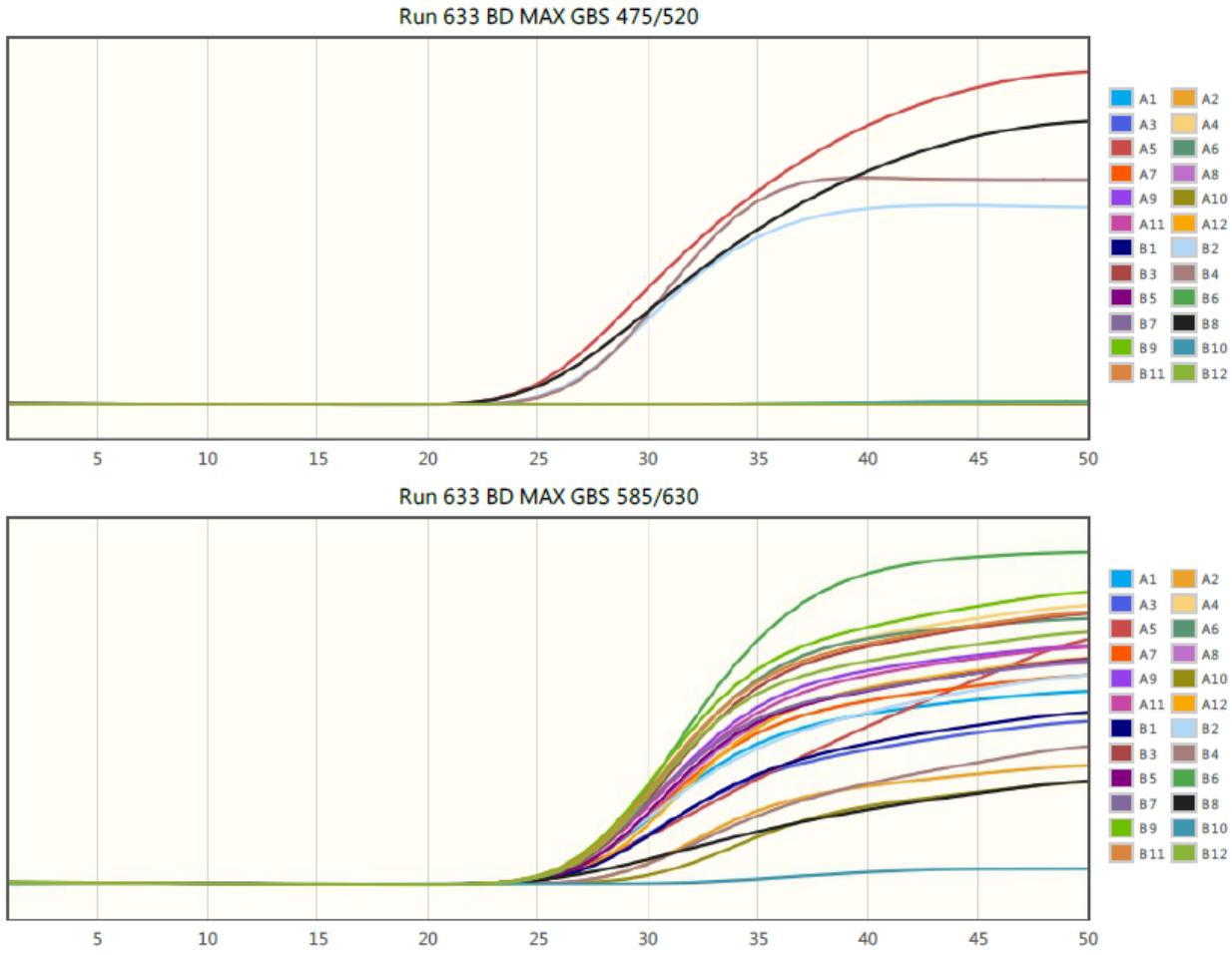


Figure 6 shows the Amplification curves in real-time PCR of templates containing GBS DNA in various concentrations. The PCR curves for each GBS DNA run with its own color. The expression of the measured "quantity" of light is labeled along the y-axis, and the number of PCR cycles (with heating and cooling) is labeled along the x-axis. The upper image shows the curves for GBS measurements, where rising curves (= amplification of the target sequence in GBS) are positive for GBS and the flat curve is negative for GBS. The lower image shows the curves for the measurement of internal control, where a control was added in advance in the kit, and therefore provides a rising curve in all samples.

The lower image shows the curves for the measurement of the internal control. This is a pre-added check in the kit and therefore should give an increasing curve in all samples. If it does not, it is either an inhibition (which can be something in the test that interferes with the PCR) or the PCR for some reason has not elapsed correctly (for example, because of technical errors).

Figure 6

Amplification curves in real-time PCR of templates containing GBS DNA in various concentrations. The PCR curves for each GBS DNA run with its own color.



Collection and culture of specimens (for Study IV)

From a population-based cohort consisting of all pregnant women (n=36,097) from the catchment area of Lillebaelt Hospital, Denmark, during the period January 2002 – December 2012 (11 years),

34,285 deliveries of singleton pregnancies were included in this study. Of those, 17.5% (6,014/34,285) had one or more urine cultures analyzed at the Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark. Maternal, obstetrical, and neonatal data were obtained from the Hospital Information System at Lillebaelt Hospital, which contains data on all hospitalized patients. Linkage of information could be performed using the unique Danish Personal Identification number (CPR). Among others, the following data were extracted: maternal age at delivery, parity, maternal body mass index (BMI), smoking habits, rupture of membranes, birth weight, mode of delivery, past reproductive career, medical diseases, previous cervical cone biopsy(ies), and previous preterm delivery(ies).

National data on prescription of antibiotics to outpatients were extracted from the Danish National Prescription Registry (DNPR) [90], which includes records on all drugs filed at the pharmacy. Antibiotic treatment administered in hospital was obtained by reviewing the patient's medical record. Results from all microbiological examinations were extracted from the Laboratory Information System (MADS) at Department of Clinical Microbiology serving the hospitals and all general practitioners in the catchment area of Lillebaelt Hospital, Denmark. Women with one or more positive bacterial urine specimens for GBS were defined as GBS-positive. Data on all births in Denmark (including gestational age at delivery) were merged from the Danish National Health Service Register [91]. The study population with in- and exclusion criteria is described in Figure 2.

The cohort used in this study was divided into three groups. Group I included women whose urine culture was positive for GBS, group II included women whose urine culture was negative for GBS, and group III included women whose urine had not been cultured.

Urine samples were collected as midstream clean catch urine. If the transportation time to the laboratory exceeded two hours, the samples were stored in refrigerators. In brief, all urine specimens were handled as follows throughout the period: 1 µl were streaked on 5% Danish blood

agar (DBA) for quantitative evaluation. In case of Beta-hemolytic colonies (≥ 1,000 CFU/mL) a representative colony was purified and identified according to conventional laboratory procedures involving a CAMP test and a commercial Latex Agglutination Test for differentiation of streptococci Lancefield groups A, B, C, F, and G. The bioMérieux's chromID CPS agar plate replaced the CAMP-test from 2009. From 2011, all GBS-like colonies were routinely confirmed as *Streptococcus agalactiae* (GBS) using the Microflex LT ™ MALDI-TOF system (Bruker Daltonik, Germany).

Data analyses

Study I, II, and III

STATA Statistics/Data Analysis software (version 14; StataCorp LP) was used for the statistical analysis. Sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and Likelihood ratio (LH) including 95% confidence intervals (CI) were calculated for both antepartum GBS screening and the intrapartum PCR assay using culture of a vaginal swab sample as *the reference standard*. Differences in proportions were compared using either the chi-square test or Fisher's exact test, and Odds ratios were calculated from the two-by-two frequency table as case versus non-case. P values less than 0.05 were considered statistically significant.

- The *sensitivity* of the test (either antepartum culture or intrapartum PCR test) is defined as the probability of the test being positive when the intrapartum vaginal culture (reference standard) is positive, calculated as: $\frac{\text{Test true positive}}{\text{Test true positive + Test false negative}}$ (Table A).
- The *specificity* of the test (either antepartum culture or intrapartum PCR test) is defined as the probability of the test being negative when the intrapartum vaginal culture is negative, calculated as: $\frac{\text{Test true negative}}{\text{Test true negative + test false positive}}$ (Table A).
- The *positive predictive value* (PPV) of the test is defined as the probability for a woman of being positive in the intrapartum vaginal culture (reference standard) when the test (antepartum culture or intrapartum PCR test) is positive, calculated as:

  $\frac{\text{true positive}}{Test\ true\ positive + Test\ false\ positive}$ (Table A).
- The *negative predictive value* (NPV) of the test is defined as the probability for a woman of being negative in the intrapartum vaginal culture (reference standard) when the test (antepartum culture or intrapartum PCR test) is negative, calculated as:

$\frac{true\ negative}{\text{Test true negative + Test false negative}}$ (Table A).

Likelihood ratio (LH) constitutes one of the best ways to measure and express diagnostic accuracy [92]. It is a statistical test that assesses the value of performing a diagnostic test. By using the sensitivity and specificity of the test we can determine whether a test result usefully changes the probability that a condition (such as intrapartum GBS) exists. The sensitivity and specificity can tell us how likely a given test result is in people with the disease, compared to how likely a given test result is in people without the disease. For example, the likelihood that a positive antepartum culture would be expected in a woman with intrapartum GBS compared to the likelihood that the same result would be expected in a woman without intrapartum GBS. LH measures the power of a test (antepartum culture) to change the pre-test into the post-test probability of a disease (intrapartum GBS-positive) being present (Table B).

The LH is a way to incorporate the sensitivity and specificity of a test into a single measure. Since sensitivity and specificity are fixed characteristics of the test itself, the likelihood ratio is independent of the prevalence of the disease in the population. (This is not true for positive predictive value). Likelihood ratio for a positive test (LH+) is defined as the probability of a woman with intrapartum GBS having a positive test (antepartum culture) divided by the probability of woman with intrapartum negative GBS having a positive test, calculated as:

- $\text{LH+} = \frac{\text{Sensitivity}}{1-\text{specificity}}$

The fact that LH is independent of the disease prevalence is important when we are testing a high number of women, where both high sensitivity and specificity are essential. Low sensitivity will give a high number of false-negatives who will not be treated with IPA, while low specificity will give a high number of false-positives who will be treated with IPA without being carriers.

Table A

Antepartum vaginal culture	Intrapartum vaginal culture (Reference standard)		
	Positive	Negative	Total
Positive	67 (true-positive)	33 (false-positive)	100
Negative	37 (false-negative)	765 (true-negative)	802
Total	104	798	902

As is shown in an example of vaginal antepartum culture (Table A):

Sensitivity: $\frac{67}{104}$ = 64% **Specificity**: $\frac{765}{798}$ = 96% **LH+**: $\frac{0.67}{1-0.96}$ = 16.8%

PPV: $\frac{67}{100}$ = 67% **NPV**: $\frac{765}{802}$ = 95%

Table B

How much do LHs change disease (intrapartum GBS-positive) likelihood?	
LHs greater than 10 or less than 0.1	cause large changes
LHs 5 - 10 or 0.1 - 0.2	cause moderate changes
LHs 2 - 5 or 0.2 - 0.5	cause small changes
LHs less than 2 or greater than 0.5	cause tiny changes
LHs = 1.0	cause no change at all

Study IV

There are a variety of regression methodologies based on the type of response variable, the type of model that is required to provide an adequate fit to the data, and the estimation method. The type of the regression model depends on the distribution of the response variable (dependent variable); if it is dichotomous, we use logistic regression. These methods allow us to assess the impact of multiple variables (covariates and factors) in the same model [93, 94]. Logistic regression is a mathematical model that provides an odds ratio controlled for possible confounders. This odds ratio is known as the adjusted odds ratio, because its value has been adjusted for the other covariates (including confounders) [95].

In the current study, the primary outcome was preterm delivery before 37 weeks of gestation. Secondary outcome was urine culture status; GBS-positive versus GBS-negative (Table 9), and cultured versus not cultured (Table 2 in Paper VI). Statistical analyses included comparisons between groups presented as dichotomous and categorical variables using univariate logistic regression **[96]** reported as Odds Ratios (ORs), and Chi-square tests (trend analyses) reported as p-values on secondary outcome. Binary multiple logistic regression analysis was performed on primary outcome and including á priori defined variables as possible confounders in the model (Table 10); the predefined confounders included age, BMI, parity, prior PTD, prior cervical cone biopsy, hypertension, pre-eclampsia, diabetes Type 1, gestational diabetes, tobacco use, inflammatory bowel disease, cervix insufficiency, and early bleeding. Potential confounders were included in the model as additional categorical variables, taking into account findings from prior knowledge, previous scientific literature, and what is known about the possible etiological mechanisms. All explanatory variables, both exposure and potential confounders, were treated in our model in the same way, as one of the main advantages of regression modelling. P values < 0.05 were considered statistically significant.

Ethics

The Regional Scientific Ethics Committee for Southern Denmark approved the experimental protocol (S-20130089), and the study was reported to the Danish Data Protection Agency (2008-58-0035). The date of issue: 6 November 2013. All participants provided written informed consent. In addition to the oral and written information with text and drawings on how women should obtain a self-administered vaginal and rectal swab sample for the culture, two instructional videos were available to all participants on the project website.

Results

The overall number of women enrolled in the three prospective studies was 902. They all had a urine test and an antepartum swab obtained as part of the culture-strategy and an intrapartum swab as part of the culture-based reference standard and the PCR-strategy. The demographic characteristics of women included in the study and those who did not, either because they refused to participate or did not submit antepartum samples despite prior acceptance, are shown in Table 1. The only significant difference between the groups was on tobacco, where the rate of smokers in the invited cohort in our study was 10.1%; 8.3% vs. 11.2% for participating and non-participating pregnant women (OR 0.3; 95% CI 0.3 – 0.4; p=0.0001). The demographic characteristics of women enrolled and tested are shown in Table 2. For Study I, growth of intrapartum vaginal culture was classified semi-quantitatively as plates having only growth of few GBS colonies (1+), some (2+), or many (3+). For Study II, the presence of risk factors for EOGBS was recorded, while for Study III, GBS bacteriuria at 35-37 weeks of gestation was classified according to the number of colony-forming units per mL (CFU/mL).

Table 1
Demographic characteristics between women who were included in the study and those who did not

	Included (N=902)		Not included (N=1441*)				
Maternal characteristics	Number	%	Number	%	OR	95% CI	P-value
Age of the mother							
Under 25	87	9.7	171	11.9	0.8	0.6 – 1.04	0.095
25-34	614	68.1	948	65.8	1.1	0.9 – 1.3	0.254
Above 35	201	22.3	322	22.4	1.0	0.8 – 1.2	0.972
Parity							
1	407	45.1	639	44.3	1.0	0.9 – 1.2	0.713
2	446	49.5	740	51.4	0.9	0.8 – 1.1	0.369
3 or more	49	5.4	62	4.3	1.3	0.9 – 1.9	0.211
Body mass index							
Under 24.9	577	64.0	908	63.0	1.0	0.9 – 1.2	0.640

25-29.9	207	23.0	329	22.8	1.0	0.8 – 1.2	0.947
30- or more	118	13.1	204	14.2	0.9	0.7 – 1.2	0.735
Tobacco							
Never smoking	827	91.7	1,280	88.8	0.3	0.3 – 0.4	0.0001
Stopped in pregnancy	27	3.0	44	3.1	1.0	0.6 – 1.6	0.934
Smoke =< 10 cigarettes	35	3.9	79	5.5	0.7	0.5 – 1.1	0.081
Smoke > 10 cigarettes	13	1.4	38	2.6	0.5	0.3 – 1.02	0.057

* Those that either refused to participate or did not submit antepartum samples despite prior acceptance.

Table 2

Demographic characteristics of women enrolled and tested in the three prospective studies; N= 902.

	GBS positive (N=128)		GBS negative (N=774)				
Maternal characteristics	Number	%	Number	%	OR	95% CI	P-value
Age of the mother							
Under 25	6	4.7	81	10.5	0.42	0.18 to 0.99	0.05
25-34	93	72.7	521	67.3	1.29	0.85 to 1.96	0.23
Above 35	29	22.7	172	22.2	1.03	0.66 to 1.60	0.91
Parity							
1	49	38.3	358	46.3	0.72	0.49 to 1.06	0.09
2	68	53.1	378	48.8	1.19	0.82 to 1.73	0.46
3 or more	11	8.6	38	4.9	1.82	0.91 to 3.66	0.37
Body mass index							
Under 24.9	72	56.3	505	65.3	0.69	0.47 to 1.00	0.05
25-29.9	35	27.3	172	22.2	1.32	0.86 to 2.01	0.20
30- or more	21	16.4	97	12.5	1.37	0.82 to 2.29	0.23
Tobacco							
Never smoking	121	94.5	706	91.2	1.67	0.75 to 3.71	0.21
Stopped in pregnancy	2	1.6	25	3.2	0.48	0.11 to 2.03	0.32
Smoke =< 10 cigarettes	3	2.3	32	4.1	0.56	0.17 to 1.85	0.34
Smoke > 10 cigarettes	2	1.6	11	1.4	1.10	0.24 to 5.03	0.90

Study I

The concordance between detection of GBS colonization analyzed by antepartum culture (rectum and vaginal) and intrapartum culture (vagina) as the reference standard is shown in Table 3. The intrapartum vaginal GBS colonization rate detected by culture was 11.5% (reference standard). By comparison, the culture-based strategy found 9.4% (85/902) GBS-positive women by combining results from antepartum vaginal and rectal swab cultures (7.4% by vaginal swab samples and 8.9% by rectal samples), and the PCR-strategy (intrapartum vaginal swab sample) found 12.2% GBS-positive women (Table 3).

Based on the reference standard, the performance characteristics of the culture-strategy and the PCR-strategy are given in Table 4. A marked difference was seen between the positive likelihood ratios (LH+) of 9.2 for the culture-strategy and 27.5 for the PCR-strategy. The positive predictive value was 55% for combined antepartum vaginal and rectal swab cultures, and 78% for PCR-strategy.

The false-negative rate by the PCR-strategy was 17% (18/104). Fourteen of these 18 false-negative samples were assessed by the semi-quantitative culture assessment, and among these 12 (86%) were classified with only few GBS colonies (data not shown) (Paper I).

We found no statistical difference between antepartum self-administered vaginal samples (11.1%) and intrapartum midwife-assisted samples (11.5%) (OR 0.96, 95% CI 0.7-1.3, P=0.77).

Table 3

Concordance between detection of GBS colonization analyzed by antepartum culture (rectum and vaginal) and intrapartum culture (vagina) as the reference standard

Antepartum culture		Intrapartum vaginal culture (reference)					
		Positive		Negative		Total = 902	
		N (%)	95% CI	N (%)	95% CI	N (%)	95% CI
Vagina or rectum	Positive	85 (9.4)	7.6-11.5	71 (7.9)	6.2-9.8	156 (17.3)	14.9-19.9
	Negative	19 (2.1)	1.3-3.3	727 (80.6)	77.9-83.1	746 (82.7)	80.1-85.1
Vagina	Positive	67 (7.4)	5.8-9.3	33 (3.7)	2.5-5.1	100 (11.1)	9.1-13.3
	Negative	37 (4.1)	2.9-5.6	765 (81.8)	82.3-87.1	802 (88.9)	86.7-90.9
Rectum	Positive	80 (8.9)	7.1-10.9	66 (7.3)	5.7-9.2	146 (16.2)	13.8-18.8
	Negative	24 (2.7)	1.7-3.9	732 (81.2)	78.4-83.7	756 (83.8)	81.2-86.2

CI= Confidence interval

Table 4

Performance of antepartum vaginal and rectal culture and intrapartum vaginal PCR test using intrapartum vaginal culture for GBS as the reference standard; N=902

	Antepartum culture For GBS						Intrapartum PCR For GBS	
	Vagina		Rectum		Vagina or rectum		Vagina	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Sensitivity	64	54-74	77	68-85	82	73-89	83	74-89
Specificity	96	94-97	92	90-94	91	89-93	97	96-98
PPV	67	55-74	55	46-63	55	46-63	78	69-86
NPV	95	94-97	97	95-98	98	96-99	98	96-99
LH+	16	11-22	9	7-12	9	7-12	27	18-41

CI=confidence interval; PPV=positive predictive value; NPV=negative predictive value; LH= Likelihood ratio

Study II

The overall number of women with either vaginal positive culture or GBS-PCR positive was 128 (14.2%). Among the 902 participants, 12.0% (108) had one or more risk factors for EOGBS (Table 5), of which 23.2% (25/108) were vaginal GBS-culture positive.

The EOGBS risk factor most strongly associated with intrapartum vaginal GBS colonization was GBS bacteriuria during pregnancy. Eighteen of the 30 pregnant women with GBS bacteriuria (60%) were vaginal GBS-culture positive (Table 5). However, seven of the 30 women (23%) were GBS-PCR positive despite being GBS-culture negative (data not shown) (Paper II).

In total, 2.7% (25/902) had both one or more risk factors and a positive vaginal GBS culture (Table 4), whereas 3.6% (32/902) had both one or more risk factors and a positive GBS-PCR test (Table 4). Among the participants with risk factors, the sensitivity of the GBS-PCR test was 92% (23/25) using the vaginal GBS culture as a reference standard (Table 6).

Ninety-four percent (101/108) of women with one or more risk factors received IAP during labor. Two women (2%) underwent an elective caesarian section and were treated routinely with cefuroxime during operation also providing IAP. For the last five (5%), the IAP could not be technically implemented for various reasons such as a quick or hectic birth (data not shown).

Table 5
Intrapartum GBS prevalence by vaginal culture and PCR test for each of the four risk factors (N=902).

	Intrapartum vaginal culture	Intrapartum PCR test
Risk factors	Positive rate % (No)	Positive rate % (No)
EOGBS in prior delivery	0% (0/1)	0% (0/1)
GBS bacteriuria	60% (18/30)	80% (24/30)
Fever (≥38.0°C)	0% (0/9)	0% (0/9)
ROM ≥18 hours	10% (7/68)	12% (8/68)
Total with risk factors	23% (25/108)	30% (32/108)
No risk factors	10% (79/794)	10% (78/794)
Total	12% (104/902)	12% (110/902)

ROM=rupture of membranes
Note: Appendix to Paper II contains 2 small errors in Table 2, which are corrected here in Table 4. We addressed this error to the journal, and a Corrigendum was published (is attached to the thesis as an extra appendix entitled: "Corrigendum to Paper II".

Table 6
Performance of risk-based screening and intrapartum PCR-GBS test individually or in combination for detection of intrapartum GBS carriage[#].

	One or more Risk factors (N=902)		PCR-GBS (N=902)		PCR-GBS if Risk-factor present (N=108)	
	% (n)	95% CI	% (n)	95% CI	% (n)	95% CI
Sensitivity	24%	16-33%	83%	74-89%	92%	74-99%
Specificity	90%	87-92%	97%	96-98%	89%	80-95%
PPV	23%	16-32%	78%	69-86%	72%	53-86%
NPV	90%	88-92%	98%	96-99%	97%	91-100%

CI=confidence interval; PPV=positive predictive value; NPV=negative predictive value
# Reference standard: Vaginal GBS colonization rate (N 104, 12%)

Study III

No demographic characteristic differences (neither as categories shown nor as continuous variables) were seen between participants who were GBS-positive and those who were GBS-negative (Table 2). The rate of GBS in urine at 35-37 weeks of gestation was 5.9%, whereas the rate of GBS in vagina at birth was 11.5% (104/902). Those with and without GBS in urine at 35-37 weeks of gestation did not differ on age of the mother, parity, body mass index, or tobacco use. The association between GBS colony-count in urine at 35-37 weeks of gestation and vaginal culture at 35-37 weeks of gestation and labor is shown in Table 7.

We evaluated the ability of GBS in urine at 35-37 weeks of gestation to predict GBS in vagina at birth. The sensitivity was 30% concerning any degree of GBS in vagina at birth and the positive predictive value (PPV) was 59% (Table 8). For high load (+3) GBS in vagina at birth, this sensitivity increased to 52% (17/33) (Table 9). The GBS colony count in case of GBS in urine at 35-37 weeks of gestation was informative. Thus, 6/17 (35%) with GBS in urine at 35-37 weeks of gestation was $<10^4$ CFU/mL had GBS in vagina at birth (Table 9). The corresponding figures for 10^4 CFU/mL were 19/27 (70%), and for $>10^4$ CFU/mL 6/9 (67%) (Table 9). GBS in vagina at 35-37 weeks of gestation predicted GBS in vagina at birth with a sensitivity of 64% (67 of 104 cases; data not shown). Furthermore, GBS in urine at 35-37 weeks of gestation predicted GBS in vagina at 35-37 weeks of gestation with a sensitivity of 48% (48/100) and a specificity of 99% (797/802).

Table 7
Association between GBS colony-count in urine at 35-37 weeks of gestation and vaginal culture at 35-37 weeks of gestation and labor.

		GBS in urine at 35-37 weeks´ gestation					
Vagina culture		Positive (N=53)		Negative (N=849)		Total (N=902)	
		N (%)	95% CI	N (%)	95% CI	N (%)	95% CI
At 35-37 weeks	Positive	48 (5.3)	3.9-7.0	52 (5.8)	4.3-7.5	100 (11.1)	9.1-13.3
	Negative	5 (0.6)	0.2-1.3	797 (88.4)	86.1-90.4	802 (88.9)	86.7-90.9

At labor	Positive	31(3.4)	2.3-4.8	73 (8.1)	6.4-10.1	104 (11.5)	9.5-13.8
	Negative	22 (2.4)	1.5-3.7	776 (86.0)	83.6-88.2	798 (88.5)	86.2-90.5

CI=confidence interval

Table 8

Performance of culture of GBS in urine at gestational week 35-37 for prediction of vaginal GBS colonization same day and at birth.

	Week 35-37*		Intrapartum**	
	% (n/N)***	95% CI	% (n/N)***	95% CI
Sensitivity	48% (48/100)	37.9 – 58.2%	30% (31/104)	21.2 - 39.6%
Specificity	99% (797/802)	98.6 – 99.8%	97% (776/798)	95.9 - 98.3%
PPV	91% (48/53)	79.6 – 95.9%	59% (31/53)	45.9 - 70.1%
NPV	94% (797/849)	92.7 – 94.9%	91% (776/849)	90.4 - 92.3%

CI=confidence interval; PPV=positive predictive value; NPV=negative predictive value

* Vaginal swab cultures obtained the same day as the urine-samples at gestational week 35-37 and ** the day of delivery, respectively.

*** number of women with GBS-positive antepartum urine samples (n) and number of women with vaginal samples with GBS (N) at week 35-37 and intrapartum, respectively

Table 9

GBS colony-counts in urine at gestational week 35-37 compared to intrapartum semi-quantitative culture[1] assessment of vaginal GBS.

	Vaginal GBS detected intrapartum					
Antepartum culture of GBS in urine	Negative	NA[2]	1+	2+	3+	Total
Negative	776	21	17	19	16	849
Colony count $<10^4$ CFU/mL	11	2	2	1	1	17
Colony count $=10^4$ CFU/mL	8	4	2	3	10	27
Colony count $>10^4$ CFU/mL	3	0	0	0	6	9
Total	798	27	21	23	33	902

[1] Semi quantitative assessment of vaginal GBS colonies: 1+: few; 2+: some; 3+: many.

[2] NA = Culture result was only registered at Department of Clinical Microbiology as positive or negative. The 27 Culture positive samples must at least correspond to 1+.

Study IV

The study population is described in Figure 2. Among the 34,285 singletons, the rate of women colonized by GBS in the cultured group was 4.1% (249/6,014; 95% CI: 3.7-4.7). The overall rate of singleton preterm delivery (PTD) was 5.8% (1,978/34,285; 95% CI: 5.5-6.0).

The demographic characteristics of GBS-positive (GI) versus GBS-negative (GII) in the cultured group of women are shown in Table 10. Corresponding data for urine-cultured women (GI/GII) versus the urine-uncultured group (GIII) are available in Paper IV.

The overall pattern shows fewer statistically significant differences between GI and GII than between GI/GII and GIII. The results are best illustrated in Figure 7 with positive (ORs above 1.00) and negative (ORs below 1.00) statistically significant differences in 25 and 4 variables, when comparing GI/GII and GIII, with the corresponding figures for comparison of GI and GII being 9 and 2. Further, analyses for trend on categorical variables also support these findings, with highly statistically significant ($p<0.001$) differences on all comparisons made between GI/GII vs. GIII (Paper IV).

We found no association on PTD and GBS bacteriuria between GBS-positive and GBS-negative women in the two cultured groups (OR=0.89; 95% CI: 0.5-1.4; p=0.610) (Table 11). After controlling for potential confounders, the PTD was still not associated with GBS bacteriuria (OR=0.99; 95% CI: 0.6-1.6; p=0.972) (Table 11). However, the two cultured groups had statistically significantly higher risk for PTD than the group with no urine specimens taken for culture (OR=1.96; 95% CI: 1.8-2.2; p<0.000) (Table 11). After controlling for potential confounders, the cultured groups I and II remained associated with PTD when compared with group III who had no urine specimens cultured during pregnancy (OR= 1.80; 95% CI: 1.6 to 2.0; P=0.000) (Table 11). Among women with positive GBS in the urine, no correlation was found to women with

early term deliveries between 37-39 weeks of gestation, when compared with women delivering at 40 weeks of gestation or later.

Table 10
Characteristics of the GBS-positive group (GI) and GBS-negative group (GII), N=6,014.

GBS status - GI versus GII								
	GI N=249		GII N=5,765		OR	95% CI	P-value	Trend P-Value
Maternal characteristics	N	%	N	%				
Age of mother (years)								
Under 25	31	12.5	985	17.1	Ref			0.604
25-34	185	74.3	3,855	66.9	1.53	1.0-2.3	0.033	
35-40	27	10.8	801	13.9	1.07	0.6-1.8	0.798	
Above 40	6	2.4	124	2.2	1.54	0.6-3.8	0.346	
BMI								
Under 18.5	16	6.7	254	4.8	1.44	0.8-2.5	0.184	**0.029**
18.5-24.9	127	53.1	2,980	56.6	Ref			
25-29.9	63	26.4	1,189	22.6	1.24	0.9-1.7	0.166	
30-34.9	18	7.5	544	10.3	0.76	0.5-1.3	0.280	
35-39.9	10	4.1	206	3.9	1.10	0.6-2.1	0.776	
40-44.9	2	0.8	68	1.3	0.67	0.2-2.8	0.579	
45 or more	3	1.3	26	0.5	2.51	0.8-8.4	0.134	
Status unknown	10		498					
Obstetric history								
Parity								
0	126	50.6	3,408	59.1	Ref			**0.000**
1	75	30.1	1,730	30.1	1.17	0.9-1.6	0.285	
2	39	15.6	528	9.2	2.00	1.4-2.9	0.000	
3	8	3.2	86	1.5	2.52	1.2-5.3	0.015	
4 or more	1	0.4	13	0.2	2.08	0.3-16.0	0.482	
Prior PTD								
0	228		5,528		Ref			**0.004**
1	12	4.8	129	2.2	2.64	0.9-7.5	0.067	
2	8	3.2	93	1.6	2.13	0.9-4.7	0.058	
3 or more	1	0.4	15	0.3	1.59	0.2-12.1	0.656	
Abortion spontaneous > 4	0	0.0	27	0.5	-	-	-	-
Prior cervical cone biopsy								
0	243		5,615		Ref			0.775

1	6	2.4	143	2.5	1.02	0.5-2.3	0.961	
2 or more	0	0.0	7	0.1	-	-	-	
Medical outcomes								
Hypertension	1	0.4	10	0.2	2.32	0.3-18.2	0.432	-
Pre-eclampsia	9	3.6	225	3.9	0.92	0.5-1.8	0.818	-
Diabetes								
No Diabetes	224		5,520		Ref			**0.000**
Type 1	2	0.8	6	0.1	7.77	1.5-38.7	0.012	
Type 2	0	0.0	3	0.1	-	-	-	
Gestational diabetes	23	9.2	236	4.1	2.38	1.5-3.7	0.000	
Tobacco use								
Never smoking	197	86.8	3,968	79.3	Ref			**0.001**
Stopped in trimester 1	11	4.9	220	4.4	1.01	0.5-1.9	0.982	
Stopped in trimester 2	7	3.1	167	3.3	0.84	0.4-1.8	0.666	
Smoke =< 5 cigarettes	5	2.2	253	5.1	0.40	0.2-1.0	0.044	
Smoke 6-10 cigarettes	4	1.8	235	4.7	0.34	0.1-0.9	0.036	
Smoke 11-20 cigarettes	1	0.4	66	1.3	0.31	0.04-2.2	0.240	
Smoke > 20 cigarettes	0	0.0	11	0.2	-	-	-	
Amount unknown	2	0.9	82	1.6	0.49	0.1-2.0	0.323	
Status unknown	22	8.8	763	13.2	0.58	0.4-0.9	0.017	
Infections								
Chorioamnionitis	0	0.0	5	0.1	-	-	-	-
Urinary tract infection	33	13.3	342	5.9	2.42	1.7-3.6	0.000	-
Pyelonephritis	0	0.0	8	0.1	-	-	-	-
Inflammatory bowel disease	4	1.6	30	0.5	3.12	1.1-8.9	0.034	-
Obstetric outcomes								
Emergency CS	36	14.5	657	11.4	1.31	0.9-1.9	0.140	-
Cervix insufficiency	3	1.2	19	0.3	3.69	1.1-12.6	0.037	-
Early bleeding	9	3.6	100	1.7	2.12	1.1-4.3	0.033	-
Abruption of Placentae	0	0.0	18	0.3	-	-	-	-
Placenta Previa	0	0.0	19	0.3	-	-	-	-
Hydronephrosis	1	0.4	9	0.2	2.58	0.3-20.4	0.370	-
Threatened preterm delivery	9	3.6	208	3.6	1.00	0.5-2.0	0.996	-
Threatened miscarriage	3	1.2	160	2.8	0.43	0.1-1.4	0.147	-

GI= cultured GBS-positive; GII= cultured GBS-negative; OR= Odds ratio; Ref= Referent category; CS= caesarean section.

Yellow color indicates positive (above 1.00) statistically significant OR, while blue color indicates the opposite.

Figure 7

Summary of differences between cultured groups (GI versus GII) and between cultured groups (GI/GII) and uncultured group (GIII) reported as statistically significant Odds ratios.

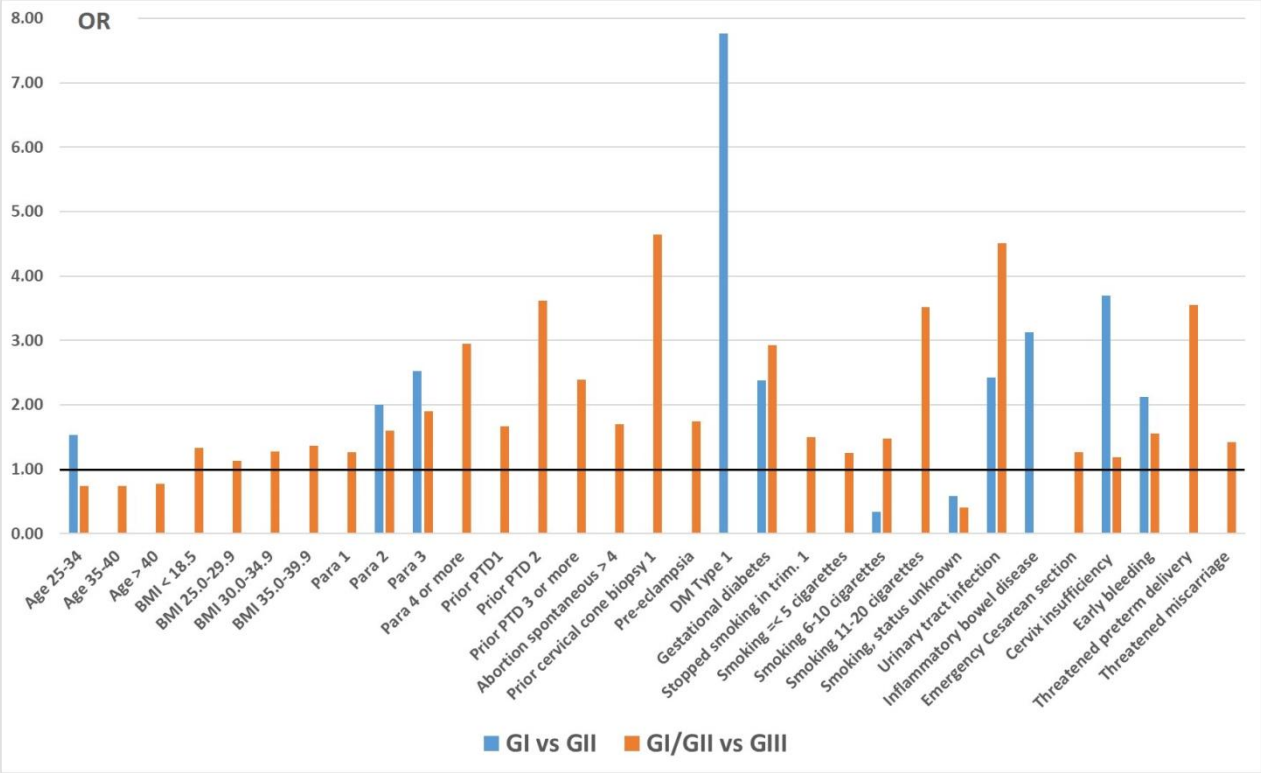


Table 11

Crude and adjusted odds ratios for preterm delivery between groups

Preterm delivery< 37 weeks´ gestation							
	N	%	N	%	Crude OR (95% CI)	Adjusted OR (95% CI)	P value
GI vs. GII	21	8.4	542	9.4	0.89 (0.5-1.4)	0.99 (0.6-1.6)	0.970
GI+GII vs. GIII	563	9.4	1,415	5.0	1.96 (1.8-2.2)	1.80 (1.6-2.0)	0.000

GI= cultured GBS-positive; GII= cultured GBS-negative; GIII= uncultured; OR= Odds ratio;
GI: N= 249; GII: N= 5,765, GI+GII: N= 6,014; GIII: N= 28,271
Possible confounders included age, BMI, parity, prior PTD, prior cervical cone biopsy, hypertension, pre-eclampsia, diabetes Type 1, gestational diabetes, tobacco use, inflammatory bowel disease, cervix insufficiency, and early bleeding.

Discussion

Main findings

In a Danish population with a low rate of early onset neonatal infection with GBS, the intrapartum PCR assay performed better than the antepartum culture for identification of GBS vaginal carriers during labor. In programs that aim to give penicillin to women with vaginal GBS colonization at labor (12% in the present study), the PCR-GBS will perform well (sensitivity 83% and specificity 97%). In programs aiming to treat only GBS-carriers among those with risk factors of EOGBS, a reduction of penicillin usage by two-thirds from 12% to 4% may be possible. Among the participants with risk factors, the sensitivity of the GBS-PCR test was 92%.

Even though the urinary GBS cell count at 35-37 weeks of gestation is strongly associated with a high load of vaginal GBS colonization intrapartum, it may not perform satisfactorily as a standalone-screening marker for risk of early-onset GBS disease.

We found no association between asymptomatic GBS bacteriuria and preterm delivery among women with singleton pregnancy and urine specimens cultured during pregnancy. The cultured group differed considerably from the group of women with no urine cultures on the vast majority of variables examined. The risk of PTD was statistical significantly associated with being in one of the two groups of women with urine specimens cultured for GBS, even after controlling for potential confounders. Previous suggestions of such association may be compromised by a selection problem for testing due to a high-risk profile of pregnancy complications in pregnant women selected for urine culture.

The importance of the study findings

In Studies I and II, we assessed three different strategies on a Danish cohort, in a country where the incidence of EOGBS is low, and the risk-based approach is still recommended, namely 1) risk-based approach, 2) culture-based screening, and 3) intrapartum PCR test.

Strategy 1: As the situation is today and in accordance with the RCOG recommendations, a substantial overtreatment is shown in offering IAP to all women in labor who have with one or more risk factors for EOGBS. Our study proposal of offering IAP to women with one or more risk factors for EOGBS and with a positive PCR test would reduce the use of IAP.

Strategy 2: Of those women with positive antepartum cultures, 46% (71/156) were no longer positive at labor, and thus would be offered unnecessary IAP. A substantial number of positive mothers for GBS at delivery (18%) were not identified as carriers by antepartum cultures. These women would not be offered antibiotics at intrapartum, and thus would be at risk of EOGBS.

Strategy 3: The universal intrapartum PCR assay performs better than the antepartum culture for identification of GBS vaginal carriers during labor. Semi-quantitation assessment of the GBS in the intrapartum vaginal samples indicate that the potential lower sensitivity of a PCR assay is primarily caused by a failure to detect vaginal colonization with low numbers of GBS, which may be of less risk for the newborn during birth [97, 98]. The false-positives of the intrapartum PCR test (n=24, 3%) were less than half of the false-positives in an antepartum culture screening (n=71, 8%). This means that more than double the women will be over-treated in antepartum screening strategy compared with the PCR assay. In addition, other bacteria of the genital tract can inhibit the growth of GBS even when using the selective broth. The supposedly false-positive results in PCR may thus actually indicate the presence of GBS in the studied material, as the sensitivity of this analytical technique could be greater than that of the bacteriological examination [99].

Furthermore, we found that antepartum urinary GBS colony number is strongly associated with a high degree of vaginal GBS colonization intrapartum, a fact that could be useful for improvements of strategy studies in the future (Study III).

Finally, we found no association between asymptomatic GBS bacteriuria and preterm delivery among women with singleton pregnancy and cultured during pregnancy. Previous suggestions of such findings can be due to a selection problem (Study IV). This may have an impact on the overuse of antibiotics because of asymptomatic GBS-bacteriuria during pregnancy, especially in cases with colony counts $<10^4$ CFU/mL.

Methodological considerations

Strengths and Limitations

Study I, II, and III

The overall participation rate is 39% with a slow startup and gradually an increasing number of participants over time in our study. For most women, the reason for not participating has been the idea of self-administered culturing, which many considered to be awkward. This is also the reason why the 179 women who did not submit antepartum samples, even though they had accepted to participate. The number of participants increased as improvement of information was made during the project period for both participants and health care personnel.

About 10% of the women presenting in labor were not ethnic Danish, and those with communication challenges were excluded.

The strengths of our studies are the size of the cohort (902 participants from a well-defined population who received no antibiotics between antepartum culture and the time of labor), and that we have combined the quantitative assessment of GBS in urine antepartum with a semi-quantitative assessment of GBS in vagina at birth in a prospective cohort design with a relatively high number of participants.

Intrapartum screening: It could also be considered as a limitation that we chose vaginal GBS colonization as the reference standard instead of rectovaginal GBS colonization, knowing that obtaining swab samples from both the rectum and vagina would improve the yield compared with only sampling the vagina [100]. Our consideration is that although the rectum in many women constitutes a GBS reservoir from which the vagina is colonized interminably, EOGBS seems to depend primarily on the actual vaginal GBS colonization during labor [101]. Furthermore, a number of studies have shown that as many as 10% of women with a negative antepartum screening result at 35-37 weeks of gestation become positive for GBS vaginal carriage at the time of delivery [3, 37, 39]. A Swedish study that used both vaginal and rectal swabs showed a GBS prevalence rate up to 25% [102].

In view of the very low risk of EOGBS, it is obvious that too many women, or possibly the wrong women, will get antibiotic prophylaxis. The purpose is thus not necessarily to find the most possible colonized women with GBS in the vagina or rectum. We believe that the amount of GBS in the vagina probably correlates best to the risk of infection to the child and the development of EOGBS, i.e. that it is not important to detect carriers with small amounts of GBS in the vagina. We find that the potential lower sensitivity of a PCR assay with a false-negative rate of 17% (18/104) is primarily caused by a failure to detect vaginal colonization with low numbers of GBS, which may be of less risk for the newborn during birth. A number of studies suggest, through indirect observation that low-level carriage of GBS is associated with a reduced transmission rate compared to women with higher bacterial burdens [85, 86].

Antepartum screening: We find it appropriate to include both vaginal and rectal swabs in antepartum screening. The lower sensitivity and specificity of antepartum culture has also been found previously; several studies have shown a low sensitivity of antenatal GBS cultures to detect colonization during labor [62, 103-105].

The main issue here is the question of who are the pregnant women that deliver babies developing EOGBS. We know there is also a human/genetic factor and a bacterial virulence factor that affect the risk of EOGBS, otherwise far more babies would become ill. Moreover, we wanted to investigate the routine clinical research setting as opposed to several other studies, where it is a microbiological research setting in terms of maximal detection of GBS. However, our antepartum recto-vaginal colonization rate has been 17.3% (156/902). We could probably find a higher rate of colonization if we had used Broth enrichment prior to culturing.

Frozen samples: It might be considered a limitation that the PCR analyses were performed retrospectively as a batch processing of frozen samples and thus only simulated a rapid on-site test. However, to create a realistic screening scenario for a rapid PCR-strategy, we used a GBS PCR assay without a delaying broth enrichment step prior to the PCR analysis.

Granada medium: The Granada medium for isolation and identification of GBS is a selective and differential culture medium designed to selectively isolate *Streptococcus agalactiae* (group B streptococcus, GBS) which differs from the standard recommended by CDC (Lim or TransVag). Unlike other chromogenic media, the Granada medium cannot detect non-hemolytic GBS, thereby potentially decreasing the sensitivity of this culture medium for GBS screening [7]. However, the frequency of non-hemolytic GBS isolates is around 5% among GBS carriers, and a rate of only 1% is observed among invasive GBS strains, which suggests that EOGBS caused by non-hemolytic GBS strains is negligible [106].

Furthermore, it should be noted that the difference in the detecting rates between the direct plating of the rectovaginal swab on the Granada medium and plating after prior Lim broth enrichment is only 4% [107].

Lim broth enrichment: The choice of a PCR assay for intrapartum vaginal GBS detection performed without a prior Lim broth enrichment was intended – and thereby also to accept a small,

statistically significant lower sensitivity (92.7% versus 99.1%) compared to the use of the same PCR test with a Lim broth inoculation of the specimen according to the study by Silbert et al. [89]. Using a prior 18-hour Lim broth enrichment step as part of the PCR assay would prohibit the use of the GBS PCR as a rapid test at the time of delivery.

It may also constitute a shortcoming of the study that omitting a prior enrichment step of the specimen is likely to reduce the number of positive cases detected by not only the PCR assay but also the intrapartum culture of the vaginal specimen. However, this approach allowed us to conduct the semi-quantitation of the GBS in the vaginal sample. These results indicate that the potential lower sensitivity of a PCR assay without a prior enrichment step with a false-negative rate of 17% (18/104) is primarily caused by a failure to detect vaginal colonization with low numbers of GBS, which may be of less risk for the newborn during birth.

We have calculated which effect the prior enrichment step would have on the sensitivity of PCR test. Under the condition that an extra 4% of GBS-positives would be found with a prior enrichment step [107] (the new reference standard), we have found that the sensitivity would decrease from 83% to 80%.

PCR Process Control: It is a limitation of the PCR-strategy that 3.4% of all specimens tested were initially undetermined for technical reasons based on the amplification status of the target and the Internal Process Control (data not shown). In such cases, a repeat testing must be conducted, which delay the definitive result and may in some cases not be in time to decide the use of preventive antibiotic prophylaxis.

Antimicrobial susceptibility testing: The PCR strategy does not allow for performing antimicrobial susceptibility testing, which may be of relevance for penicillin-allergic patients. However, susceptibility testing is not necessary in general because GBS isolates with confirmed resistance to penicillin, ampicillin, or cefazolin have not yet been described [59]. Fortunately,

efficient alternative choices exist for those with known penicillin-allergy, e.g. cefuroxime, cefaclor, and ceftriaxone. In patients with a history of severe anaphylactic reactions following cephalosporin treatment, vancomycin is an alternative antibiotic [108].

Self-administered swab sampling: Antepartum screening by GBS culture or PCR test with or without a prior Lim broth enrichment at week 35-37 during pregnancy is known to miss a substantial number of women with later intrapartum carriage of GBS [19, 103, 105, 109]. We observed no substantial difference in the number of positive findings between the antepartum vaginal swab samples obtained by the pregnant women and the intrapartum vaginal swab samples collected by midwives, thus supporting the principle of self-administered swab sampling (OR 0.96, 95% CI 0.7-1.3, P=0.77).

Self-administered PCR test: It has not been possible for the designate midwife to make the intrapartum PCR test on BDMax, which needs specialists to perform. The aim of our study was to compare the performance of an antepartum culture-based screening strategy and an intrapartum PCR assay for the prediction of intrapartum vaginal carriage of GBS in a Danish cohort, using intrapartum vaginal culture as the reference standard. The PCR analyses were performed retrospectively as a batch processing of frozen samples and thus only simulated a rapid on-site test. The real-time PCR technique for the determination of intrapartum GBS status as a point of care assay has been considered too complex to be used in the labor ward [110]. However, in a Swedish study [45] it has been shown that this option may be feasible, albeit the management in the hands of midwives and assistants could be further improved. The approach has now been successfully introduced into our labor ward using the new PCR assay GenomEra [111].

Semi-quantitative assessment: It is a minor limitation in Study III that the semi-quantitative assessment of 27 intrapartum vaginal swabs has not been recorded during the study.

Confounder control: It may also constitute a shortcoming of the study that lack of confounder control of possible variables changed from the time of the antepartum screening strategy (35-37 weeks of gestation) until the intrapartum screening strategy (delivery at 37-41 weeks of gestation) and that could influence the detection rate of GBS at delivery. Paper I aims to compare the performance of an antepartum culture-based screening strategy and an intrapartum polymerase chain reaction assay for the prediction of intrapartum vaginal carriage of group B streptococci in a Danish cohort. In other words, two strategies are applied to the same population and with a relative short time-span in pregnancy in between (1 to 6 weeks). The use of antibiotics could have been a confounder, however, those women treated with antibiotics between antepartum- and intrapartum culture test were excluded from the study [39]. Theoretically, factors could change; e.g. smoking could be reduced and may as such have changed the likelihood for GBS colonization even during this short time-span. The study setup did not detect e.g. changes in smoking during this period (from the antepartum screening until delivery), and thus, it was not possible to control for the influence of change in smoking habits during this short period in pregnancy (e.g. by stratification). One could also consider other theoretical confounders influencing the differences in the strategies evaluated, e.g. new partner, change in sexual behavior, debut of serious diseases (e.g. gestational Diabetes), etc. during this short period in pregnancy. For all of these theoretical (if existing) effects of changes in conditions for the population observed the study did not provide sufficient data to control for the potential confounders mentioned. Besides, in ten (10) representative studies addressing the same issue as Paper 1, either directly or indirectly, confounder control was not performed nor discussed by the authors [20, 109, 112-119]. Finally, the study was not a comparative patient study, but a comparison of 2 laboratory methods. The possible associations between maternal GBS colonization and age, parity, BMI, duration of ROM, and length of labor or infant gender were all out of the scope of this study.

Study IV

The homogeneity of this cohort avoids many confounders related to race/ethnicity and socio-economic factors that have been attributed to ambiguous reports from other parts of the world on this topic. The strengths of our study include the uniformly organized Danish public healthcare system [120] that allows a population-based design. More specific strengths include a) exact gestational age based on ultrasonographic measures, b) reliable information on GBS culture results, and c) the sample size with the ability to control for many potential confounding variables that might affect the outcome of PTD.

Limitations of the study are that the dataset included all observations of GBS bacteriuria before 37 weeks of gestation, and thus presented statistical challenges due to the unsystematic collection of urine samples and the differing reasons they were obtained. Women with GBS-positive urine culture did not even appear to be associated with PTD between the cultured group (GI) and the uncultured group (GII). We did not have the opportunity to differentiate colony count and a possible correlation to PTD, as many GBS-positive cultures are presumed to be derived from the vagina and rectum [85, 100]**.**

One could argue that the use of prophylactic antibiotic treatment removes an expected higher rate of PTD in a GBS-positive group and masks an otherwise clear association between GBS bacteriuria and PTD in the cultured groups. However, the present findings are in line with previous retrospective and prospective studies [83, 84].

Findings compared to studies of others

The GBS intrapartum carriage rate was only 12% compared to 10-29 % in other studies [24, 52, 103, 121-123] that comprised different populations and used other GBS detection methods based primarily on broth enrichment [23, 53, 122]. This issue is discussed under Strength and Limitations

section, Study I, II, and III. However, our findings are in line with prior studies reporting on the usefulness of a PCR-strategy in detecting intrapartum GBS [19, 20, 45, 113-115, 124].

The difference between antepartum vaginal and rectal culture carriage (11% vs. 16%, respectively) had also been shown in previous studies, supporting the hypothesis that the gastrointestinal tract is the primary reservoir of GBS, and that vaginal colonization represents spread of GBS from the rectum [24, 122].

In the Swedish study of Håkansson et al, the infant colonization rate was 68% in the GBS-positive mothers delivered vaginally and without intrapartum antibiotics [102]. In this study, 12.4% (44/359) were considered falsely negative, since GBS was isolated only from the infant. The overall sensitivity of maternal cultures could be estimated by calculating the proportion of positive maternal samples among positive infant samples (200/244 - 82%). The difference in colonization rate between the mother and the infant was also demonstrated in the study of Hansen et al., where the infant colonization rate was 80% in the GBS-positive mothers [23]. Using this estimate of sensitivity, the 'true' number of positive maternal tests for intrapartum vaginal colonization in our study could have been different.

The external validity of our studies, and some disagreement between our results and those from other studies, must be considered. Some studies detect higher GBS prevalence among women with risk factors, probably due to differences between populations, cultures, and PCR techniques, and differing risk factor criteria, e.g. the inclusion of women delivering preterm [21, 125]. However, we did not address this aspect in the study as the population consisted of pregnant women at 35-37 weeks of gestation. Previous studies have demonstrated decreased recovery of GBS following broth enrichment of specimens containing a high concentration of *Enterococcus* spp., which overgrow GBS in broth and hinder recovery upon subculture [126, 127].

GBS in urine is only a proxy variable, which we believe is a risk factor for EOGBS. The relation between GBS in urine at 35-37 weeks of gestation as a risk marker for EOGBS was thus not studied in this paper. However, GBS in urine at 35-37 weeks of gestation with its low sensitivity does not perform satisfactorily as a stand-alone or isolated screening marker for EOGBS.

The prevalence of GBS bacteriuria was comparable to that reported in other studies [128-131]. Our findings confirm the findings of Perez-Moreno and colleagues in their prospective study about GBS in urine during pregnancy as a risk factor for maternal intrapartum colonization. They found a sensitivity of 41%, a specificity of 95%, a PPV of 59%, and negative predictive value of 95% of GBS at 35-37 weeks' gestation in predicting GBS in the vagina at birth [132]. The authors concluded that GBS bacteriuria is a risk factor for intrapartum colonization, irrespective of urinary GBS concentration or of colonization status at late gestation [132]. These findings agree with our results with PPV for participants without GBS in urine at 35-37 weeks of gestation of 6% (52/849), while for colony counts < 10^4 CFU/mL it was 27% (4/15). However, Perez-Moreno et al. (2017) did not classify the growth of GBS in vagina at birth semi-quantitatively as we did in our study.

Our smoking rates were comparable to those reported in other studies performed on data collected from the national Danish Fetal Medicine Database and from The Danish Medical Birth Register [133, 134]. The smoking rate of pregnant women has actually been decreasing from 18% in 2001 to about 6% by 2016 [134]. Our findings confirm the findings of Bak and colleagues in their prospective study about maternal obesity as a source of error in gestational age estimation at 11–14 weeks of gestation for the period 2008-2012 [133]. They found in the 5 years' period a smoking rate of 9.7% (singleton pregnancies) [133]. The rate of smokers in the invited cohort in our study was 10.1%; 8.3% vs. 11.2% for participating and non-participating pregnant women (OR 0.3; 95% CI 0.3 – 0.4; p=0.0001). This could be due to the decreasing number of smokers that usually occur during pregnancy, which was shown by Ekblad and colleagues in a Danish cohort [135]; or

due to the proportion of smokers is significantly higher among citizens with low-income socioeconomic status compared to citizens with higher socioeconomic status [136] also found with lower participation rates in maternal follow-up within e.g. the Danish National Birth Cohort [134].

In Study IV, all information on exposure and outcome was collected independently of the possible association challenged in this study, meaning that any information bias is considered negligible. However, there was likely to have been a systematic selection of the individuals for urine culture, as general practitioners may be more attentive towards a high-risk profile, including GBS urinary tract infections in pregnant women having complications in current or previous pregnancy(ies). The clear differences in characteristics between the urine cultured group and the group without urine specimens cultured in this study may indicate such a behavioral pattern among general practitioners. This may explain the associations found on PTD in this and other studies [137, 138] that show statistically significant differences between urine-cultured groups and groups of pregnant women having no urine specimens cultured. Additionally, the higher rates of urine cultures among the high-risk group of women examined for GBS bacteriuria could be attributed to closer prenatal care, where more urine specimens submitted to the laboratory lead to a higher GBS detection rate. The higher caesarean section rate we found in the groups of women with urine specimens cultured is due to the above mentioned high-risk profile and not due to the GBS bacteriuria per se.

One could argue that the use of prophylactic antibiotics removes an expected higher rate of PTD in a GBS-positive group and masks an otherwise clear association between GBS bacteriuria and PTD in the cultured groups. However, the present findings are in line with previous retrospective and prospective studies [83, 84]. Anderson and colleagues conducted a retrospective cohort study and found no increased risk of PTD with asymptomatic GBS bacteriuria, although they did show an increased risk of PTD with GBS bacteriuria if additional antibiotics are administered for other

urinary tract infections, sexually transmitted infections, or upper respiratory infections [77]. A systematic review of 20 studies [83] showed a positive association between PTD and GBS colonization at the time of delivery (case–control studies: OR 1.59; 95% CI 1.03-2.44; cross-sectional meta-analyses: OR 1.75; 95% CI 1.43-2.14). However, colonization during pregnancy was not associated with PTD (cohort meta-analyses: OR 1.06; 95% CI 0.95-1.19). The authors of a Cochrane review showed that antibiotic treatment for asymptomatic bacteriuria had no significant effect on PTD [129].

In another systematic review (2009), the author did not find an association between maternal GBS colonization during pregnancy and preterm delivery. However, in the case of preterm delivery, there is an increased risk of subsequent maternal GBS colonization [83].

Clinical implications and Perspectives

Change of screening strategy in Denmark

The UK National Screening Committee does not recommend universal bacteriological screening for GBS [139]. Their view is that there is no clear evidence to show that routine testing for GBS would do more good than harm, and giving IAP to all carriers of GBS would mean that a very large number of women would receive unnecessary treatment. However, they recommend IAP to women who have tested positive for GBS during the current pregnancy (includes incidental or intentional testing).

As an argument against the risk-based approach, we found that 12.0% (108 out of 902) participants had one or more risk factors for EOGBS, of which only 3.6% (32/902) had both one or more risk factors and a positive GBS-PCR test. The remaining 76 (108-32) women would receive unnecessary treatment, while the 96 (128-32) women with either vaginal positive culture or GBS-PCR positive but without any of the risk factors would be left untreated.

Our PCR test has been shown to be at least as accurate (sensitivity of 83%) as antepartum culture-based screening (sensitivity of 82%), with the major advantage of identifying those women who are really carrying GBS during labor and thus allow for targeted IAP. Moreover, this approach enables screening of pregnant women whose babies are at higher risk for neonatal sepsis, such as those delivering preterm or not followed during pregnancy [109, 112, 125]. In our study, we found that 46% (71/156) of positive antepartum cultures had turned negative at the time of labor; these women and their infants will be over-treated. Of those women with antepartum negative cultures, 19 cases had turned to positive at the time of labor; these women will be undertreated for the risk of EOGBS. The false-positive specimens (n=24, 3%) identified by the BDMax^{TM} GBS assay may be attributable to a low concentration of GBS in the specimen or to the identification of non-beta-hemolytic GBS that may not be detectable on Granada agar plates. Alternatively, these additional positive results might correspond to a non-viable organism or a residual GBS nucleic acid in the specimen from previous carriage.

In summary, intrapartum rapid PCR should be the reference standard when 1) it is decided to only screen women with one or more risk factor, or 2) it is decided to screen systematically for GBS in pregnant women. A French research group showed that the PCR strategy is cost-neutral compared to the antepartum culture strategy if the cost of treating GBS-infected newborns is taken into account, and with the additional benefit of reducing the incidence of GBS sepsis [20]. However, this strategy does not prevent the fact that around 20–30% of fetuses will receive antibiotics prior to birth.

It is logical to offer PCR screening to all women in labor if screening-based prophylaxis should be used, as most GBS carriers have no other risk factors. Moreover, we would probably treat a larger group with antibiotics than those we had previously treated based on risk-based strategy, but the treatment would be targeted towards GBS-carriers and not the many random GBS-negative

women who used to be treated due to risk factors. A large number of women who are GBS-positive carriers will (together with their fetuses) be subjected to IAP despite no risk of EOGBS.

In countries with a low incidence of EOGBS, such as Denmark, Sweden, Norway, and Finland, a substantial reduction in antibiotic prophylaxis could be achieved at term just by combining risk factor-based screening with a rapid intrapartum PCR test for vaginal carriage of GBS.

It should be noted that there are some practical demands of an intrapartum PCR test. It should first of all be simple for midwives or nurses to perform. Staff should also be able to expect a test result within a relatively short time, to allow the decision of whether or not to administer antibiotics in a busy labor and delivery ward. In some urgent clinical cases, a PCR result may be required within less than 120 minutes, which is possible with the present PCR assay when only a few patient samples are handled at a time.

We are aware that the risk factors of EONS caused by various kinds of bacteria do not represent risk estimates of maternal GBS colonization. Moreover, the occurrence of temperature >38°C can be a sign of EONS, which frequently precedes delivery. Therefore, we recommend that broad spectrum antibiotics are administered in cases of temperature >38°C, preterm labor, or PPROM <34 weeks of gestation together with negative PCR. In the case of pregnancy with PPROM between 34-37 weeks of gestation, there should be induction of labor.

Mothers who have previously had a baby affected by early- or late-onset GBS are at increased risk of another affected baby compared with women of similar carrier status who have not had an affected baby. The reasons for this increased risk may indicate persistence of carriage of a virulent strain of GBS or a deficient immune response [140-142]. In view of this potentially increased risk, and the possibility of a false-negative PCR test, we recommend giving IAP in such cases.

The risk of EONS with other extremely virulent GBS strains that cannot be detected with accessible PCR assays or with other types of bacteria cannot be neglected. Although this approach

will lead to a restriction of the use of IAP, the benefit in terms of an impact on the incidence of EONS is not yet known and is beyond the scope of our research. Neither the molecular characterization of GBS nor its virulence structures are part of this thesis.

The value of screening urine for GBS during pregnancy

Even though high urinary GBS colonization antepartum was strongly associated with a high degree of vaginal GBS colonization intrapartum, it cannot perform satisfactorily as a standalone screening marker for risk of EOGBS. Together with other risk factors for EOGBS, antepartum colony size could be useful tool in a scenario of a combined strategy where intrapartum PCR might be quantified.

Our findings do not support the use of GBS in urine at 35-37 weeks of gestation as an isolated risk marker for EOGBS, and thus the identification of women in labor who should be offered prophylactic penicillin. However, GBS in urine at 35-37 weeks of gestation seems to perform better than other risk markers like gestational age at birth <37 weeks [143], duration of rupture of membranes >18 hours [4], temperature >38.0 °C [44, 59, 144], or delivery of a previous infant with GBS-specific EOS [24, 140]. Therefore, EOGBS prevention strategies that offer an intrapartum GBS test only to mothers at risk of having a newborn acquiring EOGBS could benefit from the inclusion of this risk marker. This would probably substantially increase the sensitivity of a selective intrapartum GBS screening strategy.

Is preterm delivery dependent on GBS bacteriuria during pregnancy?

The utility of treating GBS bacteriuria at colony counts ≤10 CFU/mL prior to 35 weeks of gestation is controversial; some favor this approach to prevent the subsequent development of pyelonephritis and to prevent preterm delivery [145]. In study IV, we found no association between asymptomatic bacteriuria and preterm delivery among women with singleton pregnancy. The clear differences in characteristics between the urine-cultured group and the group without culture in this study indicate

that this behavioral pattern among general practitioners can be one of the reasons for the statistically significant differences in PTD between urine-cultured groups and non-cultured groups in this study and other studies [137, 138]. Such comparisons are highly problematic due to selection bias. Further, the higher rates of urine cultures among this high-risk group of women cultured for GBS bacteriuria could also be attributed to a tighter prenatal care with more urine specimens submitted to the laboratory, also leading to higher detection rate. International guidelines recommend treatment if colony counts ≥10 CFU/mL is detected [7, 146].

Implementing urine culture screening of all pregnant women will optimize the opportunity to detect women at risk for transmitting GBS to their infant through the risk-based approach. This is expected to optimize the benefits of the intrapartum PCR test for GBS detection in women with EOGBS risk factors, which was recently introduced in Denmark [63, 64]. However, urine culture screening of all pregnant women may not be necessary if universal screening of all women in labor is implemented.

Conclusions

We conclude that in a Danish population of pregnant women with a low risk for their babies to acquire EOGBS, intrapartum PCR could be efficient technique to screen for vaginal carriage of GBS during labor. In programs that aim to give penicillin to all women in labor who have vaginal GBS colonization (12% in the present study), the PCR-GBS test will perform well (sensitivity 83% and specificity 97%). In programs aiming to treat only GBS-carriers among those with risk factors of EOGBS, it may be possible to reduce penicillin usage by two-thirds (from 12% to 4%). It should also be taken into account that PCR screening cannot replace the risk-based approach, which also reduces the risk of other EONS caused by *Escherichia coli* and other bacteria that are not identified by GBS screening. Antepartum urinary GBS colony size was strongly associated with a high degree

of intrapartum vaginal GBS colonization, but it cannot stand alone as screening marker for risk of EOGBS.

No association was found between asymptomatic GBS bacteriuria and PTD among women with singleton pregnancy. Previous suggestions of such findings can be due to selection bias.

Future research and considerations

- Supporting studies: During the PhD study, we had the opportunity to test the GenomEra® GBS assay with a turnaround time of only 50 minutes, which makes the assay a potential rapid on-site test for intrapartum detection of intrapartum carriage of GBS. When it was tested at Karolinska University Hospital, Stockholm, on frozen intrapartum rectovaginal samples without pre-enrichment broth, it showed a sensitivity of 93%, specificity of 93%, PPV of 89%, and NPV of 95% compared with a culture method used as the reference standard (data unpublished). We have conducted a study where we compared the diagnostic performance of BDMax™ GBS and GenomEra® and tested 100 culture-positive and 300 culture-negative frozen vaginal samples. Of the 100 culture-positive specimens, 86 were found to be positive with the BDMax™ and 73 with the GenomEra PCR assay. No statistically significant differences have been found between culture, BDMax™, and GenomEra GBS PCR assays. The sensitivity was 82%, 89%, and 83%, respectively. As a consequence of these results, we have implemented the GenomEra GBS PCR assay at our department. With a careful implementation of GenomEra, and by combining risk factor-based screening with a rapid intrapartum PCR test for recto-vaginal carriage of GBS at term, a 60% reduction in antibiotic prophylaxis is achieved (data unpublished).
- Further research on technological improvements regarding the speed of the PCR assay while maintaining its sensitivity. Even with negative PCR results, we cannot be sure that the newborn does not acquire EOGBS anyway; we can neither rule out other routes of colonization nor detect

very small amounts of GBS. It could be beneficial in developing quantitative PCR (qPCR-GBS) with low, medium, and high level/load of GBS. The findings could then indicate treatment or not, where risk factors are also taken into account in the considerations of treatment. For example, in the case of ROM, a high GBS finding could indicate treatment if ROM >6h; medium indicate treatment if ROM >12h, and 18h for low etc. Antepartum urine GBS colony count and its relation to the qPCR-GBS results could be an additional area for research.

- There is no test that can distinguish between women whose babies would be affected by GBS at birth, and those who would not. We know too little about children who have a special sensitivity or some genetic relationship for a particular type of the bacterium.
- Some women may have better transfer of maternal antibodies to the fetus than other women, e.g. the passive immunization of the newborn differs [16, 147]. This aspect might be addressed by appropriate vaccination programs or by identifying women at special risk due to an inappropriate immunoglobulin profile.
- Even without a history of previous infant with EONS, pregnant woman may be colonized by extremely virulent GBS strains. We know too little about multiplex PCR, which can distinguish between the presence of bacteria with higher virulence and aggressive serotypes and the presence of bacteria with no higher virulence and non-aggressive serotypes. The technology can identify several different genes in a single test [148].
- Further research on the cost-effectiveness and how many women should be treated in different scenarios to save one child from acquiring EOGBS.

English Summary

Introduction

Streptococcus agalactiae (group B streptococcus, GBS) is the most common cause of severe early-onset infection (EOGBS) in newborn infants (defined as GBS acquired before seven days of age). Infants with EOGBS disease generally present with respiratory distress, apnea, or other signs of sepsis within the first 24-48 hours of life. The most important risk factor for EOGBS is vaginal colonization that causes vertical transmission of bacteria to the infant during labor. Intrapartum antibiotic prophylaxis (IAP) is the most effective available intervention against EOGBS. International guidelines outline two main strategies for identifying women in labor who should be offered IAP, the *risk-based approach* and *the culture-based screening*. Recently, a European consensus conference on intrapartum GBS screening and antibiotic prophylaxis recommended IAP, based on a universal intrapartum GBS screening strategy using a rapid real-time test, polymerase chain reaction (PCR).

The reduced EOGBS rate in USA after the introduction of the culture-based screening strategy must be categorized as a success; however, one might wonder why the EOGBS rate in some other countries including Denmark is only 0.1-0.4/1,000 live births even though they have not implemented this antepartum culture-based screening program. As both strategies suffer from various limitations, the performance of an intrapartum PCR test has never been evaluated in a Danish population of pregnant women, whose risk of having a baby acquiring EOGBS is very low.

GBS bacteriuria during pregnancy is considered a marker of high load maternal colonization and has been associated with an increased risk of EOGBS. However, little is known about the association between the antepartum GBS-urine colony count and the intrapartum load of GBS in the vagina.

GBS bacteriuria is also associated with an increased risk of chorioamnionitis, which is itself a risk factor for preterm delivery, but a broader role for GBS in triggering preterm delivery is uncertain.

The association between preterm delivery and GBS bacteriuria during pregnancy remains controversial.

Aims

The aim of this study was to find the best screening method for GBS colonization, with the ultimate goal of providing a targeted prophylaxis for women at risk for 1) EOGBS and 2) preterm delivery. Specifically, we investigated four objectives in four separate studies:

1. To compare the performance of an antepartum culture-based screening strategy and an intrapartum polymerase chain reaction assay for the prediction of intrapartum vaginal carriage of group B streptococci in a Danish cohort. (Paper I).
2. To assess the performance of a polymerase chain reaction – group B streptococci test in deciding antibiotic prophylaxis in women in labor at term. (Paper II)
3. To evaluate how well GBS colony numbers in the urine antepartum at 35-37 weeks of gestation predict the load of GBS colonization of the vagina at birth. (Paper III)
4. To investigate a possible association between preterm delivery and group B streptococci detected in urine culture during pregnancy. (Paper IV)

Methods

Study I, II, and III: A prospective observational cohort study of unselected Danish pregnant women (n=902). At 35-37 weeks of gestation, each participant obtained a self-administered vaginal and rectal swab sample for culture, and delivered a midstream clean catch urine sample during a planned visit to the outpatient clinic. During labor, the midwife collected a vaginal swab sample for both culture (reference standard) and a PCR assay for GBS; a midstream clean catch urine sample was also collected. For study I, the specimens were analyzed without prior enrichment to make the culture findings comparable with the results of the PCR assay. For study II, the presence of risk factors for EOGBS was recorded: 1) Bacteriuria during current pregnancy, 2) Prior infant with

EOGBS, 3) Temperature above 38.0°C during labor, and 4) Rupture of membranes ≥18 hours. For study III, GBS bacteriuria was classified according to the number of colony-forming units per mL (CFU/mL).

Study IV: A retrospective population-based cohort (n=36,097), which was divided into three groups. Group I included women whose urine culture was positive for GBS, group II included women whose urine culture was negative for GBS, and group III included women whose urine had not been cultured.

Main findings

- The culture-strategy showed a sensitivity of 82%, positive predictive value (PPV) of 55%, and likelihood ratio (LH+) of 9.2. The PCR-strategy showed a sensitivity of 83%, PPV of 78%, and LH+ of 27.5.
- Within this cohort, 12% had EOGBS risk factors, whereas only 2.7% had both one or more risk factors and a positive vaginal GBS culture. This indicates substantial overtreatment if all women with EOGBS risk factors are offered IAP, as the situation is today. Among these 2.7%, not fewer than 2.5% had a positive intrapartum GBS-PCR, which was equivalent to a sensitivity of 92%.
- GBS bacteriuria at 35-37 weeks of gestation showed a sensitivity of 30% for any degree of vaginal GBS colonization at birth (31 of 104 cases); 19% for light (+1), 17% for medium (+2), and 52% for heavy (+3) vaginal GBS colonization. The positive predictive values for heavy vaginal colonization at birth were 2% for no CFU, 7% for $<10^4$ CFU/mL, 44% for 10^4 CFU/mL, and 67% for $>10^4$ CFU/mL.
- We found no association between PTD and GBS bacteriuria in the cultured groups (OR=0.89; 95% CI: 0.5-1.4). After controlling for potential confounders, the PTD remained not associated with GBS bacteriuria (adjusted OR=0.99; 95% CI: 0.6-1.6). Combined, the cultured groups

were associated with a statistically significant higher risk for PTD when compared with the group with no urine samples taken for culture (OR=1.96; 95% CI: 1.8-2.2 and adjusted OR=1.80; 95% CI 1.6-2.0). The cultured group differed considerably from the group of women with no urine cultures on the vast majority of variables examined.

Conclusions

Intrapartum PCR is recommended to screen for vaginal carriage of GBS during labor. In programs that aim to give penicillin to all women in labor who have vaginal GBS colonization, the PCR-GBS test will perform well (sensitivity 83% and specificity 97%). In programs aiming to treat only GBS-carriers among those with risk factors of EOGBS, it may be possible to reduce penicillin usage by two-thirds (from 12% to 4%). Antepartum urinary GBS colony size was associated with a high degree of intrapartum vaginal GBS colonization, but it cannot stand alone as screening marker for risk of EOGBS.

No association was found between asymptomatic GBS bacteriuria and PTD among women with singleton pregnancy. Previous suggestions of such findings can be due to selection bias.

Dansk resume´

Introduktion

Streptococcus agalactiae (gruppe B streptokokker, GBS) er den mest almindelige årsag til alvorlig tidlig infektion (EOGBS) hos nyfødte børn (defineret som GBS erhvervet før syv dage). Spædbørn med EOGBS sygdom frembyder almindeligvis med symptomer som åndedrætsbesvær, apnø eller andre tegn på sepsis inden for de første 24-48 timers levetid. Den vigtigste risikofaktor for EOGBS er den vaginale kolonisering, der forårsager lodret overførsel af bakterier til spædbarnet under fødslen. Intrapartum antibiotikaprofylakse (IAP) er den mest effektive tilgængelige indsats mod EOGBS. Internationale retningslinjer beskriver to hovedstrategier for identifikation af kvinder, der bør tilbydes IAP, den risikobaserede tilgang og den dyrkningsbaserede screening. For nylig anbefalede en europæisk konsensuskonference om intrapartum GBS screening og antibiotisk profylakse baseret på en universel intrapartum GBS screeningsstrategi brug af en hurtig en real-time PCR baseret (Polymerase Chain Reaction) metode.

Faldet i forekomsten af EOGBS i USA efter introduktion af dyrkningsbaseret screeningsstrategi kan betegnes som en succes; Men man kan måske undre sig over, hvorfor forekomsten af EOGBS i nogle andre lande, herunder Danmark, kun er 0,1-0,4/1.000 levendefødte, selv om de ikke har implementeret dette antepartum-dyrkningsbaserede screeningsprogram. Både risikobaserede og den dyrkningsbaserede strategi lider under forskellige begrænsninger og et alternativ såsom en intrapartum PCR test er imidlertid aldrig blevet testet på en dansk befolkning af gravide kvinder, hvor risikoen for EOGBS er meget lav.

GBS bacteriuria under graviditeten betragtes som en markør for udbredt kolonisering hos den gravide, og har været forbundet med en øget risiko for EOGBS. Imidlertid er der meget lidt viden om forbindelsen mellem GBS-urin-kolonitællingen og GBS koncentrationen i vagina under fødslen.

GBS bacteriuria er også forbundet med en øget risiko for chorioamnionitis som er i sig selv en risikofaktor for præterm fødsel, men GBS's rolle for initiering af præterm fødsel forbliver usikker. Association mellem GBS bacteriuria under graviditet og præterm fødsel forbliver imidlertid kontroversiel.

Formål

Formålet med denne undersøgelse var at finde den bedste screeningsmetode til GBS-kolonisering med det ultimative mål at give målrettet profylakse for kvinder i risiko for 1) EOGBS og 2) Præterm fødsel; specifikt undersøgte vi 4 sæt delmål i 4 separate undersøgelser:

1. At sammenligne effektiviteten af en antepartum dyrkningsbaseret screeningsstrategi og et intrapartum polymerase kædereaktion essay til forudsigelse af intrapartum vaginal kolonisering af gruppe B streptokokker (GBS) i en dansk kohorte. . (Publikation I)
2. At vurdere udførelsen af en polymerase kædereaktion - gruppe B streptokok test til at afgøre hvilke fødende, der skal tilbydes antibiotikaprofylakse. (Publikation II)
3. At vurdere, hvor godt GBS-koloniantallet i en antepartum urinprøve ved gestationsalder 35-37 uger forudsiger niveauet af GBS-koloniseringen af vagina ved fødslen. (Publikation III)
4. At undersøge en eventuel association mellem gruppe B streptokokker påvist i urinen under graviditet og præterm fødsel. (Publikation IV)

Methods

Studier I, II og III: Et prospektivt ikke selekteret observations kohorte studie af danske gravide kvinder (n=902). Ved gestationsalder 35-37 uger foretog alle deltagende gravide en selv administreret vaginal og rektal podning til dyrkning og afleverede en midt stråle urin prøve under det planlagte besøg i ambulatorium. Under fødslen indsamlede jordemødre en vaginal dyrkningspodning til både dyrkning (referencestandard) og til en PCR test for GBS og en midt stråle urinprøve blev indsamlet. Til studie I, blev prøverne analyseret uden forudgående berigelse

for at gøre dyrkningsfundene sammenlignelige med resultaterne af PCR-essayets. Til studie II, blev tilstedeværelsen af risikofaktorer for EOGBS registreret: 1) GBS bakteriuri under nuværende graviditet, 2) Tidligere nyfødt barn med EOGBS 3) Temperatur over 38,0 °C under fødslen og 4) Vandafgang ≥18 timer. Til studie III, blev GBS bakteriuri klassificeret i henhold til antallet af kolonidannende enheder pr. ml (CFU/ml).

Studie IV: En retrospektiv populationsbaseret kohorte (n=36.097). Kohorten, der blev brugt i dette studie, blev opdelt i tre grupper. Gruppe I inkluderede kvinder, hvis urindyrkning var positiv for GBS, og gruppe II omfattede kvinder, hvis urindyrkning var negativ for GBS og gruppe III bestod af kvinder, hvis urin ikke var blevet dyrket for GBS.

Hovedresultater

- Dyrkningsstrategien præsterer en sensitivitet på 82%, positiv prædiktiv værdi (PPV) på 55% og Likelihood ratio (LH +) på 9,2. PCR-strategien viste tilsvarende værdier med sensitivitet på 83%, PPV på 78% og LH + på 27,5.
- Inden for denne kohorte havde 12% EOGBS risikofaktorer, mens kun 2,7% havde både en eller flere risikofaktorer og en positiv vaginal GBS dyrkning. Dette indikerer betydelige overbehandlinger, hvis alle med EOGBS risikofaktorer tilbydes IAP som situationen er i dag. Blandt de 2,7% havde ikke færre end 2,5% et positivt intrapartum GBS-PCR svarende til en sensitivitet på 92%.
- GBS bakteriuri ved gestationsalder 35-37 uger havde en sensitivitet på 30% vedrørende alle grader af vaginal GBS kolonisering ved fødslen (31 ud af 104 tilfælde); 19% for lav (+1), 17% for middel (+2) og 52% for høj (+3) vaginal GBS kolonisering. De positive prædiktive værdier for høj vaginal kolonisering ved fødslen var 2% for ingen CFU, 7% for $<10^4$ CFU/mL, 44% for 10^4 CFU/mL og 67% for $>10^4$ CFU/mL.

- Vi fandt ingen association mellem GBS bakteriuri og præterm fødsel i de dyrkede grupper (OR=0,89; 95% CI: 0,5-1,4). Efter kontrol for potentielle konfounderes forblev GBS bakteriuri uden association med præterm fødsel (justeret OR=0,99; 95% CI: 0,6-1,6). Kombineret var de dyrkede grupper statistisk signifikant associeret med højere risiko for præterm fødsel sammenlignet med gruppen uden urin dyrkning (OR=1,96; 95% CI: 1,8-2,2 og justeret OR=1,80; 95% CI 1,6-2,0). Den dyrkede gruppe adskilte sig væsentligt fra gruppen af kvinder uden urindyrkning på langt de fleste af de undersøgte variable og synes at udgøre en særlig udvalgt gruppe.

Konklusioner

Intrapartum PCR anbefales som en screenings metode for vaginal GBS kolonisering under fødslen. I behandlingsregimer, der sigter mod at behandle alle fødende med vaginal GBS kolonisering med penicillin, vil PCR-GBS virke godt (følsomhed 83% og specificitet 97%). I behandlingsregimer, der sigter mod at behandle kun GBS bærere blandt dem med risikofaktorer for EOGBS, kan en reduktion af penicillinforbruget med to tredjedele fra 12% til 4% være mulig. Urin antepartum GBS koloniantal er forbundet med en høj grad af intrapartum vaginal GBS koloniserings, men kan ikke stå som en enkeltstående screeningsmarkør for risiko for EOGBS.

Der blev ikke fundet nogen association mellem asymptomatisk GBS i urin og præterm fødsel blandt kvinder med singleton graviditet. Tidligere fund af en sådan sammenhæng kan være på grund af et selektions-problem.

References

Reference List

1. Stoll, B.J., et al., *Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues.* Pediatrics, 2011. **127**(5): p. 817-26.
2. Daley, A.J. and S.M. Garland, *Prevention of neonatal group B streptococcal disease: progress, challenges and dilemmas.* J Paediatr Child Health, 2004. **40**(12): p. 664-8.
3. Van Dyke, M.K., et al., *Evaluation of universal antenatal screening for group B streptococcus.* N Engl J Med, 2009. **360**(25): p. 2626-36.
4. Boyer, K.M., and Gotoff, S. P., *Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis.* N Engl J Med, 1986. **314**(26): p. 1665-9.
5. Verani, J.R., and Schrag, S. J., *Group B streptococcal disease in infants: progress in prevention and continued challenges.* Clin Perinatol, 2010. **37**(2): p. 375-92.
6. Boyer, K.M. and S.P. Gotoff, *Strategies for chemoprophylaxis of GBS early-onset infections.* Antibiot Chemother (1971), 1985. **35**: p. 267-80.
7. Verani JR, M.L., Schrag SJ,. , *Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC, 2010.* MMWR Recomm Rep, 2010. **59**(RR-10): p. 1-36.
8. Schrag SJ, Whitney CG, and Schuchat A, *Neonatal group B streptococcal disease: how infection control teams can contribute to prevention efforts.* Infect Control Hosp Epidemiol, 2000. **21**(7): p. 473-83.
9. Le Doare, K. and P.T. Heath, *An overview of global GBS epidemiology.* Vaccine, 2013. **31 Suppl 4**: p. D7-12.
10. Yow, M.D., et al., *The natural history of group B streptococcal colonization in the pregnant woman and her offspring. I. Colonization studies.* Am J Obstet Gynecol, 1980. **137**(1): p. 34-8.
11. Keski-Nisula, L., Kyynarainen, H. R., Karkkainen, U., Karhukorpi, J., Heinonen, S., and Pekkanen, J., *Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth.* Acta Paediatr, 2013. **102**(5): p. 480-5.
12. Rachid, R. and T.A. Chatila, *The role of the gut microbiota in food allergy.* Curr Opin Pediatr, 2016. **28**(6): p. 748-753.
13. Mueller, N.T., et al., *Does vaginal delivery mitigate or strengthen the intergenerational association of overweight and obesity? Findings from the Boston Birth Cohort.* Int J Obes (Lond), 2017. **41**(4): p. 497-501.
14. Paun, A. and J.S. Danska, *Modulation of type 1 and type 2 diabetes risk by the intestinal microbiome.* Pediatr Diabetes, 2016. **17**(7): p. 469-477.
15. Schrag, S., Gorwitz, R., Fultz-Butts, K., Schuchat, A., *Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC.* MMWR Recomm Rep, 2002. **51**(RR-11): p. 1-22.
16. Baker, C.J. and D.L. Kasper, *Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection.* N Engl J Med, 1976. **294**(14): p. 753-6.
17. Persson, K., et al., *Longitudinal study of group B streptococcal carriage during late pregnancy.* Scand J Infect Dis, 1987. **19**(3): p. 325-9.
18. Boyer, K.M., et al., *Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures.* J Infect Dis, 1983. **148**(5): p. 802-9.
19. Davies, H.D., Miller, M. A., Faro, S., Gregson, D., Kehl, S. C., and Jordan, J. A., *Multicenter study of a rapid molecular-based assay for the diagnosis of group B Streptococcus colonization in pregnant women.* Clin Infect Dis, 2004. **39**(8): p. 1129-35.
20. El Helali, N., Nguyen, J. C., Ly, A., Giovangrandi, Y., and Trinquart, L., *Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening.* Clin Infect Dis, 2009. **49**(3): p. 417-23.

21. Alfa, M.J., et al., *Real-time PCR assay provides reliable assessment of intrapartum carriage of group B Streptococcus.* J Clin Microbiol, 2010. **48**(9): p. 3095-9.
22. Feikin, D.R., et al., *Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women.* Am J Obstet Gynecol, 2001. **184**(3): p. 427-33.
23. Hansen, S.M., Uldbjerg, N, Kilian M, and Sorensen, U.B., *Dynamics of Streptococcus agalactiae colonization in women during and after pregnancy and in their infants.* J Clin Microbiol, 2004. **42**(1): p. 83-9.
24. Schrag, S.J., Zell, E. R., Lynfield, R., Roome, A., Arnold, K. E., Craig, A. S., et al., *A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates.* N Engl J Med, 2002. **347**(4): p. 233-9.
25. Stoll, B.J. and A. Schuchat, *Maternal carriage of group B streptococci in developing countries.* Pediatr Infect Dis J, 1998. **17**(6): p. 499-503.
26. Bergeron, M.G., Ke, D., Menard, C., Picard, F. J., Gagnon, M., Bernier, M., et al., *Rapid detection of group B streptococci in pregnant women at delivery.* N Engl J Med, 2000. **343**(3): p. 175-9.
27. Regan, J.A., Klebanoff, M. A., and Nugent, R. P., *The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections and Prematurity Study Group.* Obstet Gynecol, 1991. **77**(4): p. 604-10.
28. Persson, K., et al., *Several factors influencing the colonization of group B streptococci--rectum probably the main reservoir.* Scand J Infect Dis, 1981. **13**(3): p. 171-5.
29. Foxman, B., et al., *Risk factors for group B streptococcal colonization: potential for different transmission systems by capsular type.* Ann Epidemiol, 2007. **17**(11): p. 854-62.
30. Winn, H.N., *Group B streptococcus infection in pregnancy.* Clin Perinatol, 2007. **34**(3): p. 387-92.
31. Yancey, M.K., et al., *Peripartum infection associated with vaginal group B streptococcal colonization.* Obstet Gynecol, 1994. **84**(5): p. 816-9.
32. Tyrrell, G.J., et al., *Invasive disease due to group B streptococcal infection in adults: results from a Canadian, population-based, active laboratory surveillance study--1996. Sentinel Health Unit Surveillance System Site Coordinators.* J Infect Dis, 2000. **182**(1): p. 168-73.
33. Edwards, M.S., et al., *Group B streptococcal colonization and serotype-specific immunity in healthy elderly persons.* Clin Infect Dis, 2005. **40**(3): p. 352-7.
34. Dillon, H.C., Jr., S. Khare, and B.M. Gray, *Group B streptococcal carriage and disease: a 6-year prospective study.* J Pediatr, 1987. **110**(1): p. 31-6.
35. Ohlsson, A. and V.S. Shah, *Intrapartum antibiotics for known maternal Group B streptococcal colonization.* Cochrane Database Syst Rev, 2009(3): p. CD007467.
36. Centers for Disease, a.C.a.P., *Trends in perinatal group B streptococcal disease - United States, 2000-2006.* MMWR Morb Mortal Wkly Rep, 2009. **58**(5): p. 109-12.
37. Puopolo, K.M., Madoff, L. C., and Eichenwald, E. C., *Early-onset group B streptococcal disease in the era of maternal screening.* Pediatrics, 2005. **115**(5): p. 1240-6.
38. Van Dyke, M.K., Phares, C. R., Lynfield, R., Thomas, A. R., Arnold, K. E., Craig, A. S., et al., *Evaluation of universal antenatal screening for group B streptococcus.* N Engl J Med, 2009. **360**(25): p. 2626-36.
39. Pulver, L.S., Hopfenbeck, M. M., Young, P. C., Stoddard, G. J., Korgenski, K., Daly, J., et al., *Continued early onset group B streptococcal infections in the era of intrapartum prophylaxis.* J Perinatol, 2009. **29**(1): p. 20-5.
40. Heath, P.T., Balfour, G. F., Tighe, H., Verlander, N. Q., Lamagni, T. L., Efstratiou, A. et al, *Group B streptococcal disease in infants: a case control study.* Arch Dis Child, 2009. **94**(9): p. 674-80.
41. Vergnano, S., Embleton, N., Collinson, A., Menson, E., Russell, A. B. ,Heath, P., *Missed opportunities for preventing group B streptococcus infection.* Arch Dis Child Fetal Neonatal Ed, 2010. **95**(1): p. F72-3.
42. Eastwood, K.A., et al., *Prevention of early-onset Group B Streptococcal disease - the Northern Ireland experience.* BJOG, 2015. **122**(3): p. 361-7.

43. Flidel-Rimon, O., et al., *Limitations of the risk factor based approach in early neonatal sepsis evaluations.* Acta Paediatr, 2012. **101**(12): p. e540-4.
44. Heath, P.T.B., G.; Weisner, A. M.; Efstratiou, A.; Lamagni, T. L.; Tighe, H., et al., *Group B streptococcal disease in UK and Irish infants younger than 90 days.* Lancet, 2004. **363**(9405): p. 292-4.
45. Hakansson, S., et al., *Real-time PCR-assay in the delivery suite for determination of group B streptococcal colonization in a setting with risk-based antibiotic prophylaxis.* J Matern Fetal Neonatal Med, 2014. **27**(4): p. 328-32.
46. C O'Sullivan, et al., *P3 Group B Streptococcal (GBS) disease in UK and Irish infants younger than 90 days, 2014–2015* Arch Dis Child, 2016. **2016;101:A2.**
47. *Abstracts of the RCOG (Royal College of Obstetricians and Gynaecologists) 10th International Scientific Congress. June 5-8, 2012. Kuching, Sarawak, Malaysia.* BJOG, 2012. **119 Suppl 1**: p. 2-250.
48. Hackethal, V., *RCOG Advises GBS Prophylaxis for Women in Preterm Labor - Medscape* - Sep 20, 2017.
49. Ekelund, K., and Konradsen, H.B., *Invasive group B streptococcal disease in infants: a 19-year nationwide study. Serotype distribution, incidence and recurrent infection.* Epidemiol Infect, 2004. **132**(6): p. 1083-90.
50. Hakansson, S. and K. Kallen, *Impact and risk factors for early-onset group B streptococcal morbidity: analysis of a national, population-based cohort in Sweden 1997-2001.* BJOG, 2006. **113**(12): p. 1452-8.
51. Hasseltvedt, V. and E.A. Hoiby, *Systemic streptococcal group B disease in Norway—increasing health problem.* Eurosurveillance Weekly [serial online] 2001 Oct 4 [cited 5 Oct 2001]. Available from: URL: http://www.eurosurv.org, 2011.
52. Lyytikainen, O., Nuorti, J. P., Halmesmaki, E., Carlson, P., Uotila, J., Vuento, R., et al., *Invasive group B streptococcal infections in Finland: a population-based study.* Emerg Infect Dis, 2003. **9**(4): p. 469-73.
53. Rallu, F., Barriga, P., Scrivo, C., Martel-Laferriere, V., and Laferriere, C., *Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for group B streptococcus carriage in pregnant women.* J Clin Microbiol, 2006. **44**(3): p. 725-8.
54. Bergseng, H., et al., *Real-time PCR targeting the sip gene for detection of group B Streptococcus colonization in pregnant women at delivery.* J Med Microbiol, 2007. **56**(Pt 2): p. 223-8.
55. Atkins, K.L., et al., *Evaluation of polymerase chain reaction for group B streptococcus detection using an improved culture method.* Obstet Gynecol, 2006. **108**(3 Pt 1): p. 488-91.
56. Di Renzo, G.C.M., P.; Berardi, A.; Blennow, M.; Carbonell-Estrany, X.; Donzelli, G. P., et al., *Intrapartum GBS screening and antibiotic prophylaxis: a European consensus conference.* J Matern Fetal Neonatal Med, 2015. **28**(7): p. 766-82.
57. Persson, K.C., K. K.; Christensen, P.; Forsgren, A.; Jorgensen, C.; Persson, P. H., *Asymptomatic bacteriuria during pregnancy with special reference to group B streptococci.* Scand J Infect Dis, 1985. **17**(2): p. 195-9.
58. Wood, E.G. and H.C. Dillon, Jr., *A prospective study of group B streptococcal bacteriuria in pregnancy.* Am J Obstet Gynecol, 1981. **140**(5): p. 515-20.
59. Schuchat, A., Zywicki, S. S., Dinsmoor, M. J., Mercer, B., Romaguera, J., O'Sullivan, M. J., et al., *Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study.* Pediatrics, 2000. **105**(1 Pt 1): p. 21-6.
60. Thomsen AC, Morup L, and Hansen KB, *Antibiotic elimination of group-B streptococci in urine in prevention of preterm labour.* Lancet, 1987. **1**(8533): p. 591-3.
61. Schnarr, J. and F. Smaill, *Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy.* Eur J Clin Invest, 2008. **38 Suppl 2**: p. 50-7.

62. Regan, J.A.K., M. A.; Nugent, R. P.; Eschenbach, D. A.; Blackwelder, W. C.; Lou, Y., et al., *Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group.* Am J Obstet Gynecol, 1996. **174**(4): p. 1354-60.
63. Khalil, M.R.U., N.; Thorsen, P. B.; Moller, J. K., *Intrapartum PCR assay versus antepartum culture for assessment of vaginal carriage of group B streptococci in a Danish cohort at birth.* PLoS One, 2017. **12**(7): p. e0180262.
64. Khalil, M.R.U., N.; Thorsen, P. B.; Henriksen, B.; Moller, J. K., *Risk-based screening combined with a PCR-based test for group B streptococci diminishes the use of antibiotics in laboring women.* Eur J Obstet Gynecol Reprod Biol, 2017. **215**: p. 188-192.
65. Blencowe, H., et al., *Born too soon: the global epidemiology of 15 million preterm births.* Reprod Health, 2013. **10 Suppl 1**: p. S2.
66. Goldenberg, R.L., et al., *Epidemiology and causes of preterm birth.* Lancet, 2008. **371**(9606): p. 75-84.
67. Parry, S. and J.F. Strauss, 3rd, *Premature rupture of the fetal membranes.* N Engl J Med, 1998. **338**(10): p. 663-70.
68. Pararas, M.V., C.L. Skevaki, and D.A. Kafetzis, *Preterm birth due to maternal infection: Causative pathogens and modes of prevention.* Eur J Clin Microbiol Infect Dis, 2006. **25**(9): p. 562-9.
69. Ledger, W.J., et al., *Bacteremia on an obstetric-gynecologic service.* Am J Obstet Gynecol, 1975. **121**(2): p. 205-12.
70. Fried, A.M., *Hydronephrosis of pregnancy: ultrasonographic study and classification of asymptomatic women.* Am J Obstet Gynecol, 1979. **135**(8): p. 1066-70.
71. Nicolle, L.E., et al., *Hospitalization for acute pyelonephritis in Manitoba, Canada, during the period from 1989 to 1992; impact of diabetes, pregnancy, and aboriginal origin.* Clin Infect Dis, 1996. **22**(6): p. 1051-6.
72. Farkash, E., et al., *Acute antepartum pyelonephritis in pregnancy: a critical analysis of risk factors and outcomes.* Eur J Obstet Gynecol Reprod Biol, 2012. **162**(1): p. 24-7.
73. Mazor-Dray, E., et al., *Maternal urinary tract infection: is it independently associated with adverse pregnancy outcome?* J Matern Fetal Neonatal Med, 2009. **22**(2): p. 124-8.
74. Neal, D.E., Jr., *Complicated urinary tract infections.* Urol Clin North Am, 2008. **35**(1): p. 13-22; v.
75. Hill, J.B., et al., *Acute pyelonephritis in pregnancy.* Obstet Gynecol, 2005. **105**(1): p. 18-23.
76. Luciano, A.A., et al., *Preterm labor and chorioamnionitis are associated with neonatal T cell activation.* PLoS One, 2011. **6**(2): p. e16698.
77. Anderson, B.L., et al., *Untreated asymptomatic group B streptococcal bacteriuria early in pregnancy and chorioamnionitis at delivery.* Am J Obstet Gynecol, 2007. **196**(6): p. 524 e1-5.
78. Klein, L.L., et al., *Detection of intra-amniotic infection in a rabbit model by proteomics-based amniotic fluid analysis.* Am J Obstet Gynecol, 2005. **193**(4): p. 1302-6.
79. Hecht, J.L., et al., *Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues.* Pediatr Dev Pathol, 2008. **11**(1): p. 15-22.
80. Thomas, A.A., et al., *Urologic emergencies in pregnancy.* Urology, 2010. **76**(2): p. 453-60.
81. Schnarr J and Smaill F, *Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy.* Eur J Clin Invest, 2008. **38 Suppl 2**: p. 50-7.
82. Romero, R., et al., *Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight.* Obstet Gynecol, 1989. **73**(4): p. 576-82.
83. Valkenburg-van den Berg, A.W., et al., *Association between colonization with Group B Streptococcus and preterm delivery: a systematic review.* Acta Obstet Gynecol Scand, 2009. **88**(9): p. 958-67.
84. Muller, A.E., et al., *Morbidity related to maternal group B streptococcal infections.* Acta Obstet Gynecol Scand, 2006. **85**(9): p. 1027-37.

85. McKenzie, H., et al., *Risk of preterm delivery in pregnant women with group B streptococcal urinary infections or urinary antibodies to group B streptococcal and E. coli antigens.* Br J Obstet Gynaecol, 1994. **101**(2): p. 107-13.
86. Moller, M., et al., *Rupture of fetal membranes and premature delivery associated with group B streptococci in urine of pregnant women.* Lancet, 1984. **2**(8394): p. 69-70.
87. Mittendorf, R., M.A. Williams, and E.H. Kass, *Prevention of preterm delivery and low birth weight associated with asymptomatic bacteriuria.* Clin Infect Dis, 1992. **14**(4): p. 927-32.
88. Binghuai, L., et al., *Use of MALDI-TOF mass spectrometry for rapid identification of group B Streptococcus on chromID Strepto B agar.* Int J Infect Dis, 2014. **27**: p. 44-8.
89. Silbert, S., Rocchetti, T. T., Gostnell, A., Kubasek, C., and Widen, R., *Detection of Group B Streptococcus Directly from Collected ESwab Samples by Use of the BD Max GBS Assay.* J Clin Microbiol, 2016. **54**(6): p. 1660-3.
90. Kildemoes, H.W., H.T. Sorensen, and J. Hallas, *The Danish National Prescription Registry.* Scand J Public Health, 2011. **39**(7 Suppl): p. 38-41.
91. Olivarius, N.F., et al., *The Danish National Health Service Register. A tool for primary health care research.* Dan Med Bull, 1997. **44**(4): p. 449-53.
92. McGee, S., *Simplifying likelihood ratios.* J Gen Intern Med, 2002. **17**(8): p. 646-9.
93. BA, R., *Fundamentals of Biostatistics. 4th ed. Duxbury*. 1995.
94. Draper NR, S.H., *Applied Regression Analysis. Wiley Series in Probability and Statistics. Applied Regression Analysis*. 1998.
95. Pourhoseingholi, M.A., A.R. Baghestani, and M. Vahedi, *How to control confounding effects by statistical analysis.* Gastroenterol Hepatol Bed Bench, 2012. **5**(2): p. 79-83.
96. Kirkwood, B.R.a.S., J.A.C., *Logistic regression: comparing two or more exposure groups., in Essential Medical Statistics*. Wiley-Blackwell. 2003: Blackwell Publishing Company. 189-204.
97. McNanley, A.R.G., J. C.; Hardy, D. J.; Vicino, D., *The effect of intrapartum penicillin on vaginal group B streptococcus colony counts.* Am J Obstet Gynecol, 2007. **197**(6): p. 583 e1-4.
98. Facchinetti, F.P., F.; Mordini, B.; Volpe, A., *Chlorhexidine vaginal flushings versus systemic ampicillin in the prevention of vertical transmission of neonatal group B streptococcus, at term.* J Matern Fetal Neonatal Med, 2002. **11**(2): p. 84-8.
99. de-Paris, F., Machado, A. B., Gheno, T. C., Ascoli, B. M., Oliveira, K. R., Barth, A. L., *Group B Streptococcus detection: comparison of PCR assay and culture as a screening method for pregnant women.* Braz J Infect Dis, 2011. **15**(4): p. 323-7.
100. Badri, M.S., et al., *Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women.* J Infect Dis, 1977. **135**(2): p. 308-12.
101. Benitz, W.E., Gould, J.B., and Druzin, M.L., *Risk factors for early-onset group B streptococcal sepsis: estimation of odds ratios by critical literature review.* Pediatrics, 1999. **103**(6): p. e77.
102. Hakansson, S., et al., *Group B streptococcal carriage in Sweden: a national study on risk factors for mother and infant colonisation.* Acta Obstet Gynecol Scand, 2008. **87**(1): p. 50-8.
103. Yancey, M.K., Schuchat, A., and Duff, P., *Ethical issues associated with routine screening and prophylaxis for group B streptococcus in pregnancy.* Infect Dis Obstet Gynecol, 1996. **4**(1): p. 36-42.
104. Edwards, R.K., P. Clark, and P. Duff, *Intrapartum antibiotic prophylaxis 2: positive predictive value of antenatal group B streptococci cultures and antibiotic susceptibility of clinical isolates.* Obstet Gynecol, 2002. **100**(3): p. 540-4.
105. Towers, C.V., Rumney, P. J., Asrat, T., Preslicka, C., Ghamsary, M. G., and Nageotte, M. P., *The accuracy of late third-trimester antenatal screening for group B streptococcus in predicting colonization at delivery.* Am J Perinatol, 2010. **27**(10): p. 785-90.
106. Rodriguez-Granger, J., Spellerberg, B., Asam, D., and Rosa-Fraile, M., *Non-haemolytic and non-pigmented group b streptococcus, an infrequent cause of early onset neonatal sepsis.* Pathog Dis, 2015. **73**(9): p. ftv089.

107. El Aila, N.A., Tency, I., Claeys, G., Saerens, B., Cools, P., Verstraelen, H., et al., *Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women.* BMC Infect Dis, 2010. **10**: p. 285.
108. Piccinelli, G., Biscaro, V., Gargiulo, F., Caruso, A., and De Francesco, M. A., *Characterization and antibiotic susceptibility of Streptococcus agalactiae isolates causing urinary tract infections.* Infect Genet Evol, 2015. **34**: p. 1-6.
109. de Tejada, B.M., Pfister, R. E., Renzi, G., Francois, P., Irion, O., Boulvain, M., et al., *Intrapartum Group B streptococcus detection by rapid polymerase chain reaction assay for the prevention of neonatal sepsis.* Clin Microbiol Infect, 2011. **17**(12): p. 1786-91.
110. Gray, J.W., et al., *Feasibility of using microbiology diagnostic tests of moderate or high complexity at the point - of - care in a delivery suite.* J Obstet Gynaecol, 2012. **32**(5): p. 458-60.
111. Ataker F., A.S., Lüthje P., Saeedi B., *Clinical evaluation of a molecular assay for direct and rapid screening for Streptococcus agalactiae in vaginal/rectal samples.* Poster, Karolinska University Hospital, Sweden, 2017.
112. Gavino, M., and Wang, E., *A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization.* Am J Obstet Gynecol, 2007. **197**(4): p. 388 e1-4.
113. Young, B.C., Dodge, L. E., Gupta, M., Rhee, J. S., and Hacker, M. R., *Evaluation of a rapid, real-time intrapartum group B streptococcus assay.* Am J Obstet Gynecol, 2011. **205**(4): p. 372 e1-6.
114. Mueller, M., Henle, A., Droz, S., Kind, A. B., Rohner, S., Baumann, M., et al., *Intrapartum detection of Group B streptococci colonization by rapid PCR-test on labor ward.* Eur J Obstet Gynecol Reprod Biol, 2014. **176**: p. 137-41.
115. Edwards, R.K., Novak-Weekley, S. M., Koty, P. P., Davis, T., Leeds, L. J., and Jordan, J. A., *Rapid group B streptococci screening using a real-time polymerase chain reaction assay.* Obstet Gynecol, 2008. **111**(6): p. 1335-41.
116. Abdelazim, I.A., *Intrapartum polymerase chain reaction for detection of group B streptococcus colonisation.* Aust N Z J Obstet Gynaecol, 2013. **53**(3): p. 236-42.
117. Delabaere, A., et al., *Accuracy of a rapid intrapartum group B Streptococcus test: A new immunochromatographic assay.* J Gynecol Obstet Hum Reprod, 2017. **46**(5): p. 449-453.
118. Picchiassi, E., et al., *Intrapartum test for detection of Group B Streptococcus colonization during labor.* J Matern Fetal Neonatal Med, 2017: p. 1-8.
119. Tanaka, K., et al., *Intrapartum group B Streptococcus screening using real-time polymerase chain reaction in Japanese population.* J Matern Fetal Neonatal Med, 2016. **29**(1): p. 130-4.
120. Frank, L., *Epidemiology. When an entire country is a cohort.* Science, 2000. **287**(5462): p. 2398-9.
121. Brimil, N., Barthell, E., Heindrichs, U., Kuhn, M., Lutticken, R., and Spellerberg, B., *Epidemiology of Streptococcus agalactiae colonization in Germany.* Int J Med Microbiol, 2006. **296**(1): p. 39-44.
122. Valkenburg-van den Berg, A.W., Sprij, A. J., Oostvogel, P. M., Mutsaers, J. A., Renes, W. B., Rosendaal, F. R., et al., *Prevalence of colonisation with group B Streptococci in pregnant women of a multi-ethnic population in The Netherlands.* Eur J Obstet Gynecol Reprod Biol, 2006. **124**(2): p. 178-83.
123. Werawatakul, Y., Wilailuckana, C., Taksaphan, S., Thinkumrup, J., Pragarasung, M., Chouwajaroen, P., et al., *Prevalence and risk factors of Streptococcus agalactiae (group B) colonization in mothers and neonatal contamination at Srinagarind Hospital.* J Med Assoc Thai, 2001. **84**(10): p. 1422-9.
124. Money, D., Dobson, S., Cole, L., Karacabeyli, E., Blondel-Hill, E., Milner, R., et al., *An evaluation of a rapid real time polymerase chain reaction assay for detection of group B streptococcus as part of a neonatal group B streptococcus prevention strategy.* J Obstet Gynaecol Can, 2008. **30**(9): p. 770-5.
125. Daniels, J.P., Gray, J., Pattison, H. M., Gray, R., Hills, R. K., Khan, K. S. et al, *Intrapartum tests for group B streptococcus: accuracy and acceptability of screening.* BJOG, 2011. **118**(2): p. 257-65.

126. Dunne, W.M., Jr. and C.A. Holland-Staley, *Comparison of NNA agar culture and selective broth culture for detection of group B streptococcal colonization in women.* J Clin Microbiol, 1998. **36**(8): p. 2298-300.
127. Dunne, W.M., Jr., *Comparison of selective broth medium plus neomycin-nalidixic acid agar and selective broth medium plus Columbia colistin-nalidixic acid agar for detection of group B streptococcal colonization in women.* J Clin Microbiol, 1999. **37**(11): p. 3705-6.
128. Millar, L.K., and Cox, S. M., *Urinary tract infections complicating pregnancy.* Infect Dis Clin North Am, 1997. **11**(1): p. 13-26.
129. Smaill, F., and Vazquez, J. C., *Antibiotics for asymptomatic bacteriuria in pregnancy.* Cochrane Database Syst Rev, 2007(2): p. CD000490.
130. Mignini, L.C., G.; Abalos, E.; Widmer, M.; Amigot, S.; Nardin, J. M., et al., *Accuracy of diagnostic tests to detect asymptomatic bacteriuria during pregnancy.* Obstet Gynecol, 2009. **113**(2 Pt 1): p. 346-52.
131. Sheiner, E.M.-D., E.; Levy, A., *Asymptomatic bacteriuria during pregnancy.* J Matern Fetal Neonatal Med, 2009. **22**(5): p. 423-7.
132. Perez-Moreno, M.O.P.-P., E.; Grande-Armas, J.; Centelles-Serrano, M. J.; Arasa-Subero, M.; Ochoa, N. C. and M. Led By Mo Perez-Moreno, *Group B streptococcal bacteriuria during pregnancy as a risk factor for maternal intrapartum colonization: a prospective cohort study.* J Med Microbiol, 2017. **66**(4): p. 454-460.
133. Bak, G.S., et al., *Prospective population-based cohort study of maternal obesity as a source of error in gestational age estimation at 11-14 weeks.* Acta Obstet Gynecol Scand, 2016. **95**(11): p. 1281-1287.
134. Bliddal, M., et al., *The Danish Medical Birth Register.* Eur J Epidemiol, 2018. **33**(1): p. 27-36.
135. Ekblad, M., et al., *Trends and risk groups for smoking during pregnancy in Finland and other Nordic countries.* Eur J Public Health, 2014. **24**(4): p. 544-51.
136. Christensen, A.I., et al., *What characterizes persons with poor mental health? A nationwide study in Denmark.* Scand J Public Health, 2014. **42**(5): p. 446-55.
137. Kessous, R., et al., *Bacteruria with group-B streptococcus: is it a risk factor for adverse pregnancy outcomes?* J Matern Fetal Neonatal Med, 2012. **25**(10): p. 1983-6.
138. Petersen, K.B., et al., *Increasing prevalence of group B streptococcal infection among pregnant women.* Dan Med J, 2014. **61**(9): p. A4908.
139. UK National Screening Committee, *Group B Streptococcus (GBS) Recommendation.* London: UK NSC, 2017.
140. Carstensen, H.C., K. K.; Grennert, L.; Persson, K.; Polberger, S., *Early-onset neonatal group B streptococcal septicaemia in siblings.* J Infect, 1988. **17**(3): p. 201-4.
141. Christensen, K.K., et al., *Obstetrical care in future pregnancies after fetal loss in group B streptococcal septicemia. A prevention program based on bacteriological and immunological follow-up.* Eur J Obstet Gynecol Reprod Biol, 1981. **12**(3): p. 143-50.
142. Faxelius, G., et al., *Neonatal septicemia due to group B streptococci--perinatal risk factors and outcome of subsequent pregnancies.* J Perinat Med, 1988. **16**(5-6): p. 423-30.
143. Weston, E.J.P., T.; Lewis, M. M.; Martell-Cleary, P.; Morin, C.; Jewell, B., et al., *The burden of invasive early-onset neonatal sepsis in the United States, 2005-2008.* Pediatr Infect Dis J, 2011. **30**(11): p. 937-41.
144. Oddie, S., and Embleton, N. D., *Risk factors for early onset neonatal group B streptococcal sepsis: case-control study.* BMJ, 2002. **325**(7359): p. 308.
145. Aungst, M., et al., *Low colony counts of asymptomatic group B streptococcus bacteriuria: a survey of practice patterns.* Am J Perinatol, 2004. **21**(7): p. 403-7.
146. American College of, O. and Gynecologists, *ACOG Committee Opinion No. 485: Prevention of early-onset group B streptococcal disease in newborns.* Obstet Gynecol 2011, 2011. **117:1019**.

147. Lin, F.Y., et al., *Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen.* J Infect Dis, 2004. **190**(5): p. 928-34.
148. Poyart, C., et al., *Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci.* J Clin Microbiol, 2007. **45**(6): p. 1985-8.

Appendix

Paper I

Intrapartum PCR assay versus antepartum culture for assessment of vaginal carriage of group B streptococci in a Danish cohort at birth

Paper II

Risk-based screening combined with a PCR-based test for group B streptococci diminishes the use of antibiotics in laboring women

Corrigendum to Paper II

Paper III

Number of colony forming units in urine at 35-37 weeks' gestation and the load of vaginal Group B Streptococci at birth

Paper IV

Group B streptococci cultured in urine during pregnancy associated with preterm delivery: a selection problem?

Paper I

PLOS ONE

RESEARCH ARTICLE

Intrapartum PCR assay versus antepartum culture for assessment of vaginal carriage of group B streptococci in a Danish cohort at birth

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Abstract

The aim of this study was to compare the performances of two strategies for predicting intrapartum vaginal carriage of group B streptococci (GBS). One strategy was based on an antepartum culture and the other on an intrapartum polymerase chain reaction (PCR). We conducted a prospective observational study enrolling 902 pregnant women offered GBS screening before delivery by two strategies. The Culture-strategy was based on vaginal and rectal cultures at 35–37 weeks' gestation, whereas the PCR-strategy was based on PCR assay on intrapartum vaginal swab samples. An intrapartum vaginal culture for GBS was used as the reference standard from which the performances of the 2 strategies were evaluated. The reference standard showed a GBS-prevalence of 12%. The culture-strategy performed with a sensitivity of 82%, specificity of 91%, positive predictive value (PPV) of 55%, negative predictive value (NPV) of 98%, and Likelihood ratio (LH+) of 9.2. The PCR-strategy showed corresponding values as sensitivity of 83%, specificity of 97%, PPV of 78%, NPV of 98%, and LH+ of 27.5. We conclude that in a Danish population with a low rate of early-onset neonatal infection with GBS, the intrapartum PCR assay performs better than the antepartum culture for identification of GBS vaginal carriers during labor.

Introduction

Even though early-onset neonatal infection with Group B streptococci (EOGBS) is rare, it still constitutes a health problem in countries where the prevalence of EOGBS disease is 2 in 1,000 live births, and the mortality rate is 50% [1]. As EOGBS [2] occurs only among the group of neonates who are born by the 10–35% of women colonized vaginally with GBS [3–8], the Centers for Disease Control and Prevention (CDC), USA, in 2002 recommended universal culture screening of all pregnant women between 35 and 37 weeks' gestation in order to give intrapartum antibiotics to the screen positives [9, 10]. The implementation of this strategy was

followed by a decrease in the EOGBS rate from 1.5 to 0.4/1,000 live births [9]. This decrease must be categorized as a success, however, one might wonder why the EOGBS rate in some other countries including Denmark is only 0.1–0.4/1,000 live births [11] even though they have not implemented this antepartum culture-based screening program.

A weakness of the CDC strategy based on a rectovaginal culture obtained often weeks before labor is that shifts in the GBS status [2] reduces the sensitivity to about 50% [12] and positive predictive value to about 60% [13, 14]. This explains why the majority of neonates with EOGBS in the USA are born by women with a negative test for GBS [15–17]. Furthermore, it may cause an overuse of antibiotics if a test for GBS has been positive at a preterm screening but GBS is no longer present at delivery [18, 19]. However, changes in the GBS colonization status of the mother during the period between antepartum screening and delivery may be influenced by several factors including a low colonization status of the woman, suboptimal timing of specimen collection, and inappropriate transport media for specimens, such as lack of storage at 5˚C if transportation of specimens for culture is delayed or there is inadequate laboratory processing [20].

These data call for a rapid GBS test that can be used intrapartum for better identification of women carrying GBS in the vagina at the time of delivery. Previous studies have shown that the sensitivity of the intrapartum polymerase chain reaction test (PCR) to detect GBS colonization during labor may be superior to antenatal cultures; however, these differences have not always been statistically significant [14, 21–23].

The BD Max GBS assay (BD Diagnostic Systems, Québec, Canada) performed on the BDMax™ system (BD Diagnostic Systems, Sparks, MD) is a PCR test intended for use with enriched Lim broth culture after 18 h of incubation of vaginal/rectal swab samples and can provide results for up to 24 concurrent specimens in approximately 2.5 h. The use of E-Swab samples with the BDMax™ GBS assay, eliminating the Lim broth inoculation and incubation steps, may enable a rapid detection of GBS in pregnant women at birth with a sensitivity of 93% [24]. The performance of such an intrapartum PCR-test without a prior enriched Lim broth culture, however, has never been evaluated in a Danish population of pregnant women with a low risk of their babies acquiring early-onset neonatal group B streptococcal disease.

Therefore, the aim of this study was to compare the performance of an antepartum culture based screening strategy and an intrapartum PCR assay for the prediction of intrapartum vaginal carriage of group B streptococci (GBS) in a Danish cohort, using intrapartum vaginal culture as the reference standard.

Material and methods

A total of 2,343 pregnant women attending the prenatal clinic at Lillebaelt Hospital, Kolding, Denmark (with an average of 3,200 deliveries per year) over a 15 months' period between April 2013 and June 2014 were invited to participate in this prospective observational study. One thousand three hundred sixty-four (n = 1,364) declined to participate, leaving 979 participants in the final cohort (Fig 1). Detailed information on oral antibiotic use during pregnancy was obtained from registered data in both patient hospital records and the Danish Medical Agency's Register of non-hospitalized patient use, which included records on all drug prescriptions filed at any Danish pharmacy. Five patients received antibiotics after week 35 of gestation and were therefore excluded (Fig 1). Further, sixty women withdrew from the study at the time of birth for various reasons. Twelve were lost for follow-up. Thus, 902 sets of patient samples were available for comparisons between antepartum culture (culture-based screening) and PCR analysis (PCR-based screening) with intrapartum culture.

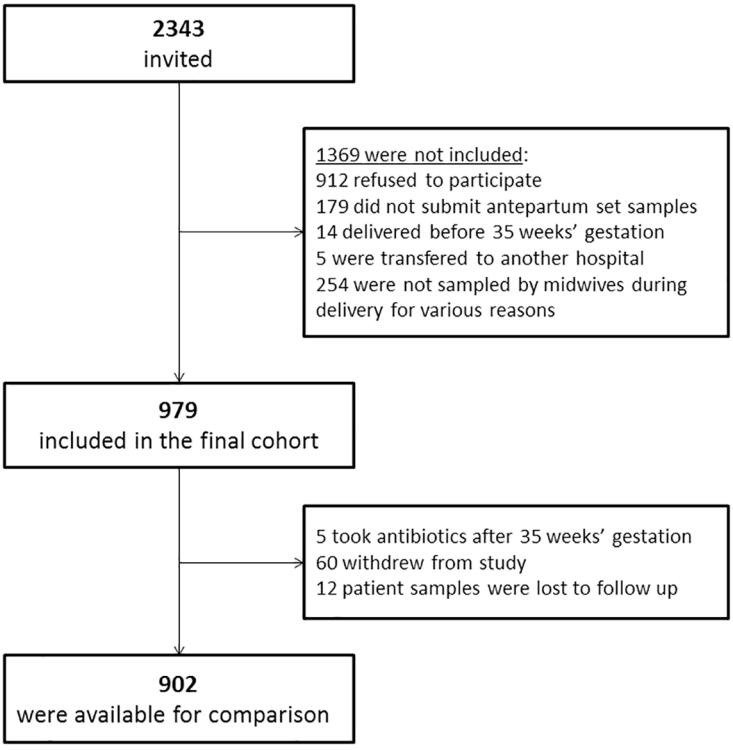

Fig 1. Flowchart of the population.

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Inclusions criteria

All pregnant women attending the prenatal Clinic at Lillebaelt Hospital, Kolding were invited to participate.

Exclusions criteria

Women who delivered preterm (< 37 weeks´ gestation)
Women who received antibiotics after 35 weeks´ gestation
Women with communication restrictions
Women under 18 years old were excluded.

Collection of specimens

All samples were collected using nylon flocked swabs submerged separately into 1 ml of E-Swab transport medium (E-Swab, Copan Diagnostics, Brescia, Italy).

- The Culture-strategy: At 35–37 weeks' gestation, each participant obtained a self-administered and time-saving vaginal and rectal swab sample for culture during a planned visit to the outpatient clinic [25, 26].
- The reference standard: During labor, the midwife collected a vaginal swab sample which was used for immediately culture of GBS.
- The PCR-strategy: The vaginal swab obtained during labor as intrapartum culture sample (reference standard), submerged into the transport medium, was frozen at minus 80˚C for later GBS PCR analysis as a batch processing.

In addition to the written information with text and drawings on how women should obtain a self-administered vaginal and rectal swab sample for the culture, two instructional videos were available to all participants on the project website. The sampling was carried out by inserting and rotating one E-Swab 1.5–2 cm inside the vagina and another one in the rectum by inserting the swab 1.5–2 cm beyond the anal sphincter. All samples were analyzed at the Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark.

Culture of specimens

Samples were cultured at the time of arrival to the laboratory; if received after 8 PM, they were kept at 4˚C until the next morning. Broth enrichment was not employed as part of a strategy to simulate and evaluate a rapid testing of the presence of GBS in the vaginal samples by both culture and PCR. Therefore, direct plating without prior enrichment of the specimen in a culture broth was carried out by streaking the E-Swab specimen on a selective Granada agar plate. The vaginal and rectal swabs from the same patient were seeded on different sides of the same Granada agar plate (BioMérieux®, Spain). The Granada agar plates were incubated immediately after seeding in the CO_2-containing atmosphere at 35˚C. The specimen tubes containing the vaginal intrapartum E-Swab sample medium were subsequently frozen at minus 80˚C for later PCR analysis. The Granada agar plates were read after one and two days of incubation.

All GBS-like colonies (identified by their orange color on Granada agar plates) were routinely confirmed as *Streptococcus agalactiae* using the Microflex LT ™ MALDI-TOF system (Bruker Daltonik, Germany). A semi-quantitative culture assessment of GBS growth was conducted in most cases. The culture was classified as having only growth of few GBS colonies (+), some (++) or many (+++) by intrapartum vaginal culture.

Polymerase chain reaction (PCR)

PCR analysis is a real-time PCR test performed on the BDMaxTM system (Becton, Dickinson and Company, USA) without enrichment of the specimen in a culture broth prior to analysis. The BDMax™ System automatically extracts the target nucleic acid and amplifies a section of the cfb gene sequence of the GBS genome if present. The BDMax™ Assay includes an Internal Process Control to monitor for the presence of potential inhibitory substances as well as system or reagent failures that may be encountered during the entire process. The results are reported by the BDMax™ software as a qualitative answer, either positive or negative for GBS. In a small number of cases, the specimens were initially undetermined because of inhibition, reagent failure or system errors, which led to additional testing by taking a new aliquot of the sample and repeating the DNA extraction and PCR assay. The PCR analyses were performed retrospectively on frozen samples as batch processing.

The results of the GBS culture and PCR tests were read by independent laboratory technicians and recorded separately.

Formalities

The study was approved by the Regional Scientific Ethical Committees for Southern Denmark (S-20130089) and the Danish Data Protection Agency (2008-58-0035). The date of issue: 6 November 2013. All participants provided written informed consent.

Statistics

STATA Statistics/Data Analysis software (version 14; StataCorp LP) was used for the statistical analysis. Sensitivity, specificity, positive predictive values (PPV), negative predictive values

(NPV), and Likelihood ratio (LH) including 95% confidence intervals (CI) were calculated for both antepartum GBS screening and the intrapartum PCR assay using culture of a vaginal swab sample as the gold standard.

Results

All 979 enrolled women had an antepartum swab obtained as part of the culture-strategy and 902 (92%) had an intrapartum swab as part of the culture-based reference standard and the PCR-strategy. The intrapartum vaginal GBS colonization rate detected by culture was 11.5% (reference standard). By comparison, the culture-based strategy found 9.4% (85/902) GBS-positive women by combining results from antepartum vaginal and rectal swab cultures (7.4% by vaginal swab samples and 8.9% by rectal samples) (Table 1), and the PCR-strategy (intrapartum vaginal swab sample) found 12.2% GBS-positive women (Table 2).

Based on the reference standard, the performance characteristics of the culture-strategy and the PCR-strategy are given in Tables 2 and 3. Notably, a marked difference between the positive likelihood ratios (LH+) of 9.2 for the culture-strategy and 27.5 for the PCR-strategy was seen. The positive predictive value was 55% for combining antepartum vaginal and rectal swab cultures and 78% for PCR-strategy.

The false negative rate by the PCR-strategy was 17% (18/104). Fourteen of these 18 false negative samples were assessed by the semi-quantitative culture assessment, and among these 12 (86%) were classified with only few GBS colonies (Table 4). On the other hand, the false positive rate was 3% (24/798).

Discussion

We evaluated two screening strategies for identification of vaginal GBS colonization in a Danish cohort of laboring women, using an intrapartum culture as the reference standard. The antepartum culture-strategy achieved a LR+ of 9.2, whereas the intrapartum PCR-strategy achieved a LR+ of 27.5.

The strength of our study is the size of the cohort consisting of 902 participants from a well-defined population, which did not receive antibiotics between antepartum culture and the time of labor. It might be considered as a limitation that the PCR analyses were performed retrospectively as a batch processing of frozen samples thus only simulated as a rapid on-site test. However, to create a realistic screening scenario for a rapid PCR-strategy we used a GBS PCR assay without a delaying broth enrichment step prior to the PCR analysis. The Granada medium for isolation and identification of GBS is a selective and differential culture medium designed to selectively isolate *Streptococcus agalactiae* (Group B streptococcus, GBS) which differs from the standard recommended by CDC (Lim or TransVag).

The choice of a PCR assay for vaginal GBS detection performed without a prior Lim broth enrichment was intended and thereby also to accept a small, however, statistically significant lower sensitivity (92.7% versus 99.1%) compared to the use of the same PCR test with a Lim broth inoculation of the specimen according to the study of Silbert et al. [24]. Using a prior 18 hours Lim broth enrichment step as part of the PCR assay would prohibit the use of the GBS PCR as a rapid test at the time of delivery.

It may constitute a shortcoming of the study that omitting a prior enrichment step of the specimen is likely to reduce the number of positive cases detected by not only the PCR assay but also the intrapartum culture of the vaginal specimen. However, this approach allowed us to conduct the semi-quantitation of the GBS in the vaginal sample. These results indicate that the potential lower sensitivity of a PCR assay without a prior enrichment step with a false

Table 1. Concordance between detection of GBS colonization analyzed by antepartum culture (rectum and vaginal) and intrapartum culture (vagina) as the reference standard.

		Intrapartum vaginal culture (reference)		
		Positive	**Negative**	**Total = 902**
Antepartum culture		**N (%)**		
Vagina or rectum	Positive	85	71	156 (17.3)
	Negative	19	727	746
Vagina	Positive	67	33	100 (11.1)
	Negative	37	765	802
Rectum	Positive	80	66	146 (16.2)
	Negative	24	732	756

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negative rate of 17% (18/104) is primarily caused by a failure to detect vaginal colonization with low numbers of GBS, which may be of less risk for the newborn during birth.

The false positive rate was only 3%, and in fact, we find it likely that these women may also be colonized with GBS, e.g., by non-hemolytic GBS isolates which may not be detectable on Granada agar plates. However, it is a limitation of the PCR-strategy that 3.4% of all specimens tested were initially undetermined for technical reasons based on the amplification status of the target and the Internal Process Control (data not shown). In such cases, a repeat testing must be conducted, which will delay the definitive result and may be in some cases not in time to decide the use of preventive antibiotic prophylaxis.

In contrast, antepartum screening by a GBS culture or PCR test with or without a prior Lim broth enrichment at week 35–37 during pregnancy is known to miss a substantial number of women with later intrapartum carriage of GBS [12, 13, 21, 27]. Furthermore, it should be noted that the difference in the detecting rates between the direct plating of the rectovaginal swab on the Granada medium and plating after prior Lim broth enrichment is only 4% [28].

Our study is the first of its kind performed in a country such as Denmark where the risk based approach is still recommended. This study is in line with prior studies reporting on the usefulness of a PCR-strategy in detecting intrapartum GBS [12, 14, 22, 29–31]. The GBS carriage rate was only 12% compared to 10–29% in other studies [27, 32–36] comprising different population and using other GBS detection methods based primarily on broth enrichment [2, 35, 37]. The difference between antepartum vaginal and rectal culture carriage (11% vs. 16%, respectively) have also been shown in previous studies, supporting the hypothesis that the gastrointestinal tract is the primary reservoir of GBS, and that vaginal colonization represents spread of GBS from rectum [34, 35].

Unlike other chromogenic media, the Granada medium cannot detect non-hemolytic GBS, thereby potentially decreasing the sensitivity of this culture medium for GBS screening [20]. However, the frequency of non-hemolytic GBS isolates is around 5% among GBS carriers, and

Table 2. Detection of GBS colonization by intrapartum polymerase chain reaction test (PCR) compared to intrapartum vaginal culture as the reference standard.

	Intrapartum vaginal culture (reference)		
	Positive	**Negative**	**Total**
Intrapartum vaginal PCR	**N (%)**		
	104 (11.5)	798 (88.5)	902 (100)
Positive	86 (82.7)	24	110 (12.2)
Negative	18	774 (98.1)	792 (87.8)

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Table 3. Performance of antepartum vaginal/rectal culture and intrapartum vaginal PCR test using intrapartum vaginal culture for GBS as the reference standard.

	Antepartum culture						Intrapartum PCR	
	For GBS						For GBS	
	Vagina		Rectum		Vagina or rectum		Vagina	
	%, n/N	95% CI	%, n/N	95% CI	%, n/N	95% CI	%, n/N	95% CI
Sensitivity	64 67/104	54–74	77 80/104	68–85	82 85/104	73–89	83 86/104	74–89
Specificity	96 765/798	94–97	92 732/798	90–94	91 727/798	89–93	97 774/798	96–98
PPV	67 67/100	55–74	55 80/146	46–63	55 85/156	46–63	78 86/110	69–86
NPV	95 765/802	94–97	97 732/756	95–98	98 727/746	96–99	98 774/792	96–99
LH+	16 65/1-96	11–22	9 77/1-92	7–12	9 82/1-91	7–12	27 83/1-97	18–41

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value; LH = Likelihood ratio

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a rate of only 1% is observed among invasive GBS strains, which suggests that EOGBS caused by non-hemolytic GBS strains is negligible [38].

The PCR strategy does not allow for performing antimicrobial susceptibility testing, which may be of relevance for penicillin-allergic patients. However, susceptibility testing is not necessary in general because GBS isolates with confirmed resistance to penicillin, ampicillin, or cefazolin have not yet been described [39]. Fortunately, efficient alternative choices exist for those with known penicillin-allergy, e.g., cefuroxime, cefaclor, and ceftriaxone. In patients with a history of severe anaphylactic reactions following cephalosporin treatment, vancomycin is an alternative antibiotic [40].

We conclude that in a Danish population of pregnant women with a low risk for their babies to acquire EOGBS, intrapartum PCR could be an efficient recommendation to screen for vaginal carriage of GBS during labor. It remains, to be evaluated, however from a medical technology perspective whether the test should be offered to all laboring women or only to those with a predefined risk. Such a medical technology evaluation must take into account 1) the fact that we have studied only proxy variables (GBS vaginal colonization) for EOGBS, 2) the overall costs, 3) the risks of maternal anaphylactic reactions and sensitization, 4) the

Table 4. Association between PCR and culture with semi-quantitative assessment of intrapartum vaginal growth of GBS in sample.

	Intrapartum vaginal culture (reference) (N = 902)				
	Positive				Negative
Intrapartum vaginal PCR	Semi-quantification				
	+	++	+++	Not assessed	
Positive	10	17	36	23	24
Negative	12	1	1	4	774

$^{+++}$ = many

$^{++}$ = some

$^{+}$ = growth of few GBS colonies

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possible adverse effects of antibiotics on the microbiome of the mother and the newborn [41], and 5) the risk of promoting drug resistance among the bacteria.

It should be noted that there are some practical demands of an intrapartum PCR test. It should first of all be simple for midwives or nurses to perform, and they should also be able to expect a test result made available within a relatively short time, which is necessary for the decision whether or not to administer antibiotics in a busy labor and delivery ward. In some urgent clinical cases, a PCR result may be required within less than 120 minutes, which is possible with the present PCR assay when a few patient samples are handled at a time.

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Reference

1. Wilkinson H.W., Facklam R.R., and Wortham E.C., Distribution by serological type of group B streptococci isolated from a variety of clinical material over a five-year period (with special reference to neonatal sepsis and meningitis). Infect Immun, 1973. 8(2): p. 228–35. PMID: 4725698
2. Hansen S.M., Uldbjerg N, Kilian M, and Sorensen U.B., Dynamics of Streptococcus agalactiae colonization in women during and after pregnancy and in their infants. J Clin Microbiol, 2004. 42(1): p. 83–9. https://doi.org/10.1128/JCM.42.1.83-89.2004 PMID: 14715736
3. Verani J.R., and Schrag S. J., Group B streptococcal disease in infants: progress in prevention and continued challenges. Clin Perinatol, 2010. 37(2): p. 375–92. https://doi.org/10.1016/j.clp.2010.02.002 PMID: 20569813

4. Stoll B.J., and Schuchat A., Maternal carriage of group B streptococci in developing countries. Pediatr Infect Dis J, 1998. 17(6): p. 499–503. PMID: 9655542
5. Bergeron M.G., Ke D., Menard C., Picard F. J., Gagnon M., Bernier M., et al., Rapid detection of group B streptococci in pregnant women at delivery. N Engl J Med, 2000. 343(3): p. 175–9. https://doi.org/10.1056/NEJM200007203430303 PMID: 10900276
6. Regan J.A., Klebanoff M. A., and Nugent R. P., The epidemiology of group B streptococcal colonization in pregnancy. Vaginal Infections and Prematurity Study Group. Obstet Gynecol, 1991. 77(4): p. 604–10. PMID: 2002986
7. Schuchat A., Epidemiology of group B streptococcal disease in the United States: shifting paradigms. Clin Microbiol Rev, 1998. 11(3): p. 497–513. PMID: 9665980
8. Benitz W.E., Gould J.B., and Druzin M.L., Risk factors for early-onset group B streptococcal sepsis: estimation of odds ratios by critical literature review. Pediatrics, 1999. 103(6): p. e77. PMID: 10353974
9. Centers for Disease, a.C.a.P., Trends in perinatal group B streptococcal disease—United States, 2000–2006. MMWR Morb Mortal Wkly Rep, 2009. 58(5): p. 109–12. PMID: 19214159
10. Phares C.R., Lynfield R., Farley M. M., Mohle-Boetani J., Harrison L. H., Petit S., et al., Epidemiology of invasive group B streptococcal disease in the United States, 1999–2005. JAMA, 2008. 299(17): p. 2056–65. https://doi.org/10.1001/jama.299.17.2056 PMID: 18460666
11. Ekelund K., and Konradsen H.B., Invasive group B streptococcal disease in infants: a 19-year nationwide study. Serotype distribution, incidence and recurrent infection. Epidemiol Infect, 2004. 132(6): p. 1083–90. PMID: 15635965
12. Davies H.D., Miller M. A., Faro S., Gregson D., Kehl S. C., and Jordan J. A., Multicenter study of a rapid molecular-based assay for the diagnosis of group B Streptococcus colonization in pregnant women. Clin Infect Dis, 2004. 39(8): p. 1129–35. https://doi.org/10.1086/424518 PMID: 15486835
13. Towers C.V., Rumney P. J., Asrat T., Preslicka C., Ghamsary M. G., and Nageotte M. P., The accuracy of late third-trimester antenatal screening for group B streptococcus in predicting colonization at delivery. Am J Perinatol, 2010. 27(10): p. 785–90. https://doi.org/10.1055/s-0030-1254237 PMID: 20458663
14. El Helali N., Nguyen J. C., Ly A., Giovangrandi Y., and Trinquart L., Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis, 2009. 49(3): p. 417–23. https://doi.org/10.1086/600303 PMID: 19580414
15. Puopolo K.M., Madoff L. C., and Eichenwald E. C., Early-onset group B streptococcal disease in the era of maternal screening. Pediatrics, 2005. 115(5): p. 1240–6. https://doi.org/10.1542/peds.2004-2275 PMID: 15867030
16. Van Dyke M.K., Phares C. R., Lynfield R., Thomas A. R., Arnold K. E., Craig A. S., et al., Evaluation of universal antenatal screening for group B streptococcus. N Engl J Med, 2009. 360(25): p. 2626–36. https://doi.org/10.1056/NEJMoa0806820 PMID: 19535801
17. Pulver L.S., Hopfenbeck M. M., Young P. C., Stoddard G. J., Korgenski K., Daly J., et al., Continued early onset group B streptococcal infections in the era of intrapartum prophylaxis. J Perinatol, 2009. 29 (1): p. 20–5. https://doi.org/10.1038/jp.2008.115 PMID: 18704032
18. Langhendries J.P., [Early bacterial colonisation of the intestine: why it matters?]. Arch Pediatr, 2006. 13(12): p. 1526–34. https://doi.org/10.1016/j.arcped.2006.09.018 PMID: 17079124
19. Romero R., and Korzeniewski S. J., Are infants born by elective cesarean delivery without labor at risk for developing immune disorders later in life? Am J Obstet Gynecol, 2013. 208(4): p. 243–6. https://doi.org/10.1016/j.ajog.2012.12.026 PMID: 23273890
20. Verani J.R., McGee L., Schrag S. J., Division of Bacterial Diseases, National Center for Immunization, Respiratory Diseases, Centers for Disease Control and Prevention., Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. MMWR Recomm Rep, 2010. 59(RR-10): p. 1–36. PMID: 21088663
21. de Tejada B.M., Pfister R. E., Renzi G., Francois P., Irion O., Boulvain M., et al., Intrapartum Group B streptococcus detection by rapid polymerase chain reaction assay for the prevention of neonatal sepsis. Clin Microbiol Infect, 2011. 17(12): p. 1786–91. https://doi.org/10.1111/j.1469-0691.2010.03378.x PMID: 20860701
22. Edwards R.K., Novak-Weekley S. M., Koty P. P., Davis T., Leeds L. J., and Jordan J. A., Rapid group B streptococci screening using a real-time polymerase chain reaction assay. Obstet Gynecol, 2008. 111 (6): p. 1335–41. https://doi.org/10.1097/AOG.0b013e31817710ee PMID: 18515517
23. Gavino M., and Wang E., A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization. Am J Obstet Gynecol, 2007. 197 (4): p. 388 e1–4.

24. Silbert S., Rocchetti T. T., Gostnell A., Kubasek C., and Widen R., Detection of Group B Streptococcus Directly from Collected ESwab Samples by Use of the BD Max GBS Assay. J Clin Microbiol, 2016. 54 (6): p. 1660–3. https://doi.org/10.1128/JCM.00445-16 PMID: 27053670

25. Price D., Shaw E., Howard M., Zazulak J., Waters H., and Kaczorowski J., Self-sampling for group B streptococcus in women 35 to 37 weeks pregnant is accurate and acceptable: a randomized cross-over trial. J Obstet Gynaecol Can, 2006. 28(12): p. 1083–8. PMID: 17169231

26. Mercer B.M., Taylor M. C., Fricke J. L., Baselski V. S., and Sibai B. M., The accuracy and patient preference for self-collected group B Streptococcus cultures. Am J Obstet Gynecol, 1995. 173(4): p. 1325–8. PMID: 7485347

27. Yancey M.K., Schuchat A., and Duff P., Ethical issues associated with routine screening and prophylaxis for group B streptococcus in pregnancy. Infect Dis Obstet Gynecol, 1996. 4(1): p. 36–42. https://doi.org/10.1155/S1064744996000099 PMID: 18476063

28. El Aila N.A., Tency I., Claeys G., Saerens B., Cools P., Verstraelen H., et al., Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect Dis, 2010. 10: p. 285. https://doi.org/10.1186/1471-2334-10-285 PMID: 20920213

29. Young B.C., Dodge L. E., Gupta M., Rhee J. S., and Hacker M. R., Evaluation of a rapid, real-time intrapartum group B streptococcus assay. Am J Obstet Gynecol, 2011. 205(4): p. 372 e1–6.

30. Mueller M., Henle A., Droz S., Kind A. B., Rohner S., Baumann M., et al., Intrapartum detection of Group B streptococci colonization by rapid PCR-test on labor ward. Eur J Obstet Gynecol Reprod Biol, 2014. 176: p. 137–41. https://doi.org/10.1016/j.ejogrb.2014.02.039 PMID: 24680393

31. Money D., Dobson S., Cole L., Karacabeyli E., Blondel-Hill E., Milner R., et al., An evaluation of a rapid real time polymerase chain reaction assay for detection of group B streptococcus as part of a neonatal group B streptococcus prevention strategy. J Obstet Gynaecol Can, 2008. 30(9): p. 770–5. PMID: 18845045

32. Brimil N., Barthell E., Heindrichs U., Kuhn M., Lutticken R., and Spellerberg B., Epidemiology of Streptococcus agalactiae colonization in Germany. Int J Med Microbiol, 2006. 296(1): p. 39–44. https://doi.org/10.1016/j.ijmm.2005.11.001 PMID: 16361113

33. Lyytikainen O., Nuorti J. P., Halmesmaki E., Carlson P., Uotila J., Vuento R., et al., Invasive group B streptococcal infections in Finland: a population-based study. Emerg Infect Dis, 2003. 9(4): p. 469–73. https://doi.org/10.3201/eid0904.020481 PMID: 12702228

34. Schrag S.J., Zell E. R., Lynfield R., Roome A., Arnold K. E., Craig A. S., et al., A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. N Engl J Med, 2002. 347(4): p. 233–9. https://doi.org/10.1056/NEJMoa020205 PMID: 12140298

35. Valkenburg-van den Berg A.W., Sprij A. J., Oostvogel P. M., Mutsaers J. A., Renes W. B., Rosendaal F. R., et al., Prevalence of colonisation with group B Streptococci in pregnant women of a multi-ethnic population in The Netherlands. Eur J Obstet Gynecol Reprod Biol, 2006. 124(2): p. 178–83. https://doi.org/10.1016/j.ejogrb.2005.06.007 PMID: 16026920

36. Werawatakul Y., Wilailuckana C., Taksaphan S., Thinkumrup J., Pragarasung M., Chouwajaroen P., et al., Prevalence and risk factors of Streptococcus agalactiae (group B) colonization in mothers and neonatal contamination at Srinagarind Hospital. J Med Assoc Thai, 2001. 84(10): p. 1422–9. PMID: 11804252

37. Rallu F., Barriga P., Scrivo C., Martel-Laferriere V., and Laferriere C., Sensitivities of antigen detection and PCR assays greatly increased compared to that of the standard culture method for screening for group B streptococcus carriage in pregnant women. J Clin Microbiol, 2006. 44(3): p. 725–8. https://doi.org/10.1128/JCM.44.3.725-728.2006 PMID: 16517846

38. Rodriguez-Granger J., Spellerberg B., Asam D., and Rosa-Fraile M., Non-haemolytic and non-pigmented group b streptococcus, an infrequent cause of early onset neonatal sepsis. Pathog Dis, 2015. 73(9): p. ftv089. https://doi.org/10.1093/femspd/ftv089 PMID: 26449711

39. Schuchat A., Zywicki S. S., Dinsmoor M. J., Mercer B., Romaguera J., O'Sullivan M. J., et al., Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. Pediatrics, 2000. 105(1 Pt 1): p. 21–6. PMID: 10617699

40. Piccinelli G., Biscaro V., Gargiulo F., Caruso A., and De Francesco M. A., Characterization and antibiotic susceptibility of Streptococcus agalactiae isolates causing urinary tract infections. Infect Genet Evol, 2015. 34: p. 1–6. https://doi.org/10.1016/j.meegid.2015.07.001 PMID: 26144658

41. Keski-Nisula L., Kyynarainen H. R., Karkkainen U., Karhukorpi J., Heinonen S., and Pekkanen J., Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth. Acta Paediatr, 2013. 102(5): p. 480–5. https://doi.org/10.1111/apa.12186 PMID: 23398392

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Full length article

Risk-based screening combined with a PCR-based test for group B streptococci diminishes the use of antibiotics in laboring women

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ABSTRACT

Objective: To assess the performance of a polymerase chain reaction – group B streptococci test (PCR-GBS test) – in deciding antibiotic prophylaxis in term laboring women.
Study design: In this observational study, we enrolled 902 unselected Danish term pregnant women. During labor, midwives obtained vaginal swabs that were used for both GBS cultures (reference standard) and for the PCR-GBS test. Furthermore, we recorded the presence of risk factors for EOGBS (Early Onset Group B Streptococcal disease): (1) Bacteriuria during current pregnancy, (2) Prior infant with EOGBS (3) Temperature above 38.0 °C during labor, and (4) Rupture of membranes ≥18 h.
Results: The prevalence of GBS carriers was 12% (104 of 902), the sensitivity of the PCR-GBS test 83% (86 of 104), and the specificity 97% (774 of 798). Among the 108 with one or more EOGBS-risk factors, GBS was present in 23% (25 of 108), the sensitivity 92% (23 of 25), and the specificity 89% (74 of 83).
Conclusion: In programs that aim to treat all laboring women with vaginal GBS-colonization (12% in the present study) with penicillin, the PCR-GBS will perform well (sensitivity 83% and specificity 97%). In programs aiming to treat only GBS-carriers among those with risk factors of EOGBS, a reduction of penicillin usage by two-thirds from 12% to 4% may be possible.

1.4 Introduction

In order to reduce the risk of "early-onset" neonatal infection with GBS (EOGBS), international guidelines recommend two strategies for identification of laboring women who should be offered intrapartum antibiotics prophylaxes (IAP): the *risk-based approach* and *the culture-based screening* [1,2]. In the UK the Royal College of Obstetricians and Gynaecologists (RCOG) recommend the *risk based approach* and have defined the following 5 risk factors: (1) previous infant with EOGBS, (2) GBS bacteriuria during the current pregnancy, (3) temperature >38.0 °C, (4) rupture of membranes (ROM) ≥18 h, or (5) delivery at <37 weeks' gestation. They claim that 66% of EOGBS neonates are born to mothers with one or more of these risk factors [3,4]. RCOG has further recommended (2012) IAP to women with GBS vaginal carriage detected during the current pregnancy [1]. On the other hand, the Centers for Disease Control and Prevention, USA, (CDC), recommends universal *screening at* 35–37 *weeks' gestation for GBS recto-vaginal colonization*, as they find that the culture-screening was >50% more effective than the risk-based approach of preventing EOGBS disease [5]. In the USA, the introduction of a culture-based screening strategy was followed by a decrease in the EOGBS incidence from 1.7 to 0.4 cases per 1000 births [2]. However, it is of concern that the false positive rate is high and as many as two-thirds of EOGBS newborns are born to GBS screen negative mothers [6–8].

It is also of concern that a substantial fraction of the laboring women who are at risk according to the RCOG definition [1] are GBS negative. This calls for a GBS test that can be used intrapartum in order to refrain from IAP in the GBS negative women. Therefore,

Abbreviations: ACOG, American College of Obstetricians and Gynecologists; CDC, Centers for Disease Control and Prevention, USA; EO, Early-onset; EOGBS, Early onset of neonatal group B streptococcal disease; GBS, Group B streptococci; IAP, Intrapartum antibiotic prophylaxis; NPV, Negative predictive value; PCR, Polymerase chain reaction; PPV, Positive predictive value; RCOG, Royal College of Obstetricians and Gynecologists, Great Britain; PTD, Preterm delivery; ROM, Rupture of membranes.

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polymerase chain reaction tests (PCR) have been used as an intrapartum GBS assay in several studies [9–11]; however, they have not been evaluated in a low-risk population like that in Denmark with an EOGBS incidence of 0.34-0.37 cases per 1000 live births [12].

The aim of this study is to assess the performance of the risk-based approach in combination with a PCR assay in detecting vaginal carriage of GBS in unselected Danish laboring women at term. The perspective is a diminished use of IAP.

Material and methods

Study design

The study was approved by the Regional Scientific Ethical Committees for Southern Denmark (S-20130089) and the Danish Data Protection Agency (2008-58-0035). All participants provided written informed consent.

In this prospective observational study, we invited 2343 unselected pregnant women at 29 weeks gestation attending the prenatal Clinic at Lillebaelt Hospital, Kolding (approximately 3200 deliveries per year) between April 2013 and June 2014. Nine hundred and two (n = 902) invited women were enrolled, while 1441 did not participate. A flowchart of the population is shown in Fig. 1. The attending midwives at the delivery ward recorded the presence of risk factors for EOGBS (1) GBS bacteriuria during current pregnancy, (2) prior infant with EOGBS (3) temperature above 38.0 °C during labor, and (4) ROM ≥18 h. Detailed information on oral antibiotic use during pregnancy was obtained from the registered data in medical records and delivered from the Danish Medical Agency's Register of non-hospitalized patient use, which included records on all drug prescriptions filled at any Danish pharmacy [13].

Inclusion criteria

- All pregnant women attending the prenatal Clinic at Lillebaelt Hospital, Kolding.

Exclusion criteria

- Women treated with antibiotics after 35 weeks' gestation.
- Preterm labor (before 37 + 0 weeks gestation).
- Age under 18 years.
- Women with a communication barrier.

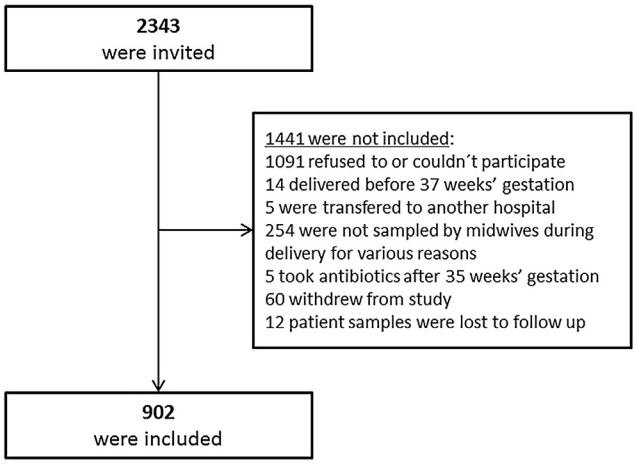

Fig. 1. Flow chart of patient inclusion.

Collection and culture of specimens

During labor, the midwife obtained vaginal swabs (E-Swab, Copan Diagnostics, Brescia, Italy). The vaginal swab was rotated 1.5–2 cm inside the vagina.

If received at the laboratory (Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark) between 7 A.M. and 8 P.M., the samples were cultured immediately; otherwise, they were kept at 4 °C until the next morning. Direct plating was carried out by streaking the E-Swab specimen on a selective Granada agar plate (BioMérieux®, Spain). The Granada agar plates were incubated immediately after seeding in the CO_2-containing atmosphere at 35 °C. The specimen tubes containing the vaginal intrapartum E-Swab sample medium were subsequently frozen at minus 80 °C for later PCR analysis.

The Granada agar plates were read twice after one and two days of incubation, respectively. All GBS-like colonies (identified by their orange color) were routinely confirmed as *Streptococcus agalactiae* using the Microflex LT ™ MALDI-TOF system (Bruker Daltonik, Germany).

Polymerase chain reaction (PCR)

The intrapartum vaginal PCR analysis was performed on BDMax™ (Becton, Dickinson and Company, USA) without enriching the specimen in a culture broth prior to analysis. The BDMax™ System automatically extracts the target nucleic acid and amplifies a section of the cfb gene sequence of the GBS chromosome (Becton, Dickinson), if present. The BDMax™ Assay includes an Internal Process Control to monitor for the presence of potential inhibitory substances as well as system or reagent failures that may be encountered during the entire process. The results are interpreted and produced by the BDMax™ software as a qualitative answer, either positive or negative for GBS. In a small number of cases (3.4% of all specimens), the specimens were initially undetermined because of inhibition, reagent failure or system errors, which led to additional testing by taking a new aliquot of the sample and repeating the DNA extraction and PCR assay. The PCR analyses were performed retrospectively on frozen samples as batch processing. The results of the GBS culture and PCR tests were read by independent laboratory technicians at Department of Clinical Microbiology and recorded separately.

Statistical analysis

STATA Statistics/Data Analysis software (version 14; StataCorp LP) was used for the statistical analysis. Sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated for both antepartum screening and the PCR technique using the intrapartum culture as the gold standard. Differences of P value less than 0.05 were considered statistically significant.

Results

Nine hundred and two unselected women in labor at term were included. The demographic characteristics of the women tested intrapartum with both vaginal culture and PCR test are shown in Table 1. Mothers' age under 25 years was negatively associated with the status of intrapartum vaginal GBS colonization (OR 0.42; 95% CI: 0.18 to 0.99; $P < 0.05$).

Among participants (Table 2), 11.5% were vaginal GBS culture-positive (104/902) and 12.2% (110/902) were GBS-PCR positive. The

Table 1
Demographic characteristics of women tested positive intrapartum with both vaginal culture and PCR test (n = 128) or negative for GBS (n = 774).

Maternal characteristics	GBS positive (N = 128)		GBS negative (N = 774)				
	Number	%	Number	%	OR	95% CI	P-value
Age of the mother							
Under 25	6	4.7	81	10.5	0.42	0.18 to 0.99	0.05
25–34	93	72.7	521	67.3	1.29	0.85 to 1.96	0.23
Above 35	29	22.7	172	22.2	1.03	0.66 to 1.60	0.91
Parity							
1	49	38.3	358	46.3	0.72	0.49 to 1.06	0.09
2	68	53.1	378	48.8	1.19	0.82 to 1.73	0.46
3 or more	11	8.6	38	4.9	1.82	0.91 to 3.66	0.37
Body mass index							
Under 24.9	72	56.3	505	65.3	0.69	0.47 to 1.00	0.05
25–29.9	35	27.3	172	22.2	1.32	0.86 to 2.01	0.20
30- or more	21	16.4	97	12.5	1.37	0.82 to 2.29	0.23
Tobacco							
Never smoking	121	94.5	706	91.2	1.67	0.75 to 3.71	0.21
Stopped in pregnancy	2	1.6	25	3.2	0.48	0.11 to 2.03	0.32
Smoke = < 10 cigarettes	3	2.3	32	4.1	0.56	0.17 to 1.85	0.34
Smoke > 10 cigarettes	2	1.6	11	1.4	1.10	0.24 to 5.03	0.90

Table 2
Intrapartum GBS prevalence by vaginal culture and PCR test for each of the four risk factors (N = 902).

	Intrapartum vaginal culture	Intrapartum PCR test
Risk factors	Positive rate% (No)	Positive rate% (No)
EOGBS in prior delivery	0% (0/1)	0% (0/1)
GBS bacteriuria	60% (18/30)	80% (24/30)
Fever (≥38.0 °C)	0% (0/1)	0% (0/1)
ROM ≥18 h	10% (7/68)	12% (8/68)
Total with risk factors	23% (25/83)	30% (32/108)
No risk factors	10% (79/794)	10% (78/794)
Total	12% (**104/902**)	12% (**110/902**)

ROM = rupture of membranes.

overall number of women with either vaginal positive culture or GBS-PCR positive was 128 (14.2%) (Table 1). Among the 902 participants, 12.0% (108) had one or more risk factors concerning EOGBS (Table 2), of which 23.2% (25/108) were vaginal GBS-culture positive. The EOGBS risk factor most strongly associated with intrapartum vaginal GBS colonization was GBS bacteriuria during pregnancy. Eighteen of the 30 pregnant women with GBS bacteriuria (60%) were vaginal GBS-culture positive (Table 2). However, seven of the 30 women (23%) were GBS-PCR positive despite being GBS-culture negative (data not shown).

In total, 2.7% (25/902) had both 1 or more risk factors and a positive vaginal GBS culture, whereas 3.6% (32/902) had both 1 or more risk factors and a positive GBS-PCR test (Table 3). Among the participants with risk factors, the sensitivity of the GBS-PCR test was 92% (23/25) using the vaginal GBS culture as a reference standard (Table 3).

Ninety-four percent (101/108) of women with one or more risk factors received IAP during labor. Two women (2%) underwent an elective caesarian section and were treated routinely with cefuroxime during operation also providing IAP. For the last five (5%), the IAP could not be technically implemented for various reasons such as quick or hectic birth (data not shown).

Discussion

We studied an unselected Danish cohort of women in labor at term of pregnancy. Within this cohort, 12% had EOGBS-risk factors whereas only 2.7% had both 1 or more risk factors and a positive vaginal GBS culture. This indicates substantial overtreatments if all with EOGBS risk factors are offered IAP in accordance with the RCOG recommendations. Among the 2.7%, not fewer than 2.5% had a positive intrapartum GBS-PCR equivalent to a sensitivity of 92%. Thus, a strategy offering IAP only to those laboring women with both 1 or more risk factors and a positive GBS-PCR may be safe and substantially reduce the use of IAP.

The strength of our study is the prospective cohort design and the relatively high number of participants, which allowed us to draw robust conclusions about detection of GBS colonization

Table 3
Performance of risk-based screening and intrapartum PCR-GBS test individually or in combination for detection of intrapartum GBS carriage[#].

	One or more Risk factors (N = 902)		PCR-GBS (N = 902)		PCR-GBS if Risk-factor present (N = 108)	
	% (n)	95% CI	% (n)	95% CI	% (n)	95% CI
Sensitivity	24% (25/104)	16–33%	83% (86/104)	74–89%	92% (23/25)	74–99%
Specificity	90% (715/798)	87–92%	97% (774/798)	96–98%	89% (74/83)	80–95%
PPV	23% (25/108)	16–32%	78% (86/110)	69–86%	72% (23/32)	53–86%
NPV	90% (715/794)	88–92%	98% (774/792)	96–99%	97% (74/76)	91–100%

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value.
# Reference standard: Vaginal GBS colonization rate (N 104, 12%).

during labor. Furthermore, in order to create a realistic real-time screening scenario for rapid intrapartum testing during labor, we used a GBS-PCR assay without a time-consuming broth enrichment step prior to the PCR analysis.

It might be considered as a limitation of the study that the PCR analyses were performed as a batch processing of frozen samples. However, we chose this design as the purpose was to test the principle and not a specific product for rapid GBS-PCR testing.

It could also be considered as a limitation that we chose vaginal GBS-colonization as the reference standard instead of rectovaginal GBS-colonization, knowing that obtaining swab samples from both the rectum and vagina improves the yield compared with only sampling the vagina [14]. Our consideration is that although the rectum in many women constitutes a GBS-reservoir from which the vagina is colonized interminably, EOGBS seems to depend primarily on the actual vaginal GBS colonization during labor [15]. A number of studies have shown that as many as 10% of women with a negative antepartum screening result at 35–37 weeks' gestation become positive for GBS vaginal carriage at the time of delivery [6–8].

Furthermore, the Granada medium for culture of GBS is a selective and differential culture medium designed to selectively isolate *Streptococcus agalactiae* (Group B streptococcus, GBS), which differs from the standard recommended by CDC (Lim or TransVag). Not using a prior enrichment step of the specimen in addition to inoculation into selective broth may reduce the number of positive cases; however, the difference in the detection rates between direct plating of the rectovaginal swab on Granada medium with and without prior Lim broth enrichment is as low as 4% [16].

The rationale for the risk-based strategy is challenged by the data in Table 2 showing that 9% (79/902) of our population was GBS-colonized even though they did not have risk factors. However, the risk based strategy does probably reduce the EOGBS incidence because it identifies those fetuses exposed to heavy GBS-colonization (e.g. maternal GBS-uria, Table 1) or long term exposure to GBS (e.g. ROM > 18 h) [3,5,17–21]. On the other hand, in populations treated according to a risk based strategy, 25% of newborns with EOGBS are delivered by women without risk factors [3–5,22–24], and in populations treated according to recto-vaginal cultures obtained several weeks before delivery, 65% of EOGBS newborns are born to screen negative mothers [6–8]. Only considering the IAP of those laboring women with both a positive PCR and the presence of 1 or more risk factors (n = 32; 4% of the total population of laboring women) would leave 78 women with a positive GBS PCR without antibiotic prophylaxis. One could therefore argue that all GBS-PCR positive women (12% of our population) should have IAP. However, this should be evaluated with the proper medical technology taking into account the following issues: (1) the overall costs, (2) the risks of maternal anaphylactic reactions and sensitization, (3) the possible adverse effects of antibiotics on the microbiome of the mother and the newborn [25], and (4) the risk of promoting drug resistance among the bacteria.

The external validity of our study and some disagreement between our results and those from other studies must be considered. Some studies detect higher GBS prevalence among women with risk factors, probably due to differences between populations, different culture and PCR techniques, and different risk factor criteria, e.g. the inclusion of women delivering preterm [26,27].

Furthermore, one must remember that the EOGBS challenges include a number of important perspectives which we must elucidate. These include varying numbers of virulence factors of different GBS strains [28], a factor which might be addressed in future more specific GBS-PCR assays [29]. Furthermore, some women may have better transfer of maternal antibodies to the fetus than other women, e.g. the passive immunization of the newborn differs [30,31]. This aspect might be addressed by appropriate vaccination programs or by identification of women at special risk due to an inappropriate profile of immunoglobulins.

It should be noted that there are some practical demands of an intrapartum PCR test. It should be simple for midwives or nurses to perform, as well as provide a test result within a relatively short period of time (in some urgent clinical cases within less than 120 min as for the present PCR assay), which is necessary for the decision whether or not to administer antibiotics in a busy labor and delivery ward.

In conclusion, in countries including Denmark with a low incidence of EOGBS, a substantial reduction in antibiotic prophylaxis of two-thirds could be achieved at term by combining a risk factor based screening with a rapid intrapartum PCR test for vaginal carriage of GBS.

Conflict of interest

All the authors declare that there is no conflict of interest.

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References

[1] Abstracts of the RCOG (Royal College of Obstetricians and Gynaecologists) 10th International Scientific Congress. June 5–8, 2012. Kuching, Sarawak, Malaysia. BJOG . 2012;119 Suppl 1:2-250.
[2] Verani JR, McGee L, Schrag SJ, Division of Bacterial Diseases NCfI, Respiratory Diseases CfDC, Prevention. Prevention of perinatal group B streptococcal disease–revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59 (RR-10):1-36.
[3] Heath PT, Balfour GF, Tighe H, Verlander NQ, Lamagni TL, Efstratiou A, et al. Group B streptococcal disease in infants: a case control study. Arch Dis Child 2009;94(9):674–80.
[4] Vergnano S, Embleton N, Collinson A, Menson E, Russell AB, Heath P. Missed opportunities for preventing group B streptococcus infection. Arch Dis Child Fetal Neonatal Ed 2010;95(1):F72–3.
[5] Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. MMWR Recomm Rep 2002;51(RR-11):1–22.
[6] Puopolo KM, Madoff LC, Eichenwald EC. Early-onset group B streptococcal disease in the era of maternal screening. Pediatrics 2005;115(5):1240–6.
[7] Van Dyke MK, Phares CR, Lynfield R, Thomas AR, Arnold KE, Craig AS, et al. Evaluation of universal antenatal screening for group B streptococcus. N Engl J Med 2009;360(25):2626–36.
[8] Pulver LS, Hopfenbeck MM, Young PC, Stoddard GJ, Korgenski K, Daly J, et al. Continued early onset group B streptococcal infections in the era of intrapartum prophylaxis. J Perinatol 2009;29(1):20–5.
[9] El Helali N, Nguyen JC, Ly A, Giovangrandi Y, Trinquart L. Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis 2009;49(3):417–23.
[10] Edwards RK, Novak-Weekley SM, Koty PP, Davis T, Leeds LJ, Jordan JA. Rapid group B streptococci screening using a real-time polymerase chain reaction assay. Obstet Gynecol 2008;111(6):1335–41.
[11] Young BC, Dodge LE, Gupta M, Rhee JS, Hacker MR. Evaluation of a rapid, real-time intrapartum group B streptococcus assay. Am J Obstet Gynecol 2011;205 (4)e1–6 372.
[12] Jordan HT, Farley MM, Craig A, Mohle-Boetani J, Harrison LH, Petit S, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease: a multistate, population-based analysis. Pediatr Infect Dis J 2008;27(12):1057–64.
[13] Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90.
[14] Badri MS, Zawaneh S, Cruz AC, Mantilla G, Baer H, Spellacy WN, et al. Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women. J Infect Dis 1977;135(2):308–12.

[15] Benitz WE, Gould JB, Druzin ML. Risk factors for early-onset group B streptococcal sepsis: estimation of odds ratios by critical literature review. Pediatrics 1999;103(6):e77.
[16] El Aila NA, Tency I, Claeys G, Saerens B, Cools P, Verstraelen H, et al. Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infect Dis 2010;10:285.
[17] American College of O, Gynecologists. ACOG committee opinion: number 279, December 2002. Prevention of early-onset group B streptococcal disease in newborns. Obstet Gynecol 2002;100(6):1405–12.
[18] Oddie S, Embleton ND. Risk factors for early onset neonatal group B streptococcal sepsis: case-control study. BMJ 2002;325(7359):308.
[19] Wood EG, Dillon Jr. HC. A prospective study of group B streptococcal bacteriuria in pregnancy. Am J Obstet Gynecol 1981;140(5):515–20.
[20] Regan JA, Klebanoff MA, Nugent RP, Eschenbach DA, Blackwelder WC, Lou Y, et al. Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group. Am J Obstet Gynecol 1996;174(4):1354–60.
[21] Feikin DR, Thorsen P, Zywicki S, Arpi M, Westergaard JG, Schuchat A. Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women. Am J Obstet Gynecol 2001;184(3):427–33.
[22] Goodman JR, Berg RL, Gribble RK, Meier PR, Fee SC, Mitchell PD. Longitudinal study of group B streptococcus carriage in pregnancy. Infect Dis Obstet Gynecol 1997;5(3):237–43.
[23] Main EK, Slagle T. Prevention of early-onset invasive neonatal group B streptococcal disease in a private hospital setting: the superiority of culture-based protocols. Am J Obstet Gynecol 2000;182(6):1344–54.
[24] Gilson GJ, Christensen F, Romero H, Bekes K, Silva L, Qualls CR. Prevention of group B streptococcus early-onset neonatal sepsis: comparison of the Center for Disease Control and prevention screening-based protocol to a risk-based protocol in infants at greater than 37 weeks' gestation. J Perinatol 2000;20(8 Pt. (1)):491–5.
[25] Keski-Nisula L, Kyynarainen HR, Karkkainen U, Karhukorpi J, Heinonen S, Pekkanen J. Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth. Acta Paediatr 2013;102(5):480–5.
[26] Alfa MJ, Sepehri S, De Gagne P, Helawa M, Sandhu G, Harding GK. Real-time PCR assay provides reliable assessment of intrapartum carriage of group B Streptococcus. J Clin Microbiol 2010;48(9):3095–9.
[27] Daniels JP, Gray J, Pattison HM, Gray R, Hills RK, Khan KS, et al. Intrapartum tests for group B streptococcus: accuracy and acceptability of screening. BJOG 2011;118(2):257–65.
[28] Rajagopal L. Understanding the regulation of Group B Streptococcal virulence factors. Future Microbiol 2009;4(2):201–21.
[29] Maisey HC, Doran KS, Nizet V. Recent advances in understanding the molecular basis of group B Streptococcus virulence. Expert Rev Mol Med 2008;10:e27.
[30] Baker CJ, Kasper DL. Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection. N Engl J Med 1976;294(14):753–6.
[31] Lin FY, Weisman LE, Azimi PH, Philips 3rd JB, Clark P, Regan J, et al. Level of maternal IgG anti-group B streptococcus type III antibody correlated with protection of neonates against early-onset disease caused by this pathogen. J Infect Dis 2004;190(5):928–34.

Corrigendum to Paper II

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Corrigendum

Corrigendum to "Risk-based screening combined with a PCR-based test for group B streptococci diminishes the use of antibiotics in laboring women" [Eur J Obstet Gynecol Reprod Biol 215 (August) (2017) 188–192]

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The authors regret in finding 2 minor errors in Table 2. The errors do not affect the results or the conclusion.
Here is the published table, and the errors are marked with yellow and corrected in red:
The authors would like to apologise for any inconvenience caused.
Table 2 Intrapartum GBS prevalence by vaginal culture and PCR test for each of the four risk factors (N = 902).

	Intrapartum vaginal culture	**Intrapartum PCR test**
Risk factors	**Positive rate % (No)**	**Positive rate % (No)**
EOGBS in prior delivery	0% (0/1)	0% (0/1)
GBS bacteriuria	60% (18/30)	80% (24/30)
Fever (≥38.0°C)	0% (0/1) **9**	0% (0/1) **9**
ROM ≥18 hours	10% (7/68)	12% (8/68)
Total with risk factors	23% (25/83) **108**	30% (32/108)
No risk factors	10% (79/794)	10% (78/794)
Total	12% (**104/902**)	12% (**110/902**)

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Paper III

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Full length article

Number of colony forming units in urine at 35–37 weeks' gestation as predictor of the vaginal load of Group B *Streptococci* at birth

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ABSTRACT

Objective: To evaluate GBS colony numbers in the urine at 35–37 weeks' gestation to predict the load of GBS-colonization of the vagina at birth.
Study design: In this prospective observational study, we included 902 unselected pregnant women. Exposure was GBS colony forming units (CFU) per mL urine at 35–37 weeks' gestation. Outcome was vaginal GBS colonization at birth as assessed by a semi-quantitative culture of a vaginal swab sample (negative, +1, +2, +3).
Results: Bacteriuria with GBS at 35–37 weeks' gestation performed with a sensitivity of 30% concerning any degree of vaginal GBS colonization at birth (31 of 104 cases); 19% for light (+1), 17% for medium (+2), and 52% for high load (+3) vaginal GBS colonization. The colony count in case of GBS bacteriuria at 35–37 weeks' gestation performed with positive predictive values of 35% for $<10^4$ CFU/mL, 70% for 10^4 CFU/mL, and 67% for $>10^4$ CFU/mL.
Conclusion: Even though the urinary GBS CFU at 35–37 weeks' gestation is strongly associated with a high load of vaginal GBS colonization intrapartum, it may not perform satisfactorily as a standalone-screening marker for risk of early-onset GBS disease.

Introduction

Bacteriuria with Group B *Streptococci* (GBS) during pregnancy may constitute a marker for a high load of genital tract colonization with GBS, and it constitutes a risk factor for early-onset GBS disease (EOGBS) [1–3]. Therefore, identification of GBS in urine might be a useful screening tool for identification of women at risk of transferring GBS to their infant at birth.

We know only little about the association between the antepartum GBS-urinary colony count and the load of GBS in the vagina intrapartum. If this association is strong, quantification of GBS in the urine may constitute an effective tool for assessing the risk of EOGBS and minimizing the number of women who should be screened for vaginal GBS colonization intrapartum by a polymerase chain reaction (PCR) test. If the quantitative association is very strong, the antepartum GBS screening of urine might even replace the intrapartum vaginal PCR-GBS test, which is usually not quantitative [4,5] and is perhaps too sensitive, thus finding small and clinical insignificant numbers of GBS in the vagina.

The aims of this study were to assess the performance of screening for GBS in urine at 35–37 weeks' gestation to identify women with vaginal GBS colonization during labor, and furthermore, to evaluate whether the urinary GBS colony-count provides further information regarding the load of GBS in the vagina.

Material and methods

Study design

In this prospective observational study, we included 902 pregnant women at a gestational age of 29 weeks [4]. Detailed information on oral antibiotic use during pregnancy was obtained from the registered data in medical records and delivered from the

Abbreviations: CI, confidence interval; CFU/mL, colony-forming units per mL; EOGBS, early onset of neonatal group B streptococcal disease; GBS, Group B *Streptococci*; $GBS_{urine35-37\ weeks}$, GBS in urine at 35–37 weeks' gestation; $GBS_{vaginabirth}$, GBS in vagina at labor; $GBS_{vagina35-37weeks}$, GBS in vagina at 35–37 weeks' gestation; IAP, intrapartum antibiotic prophylaxis; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value.

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Danish Medical Agency's Register of non-hospitalized patient use, which included records on all drug prescriptions filed at any Danish pharmacy [6].

Inclusion criteria

- Pregnant women attending the prenatal Clinic at Lillebaelt Hospital, Kolding, Denmark. All pregnant women in the catchment area of Lillebaelt Hospital give birth at this clinic, as there are no private or other public alternatives. Only very complicated pregnancies like extreme preterm births are referred to University Hospitals.

Exclusion criteria

- Women treated with antibiotics after 35 weeks' gestation.
- Preterm labor (before 37^{+0} weeks gestation).
- Age under 18 years.
- Women with a communication barrier such as language or mental health conditions.

Collection and culture of specimens

At 35–37 weeks' gestation, each participant delivered a Clean Catch Midstream urine specimen for conventional quantitative culture during the planned visit to the midwife outpatient clinic. Urine samples were seeded on a 5% blood agar plate at Department of Clinical Microbiology, Vejle Hospital, Denmark and read after incubation at 35 °C for 24 or 48 h depending on the initial growth of bacteria. GBS was identified as described below and the bacteriuria classified according to the number of colony-forming units per mL (CFU/mL). Low colony counts refer to $<10^4$ CFU/mL, and high colony counts refer to $\geq 10^4$ CFU/mL.

A vaginal ESwab sample was obtained from each participant by self-administered sample collection at 35–37 weeks' gestation and during labor by the midwife. Samples were cultured for GBS at the time of arrival to the laboratory; if received after 8 PM, they were kept at 4 °C until the next morning. Direct plating without prior enrichment of the specimen in a culture broth was carried out by streaking the ESwab specimen on a selective Granada agar plate. The vaginal swabs from the same patient were seeded on split sides of the same Granada agar plate (BioMérieux®, Spain). The Granada agar plates were incubated immediately after the seeding in a 35 °C in CO_2-containing atmosphere. All samples were analyzed at the Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark. All GBS-like colonies (identified by their orange color on Granada agar plates) were routinely confirmed as *Streptococcus agalactiae* using the Microflex LT ™ MALDI-TOF system (Bruker Daltonik, Germany). Growth was classified semi-quantitatively as plates having only growth of a few GBS colonies (1 +), some (2 +) or many (3 +). Twenty-seven culture tests were mistakenly not recorded with a semi-quantitative assessment result: 21 were urine culture negative at 35–37 weeks' gestation, 6 were urine culture positive, of which 2 had a colony count $<10^4$ CFU/mL and 4 had a colony count = 10^4 CFU/mL.

Ethics

The study was approved by the Regional Scientific Ethical Committees for Southern Denmark (S-20130089) and the Danish Data Protection Agency (2008-58-0035). All participants provided written informed consent.

Statistics

STATA Statistics/Data Analysis software (version 14; StataCorp LP) was used for the statistical analysis. The results of the categorical variables were expressed as percentages, with a 95% corresponding confidence interval (CI). Differences in proportions were compared using either the chi-square test or Fisher's exact test. P values below 0.05 were considered statistically significant. Odds ratios are used to assess associations. Sensitivity, specificity, positive predictive value (PPV) and negative predicative value (NPV) of both antenatal vaginal cultures and urine cultures were calculated to evaluate their accuracy in predicting GBS colonization at the time of delivery.

Results

Within the population of 902 unselected pregnant women, the rate of GBS-uria at 35–37 weeks' gestation ($GBS_{urine35-37weeks}$) was 5.9% (53/902), whereas the rate of GBS in vagina at birth

Table 1
Demographic characteristics of the participants.

Number of participants = 902	$GBS_{urine\ 35-37\ weeks}$						
	Positive (N = 53)		Negative(N = 849)		OR	95% CI	P-value
Maternal characteristics	Number	%	Number	%			
Age of the mother							
Under 25	3	5.7	84	9.9	0.55	0.17–1.79	0.32
25–34	41	77.4	573	67.5	1.65	0.85–3.18	0.14
35 and above	9	17	192	22.6	0.7	0.34–1.46	0.34
Parity							
1	22	41.5	385	45.4	0.81	0.49–1.50	0.54
2	26	49.1	420	49.5	0.99	0.57–1.73	0.95
3 or more	5	9.4	44	5.2	1.91	0.72–5.03	0.19
Body mass index							
Under 24.9	33	62.3	544	64.1	0.93	0.52–1.64	0.79
25–29.9	13	24.5	194	22.9	1.1	0.58–2.09	0.78
30- or more	7	13.2	111	13.1	1.01	0.45–2.30	0.98
Tobacco							
Never smoking	48	90.6	779	91.8	0.86	0.33–2.24	0.76
Stopped in pregnancy	3	5.7	24	2.8	2.06	0.60–7.08	0.25
Smoke ≤ 10 cigarettes	1	1.9	34	4	0.46	0.06–3.43	0.45
Smoke > 10 cigarettes	1	1.9	12	1.4	1.34	0.17–10.5	0.78

($GBS_{vaginabirth}$) was 11.5% (104/902). Those with and without $GBS_{urine35–37weeks}$ did not differ concerning age of the mother, parity, body mass index, and tobacco use (Table 1).

The sensitivity of $GBS_{urine35–37weeks}$ to predict any degree of $GBS_{vaginabirth}$ was 30% (31/104), and the corresponding positive predictive value (PPV) was 59% (31/53). The negative predictive value (NPV) was 91% (776/849) (Table 2).

The GBS colony count in the case of $GBS_{urine35–37weeks}$ was further informative. Thus, 6/17 (35%) with $GBS_{urine35–37weeks}$ was $<10^4$ CFU/mL had $GBS_{vaginabirth}$ (Table 3). The corresponding figures for 10^4 CFU/mL were 19/27 (70%), and for $>10^4$ CFU/mL 6/9 (67%). A high load (+3) $GBS_{vaginabirth}$ was predicted with a sensitivity of 52% (17/33) by $GBS_{urine35–37weeks}$ (Table 3).

$GBS_{vagina35–37weeks}$ predicted $GBS_{vaginabirth}$ with a sensitivity of 64% (67 of 104 cases; data not shown). Furthermore, $GBS_{urine35-37weeks}$ predicted $GBS_{vagina35–37weeks}$ with a sensitivity of 48% (48/100) and a specificity of 99% (797/802).

Discussion

In a cohort of 902 unselected pregnant women, we found that $GBS_{urine35–37weeks}$ predicted any degree of $GBS_{vaginabirth}$ with a sensitivity of only 30% and a high load (+3) $GBS_{vaginabirth}$ with a sensitivity of 52%. However, the corresponding negative predictive value (NPV) of $GBS_{urine35–37weeks}$ was 91%.

The strength of our study is the prospective cohort design with a quantitative assessment of possible GBS bacteriuria antepartum for all women combined with a semi-quantitative assessment of GBS in the vagina at birth. Furthermore, a relatively high number of unselected pregnant women participated in the study. It is a minor limitation that the assessments of 27 intrapartum vaginal swabs in the study were not assessed semi-quantitatively.

The relatively low sensitivity of $GBS_{urine35–37weeks}$ in predicting vaginal $GBS_{vaginabirth}$ can be explained by at least two factors. Firstly, $GBS_{urine35–37weeks}$ predicts only 48% ($GBS_{vagina35–37weeks}$), i.e. only half the women with GBS in the vagina have GBS in the urine on the day of sampling. Secondly, even $GBS_{vagina35–37weeks}$ predicted only 64% of $GBS_{vaginabirth}$, i.e. at least 36% changed their vaginal GBS status within these few weeks, a substantial change which has also been noted by others [7].

The prevalence of GBS_{urine} was comparable to that reported in other studies [8–11]. Our findings confirm the findings of Perez-Moreno et al. in their prospective study on GBS_{urine} during pregnancy as a risk factor for maternal intrapartum colonization. They found a sensitivity of 41%, a specificity of 95%, a PPV of 59%, and NPV of 95% of $GBS_{urine35–37weeks}$ in predicting $GBS_{vaginabirth}$ [12]. The authors concluded that GBS bacteriuria is a risk factor for intrapartum colonization, irrespective of urinary GBS concentration or colonization status at late gestation [12]. These findings agree with our results. However, Perez-Moreno et al. did not classify the growth in $GBS_{vaginabirth}$ semi-quantitatively, therefore missing the added information in detecting a high level of vaginal colonization with GBS at birth.

Several guidelines recommend intrapartum antibiotics prophylaxis in case of significant GBS_{urine} during pregnancy, as this condition is regarded as a sign of high load of genital tract colonization [13–15]. However, the guidelines do not agree on the definition of significant GBS_{urine}. Thus, RCOG and a recent European guideline define significant GBS_{urine} as any degree of GBS_{urine} [13,14], whereas CDC uses a definition of $\geq 10^4$ CFU/mL [16].

Our findings do not support the use of$GBS_{urine35–37weeks}$ as an isolated risk marker for EOGBS, and as a result, for identification of laboring women who should be offered prophylactic penicillin at birth. However, $GBS_{urine35-37weeks}$ seems to perform better than other risk markers like gestational age at birth <37 weeks [17], duration of rupture of membranes >18 h [18], temperature >38.0 °C [19–21], or delivery of a previous infant with GBS-specific EOS [22,23]. Therefore, EOGBS-prevention strategies offering an intrapartum GBS test only to mothers at risk of having a new-born acquiring EOGBS could benefit from the inclusion of this risk marker. This would probably substantially increase the sensitivity of a selective intrapartum GBS screening strategy.

Rapid tests for vaginal colonization by GBS are based on PCR. They do not offer quantitative results and they may be too sensitive, thus identifying low loads of vaginal GBS colonization, which is likely to be associated with a low risk of EOGBS. A number of studies therefore suggest through indirect observation that low level carriage of GBS is associated with a reduced transmission rate compared to that in patients with higher bacterial burdens [24,25].

We conclude that $GBS_{urine35–37weeks}$ is associated with a high load of $GBS_{vaginabirth}$; however, $GBS_{urine35–37weeks}$ does not perform satisfactorily as a standalone screening marker for risk of EOGBS.

Table 2
$GBS_{urine35–37weeks}$ for the prediction of $GBS_{vagina\ birth}$.

$GBS_{urine35–37weeks}$	$GBS_{vagina\ birth}$	95% CI
Sensitivity	30% (31/104)	21.23% to 39.57%
Specificity	97% (776/798)	95.86% to 98.26%
PPV	59% (31/53)	45.91% to 70.05%
NPV	91% (776/849)	90.36% to 92.34%

CI = confidence interval; PPV = positive predictive value; NPV = negative predictive value; # Reference standard: Vaginal GBS colonization rate (N 104; 12%).

Table 3
GBS colony-counts in $GBS_{urine35–37weeks}$ compared to $GBS_{vagina\ birth}$ semi-quantitative culture[a] assessment.

$GBS_{urine35–37weeks}$	$GBS_{vagina\ birth}$					
	Negative	NA[b]	1+	2+	3+	Total
Negative	776	21	17	19	16	849
Colony count $< 10^4$ CFU/mL	11	2	2	1	1	17
Colony count = 10^4 CFU/mL	8	4	2	3	10	27
Colony count $> 10^4$ CFU/mL	3	0	0	0	6	9
Total	798	27	21	23	33	**902**

[a] Semi quantitative assessment of vaginal GBS colonies: 1+: few; 2+: some; 3+: many.
[b] NA = 27 were not assessed semi-quantitatively.

Contribution to authorship

All authors have

1. Made substantial contributions to conception, design, and interpretation of data.
2. Drafted the article and revised it critically for important intellectual content.
3. Given final approval of the version to be published.
4. Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest

All the authors declare that there is no conflict of interest.

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References

[1] Persson K, Christensen KK, Christensen P, Forsgren A, Jorgensen C, Persson PH. Asymptomatic bacteriuria during pregnancy with special reference to group B *Streptococci*. Scand J Infect Dis 1985;17(2):195–9.

[2] Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. Eur J Clin Invest 2008;38(Suppl 27):50–7.

[3] Regan JA, Klebanoff MA, Nugent RP, Eschenbach DA, Blackwelder WC, Lou Y, et al. Colonization with Group B *Streptococci* in pregnancy and adverse outcome. VIP Study Group. Am J Obstet Gynecol 1996;174(4):1354–60.

[4] Khalil MR, Uldbjerg N, Thorsen PB, Moller JK. Intrapartum PCR assay versus antepartum culture for assessment of vaginal carriage of Group B *Streptococci* in a Danish cohort at birth. PLoS One 2017;12(7):e0180262.

[5] Khalil MR, Uldbjerg N, Thorsen PB, Henriksen B, Moller JK. Risk-based screening combined with a PCR-based test for Group B *Streptococci* diminishes the use of antibiotics in laboring women. Eur J Obstet Gynecol Reprod Biol 2017;215:188–92.

[6] Schmidt MS, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. Clin Epidemiol 2015;7:449–90.

[7] Hansen SM, Uldbjerg N, Kilian M, Sorensen UB. Dynamics of Streptococcus agalactiae colonization in women during and after pregnancy and in their infants. J Clin Microbiol 2004;42(1):83–9.

[8] Millar LK, Cox SM. Urinary tract infections complicating pregnancy. Infect Dis Clin North Am 1997;11(1):13–26.

[9] Smaill F, Vazquez JC. Antibiotics for asymptomatic bacteriuria in pregnancy. Cochrane Database Syst Rev 2007;2:D000490.

[10] Mignini L, Carroli G, Abalos E, Widmer M, Amigot S, Nardin JM, et al. Accuracy of diagnostic tests to detect asymptomatic bacteriuria during pregnancy. Obstet Gynecol 2009;113(2 Pt 1):346–52.

[11] Sheiner E, Mazor-Drey E, Levy A. Asymptomatic bacteriuria during pregnancy. J Matern Fetal Neonatal Med 2009;22(5):423–7.

[12] Perez-Moreno MO, Picó-Plana E, Grande-Armas J, Centelles-Serrano MJ, Arasa-Subero M, Ochoa NC, et al. Group B streptococcal bacteriuria during pregnancy as a risk factor for maternal intrapartum colonization: a prospective cohort study. J Med Microbiol 2017;66(4):454–60.

[13] Abstracts of the RCOG (Royal College of Obstetricians and Gynaecologists) 10th International Scientific Congress. June 5–8, 2012. Kuching, Sarawak, Malaysia. BJOG, 2012. 119 Suppl 1: p. 2–250.

[14] Di Renzo GC, Melin P, Berardi A, Blennow M, Carbonell-Estrany X, Donzelli GP, et al. Intrapartum GBS screening and antibiotic prophylaxis: a European Consensus Conference. J Matern Fetal Neonatal Med 2015;28(7):766–82.

[15] Verani JR, Schrag SJ. Group B streptococcal disease in infants: progress in prevention and continued challenges. Clin Perinatol 2010;37(2):375–92.

[16] Verani JR, McGee L, Schrag SJ. Division of bacterial diseases, national center for immunization, respiratory diseases, centers for disease control and prevention., prevention of perinatal group B streptococcal disease–revised guidelines from CDC. MMWR Recomm Rep 2010;10:1–36.

[17] Weston EJ, Pondo T, Lewis MM, Martell-Cleary P, Morin C, Jewell B, et al. The burden of invasive early-onset neonatal sepsis in the United States, 2005–2008. Pediatr Infect Dis J 2011;30(11):937–41.

[18] Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. N Engl J Med 1986;314(26):1665–9.

[19] Schuchat A, Zywicki SS, Dinsmoor MJ, Mercer B, Romaguera J, O'Sullivan MJ, et al. Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. Pediatrics 2000;105(1 Pt 1):21–6.

[20] Oddie S, Embleton ND. Risk factors for early onset neonatal group B streptococcal sepsis: case-control study. BMJ 2002;325(7359):308.

[21] Heath PT, Balfour G, Weisner AM, Efstratiou A, Lamagni TL, Tighe H, et al. Group B streptococcal disease in UK and Irish infants younger than 90 days. Lancet 2004;363(9405):292–4.

[22] Carstensen H, Christensen KK, Grennert L, Persson K, Polberger S. Early-onset neonatal group B streptococcal septicaemia in siblings. J Infect 1988;17(3):201–4.

[23] Schrag SJ, Zell ER, Lynfield R, Roome A, Arnold KE, Craig AS, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. N Engl J Med 2002;347(4):233–9.

[24] McNanley AR, Glantz JC, Hardy DJ, Vicino D. The effect of intrapartum penicillin on vaginal group B streptococcus colony counts. Am J Obstet Gynecol 2007197(6) 583 e1-e4.

[25] Facchinetti F, Piccinini F, Mordini B, Volpe A. Chlorhexidine vaginal flushings versus systemic ampicillin in the prevention of vertical transmission of neonatal group B streptococcus, at term. J Matern Fetal Neonatal Med 2002;11(2):84–8.

Paper IV

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Group B streptococci cultured in urine during pregnancy associated with preterm delivery: a selection problem?

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ORIGINAL ARTICLE

Group B streptococci cultured in urine during pregnancy associated with preterm delivery: a selection problem?

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ABSTRACT

Objective: To investigate an association between Group B streptococci (GBS) in urine culture during pregnancy and preterm delivery.

Methods: A population-based cohort consisted of all the pregnant women ($n = 36,097$) from the catchment area of Lillebaelt Hospital, Denmark, during the period January 2002 –December 2012. The cohort of 34,285 singleton pregnancies used in this study was divided into three groups. Group I ($N = 249$) included women whose urine culture was positive for GBS; group II ($N = 5765$) included women whose urine culture was negative for GBS; and group III ($N = 28\ 271$) included women whose urine had not been cultured during pregnancy. Primary outcome was preterm delivery before 37 weeks' gestation (PTD).

Results: We did not find an association between PTD and GBS bacteriuria in the cultured groups (odds ratios (OR) = 0.89; 95% CI: 0.5–1.4) (Table 1). After controlling for potential confounders, the PTD remained not associated with GBS bacteriuria (adjusted OR = 0.99; 95% CI: 0.6–1.6). Combined, the cultured groups (I and II) were associated with a statistically significant higher risk for PTD, when compared with the group with no urine specimens taken for culture (OR = 1.96; 95% CI: 1.8–2.2 and adjusted or 1.80; 95% CI 1.6–2.0). The cultured group of women differed considerably from the group of women with no urine specimens taken for culture on the vast majority of variables examined.

Conclusions: No association between asymptomatic GBS bacteriuria and preterm delivery among women with singleton pregnancy and urine specimens cultured during pregnancy was found. Previous suggestions of such association may have been compromised by a selection problem for testing due to a high-risk profile of pregnancy complications in pregnant women selected for urine culture.

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Asymptomatic bacteriuria; group B streptococcus; preterm delivery

Introduction

Group B streptococci (GBS) are considered a marker for genital tract colonization, which in some studies have been a risk factor for preterm prelabor rupture of the membranes and preterm delivery [1–4]. Treatment and follow-up to prevent recolonization in pregnant women with GBS in the urine has been reported to reduce the incidence of preterm delivery (PTD, delivery before 37 weeks' gestation) [3]; however, this presumed reduction could not be confirmed in the Cochrane review and other studies [5–7]. Antibiotic treatment of pregnant women with GBS-positive urine culture has also been used in some antenatal clinics in order to reduce the risk of chorioamnionitis [8], pyelonephritis, and low-birth weight [9].

In Denmark, a prenatal dipstick urine-analysis is carried out routinely as standard procedure by the general practitioner, and specimens are collected according to national guidelines and performed on medical indications and as general screening [10]. A urine specimen will be submitted for culture, if the urine dipstick test-result is positive for the leukocyte esterase and/or nitrite test. However, several microorganisms including GBS are nitrite negative [11], and most patients with asymptomatic bacteriuria do not exhibit an inflammatory reaction with a leukocyte response in the urine [12–14], rendering the dipstick used in antenatal care testing inadequate in detecting GBS [11,15]. Urine specimens are also submitted for culture, if women have a history of previous PTD or GBS infection in a prior pregnancy, or they exhibit

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medical indications like symptoms of urinary tract infection, or if a variety of other maternal and obstetrical reasons are present. If GBS is found, the woman is treated with antibiotics regardless of colony counts, and usually penicillin is prescribed.

GBS bacteriuria is only occasionally associated with urinary tract infection [16]. Antibiotic treatment may not eliminate GBS from the urinary tract, the vagina, or the rectum, and subsequent recolonization is common [17] contributing to recurrent presence of GBS in urine [7]. Several authors have even postulated that antibiotic administration may alter vaginal flora, allowing heavy growth of other potentially pathogenic organisms in the upper genital tract, which may lead to PTD [6,8].

The objective of this cohort study was to investigate a possible association between GBS cultured in urine during pregnancy and PTD in a Danish cohort of pregnant women.

Materials and methods

From a population-based cohort consisting of all pregnant women ($n = 36\ 097$) from the catchment area of Lillebaelt Hospital, Denmark, during the period January 2002–December 2012 (11 years), 34,285 deliveries of singleton pregnancies were included in this study. Of those, 17.5% (6014/34,285) had one or more urine culture analysed at the Department of Clinical Microbiology, Lillebaelt Hospital, Vejle, Denmark. Maternal, obstetrical, and neonatal data were obtained from the Hospital Information System at Lillebaelt Hospital, which contains data on all hospitalized patients, and linkage of information could be performed using the unique Danish Personal Identification number (CPR). Among others, the following data were extracted: maternal age at delivery, parity, maternal body mass index (BMI), smoking habits, rupture of membranes, birth weight, mode of delivery, past reproductive career, medical diseases, previous cervical cone biopsy(ies), and previous preterm delivery(ies).

National data on prescription of antibiotics to outpatients were extracted from the Danish National Prescription Registry (DNPR) [18], which includes records on all drugs filed at the pharmacy. Antibiotic treatment administered in the hospitals was obtained by reviewing the patient's medical record. Results from all microbiological examinations were extracted from the Laboratory Information System (MADS) at Department of Clinical Microbiology serving the hospitals and all general practitioners in the catchment area of Lillebaelt Hospital, Denmark. Women with one or more positive bacterial urine specimens for GBS were defined as GBS-positive.

The study-population with in- and exclusion criteria is described in Figure 1. Data containing information on among others gestational age at delivery on all births in Denmark were merged from The Danish National Health Service Register [19].

The cohort used in this study was divided into three groups. Group I included women with GBS-positive urine specimens, group II included women whose urine culture was negative for GBS, and group III comprised women without urine specimens submitted for culture during pregnancy (Figure 1).

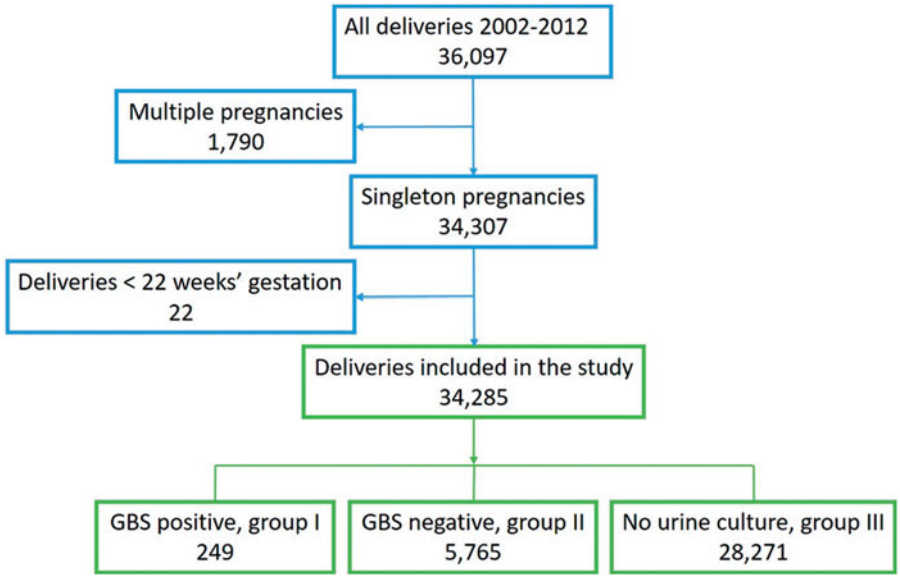

Figure 1. Flowchart describing the cohort.

Microbiological cultures

Urine specimens were collected according to the procedure for midstream clean-catch urine specimens with minimal risk of contamination; women were instructed to collect midstream urine of the urine voided after local disinfection of the meatus and the adjacent mucosa with cotton balls with water while spreading the labia during urinating. If the transportation time to the laboratory exceeded 2 h, the specimens were stored in refrigerators. In brief, all urine specimens were handled throughout the period as follows: 1 μL were streaked on 5% Danish blood agar (DBA) for quantitative evaluation. In case of beta-haemolytic colonies (≥1000 CFU/mL), a representative colony was purified and identified according to conventional laboratory procedures involving a CAMP test and a commercial Latex Agglutination Test for differentiation of streptococci Lancefield groups A, B, C, F, and G. The BioMérieux's chromID CPS agar plate replaced the CAMP-test from 2009 and onwards. From 2011 all GBS-like colonies were routinely confirmed as Streptococcus agalactiae (GBS) by identification using the Microflex LT^{TM} MALDI-TOF system (Bruker, Daltonik, Germany).

Statistics

For statistical analyses Stata Statistics/Data analysis software (version 14; Stata Corp LP, College Station, TX, USA) was used. The primary outcome was preterm delivery before 37 weeks' gestation. Secondary outcome was urine culture-status; Table 1 for GBS positive versus GBS negative and Table 2 for cultured versus not cultured. Statistical analyses included comparisons between groups presented as dichotome and categorical variables using univariate logistic regression [20] reported as odds ratios (ORs) and chi-square tests (trend analyses) reported as *p*-values on secondary outcome (Tables 1 and 2). Binary multiple logistic regression analysis was performed on primary outcome and including *á priori* defined variables as possible confounders in the model (Table 3); the predefined confounders included age, BMI, parity, prior PTD, prior cervical cone biopsy, hypertension, preeclampsia, diabetes type 1, gestational diabetes, tobacco use, inflammatory bowel disease, cervix insufficiency, and early bleeding. *p* values <.05 were considered statistically significant.

Ethics

The study was approved by the Regional Scientific Ethical Committees for Southern Denmark (S-20130089) and the Danish Data Protection Agency (2008-58-0035).

Results

The cohort is described in Figure 1. Among 34 285 singletons, 6014 had a urine culture and among these 249 (4.9%) were colonized by GBS. The overall rate of singleton preterm delivery (PTD) was 5.8% (1978/34 285).

The demographic characteristics of GBS-positive (GI) versus GBS-negative (GII) in the cultured group of women are shown in Table 1, and the corresponding results of cultured women (GI/GII) versus the uncultured group (GIII) are shown in Table 2. All are described further.

Maternal characteristics

There were almost no statistically significant differences in comparisons between GI versus GII, while there were statistically significant differences in both Age and BMI between GI/GII and GIII (GI/GII versus GIII, $p < .001$ for trend, respectively).

Obstetric history

Few and less prominent statistically significant differences were found between GI and GII, while there were statistically significant differences in all variables between GI/GII and GIII (including parity, prior PTD, abortion spontaneous >4, and prior cervical cone biopsy).

Medical outcomes

For this category the differences between the groups were weaker; however, the pattern from above was repeated with more statistically significant differences in variables when comparing GI/GII with GIII than, when groups GI and GII were compared. However, the findings were challenged by relative low numbers, when comparing groups GI and GII; large span confidence interval, e.g. diabetes type 1 with OR of 7.77 and 95% CI 1.5–38.7.

Infections

There were statistically significant differences in urinary tract infection (UTI) for both comparisons (GI versus GII and GI/GII versus GIII). This was expected, as group GI with a positive GBS urine culture would be more likely to be diagnosed with UTI, when compared with

Table 1. Characteristics of the GBS-positive group (GI) and GBS-negative group (GII), $N = 6014$.

GI versus GII	GI $N = 249$		GII $N = 5765$					
GBS status	*N*	%	*N*	%	OR	95% CI	*p* value	Trend *p* value
Maternal characteristics								
Age of mother (years)								
Under 25	31	12.5	985	17.1	Ref			.604
25–34	185	74.3	3855	66.9	*1.53*	1.0–2.3	.033	
35–40	27	10.8	801	13.9	1.07	0.6–1.8	.798	
Above 40	6	2.4	124	2.2	1.54	0.6–3.8	.346	
BMI								
Under 18.5	16	6.7	254	4.8	1.44	0.8–2.5	.184	**.029**
18.5–24.9	127	53.1	2980	56.6	Ref			
25–29.9	63	26.4	1189	22.6	1.24	0.9–1.7	.166	
30–34.9	18	7.5	544	10.3	0.76	0.5–1.3	.280	
35–39.9	10	4.1	206	3.9	1.10	0.6–2.1	.776	
40–44.9	2	0.8	68	1.3	0.67	0.2–2.8	.579	
45 or more	3	1.3	26	0.5	2.51	0.8–8.4	.134	
Status unknown	10		498					
Obstetric history								
Parity								
0	126	50.6	3408	59.1	Ref			**.000**
1	75	30.1	1730	30.1	1.17	0.9–1.6	.285	
2	39	15.6	528	9.2	*2.00*	1.4–2.9	.000	
3	8	3.2	86	1.5	*2.52*	1.2–5.3	.015	
4 or more	1	0.4	13	0.2	2.08	0.3–16.0	.482	
Prior PTD								
0	228		5528		Ref			**.004**
1	12	4.8	129	2.2	2.64	0.9–7.5	.067	
2	8	3.2	93	1.6	2.13	0.9–4.7	.058	
3 or more	1	0.4	15	0.3	1.59	0.2–12.1	.656	
Abortion spontaneous >4	0	0.0	27	0.5	–	–	–	–
Prior cervical cone biopsy								
0	243		5615		Ref			.775
1	6	2.4	143	2.5	1.02	0.5–2.3	.961	
2 or more	0	0.0	7	0.1	–	–	–	
Medical outcomes								
Hypertension	1	0.4	10	0.2	2.32	0.3–18.2	.432	–
Pre-eclampsia	9	3.6	225	3.9	0.92	0.5–1.8	.818	–
Diabetes								
No diabetes	224		5520		Ref			**.000**
Type 1	2	0.8	6	0.1	*7.77*	1.5–38.7	.012	
Type 2	0	0.0	3	0.1	–	–	–	
Gestational diabetes	23	9.2	236	4.1	*2.38*	1.5–3.7	.000	
Tobacco use								
Never smoking	197	86.8	3968	79.3	Ref			**.001**
Stopped in trimester 1	11	4.9	220	4.4	1.01	0.5–1.9	.982	
Stopped in trimester 2	7	3.1	167	3.3	0.84	0.4–1.8	.666	
Smoke =<5 cigarettes	5	2.2	253	5.1	0.40	0.2–1.0	.044	
Smoke 6–10 cigarettes	4	1.8	235	4.7	***0.34***	0.1–0.9	.036	
Smoke 11–20 cigarettes	1	0.4	66	1.3	0.31	0.04–2.2	.240	
Smoke >20 cigarettes	0	0.0	11	0.2	–	–	–	
Amount unknown	2	0.9	82	1.6	0.49	0.1–2.0	.323	
Status unknown	22	8.8	763	13.2	***0.58***	0.4–0.9	.017	
Infections								
Chorioamnionitis	0	0.0	5	0.1	–	–	–	–
Urinary tract infection	33	13.3	342	5.9	*2.42*	1.7–3.6	.000	–
Pyelonephritis	0	0.0	8	0.1	–	–	–	–
Inflammatory bowel disease	4	1.6	30	0.5	*3.12*	1.1–8.9	.034	–
Obstetric outcomes								
Emergency CS	36	14.5	657	11.4	1.31	0.9–1.9	.140	–
Cervix insufficiency	3	1.2	19	0.3	*3.69*	1.1–12.6	.037	–
Early bleeding	9	3.6	100	1.7	*2.12*	1.1–4.3	.033	–
Abruption of placentae	0	0.0	18	0.3	–	–	–	–
Placenta previa	0	0.0	19	0.3	–	–	–	–
Hydronephrosis	1	0.4	9	0.2	2.58	0.3–20.4	.370	–
Threatened preterm delivery	9	3.6	208	3.6	1.00	0.5–2.0	.996	–
Threatened miscarriage	3	1.2	160	2.8	0.43	0.1–1.4	.147	–

GI: cultured GBS-positive; GII: cultured GBS-negative; OR: Odds ratio; Ref: Referent category; CS: cesarean section.
Italic values indicate positive (above 1.00) statistically significant OR, while bold italic values indicate the opposite.
Bold values indicate that the odds of event in GI is statistically significant lower than the odds of event in GII.

Table 2. Characteristics of the cultured group (GI/II) and uncultured group (GIII), $N = 34,285$.

GI + GII versus GIII	GI + GII $N = 6014$		GIII $N = 28,271$					
Cultured versus uncultured	*N*	%	*N*	%	OR	95% CI	*p* value	Trend *p* value
Maternal characteristics								
Age of mother (years)								
Under 25	1016	16.9	3696	13.1	Ref			**.000**
25–34	4040	67.2	19,905	70.4	***0.74***	0.7–0.8	.000	
35–40	828	13.8	4053	14.3	***0.74***	0.7–0.8	.000	
Above 40	130	2.2	617	2.2	***0.77***	0.6–0.9	.010	
BMI								
Under 18.5	270	4.9	854	4.1	*1.33*	1.2–1.6	.000	**.000**
18.5–24.9	3,107	56.4	12,871	60.8	Ref			
25–29.9	1,252	22.7	4597	21.7	*1.13*	1.1–1.2	.001	
30–34.9	562	10.2	1844	8.7	*1.27*	1.2–1.4	.000	
35–39.9	216	3.9	670	3.7	*1.36*	1.2–1.6	.000	
40–44.9	70	1.3	243	1.2	1.21	0.9–1.6	.160	
45 or more	29	0.5	85	0.4	1.49	0.9–2.3	.059	
Status unknown	508		7107					
Obstetric history								
Parity								
0	3534	58.8	18,600	65.8	Ref			**.000**
1	1805	30.0	7518	26.6	*1.26*	1.2–1.4	.000	
2	567	9.4	1868	6.6	*1.60*	1.4–1.8	.000	
3	94	1.6	260	0.9	*1.90*	1.5–2.4	.000	
4 or more	14	0.2	25	0.1	*2.95*	1.5–5.7	.001	
Prior PTD								
0	5756		27,837		Ref			**.000**
1	141	2.3	258	0.9	*1.67*	1.2–2.4	.005	
2	101	1.7	144	0.5	*3.62*	2.7–4.8	.000	
3 or more	16	0.3	32	0.1	*2.39*	1.3–4.4	.005	
Abortion spontaneous >4	27	0.3	75	0.3	*1.70*	1.1–2.6	.000	–
Prior cervical cone biopsy								
0	5858		28,117		Ref			**.000**
1	149	2.5	154	0.5	*4.64*	3.7–5.8	.000	
2 or more	7	0.1	0	0.0	–	–	–	
Medical outcomes								
Hypertension	11	0.2	64	0.2	0.81	0.4–1.5	.513	–
Pre-eclampsia	234	4.1	643	2.3	*1.74*	1.5–2.0	.000	–
Diabetes								
No diabetes	5744		27,819		Ref			**.000**
Type 1	8	0.1	19	0.1	1.98	0.9–4.5	.105	
Type 2	3	0.1	4	0.01	3.53	0.8–15.8	.099	
Gestational diabetes	259	4.3	429	1.5	*2.92*	2.5–3.4	.000	
Tobacco use								
Never smoking	4165	79.7	17,071	84.1	Ref			**.000**
Stopped in trimester 1	231	4.4	631	3.1	*1.50*	1.3–1.8	.000	
Stopped in trimester 2	174	3.3	655	3.2	1.09	0.9–1.3	.328	
Smoke = <5 cigarettes	258	4.9	846	4.2	*1.25*	1.1–1.4	.002	
Smoke 6–10 cigarettes	239	4.6	667	3.3	*1.47*	1.3–1.7	.000	
Smoke 11–20 cigarettes	67	1.3	78	0.4	*3.52*	2.5–4.9	.000	
Smoke >20 cigarettes	11	0.2	38	0.2	1.19	0.6–2.3	.618	
Amount unknown	84	1.6	313	1.5	1.10	0.9–1.4	.443	
Status unknown	785	13.1	7972	28.2	***0.40***	0.4–0.4	.000	
Infections								
Chorioamnionitis	5	0.1	23	0.1	1.02	0.4–2.7	.965	–
Urinary tract infection	375	6.2	411	1.5	*4.51*	3.9–5.2	.000	–
Pyelonephritis	8	0.1	20	0.1	1.88	0.8–4.3	.131	–
Inflammatory bowel disease	34	0.6	155	0.6	1.03	0.7–1.5	.871	–
Obstetric outcomes								
Emergency CS	693	11.5	2643	9.4	*1.26*	1.2–1.4	.000	–
Cervix insufficiency	22	0.4	33	0.1	*1.18*	1.8–5.4	.000	–
Early bleeding	109	1.8	332	1.2	*1.55*	1.3–1.9	.000	–
Abruption of placentae	18	0.3	55	0.2	1.54	0.9–2.6	.112	–
Placenta previa	19	0.3	121	0.4	0.74	0.5–1.2	.218	–
Hydronephrosis	10	0.2	28	0.1	1.68	0.8–3.5	.159	–
Threatened preterm delivery	217	3.6	295	1.0	*3.55*	3.0–4.2	.000	–
Threatened miscarriage	163	2.7	545	1.9	*1.42*	1.2–1.7	.000	–

GI: cultured GBS-positive; GII: cultured GBS-negative; GIII: uncultured; OR: Odds ratio; Ref: Referent category; CS: cesarean section.
Italic values indicate positive (above 1.00) statistically significant OR, while bold italic values indicate the opposite.
Bold values indicate that the odds of event in GI+GII is statistically significant lower than the odds of event in GIII.

group GII with a negative GBS urine culture. Further, groups GI/GII, when compared with group GIII, would be more likely to be diagnosed with UTI due to the fact that they have had a urine culture performed. Inflammatory bowel disease was also different between the groups GI and GII (OR 3.12 95% CI 1.1–8.9).

Obstetric outcomes

Few statistically significant differences were found between GI and GII, while almost all variables were different between GI/GII and GIII (including emergency cesarean section, cervix insufficiency, early bleeding, threatened preterm delivery, and threatened miscarriage).

Table 3. Crude- and Adjusted odds ratios for preterm delivery between groups.

Preterm delivery <37 weeks' gestation	N	%	N	%	Crude OR (95% CI)	Adjusted OR (95% CI)
GI vs. GII	21	8.4	542	9.4	0.89 (0.5–1.4)	0.99 (0.6–1.6)
GI + GII vs. GIII	563	9.4	1,415	5.0	1.96 (1.8–2.2)	1.80 (1.6–2.0)

GI = cultured GBS-positive; GII = cultured GBS-negative; GIII = uncultured; OR = Odds ratio; GI: N = 249; GII: N = 5,765, GI+GII: N = 6,014; GIII: N = 28,271.

Summary

The overall pattern shows considerably fewer statistically significant differences between GI and GII than between GI/GII and GIII. The results are best illustrated in Figure 2 with positive (ORs above 1.00) and negative (ORs below 1.00) statistically significant differences in twenty-five and four variables, when comparing GI/GII and GIII, while the corresponding figures for comparison of GI and GII are nine and two, respectively. Further, analyses for trend on categorical variables also support these findings with highly statistically significant ($p < .001$) differences on all comparisons made in Table 2 (GI/GII versus GIII).

We did not find an association on PTD and GBS bacteriuria between the GBS-positive and GBS-negative in the two groups of women with urine specimens cultured (OR = 0.89; 95% CI: 0.5–1.4) (Table 3). After controlling for potential confounders, the PTD did not remain associated with GBS bacteriuria (adjusted OR = 0.99; 95% CI: 0.6–1.6). However, the two cultured groups were associated with statistically significant higher risk for PTD than the group with no urine specimens taken for culture (OR = 1.96; 95% CI: 1.8–2.2) (Table 3). After controlling for potential confounders, the cultured groups I and II

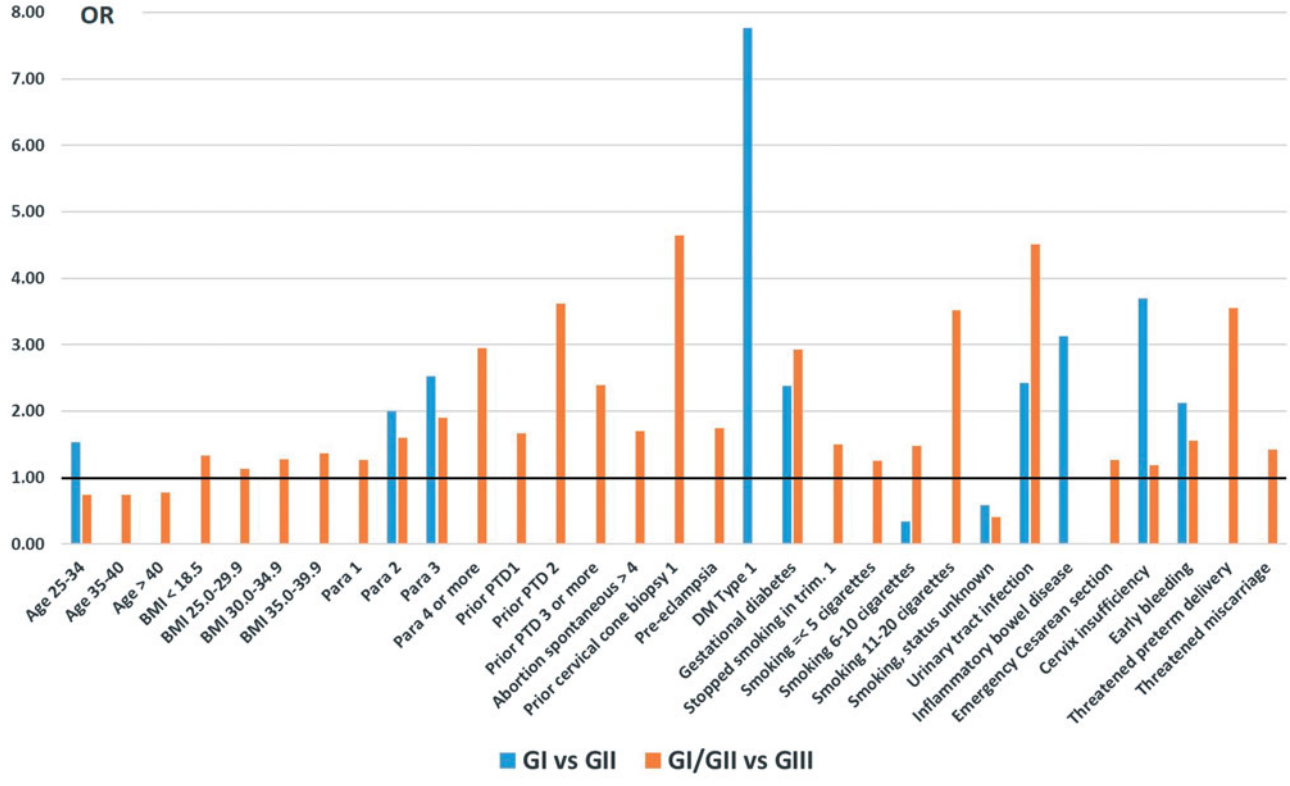

Figure 2. Summary of differences between cultured groups (GI versus GII) and between cultured groups (GI/GII) and uncultured group (GIII) reported as statistically significant odds ratios.

remained associated with PTD, when compared with the group III having no urine specimens cultured during pregnancy (adjusted OR = 1.80; 95% CI: 1.6–2.0) (Table 3).

Among women with positive GBS in urine, no correlation was found to women with early term deliveries between 37 and 39 weeks' gestation, when compared with women delivering in 40 weeks' gestation or later.

Discussion

In a Danish cohort of pregnant women, we investigated the possible association between GBS culture in urine during pregnancy and PTD. No statistically significant association between GBS bacteriuria and PTD was found in the cultured group even after controlling for potential confounders. The two groups of women with urine specimens cultured during pregnancy (GBS-positive and GBS-negative) differed considerably from the group of women with no urine specimens collected for culture during pregnancy on the vast majority of variables examined. The risk of PTD was statistically significant, associated with being a member of the two groups of women with urine specimens cultured for GBS even after controlling for potential confounders and is most likely due to the selection of pregnant women for culturing urine specimens.

All information on exposure and outcome was collected independently of the possible association challenged in this study, meaning that any information bias is considered negligible. However, a systematic selection of the individuals for culture examination of urine specimens is most likely, as general practitioners may be more attentive towards a high-risk profile including urinary tract infections with GBS in pregnant women having complications in current or previous pregnancy(ies). The clear differences in characteristics between the urine cultured group and the group without urine specimens cultured in this study may indicate this behavioural pattern among general practitioners, and this may explain the associations found on PTD in this study and other studies [21,22] showing statistically significant differences, when comparing urine-cultured groups with groups of pregnant women having no urine specimens cultured. Additionally, the higher rates of urine cultures among the high-risk group of women examined for GBS bacteriuria could also be attributed to a tighter prenatal care with more urine specimens submitted to the laboratory leading to a higher GBS detection rate. The higher cesarean-section rate we found in the groups of women with urine specimens cultured is due to the above-mentioned high-risk profile and not due to the GBS bacteriuria per-se.

The strengths of our study include the uniformly organized Danish public healthcare system data [23] allowing for a population-based design compared to other populations; specific strengths include: (a) exact gestational age based on ultrasonographic measures, (b) reliable information on GBS culture results, and (c) the sample size with the ability to control for many potential confounding variables that might affect the outcome of PTD.

Limitations of the study encompass the facts that the dataset included all observations of GBS bacteriuria before 37 weeks' gestation and it was obtained at different gestational ages. Thus, statistical challenges were presented due to the unsystematic collection of urine specimens and the different reasons for why they were obtained. We did not differentiate between colony counts in urine cultures and a possible association with PTD, as many GBS-positive cultures are presumed to be derived from the vagina and rectum [17,24].

Changes in bacterial flora during pregnancy increase the risk for GBS and other bacteria to ascend through the cervix, which may cause inflammation that increases uterine contractility and, thus, may result in PTD [25–28]. Pregnant women are also at increased risk of bacteria ascending to the kidneys causing pyelonephritis, which is associated with PTD [29–31]. GBS is a poor immunogenic pathogen, and in the absence of systemic inflammation is highly unlikely to lead to PTD [32]. One could argue that the use of prophylactic antibiotic treatment removes an expected higher rate of PTD in a GBS-positive group and masking an otherwise clear association between GBS bacteriuria and PTD in the cultured groups. However, the present findings are in line with previous retrospective and prospective studies [6,7]. Anderson and colleagues show in a retrospective cohort study no increased risk of PTD with asymptomatic GBS bacteriuria although they did show an increased risk for PTD with GBS bacteriuria, if additional antibiotics are administered for other urinary tract infections, sexually transmitted infections, or upper respiratory infections [8]. A systematic review of 20 studies [6] demonstrated that PTD was positively associated with GBS colonization at the time of delivery (case-control studies: OR 1.59; 95% CI 1.03–2.44; cross-sectional meta-analyses: OR 1.75; 95% CI 1.43–2.14). However, colonization during pregnancy was not associated with PTD (cohort meta-analyses: OR 1.06; 95% CI 0.95–1.19). In a Cochrane review, the authors show that antibiotic treatment for

asymptomatic bacteriuria has no effect on the reduction of the rates of PTD [9].

Antenatal management of pregnant women with asymptomatic GBS bacteriuria is unclear. GBS colonization is not eliminated by treatment with antibiotics, and subsequent recolonization from the vagina and rectum commonly contributes to the recurrent presence of GBS in urine [17]. Muller and colleagues found no evidence to support the treatment of GBS bacteriuria with low-colony counts for the prevention of pyelonephritis [7]. In another study by Anderson and colleagues a correlation between GBS colony count and severity of chorioamnionitis was shown; however, sample size was inadequate to determine significant differences in the risk of chorioamnionitis, by treatment or lack of treatment, stratified by colony counts [8]. Because of the retrospective nature of the beforementioned studies and analyses, it is not possible to make recommendations regarding the use of antibiotics among unselected pregnant women. However, according to the CDC (Centers for Disease Control and Prevention, USA) guidelines, women with GBS isolated from the urine at any point during pregnancy should be treated; this was revised in 2010 and endorsed by the ACOG (American College of Obstetricians and Gynecologists) and AAP (American Academy of Pediatrics). In asymptomatic women with urinary colony counts $<$100,000 CFU/mL, antimicrobial agents are not recommended before the intrapartum period since such treatment is not effective in eliminating GBS carriage or preventing neonatal disease and can cause adverse consequences [1]. Women with documented GBS bacteriuria should not be rescreened by genital tract culture or urinary culture in the third trimester, as they are presumed to be GBS colonized [33].

Conclusions

No association between asymptomatic GBS bacteriuria and PTD among women with a singleton pregnancy was found. The group of pregnant women with cultured urine specimens seems to represent a population selected for testing due to a high-risk profile of pregnancy complications in general and may represent a selection problem when comparing pregnant women cultured for GBS bacteriuria with women not cultured for possible associations with PTD.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59(RR-10):1–36.

[2] Schrag SJ, Whitney CG, Schuchat A. Neonatal group B streptococcal disease: how infection control teams can contribute to prevention efforts. Infect Control Hosp Epidemiol. 2000;21(7):473–483.

[3] Thomsen AC, Mørup L, Hansen KB. Antibiotic elimination of group-B streptococci in urine in prevention of preterm labour. Lancet. 1987;1(8533):591–593.

[4] Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. Eur J Clin Invest. 2008;38(Suppl 2):50–57.

[5] Romero R, Oyarzun E, Mazor M, et al. Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. Obstet Gynecol. 1989;73(4):576–582.

[6] Valkenburg-van den Berg AW, Sprij AJ, Dekker FW, et al. Association between colonization with Group B Streptococcus and preterm delivery: a systematic review. Acta Obstet Gynecol Scand. 2009;88(9):958–967.

[7] Muller AE, Oostvogel PM, Steegers EA, et al. Morbidity related to maternal group B streptococcal infections. Acta Obstet Gynecol Scand. 2006;85(9):1027–1037.

[8] Anderson BL, Simhan HN, Simons KM, et al. Untreated asymptomatic group B streptococcal bacteriuria early in pregnancy and chorioamnionitis at delivery. Am J Obstet Gynecol. 2007;196(6):524.e1–524.e5.

[9] Smaill FM, Vazquez JC. Antibiotics for asymptomatic bacteriuria in pregnancy. Cochrane Database Syst Rev. 2007;2:CD000490.

[10] Greve VH, Henriksen TB, Johansen HK, et al. Danish Society of Obstetrics and Gynaecology GBS Guideline, in DSOG. 2012. p. 1–13. Availabel from: http://www.dsog.dk/dsog/in-english/.

[11] Semeniuk H, Church D. Evaluation of the leukocyte esterase and nitrite urine dipstick screening tests for detection of bacteriuria in women with suspected uncomplicated urinary tract infections. J Clin Microbiol. 1999;37(9):3051–3052.

[12] Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Phys. 2005;71(6):1153–1162.

[13] Demilie T, Beyene G, Melaku S, et al. Diagnostic accuracy of rapid urine dipstick test to predict urinary tract infection among pregnant women in Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. BMC Res Notes. 2014;7:481.

[14] Jido TA. Urinary tract infections in pregnancy: evaluation of diagnostic framework. Saudi J Kidney Dis Transpl. 2014;25(1):85–90.
[15] Mignini L, Carroli G, Abalos E, et al. Accuracy of diagnostic tests to detect asymptomatic bacteriuria during pregnancy. Obstet Gynecol. 2009;113(2 Pt 1):346–352.
[16] Hassan IA, Onon TS, Weston D, et al. A quantitative descriptive study of the prevalence of carriage (colonisation) of haemolytic streptococci groups A, B, C and G in pregnancy. J Obstet Gynaecol. 2011;31(3):207–209.
[17] McKenzie H, Donnet ML, Howie PW, et al. Risk of preterm delivery in pregnant women with group B streptococcal urinary infections or urinary antibodies to group B streptococcal and E. coli antigens. Br J Obstet Gynaecol. 1994;101(2):107–113.
[18] Kildemoes HW, Sørensen HT, Hallas J, et al. The Danish National Prescription Registry. Scand J Public Health. 2011;39(7 Suppl):38–41.
[19] Olivarius NF, Hollnagel H, Krasnik A, et al. The Danish National Health Service register. A tool for primary health care research. Dan Med Bull. 1997;44(4):449–453.
[20] Kirkwood BR, Sterne JAC. Logistic regression: comparing two or more exposure groups. In: Essential medical statistics. John Wiley and Sons Ltd, Wiley-Blackwell; 2003. p. 189–204. ISBN: 978-0-865-42871-3
[21] Kessous R, Weintraub AY, Sergienko R, et al. Bacteruria with group-B streptococcus: is it a risk factor for adverse pregnancy outcomes? J Matern Fetal Neonatal Med. 2012;25(10):1983–1986.
[22] Petersen KB, Johansen HK, Rosthøj S, et al. Increasing prevalence of group B streptococcal infection among pregnant women. Dan Med J. 2014;61(9):A4908.
[23] Frank L. Epidemiology. When an entire country is a cohort. Science. 2000;287(5462):2398–2399.
[24] Badri MS, Zawaneh S, Cruz AC, et al. Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women. J Infect Dis. 1977;135(2):308–312.
[25] Blencowe H, Cousens S, Chou D, et al. Born too soon: the global epidemiology of 15 million preterm births. Reprod Health. 2013;10(Suppl 1):S2.
[26] Goldenberg RL, Culhane JF, Iams JD, et al. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84.
[27] Parry S, Strauss JF, 3rd. Premature rupture of the fetal membranes. N Engl J Med. 1998;338(10):663–670.
[28] Pararas MV, Skevaki CL, Kafetzis DA. Preterm birth due to maternal infection: causative pathogens and modes of prevention. Eur J Clin Microbiol Infect Dis. 2006;25(9):562–569.
[29] Farkash E, Weintraub AY, Sergienko R, et al. Acute antepartum pyelonephritis in pregnancy: a critical analysis of risk factors and outcomes. Eur J Obstet Gynecol Reprod Biol. 2012;162(1):24–27.
[30] Mazor-Dray E, Levy A, Schlaeffer F, et al. Maternal urinary tract infection: is it independently associated with adverse pregnancy outcome? J Matern Fetal Neonatal Med. 2009;22(2):124–128.
[31] Neal DE Jr. Complicated urinary tract infections. Urol Clin North Am. 2008;35(1):13–22; v.
[32] Luciano AA, Yu H, Jackson LW, et al. Preterm labor and chorioamnionitis are associated with neonatal T cell activation. PLoS One. 2011;6(2):e16698.
[33] Allen VM, Yudin MH, Infectious Diseases Committee. Management of group B streptococcal bacteriuria in pregnancy. J Obstet Gynaecol Can. 2012;34(5):482–486.