Title

Timing of exposure assessment in studies on Group B streptococcus colonisation and preterm birth

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Abstract

Background: Maternal colonisation by the bacterium Group B Streptococcus (GBS) increases risk of preterm birth, a condition that has an important impact on the health of children. However, research studies that quantify the effect of GBS colonisation on preterm birth have reported variable estimates of the effect measure.

Methods: We performed a simulated cohort study of pregnant women to assess how timing of exposure (GBS colonisation) assessment might influence results of studies that address this question. We used published data on longitudinal maternal GBS colonisation and on the distribution of preterm births by gestational age to inform parameters used in the simulations.

Results: Assuming that the probability of preterm birth is higher during weeks when pregnant women are colonised by GBS, our results suggest that studies that assess exposure status early during pregnancy are more likely to estimate an association between GBS colonisation and preterm birth that is closer to the null, compared to studies that assess exposure either at birth or during gestational weeks matched to preterm births. In sensitivity analyses assuming different colonisation acquisition rates and diagnostic sensitivities, we observed similar results.

Conclusions: Accurate quantification of the effect of maternal GBS colonisation on the risk of preterm birth is necessary to understand the full health burden linked to this bacterium. In this study, we investigated one possible explanation, related to the timing of exposure assessment, for the variable findings of previous observational studies. Our findings will inform future research on this question.

Keywords

preterm birth, exposure, simulation, Group B streptococcus, diagnostic methods

Key messages

- Timing of GBS colonisation assessment might explain some of the variation in results of observational studies on preterm birth and GBS.
- In simulations informed by published longitudinal data, analyses using exposure information from early during pregnancy estimate an association between preterm birth and GBS that is closer to the null compared to studies that assess exposure at birth or during gestational weeks that match preterm births.
- Careful consideration of the timing of exposure assessment will benefit future research on this question, with important public health implications.

Background

Colonisation of the maternal genito-urinary tract with Group B Streptococcus (GBS) has been linked to increased risk of preterm birth (1-3), a major cause of morbidity and mortality both during childhood and later in life (4-6). Epidemiological evidence for an association between GBS colonisation and preterm birth was summarised in a meta-analysis published in 2017 (1), and considerable heterogeneity was observed in the magnitude of the association. Several possible explanations have been proposed for this variation, including differences in study design, variable frequencies of GBS serotypes, errors in measurement of gestational age at birth and variable timing in assessment of maternal GBS colonisation. Regarding the latter, whilst many studies determined maternal GBS colonisation status at birth, some assessed GBS colonisation status during early pregnancy. **Table 1** presents the timing of GBS culture in the studies included in the previous systematic review.

In this study, we assessed whether the timing of microbiological testing for GBS colonisation could meaningfully contribute to the observed heterogeneity in estimates. Consistent with this hypothesis, in a Danish study, by Feikin and colleagues (7), different analyses were reported: one with maternal GBS colonisation status assessed at birth (estimated odds ratio: 3.0 [95% confidence interval 1.4-6.8]); and a second analysis that used as the exposure variable GBS colonisation status assessed before 24 weeks of gestation. The latter quantified a weaker association (relative risk: 1.1, 95% confidence interval 0.5-2.1). Furthermore, a few studies (8, 9) assessed within-individual changes in GBS colonisation status during pregnancy and observed that for a substantial fraction of pregnant women carriage varied over time: for example, in a study performed in South Africa (9), whilst 50.3% of pregnant women had culture negative results for all samples collected during study visits and 13.8% had positive culture

results in all four study visits, for the other study participants GBS colonisation status changed at least once during follow-up. A similar pattern - that is, transitory GBS colonisation during pregnancy - was observed in a study in the United States (8).

To investigate the extent to which the time-varying nature of the exposure could influence estimates in studies that quantify the association between maternal GBS colonisation and preterm birth, we simulated cohorts of pregnant women and performed statistical analyses that are based on different timings of exposure measurement.

Table 1. Epidemiological studies on maternal GBS colonisation and preterm birth included in a previous systematic review (1). Studies are sorted by year of publication.

Study	Year	Country	Time of screening	Risk ratio/Odds ratio	95% confidence interval
Regan	1981	USA	at delivery	3.03	2.40-3.83
Gerards	1982	Netherlands	before 20th week, at 28th and 34th week's gestation and at delivery	0.74	0.40-1.36
Minkoff	1984	USA	first prenatal visit (13.8 +/- 3.6 weeks' gestation)	1.96	0.82-4.66
Lamont	1986	UK	at admission for preterm labour or 24 h before elective delivery (controls)	3.48	0.18-66.92
Hastings	1986	UK	at booking, 28 and 36 weeks' gestation and during labour	1.01	0.68-1.49
Sweet	1987	USA	initial prenatal visit and repeated at 30 to 34 weeks' gestation	1.18	0.85-1.65
Joshi	1987	Canada	at delivery	2.85	1.68-4.86
Martius	1988	USA	at delivery	1.79	0.83-3.87
Matorras	1989	Spain	samples from range of 17th to 42nd weeks' gestation	0.91	0.57-1.46
Regan	1996	USA	at 23 to 26 weeks' gestation	1.04	0.91-1.18
Citernesi	1996	Italy	at delivery	1.34	0.85-2.11
Allen	1999	Canada	early third trimester (26-28 weeks gestational age)	2.16	1.38-3.27
Garland	2000	Australia	at week 28 or 32 of gestation	0.63	0.48-0.83
Feikin (cont)	2001	Denmark	Controls had specimens taken during routine visits in prenatal clinic; cases specimens taken during labour	1.91	0.88-4.15
Feikin	2001	Denmark	at enrollment ≤ 24 weeks' gestation	0.97	0.49-1.98
Feikin (cont)	2001	Denmark	at delivery	1.95	1.01-3.77
Tsui	2002	Hong Kong	at first or second trimester	1.09	0.50-2.37
Kovachev	2003	Bulgaria	24 weeks' gestation	5.61	2.78-11.34
Tsolia	2003	Greece	during follow-up exam (≥ 35 wks) or at labour	1.12	0.50-2.51
Wilk	2003	Poland	at delivery	2.31	1.02-5.22
Gojnic	2005	Serbia and Montenegro	at delivery	2.43	0.44-13.54
Daskalakis	2006	Greece	22 and 25 weeks' gestation	0.44	0.20-0.97
Jones	2006	UK	between 34 weeks to full term	0.7	0.05-9.54
Aali	2007	Iran	labour/delivery	1.89	0.71-5.01
Mikhova	2007	Bulgaria	not specified	3.29	0.60-17.95
Hakansson	2008	Sweden	at delivery	0.68	0.43-1.07
Seoud	2010	Lebanon	at delivery	0.94	0.55-1.61
Discacciati	2011	Brazil	at labour	5.33	0.57-49.97
Hassanzadeh	2011	Iran	at delivery	1.46	0.41-5.22
Choi	2012	Korea	at delivery	0.45	0.16-1.21
Kessous	2012	Israel	not specified	0.75	0.64-0.87
Seyyed	2013	Iran	at admission to hospital for labour	2.53	1.55-4.13
Agger	2014	USA	11.5 (± 3.7) weeks' gestation	0.41	0.17-0.95
Kim	2015	Korea	last trimester	1.9	0.48-7.55
Schwab	2016	Indonesia	second trimester of pregnancy	3.39	1.15-10.03
Seale	2016	Kenya	at delivery	0.86	0.75-0.98
LeDoare	unpublished data	Gambia	at delivery	1.07	0.87-1.31

Methods

Our simulations were parameterised using published data from previous studies that reported repeated maternal GBS colonisation assessments during pregnancy, and with data on the distribution of preterm births by gestational age (10). Our simulation approach consisted of two steps: first, we simulated longitudinal data on latent (actual) and observed (culture result) GBS colonisation status; we then applied gestational week- and GBS status-specific preterm birth hazards (i.e. probability of birth occurring in a given week conditional on it not having occurred earlier during gestation) to the simulated cohorts of pregnant women.

Maternal GBS colonisation

In simulating longitudinal maternal GBS colonisation data, we firstly randomly assigned, for each participant in the hypothetical cohort, an initial latent GBS colonisation status. GBS acquisition and clearance probabilities were then used to generate data on GBS colonisation status in follow-up assessments. Culture results were simulated based on the latent GBS state and on assay sensitivity and false positive probability. For these simulation steps, we used point estimates from a recent analysis (11) that used Bayesian Hidden Markov models and that quantified GBS acquisition and clearance probabilities between study visits, whilst accounting for and estimating culture sensitivity. That analysis used published data from two epidemiological studies; in the *Results* section, we present simulations for scenarios in which GBS colonisation parameters, but not preterm birth-related parameters (see next subsection), are based on these two studies (Study A (8) and Study B (9)). In Study A, GBS cultures were performed at the end of the first trimester and, on average, in gestational weeks 27 and 37; in Study B, microbiological testing was performed on average in gestational weeks 22, 27, 32 and 37. As these studies did not assess maternal GBS colonisation during each gestational week, in

the simulations we assumed that: for pregnant women with the same GBS latent status in two consecutive study visits, the latent status remained unchanged in all gestational weeks between the two visits; for pregnant women whose GBS latent status changed between consecutive visits, the change occurred in the mid week between the two study visits. **Figure 1** illustrates the data generating procedure for the simulated GBS colonisation datasets; information on parameter values is presented in **Table 2**. In **Supplementary Figures S1** and **S2**, we present results of simulations together with published data that were used in the two steps discussed in this and in the next subsection.

Figure 1. GBS colonisation data generating process. Panel A depicts a Markov model that is assumed to represent GBS colonisation dynamics; there are two possible latent states. In A, the continuous lines represent possible state transitions, and the dashed lines, possible observations. Panel B illustrates a sequence of latent states and of observations for a hypothetical individual; the probabilities of the two observable states (culture positive and culture negative) depend only on the latent state during the same time interval.

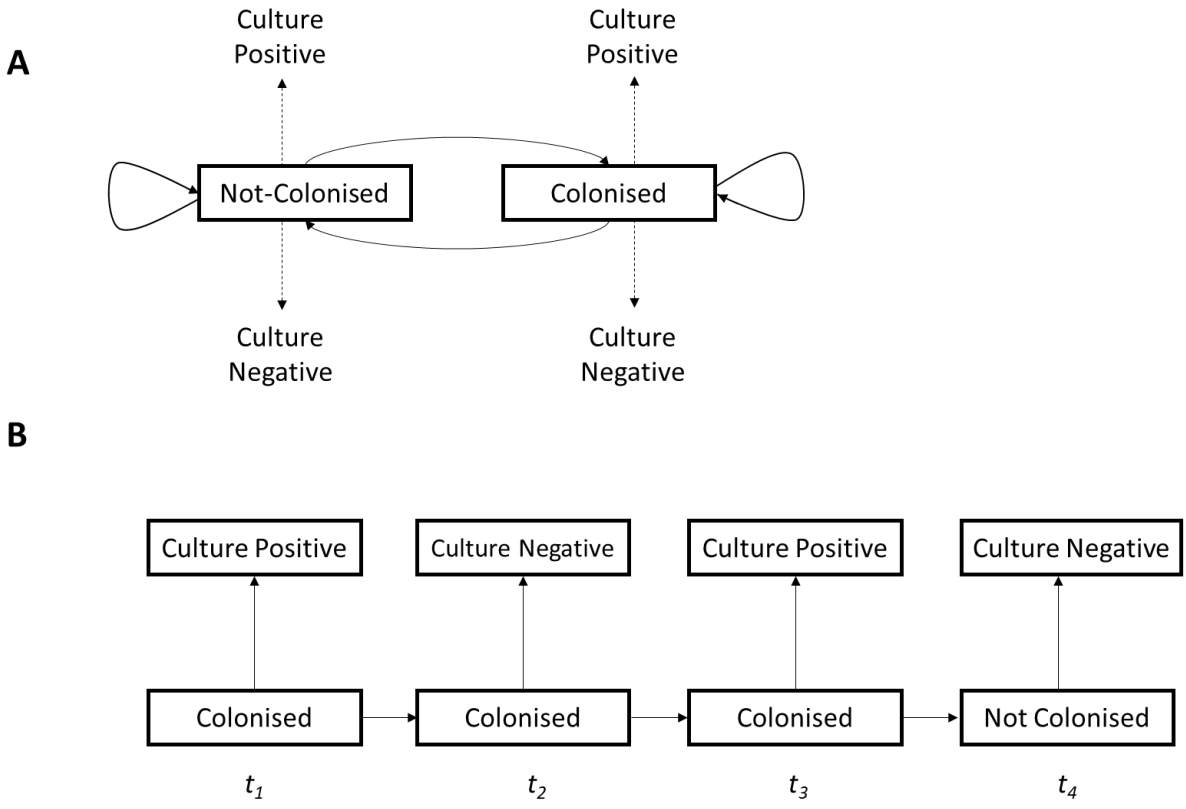


Table 2. Parameter values assumed for simulations shown in **Figure 1**. Transition probabilities between latent states are described in (11).

Parameter	**Value**
Number of pregnant women in each simulated cohort	40,000
Overall preterm birth frequency (r)	0.1
Proportion of all preterm births in gestational age category j (p_j)	See reference (10)
Multiplicative effect of GBS colonisation on the hazard of birth	1.5
Sensitivity of culture for GBS colonisation	79% (Scenario A), 86% (Scenario B)
False positive culture results	1% (Scenario A), 3% (Scenario B)
Timing of GBS colonisation assessment in analyses that use early pregnancy information	Gestational week 12 (Scenario A), gestational week 22 (Scenario B)

Simulating preterm births

We used a published distribution of preterm births by gestational age categories, and calculated the hazard of preterm birth in each gestational week – that is, the probability of birth occurring in a particular gestational week conditional on birth not having occurred earlier during pregnancy.

To calculate the hazard of preterm birth at each gestational age, we used the following formulas:

$$S_k = 1 - \sum_{j=1}^{k} p_j \, r \quad (1)$$

$$\sigma_k = S_k \, / \, S_{k-1} \quad (2)$$

where p_j is the proportion of preterm births that occur in gestational age category *j*, and *r* is the overall preterm birth frequency (i.e., the proportion of births before 37 weeks of gestation). σ_k is the probability that birth does not occur during gestational age category *k* conditional on the birth not having occurred by category $k - 1$. The probability of birth not occurring in a particular gestational week *t* (w_t) was calculated using the following formula:

$$w_t = \sqrt[n_k]{\sigma_k} \quad (3)$$

where n_k corresponds to the number of weeks in the gestational age category *k*, that includes week *t*. By using this equation we assume that the probability of birth not occurring during each week of a gestational age interval is the same; for example, the probability of a pregnant woman not giving birth during a 3-week interval k would be $w_t \,.\, w_t \,.\, w_t = w_t^3 = \sigma_k$.

In simulations, we assumed that maternal GBS colonisation multiplicatively increased the gestational week-specific hazard of preterm birth only during weeks when the latent state corresponded to GBS colonisation – in other words, we assumed that the increase in the hazard of preterm birth only occurs when pregnant women are colonised and there are no carry-over effects during non-colonised periods.

Statistical analysis of simulated data

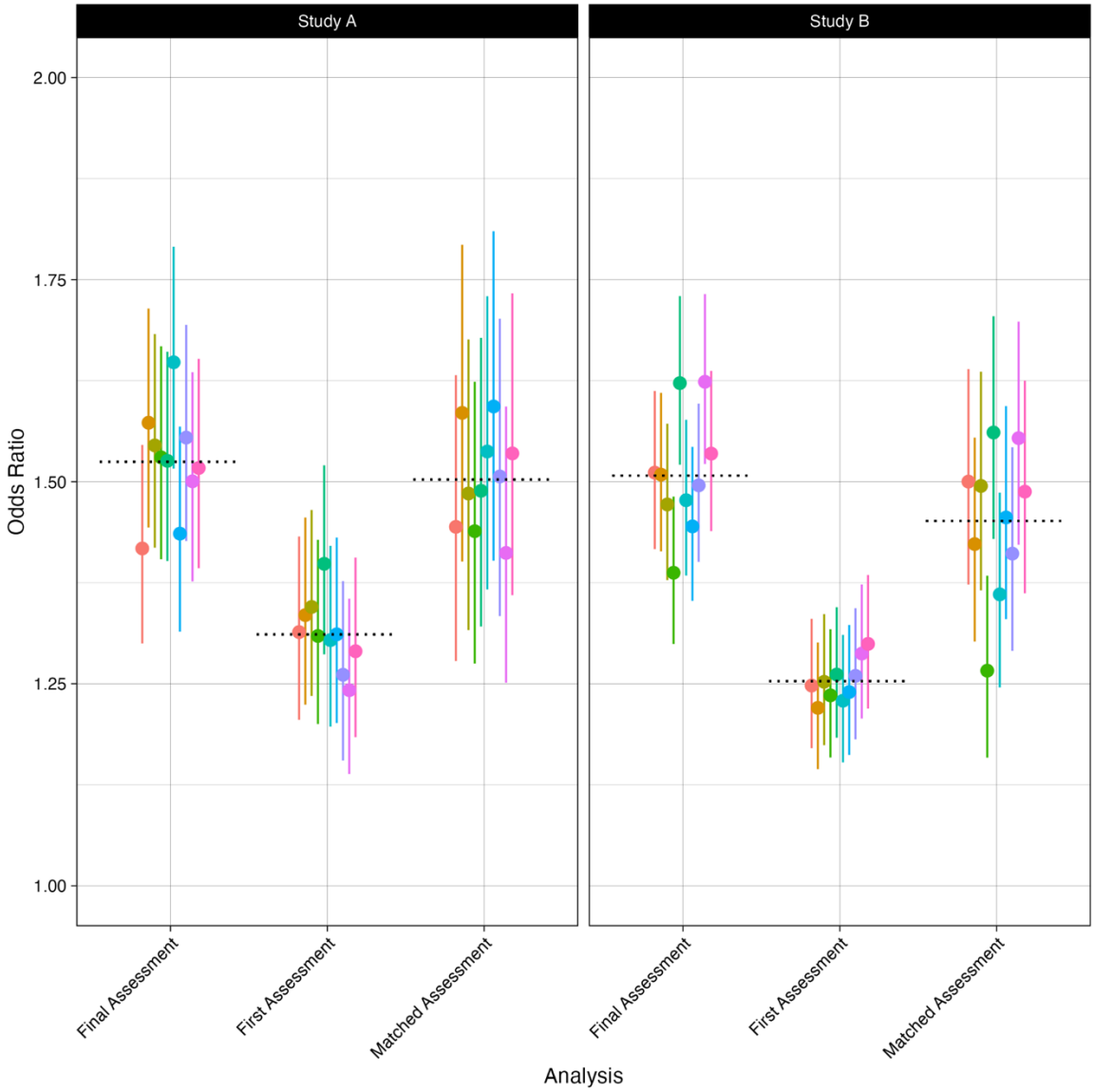
We performed three alternative analyses that have been applied to study this association: the first analysis used culture results from early in pregnancy as a measure of exposure; the second analysis used culture results at birth for both preterm and term births; finally, the third approach, which is equivalent to a case-control study with density sampling (12), used culture

results at birth for preterm births and culture results at the corresponding gestational week for matched pregnant women. In **Figure 2**, we refer to these three different analyses as *First Assessment*, *Final Assessment* and *Matched Assessment*, respectively. Logistic regression was used in each of these analyses of the simulated data and odds ratios were estimated with corresponding 95% confidence intervals; the only covariate used in these models was the culture-defined GBS colonisation status. Python and R were used in data generation, model fitting and visualisation of results. In addition to the results of analyses of simulations presented in the *Results* section, sensitivity analyses performed using data generated based on different assumptions are shown in the *Supplementary Material*.

Results

We assumed that the frequency of preterm births in the group of women who were not colonised by GBS during pregnancy was 10%. We also assumed that GBS colonisation multiplicatively affected the hazard of birth, increasing it by 50%. In **Figure 2**, we compare the results of the three statistical analyses described in the *Methods* section; results of analyses of 10 repeated simulations, with 40,000 pregnant women in each simulated cohort, are shown. We observe that, for the two scenarios that correspond to different sets of GBS colonisation-related parameter values, analyses based on GBS culture results from early pregnancy (*First Assessment* analysis) estimated associations between GBS colonisation and preterm birth that were closer to the null compared to the other two types of analyses. The other two approaches, that assessed GBS colonisation status either at birth or during gestational weeks matched to preterm births (*Final Assessment* and *Matched Assessment*, respectively), estimated odds ratios (averaged over the 10 simulations) of 1.52 and 1.50 for the scenario A, and 1.51 and 1.45 for the scenario B (see legend of **Figure 2**); the corresponding average odds ratios for the analysis that used culture results obtained early during pregnancy were 1.31 and 1.25, respectively. Results were qualitatively similar when simulations assumed higher and lower culture sensitivities, and with GBS colonisation acquisition rates that were twice as high or low as in the primary analysis (see **Supplementary Figure S3**). We also performed a sensitivity analysis where GBS colonisation was assumed to affect preterm birth hazard only from week 32 of gestation onwards; results were similar to those of our primary analysis (**Supplementary Figure S4**).

Figure 2. Analyses of simulated cohorts of pregnant women for two scenarios that assumed GBS colonisation-related parameter values based on published data from different studies (Study A (8); Study B (9)). The association between GBS colonisation and preterm birth for the three different types of analyses (x-axes; see *Methods* section for information about these approaches) is shown in the y-axes. For each scenario and each simulated cohort, results of the different analyses are shown with the same colour.



Discussion

An effect of GBS colonisation on preterm birth means that, in addition to the direct burden caused by this bacterium that is related to invasive disease, GBS increases the incidence of a condition that has an important negative impact on the health of children (4-6). Although experimental studies have suggested possible mechanistic processes involved in the GBS-related increase in risk of preterm birth (13, 14), it is unclear why, in observational studies, there is considerable heterogeneity in effect measure estimates. In **Table 1**, we present information on previous studies included in a systematic review on this association; however, between study comparisons are complicated by differences in several aspects of study design, in study populations, in pathogen populations, and by variation in the diagnostic procedures employed. We investigated one possible explanation for the observed heterogeneity: the variable timing of exposure measurement. Using simulations informed by published data, we observed a stronger association between maternal GBS colonisation and preterm birth when colonisation was measured at birth or during gestational weeks matched to preterm births, compared to when GBS cultures were performed during early pregnancy. Our analyses will be useful when designing future studies and interpreting systematic reviews on this question. In particular, assuming that the effect of GBS colonisation on preterm birth occurs only during gestational weeks when pregnant women are colonised, both the *Final Assessment* and the *Matched Assessment* capture more relevant exposure information than the *First Assessment* approach.

A limitation in this work was the use of a binary variable to represent the exposure of interest. In fact, as with the risk of early onset invasive disease caused by GBS (15), it is likely that the density of maternal GBS colonisation is a key factor in the pathogenesis of GBS-related

preterm birth (see study by Regan and colleagues (16), for example). This information, however, was not reported in the studies with published longitudinal data. Another limitation of our work is that we did not incorporate GBS serotype-specific information; this could have led to additional insights especially if increases in preterm birth risk are linked to specific GBS serotypes. Note also that our estimates of the gestational age-specific hazards of birth were informed by data from one large study, performed in Canada. Although the distribution of preterm births by gestational age reported in that study is consistent with global estimates (see Table 1 in (6)), the hazard might vary in different settings. The approach reported here could be adapted by investigators to use local data on preterm birth distribution by gestational age, whenever those data are available. Finally, to our knowledge there are no published studies that report both longitudinal data that would be sufficient to allow estimation of transition probabilities between GBS colonisation and non-colonised states and that also report the frequency, and gestational age distribution, of preterm births in the same population, and by GBS colonisation trajectory; these two components of the simulation were thus informed by separate studies.

Although future clinical trials of maternal vaccines that target GBS (17, 18) might provide additional evidence for the effect of GBS colonisation on preterm birth, prospective observational studies are urgently needed both to more precisely estimate, whilst accounting for the different factors that might influence this effect, this component of the GBS-related disease burden and to inform the design of these vaccine trials. These observational studies will likely benefit from more frequent assessment of GBS colonisation throughout pregnancy, with diagnostic methods that allow sensitive detection and quantification, and that discriminate between GBS serotypes.

Ethics approval: ethical approval not needed as only previously published and simulated data were used.

Data availability: All data used to inform simulation parameters are available in the publications cited in the manuscript.

Supplementary data: Supplementary data are available at IJE online.

Authors' contributions: BPG conceived the study and developed the simulation code. SRP performed the analysis. PP summarised previous studies on GBS colonisation and preterm births. EHP provided statistical input. All authors contributed to the writing and approved the final version of the manuscript.

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