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Citation: Husain, S. M., Wilks, M., Mupita, M., Reddy, S. P., Hennessy, E. M., Macfarlane, A. J. & Millar, M. R. (2014). Diversity and stability of cultured vaginal lactobacilli in pregnant women from a multi-ethnic urban UK population. *Journal Of Applied Microbiology*, 117(1), pp. 258-265. doi: 10.1111/jam.12506

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Diversity and stability of cultured vaginal lactobacilli in pregnant women from a multi-ethnic urban UK population

Journal:	<i>Applied Microbiology</i>
Manuscript ID:	JAM-2013-2385.R2
Journal Name:	Journal of Applied Microbiology
Manuscript Type:	JAM - Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Husain, Shahid; Barts and the London School of Medicine & Dentistry, Centre for Paediatrics Wilks, Mark; Barts Health NHS Trust, Department of Microbiology Mupita, Mary; Homerton University Hospital, Department of Midwifery Reddy, Srinivasulu; Barts Health NHS Trust, Department of Microbiology Hennessy, Enid; Barts and the London School of Medicine and Dentistry, Wolfson Institute Macfarlane, Alison; City University London, School of Health Sciences Millar, Michael; Barts and the London NHS Trust, Department of Infection
Key Words:	Lactobacillus, Diagnosis, Disease processes

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Title

Diversity and stability of cultured vaginal lactobacilli in pregnant women from a multi-ethnic urban UK population

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Running headline

Vaginal lactobacilli

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5
6 33 **Abstract**

7
8 34 **Aims**

9
10 35 To determine the diversity and stability of cultured vaginal lactobacilli in a multi-ethnic population of
11 36 pregnant women.

12
13 37 **Methods and Results**

14
15 38 A single centre, prospective, cohort study was performed in a tertiary perinatal centre in East London,
16 39 UK. Self-collected vaginal swabs at 13 and 20 weeks gestation were obtained from women attending
17 40 for routine antenatal care and cultured for lactobacilli. In women who provided both swabs, 37 of 203
18 41 (18%) had no lactobacilli cultured at either time. Only 53 (26%) had the same species at both times.
19 42 Black women were less likely to have lactobacilli cultured at 13 weeks ($p = 0.014$) and Black and
20 43 Asian women were less likely to have lactobacilli cultured at 20 weeks ($p = 0.002$) compared with
21 44 those in the White and Other groups.

22
23 45 **Conclusions**

24 46 Significant differences exist between ethnic groups in the carriage and stability of vaginal lactobacilli.

25
26 47 **Significance and Impact of Study**

27 48 These differences have implications for the design of interventions aimed at normalising the vaginal
28 49 microbiota in pregnant women.

29
30 50 **Keywords**

31 51 Lactobacilli, vaginal microbiota, pregnancy, preterm birth

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53 Introduction

54 Preterm birth (PTB) makes a major contribution to infant mortality and long-term disability (Moser *et al.*
55 2007; Saigal and Doyle 2008). The mechanisms and causes of spontaneous PTB are poorly
56 understood but known associations include ethnicity, low socio-economic status, a short interval
57 between pregnancies, poor nutritional status, previous history of PTB, intrauterine infection and
58 ethnicity (Goldenberg *et al.* 2008). In the US, the rate of PTB in Black women is 2-3 times that of white
59 mothers (Adams *et al.* 2000; Collins *et al.* 2007; Kistka *et al.* 2007; Goldenberg *et al.* 2008). Similar
60 but more complex patterns have been observed in Europe. The rate of PTB is higher in Black women
61 but differences in PTB are seen between Black Caribbean and Black African groups, and within Black
62 African subgroups. Studies in North Paris, East London, North West England, and England and Wales
63 as a whole have found higher rates of PTB among women from the Caribbean and West Africa
64 compared with women from Northern Africa (Zeitlin *et al.* 2004; Macfarlane *et al.* 2005; Balchin and
65 Steer 2007; Datta-Nemdharry *et al.* 2012). A review of ethnic disparities in PTB pointed out that both
66 social and biological factors are likely to play a part (Kramer and Hogue 2009).

67
68 Bacterial vaginosis (BV) is associated with PTB (Gibbs *et al.* 1992; Taylor *et al.* 1997). It is
69 characterised by both the absence of lactobacilli and by the presence of large numbers of anaerobic
70 species. Lactobacilli, principally the strains that produce higher levels of H₂O₂, appear to protect
71 against vaginal colonisation by pathogenic species, particularly those causing BV (Klebanoff *et al.*
72 1991; Hawes *et al.* 1996). There is some evidence that vaginal colonisation with H₂O₂ producing
73 lactobacilli reduces the risk of chorioamnionitis and PTB (Reid and Bocking 2003; Wilks *et al.* 2004;
74 Mosbah and Mesbah 2009). In the US, BV is commoner in Black women (Antonio *et al.* 2009; Uscher-
75 Pines and Hanlon 2009) and is significantly associated with PTB of a low birthweight baby in this
76 ethnic group (Hittie *et al.* 2007). Despite substantial evidence linking bacterial vaginosis with PTB, the
77 results of trials of antibiotic treatment of BV in pregnancy have not produced clear evidence of benefit
78 (Nygren *et al.* 2008; Brocklehurst 2013).

79
80 Ethnic differences in the vaginal microbiota of sexually-active, non-pregnant women have been
81 described in the US (Ravel *et al.* 2011). Previous cross-sectional (Wilks *et al.* 2004; Kiss *et al.* 2007;
82 Mosbah and Mesbah 2009) and longitudinal (Verstraelen *et al.* 2007; Verstraelen *et al.* 2009) studies

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3 83 have reported on the presence and stability of vaginal lactobacilli in pregnant women who were
4 84 predominantly White. Similar studies on pregnant women from multi-ethnic backgrounds have not
5 85 reported before. The aims of this study were to determine the prevalent types and stability of vaginal
6 86 lactobacilli in pregnant women from a multi-ethnic population in East London, UK using standard
7 87 laboratory techniques.
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14 89 **Material and Methods**

15 90 This single centre, prospective, cohort study was performed with the approval of the Redbridge &
16 91 Waltham Forest Local Research Ethics Committee which formed part of the UK National Research
17 92 Ethics Service (REC reference number 08/H0701/26). The study population consisted of women
18 93 attending the antenatal clinic at Homerton University Hospital NHS Foundation Trust (HUH), London
19 94 between September 2008 and February 2009. Women referred to the antenatal clinic at HUH received
20 95 an information leaflet about the study with the appointment letter for their first antenatal clinic visit.
21 96 Participation involved permitting access to hospital obstetric and neonatal records, contact with the GP
22 97 if required to enquire about prescribed medications, agreeing to self-collect vaginal swabs on two
23 98 occasions, and permission to retain the specimens. Antibiotic usage during the period of pregnancy
24 99 was determined by asking the participant. Ethnicity was self-defined by the participants and results
25 100 were analysed by grouping ethnicity into the categories used in England, based on categories used in
26 101 the 2001 population census: White (British, Irish and other White), Black (Caribbean, African, other
27 102 Black and mixed Black and White), Asian (Indian, Pakistani, Bangladeshi, other Asian and mixed
28 103 Asian and white) and Other (Chinese, other and not known).
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43 104

44 105 The women in the study provided two self-collected swabs: the first at the time of the first antenatal
45 106 clinic appointment at approximately 13 weeks gestation (swab A) and the second when the women
46 107 attended for a routine ultrasound anomaly scan at approximately 20 weeks gestation (swab B).
47 108 Women were provided with a sheet of written instructions and diagrams that described how to self-
48 109 collect a vaginal swab. Briefly, women were asked to wash their hands, gently part their labia, remove
49 110 the sterile swab from its plastic tube, insert the 'cotton-bud' end of the swab into their vagina to
50 111 approximately half the swab length (about 6 cm), gently twist the swab about three times, part their
51 112 labia, remove the swab and place it back into its plastic tube. The swab was then extracted into 3 mls
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3 113 brain heart infusion broth (BHI) containing 10% glycerol and 0.005% cysteine hydrochloride and
4 114 stored at -70°C. After the women gave birth, maternal and neonatal hospital records were reviewed
5 115 and data on maternal demographics, gestational age at birth, birth outcome, and birthweight collected.
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10 117 ***Culture and identification of lactobacilli***

11 118 Members of staff performing the microbiological assays were blinded to the clinical characteristics of
12 119 the study population. Thawed vaginal secretions were vortexed for 10 secs, inoculated onto MRS agar
13 120 (Unipath, Basingstoke, UK) and incubated for 48 h at 35 °C in an atmosphere of 10% CO₂, 10% H₂
14 121 and 80% N₂. Single colonies from recovered cultures were subcultured onto blood agar plates (5%
15 122 horse blood, Oxoid, Basingstoke UK) and used for DNA extraction as described below and for
16 123 determination of H₂O₂ production. H₂O₂ production was measured using a semi-quantitative assay
17 124 (Merckoquant Peroxide Test, Merck, Leics, UK) as described previously.¹⁷ Results from this test are
18 125 expressed in bands of H₂O₂ production: negative, 1-3, 3-10, 10-30 and 30-100 mg l⁻¹.
19 126

20 127 Following DNA extraction using a QIAamp DNA minikit (Qiagen, Manchester, UK), lactobacilli were
21 128 identified to species level by 16S rDNA sequencing or matrix assisted laser desorption ionisation time
22 129 of flight (MALDI-TOF) analysis. For 16S rDNA sequencing, a 1,350-bp fragment of 16S rRNA gene
23 130 was amplified using oligonucleotide primers 5'-GAA CGC TGG CGG CGT GCC (Z1-forward) and 5'-
24 131 TCC GCG ATT ACT AGC GAT TCC (Z2-reverse). During the course of the study, MALDI-TOF mass
25 132 spectrometry was introduced into the laboratory and validated for the identification of lactobacilli using
26 133 standard strains. For MALDI-TOF analysis, a single colony of a fresh culture was lysed with 70%
27 134 ethanol, extracted with acetonitrile and formic acid, overlaid with hydroxy cinnamic acid matrix and
28 135 analysed using a Bruker Microflex mass spectrometer running MALDI-TOF Biotyper 2.0 analysis
29 136 software.
30 137

31 138 ***Statistics***

32 139 The data from the swabs and the clinical information were merged and checked for obvious errors.
33 140 The analyses were performed using Stata 10. Log_e transformations of H₂O₂ were analysed by the
34 141 Kruskal-Wallis test followed by Sidak's adjustment for multiple comparisons. Associations were tested
35 142 using chi-squared or Fisher's exact tests for tables. Logistic regression was used to investigate
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3 143 associations with PTB, and any or specific lactobacilli carriage. For comparisons of White v Black, and
4 144 White v Asian, a Bonferroni correction assuming 3 potential comparisons was made. The other group
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6 145 was not included because it is heterogeneous and small. This is a conservative correction. No
7
8 146 adjustments were made for White v all others or Black v all others. All p-values are two sided and
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10 147 confidence intervals are 95%.

11 148

12 149 **Results**

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14 150 The base line characteristics of the recruited women are shown in Table 1. Of the 293 women
15
16 151 recruited to the study, gestational age and birth weights of live births were unavailable in 46 women (9
17
18 152 had a miscarriage or termination of pregnancy and 37 moved out of area). A second swab was not
19
20 153 obtained from 90 women mainly because of researcher non-availability when these women attended
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22 154 for their routine ultrasound anomaly scan.

23 155

24
25 156 Overall, 75% of women were colonised with any lactobacillus at either of the sampling times (Table 2).

26
27 157 The mean (SD) number of species of lactobacilli isolated from swab A was 1.15 (0.92) compared with
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29 158 1.14 (0.87) from swab B (data not shown). The statistically significant effects of ethnic group on
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31 159 isolation of any lactobacillus in swab A and in Swab B among mothers with both swabs is associated
32
33 160 with a significant reduction in carriage for Black compared to White mothers ($p = 0.006$ for both
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35 161 comparisons). Indian women had very similar reduced carriage for any lactobacilli in swab B as Black
36
37 162 mothers, but the results are not significant because of smaller numbers ($p = 0.105$, chi-squared after
38
39 163 Bonferroni correction). Compared with the White women in the study, the reductions in lactobacilli
40
41 164 carriage appeared to be because fewer Black and Indian women were colonised with *L. crispatus* ($p =$
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43 165 0.12 and $p = 0.19$, respectively), fewer Black women were colonised with *L. gasseri* ($p = 0.32$) and
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45 166 fewer Indian women with *L. jensenii* ($p = 0.12$) but none of these associations were significant
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47 167 (Fisher's exact test adjusted for 3 comparisons using Bonferroni's test for multiple comparisons).

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51 169 Delivery of the fetus between 22⁺⁰ and 36⁺⁶ completed weeks of gestation occurred in 9 (5%) of 181
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53 170 women who were lactobacillus positive at the first swab and 6 (9%) of 66 women who were negative
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55 171 ($p = 0.23$). Delivery during this range of gestational age was lower in White women (2.4%) compared
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57 172 to all others (9.9%) ($p = 0.016$, Fisher's exact test). Excluding 4 multiple pregnancies which are

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173 themselves associated with PTB, non-White women were at increased risk with 9 PTBs (7.8%) from
174 144 births compared to White women with 2 PTBs(1.6%) from 122 births ($p = 0.030$, Fisher's exact
175 test). The odds ratio for PTB for non-White women after adjustment for lactobacilli carriage at swab A,
176 is 5.0 (CI 1.05 – 24, $p = 0.045$) while that for presence of any lactobacilli at swab A was not significant
177 (OR = 0.7, CI .21 - 3.7, $p=0.64$ after adjustment for non-White ethnic group).

178
179 The amount of H_2O_2 produced by *L. jensenii* was significantly higher than other common species
180 (Table 3). A one-way analysis of variance comparing the $\log_e H_2O_2$ produced showed that *L. jensenii*
181 was highly significantly different from *L. crispatus*, *gassarii* and *vaginalis*, ($p < 0.001$ after Sidak's
182 adjustment for multiple comparisons). Similarly, regression analysis of the $\log_e H_2O_2$ produced showed
183 that *L. jensenii* had nearly six times the level of H_2O_2 production as *L. crispatus*, *gassarii* and *vaginalis*
184 (5.9, CI 4.3 to 8.1, $p < 0.001$).

185
186 Isolates of the same species were assumed to be the same strain of that species and the data were
187 analysed to obtain basic information on the stability of lactobacillus carriage. The proportions of
188 women who had specific strains at swab A and swab B were very similar but this masks a high
189 turnover in species in individual women (Table 4). In women who provided both swab samples, 37
190 (18%) of 203 did not have lactobacilli isolated at either time, 53 (26%) had the same lactobacillus
191 species isolated at both times, 71 (35%) gained a new species, and 68 (45%) of 150 who had a
192 lactobacillus isolated at the first sampling time lost a species. In total, 90 of 203 (44%, CI 37 to 51%)
193 had the same strains (or none) at both time points. Using multivariate analysis, Black women were
194 less likely to gain a new species (OR 0.49, CI 0.25 to 0.98, $p = 0.043$) compared with all other ethnic
195 groups combined. There were significant differences in the proportions of different ethnic groups
196 losing either any species ($p = 0.008$) or all species ($p = 0.005$), with over 20% of Asian and Black
197 women losing all species compared with only 4% of White women.

198
199 Antibiotic usage occurred in the preceding month in 18 of 293 (6%) women who provided a swab A
200 and 11 of 203 (5%) of those who provided a swab B. The oral antibiotics used were amoxicillin,
201 cefalexin and co-amoxiclav. Of the 150 women who provided both swabs and had lactobacilli in swab
202 A, 6 received antibiotics between swabs A and B and none of them lost any strains, while 65 of the

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3 203 other 144 who did not report antibiotic usage did lose a species. This difference is significant ($p =$
4 204 0.029, Fisher's exact test) and suggests that those receiving oral antibiotics were less likely to lose a
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6 205 species. The binomial exact one-sided confidence interval for the proportions losing a strain if they
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8 206 had received oral antibiotics is 0 - 46%. This suggests that of similar women given oral antibiotics
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10 207 fewer than half would be expected to lose a strain of Lactobacilli over the period.
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13 14 209 **Discussion**

15
16 210 In this study, we found significant differences in cultured vaginal lactobacilli between ethnic groups at
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18 211 two time points during pregnancy. Black women were less likely to have vaginal lactobacilli at 13 and
19
20 212 20 weeks of gestation compared with White women. There was a high turnover of vaginal lactobacilli
21
22 213 species in individual women.
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25 214
26 215 To our knowledge, this is the first report to present longitudinal data on vaginal lactobacillus
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28 216 colonisation during pregnancy in an ethnically diverse population. Vaginal colonisation was
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30 217 determined using standard laboratory techniques only. We did this because interventions involving the
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32 218 administration of live lactobacilli (Vangelista *et al.* 2010; Yamamoto *et al.* 2013) require amongst other
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34 219 properties that the strains are easily culturable to allow manufacture of adequate quantities of the
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36 220 product and to allow the ready detection of the organism after administration not only to determine the
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38 221 success of colonisation but also for reasons of safety. Therefore, no attempt was made to identify
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40 222 strains such as *L. iners* that are often difficult to recover in culture and require molecular methods of
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42 223 detection.
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44 224
45 225 Our findings are in agreement with recent reports of ethnic variation in vaginal lactobacillus
46
47 226 colonisation in non-pregnant women (Zhou *et al.* 2007). Three quarters of women in our study were
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49 227 found to be colonised with vaginal lactobacilli at both times of swabbing and this result is in agreement
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51 228 with previous cross-sectional reports (Bayó *et al.* 2002; Zhou *et al.* 2007). However, Black women at
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53 229 the time of both swabs A and B, and Asian women at the time of swab B, were less likely to have
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55 230 vaginal lactobacillus colonisation. The ethnic differences in vaginal microbiota found in this study and
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57 231 others may be due to a number of reasons including genetic influences on the immune system and
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59 232 differences in nutritional factors and cultural practices. The distribution pattern of the most common
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233 lactobacillus species varies between studies for reasons that are unclear. In earlier studies, the
234 unreliability of biochemical identification methods made reliable speciation of lactobacilli unreliable
235 (Wilks *et al.* 1984), but advances in the identification of lactobacilli by molecular methods such as 16S
236 rDNA sequencing or MALDI-TOF suggests that reported differences in detected species are not due
237 to technical factors.

238

239 In this study, mean gestational age of live births did not differ between the ethnic groups, although as
240 expected the birth weight of Asian babies was lower than that of the other groups (Leon and Moser
241 2012). PTB occurred significantly more frequently in non-White women but not significantly more in
242 the absence of lactobacilli in swab A. Reports in the literature suggest an association between preterm
243 labour and reduced frequency of vaginal lactobacillus colonisation or BV (Hitti *et al.* 2007; Donders *et*
244 *al.* 2009; Mosbah and Mesbah 2009). These findings have prompted trials both with antibiotics and
245 probiotics designed to modify the vaginal microbiota with the objective of improving pregnancy
246 outcome. Antibiotics administered to pregnant women can eradicate BV but are unable to reduce the
247 risk of preterm labour and birth (Lams *et al.* 2008; Brocklehurst *et al.* 2013). Oral or vaginal
248 administration with probiotic strains of lactobacilli has often been successful in establishing
249 colonisation of the vagina by the probiotic strain but studies have not been sufficiently powered to
250 determine an effect on preterm birth (Othman *et al.* 2007). If there are ethnic differences in the vaginal
251 microbiota, any interventions designed to restore the normal microbiota must take this into account in
252 addition to viability, dosage and strain/species of lactobacilli.

253

254 H₂O₂ production by vaginal lactobacilli is considered to be an important defence mechanism against
255 vaginal colonisation by undesirable microorganisms. In a previous study we showed that the presence
256 of H₂O₂ producing lactobacilli in the vagina of women who were at risk of PTB was associated with
257 reduced risk of adverse birth outcomes (Wilks *et al.* 2004). The explanation for this finding is unclear
258 because in vitro experiments have shown that the microbicidal activity of H₂O₂ is blocked by
259 cervicovaginal fluid and semen (O'Hanlon *et al.* 2010). However, these findings may not be applicable
260 in vivo where, for example, H₂O₂ producing lactobacilli may produce concentrations of H₂O₂ in their
261 immediate vicinity that are sufficiently high to prevent adherence of a potential pathogen to the vaginal
262 mucosa and thus prevent colonisation. In addition, it may be that H₂O₂ producing lactobacilli strains

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3 263 produce other microbicidal factors such as lactic acid or bacteriocins that prevent proliferation of
4 264 pathogenic in the vagina.

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8 266 In this study, approximately 5-6% of women received antibiotics in the month preceding either of the
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10 267 swab samples. Our figures are similar to that reported in a longitudinal study in the UK which also
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12 268 used self-reported data and showed that 8% of women reported antibiotic use in early pregnancy and
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14 269 5% at 32 weeks gestation (Headley *et al.* 2004). By contrast, Petersen and colleagues used
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16 270 prescribing information recorded in a primary care database in South West London and found that
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18 271 14% of women received at least one antibiotic in each trimester (Petersen *et al.* 2010). Taken together
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20 272 the data suggest that either the use of self-reporting underestimates the consumption of antibiotics
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22 273 during pregnancy or regional differences exist in the prescribing habits of GPs. In a study of non-
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24 274 pregnant women, use of antibiotics was associated with loss of vaginal lactobacillus strains (Vallor *et*
25
26 275 *al.* 2001). However, we found that vaginal lactobacillus colonisation was relatively unperturbed by
27
28 276 exposure to oral antibiotic administration even though lactobacilli show *in vitro* sensitivity to some of
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30 277 the antibiotics ingested by the women in this study (Hamilton-Miller and Shah 1994).

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32 279 While this observational study was not powered to detect independent effects of ethnicity and
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34 280 lactobacillus colonisation on PTB, the combined results from this and previous studies warrant further
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36 281 research to investigate their effects on PTB. Two significant advances in recent years have made it
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38 282 more practical to undertake large studies in which multiple samples could be taken during pregnancy
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40 283 from different ethnic groups. Firstly, the validity of collecting self-taken swabs, enabling easier patient
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42 284 recruitment, is now well-established (Strauss *et al.* 2005; Srinivasan *et al.* 2010) and secondly the
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44 285 ready availability of molecular methods for the in-depth analysis of samples at relatively low cost.
45
46 286 Further research along these lines will allow examination of the effects of ethnic, dietary and other
47
48 287 factors on the vaginal microbiota and provide a more robust framework for interventions.

49 288

50 289 **Acknowledgements**

51
52 290 We thank the women who participated in this study. We also thank the staff of the midwifery and
53
54 291 antenatal ultrasonography departments at Homerton University Hospital for their help in facilitating this
55
56 292 study. Angela Whiley, Simon Warwick and Najeema Begum helped with the culture and identification
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293 of the lactobacilli. This study was supported by funds allocated to Team Hackney from the UK
294 government's Neighbourhood Renewal Fund.

295

296 **Conflict of Interest**

297 No conflict of interest declared.

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Table 1 Maternal ethnicity and age, gestational age at time of vaginal swabs A and B, and gestational age and birth weight of live births

	White	Black	Asian	Other	All
Number recruited to study (%)	158 (54)	89 (30)	32 (11)	14 (5)	293 (100)
Maternal age (years)	31.4 (5.8)	28.9 (6.1)	28.3 (4.3)	31.7 (7.0)	30.3 (5.9)
Gestational age swab A (w)	12.5 (2.0)	12.8 (2.0)	13.4 (2.3)	14.4 (2.1)	12.9 (2.2)
Gestational age swab B (w)	19.3 (2.6)	19.8 (3.2)	20.5 (0.8)	20.2 (0.4)	19.7 (2.7)
Gestational age of live births (w)	40.0 (1.9)	39.3 (2.6)	38.8 (2.6)	39.8 (2.8)	39.1 (4.0)
Birth weight of live births (kg)	3.47 (0.50)	3.39 (0.53)	3.05 (0.50)	3.17 (0.55)	3.34 (0.57)

Data are shown as mean (SD) unless otherwise indicated

Table 2 Lactobacilli in women who provided swabs A and B

	White	Black	Asian	Other	Total	p-value *
Women with swab A	158	89	32	14	293	
Any lactobacillus in swab A	128 (81)	56 (63)	24 (75)	12 (86)	220 (75)	0.014
Women with swabs A and B	108	64	21	10	203	
Any lactobacillus in swab A	84 (78)	41 (64)	16 (76)	9 (90)	150 (74)	0.165
Any lactobacillus in swab B	89 (82)	39 (61)	13 (62)	10 (100)	151 (74)	0.002
<i>L. jensenii</i>						
Sample A	41 (49)	21 (51)	3 (19)	2 (22)	67 (45)	0.058
Sample B	36 (40)	18 (46)	1 (8)	2 (20)	57 (38)	0.051
<i>L. crispatus</i>						
Sample A	37 (44)	10 (24)	3 (19)	5 (56)	55 (37)	0.042
Sample B	40 (45)	10 (26)	2 (15)	3 (30)	55 (36)	0.062
<i>L. gasseri</i>						
Sample A	32 (38)	8 (20)	8 (50)	3 (33)	51 (34)	0.098
Sample B	34 (38)	9 (23)	7 (54)	3 (30)	53 (35)	0.174
<i>L. vaginalis</i>						
Sample A	14 (17)	9 (22)	3 (19)	0 (0)	26 (17)	0.514
Sample B	19 (21)	8 (21)	1 (8)	1 (10)	29 (19)	0.717
Other lactobacilli						
Sample A	14 (17)	9 (22)	6 (38)	1 (11)	30 (20)	0.307
Sample B	17 (19)	10 (26)	5 (38)	3 (30)	35 (23)	0.559

Data are shown as number (%). * chi-square test for types of lactobacilli; Fisher's exact test for individual 4 (ethnicity) x 2 (yes/no) tables for each row.

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Table 3 H₂O₂ production by Lactobacilli isolated from swab A

Lactobacillus species (isolates tested)	H ₂ O ₂ production *	
	Median	Interquartile range
<i>L. jensenii</i> (175)	10 - 30	3 – 10 to 30 – 100
<i>L. crispatus</i> (177)	1 - 3	0 – 1 to 3 – 10
<i>L. gasseri</i> (177)	1 - 3	1 – 3 to 3 – 10
<i>L. vaginalis</i> (68)	1 - 3	1 – 3 to 3 – 10
<i>Other strain</i> (115)	0 - 1	0 – 1 to 1 – 3

* There was significant interspecies variation in H₂O₂ production (p = 0.0001, Kruskal-Wallis test).

Table 4 Gain and loss of lactobacilli between swabs A and B

Ethnicity	White	Black	Asian	Other	Total	
Total number of women	108	64	21	10	203	
Gain of lactobacilli						p-value *
Any lactobacillus species	45 (41.7)	15 (23.4)	7 (33.3)	4 (40.0)	71 (35.0)	0.111
<i>L. jensenii</i>	7 (10.5)	1 (2.3)	0 (0.0)	1 (12.5)	9 (6.6)	0.204
<i>L. crispatus</i>	11 (15.5)	2 (3.7)	0 (0.0)	0 (0.0)	13 (8.8)	0.071
<i>L. gasseri</i>	12 (15.8)	4 (7.1)	2 (15.4)	0 (0.0)	18 (11.8)	0.336
<i>L. vaginalis</i>	12 (12.8)	3 (5.5)	1 (5.6)	1 (10.0)	17 (9.6)	0.473
Any other lactobacillus species	12 (11.1)	5 (7.8)	4 (19.1)	2 (20)	23 (11.3)	0.70
Loss of lactobacilli						
Any lactobacillus species	38 (45.2)	14 (34.2)	13 (81.3)	3 (33.3)	68 (45.3)	0.012
<i>L. jensenii</i>	12 (29.3)	4 (19.1)	2 (66.7)	1 (50.0)	19 (28.4)	0.316
<i>L. crispatus</i>	8 (21.6)	2 (20.0)	1 (33.3)	2 (40.0)	13 (23.6)	0.788
<i>L. gasseri</i>	10 (31.3)	3 (37.5)	3 (37.5)	0 (0.0)	16 (31.4)	0.648
<i>L. vaginalis</i>	7 (50.0)	4 (44.4)	3 (100.0)	0 (0.0)	14 (53.9)	0.226
Any other lactobacillus species	10 (71.4)	4 (44.4)	5 (83.3)	0 (0.0)	19 (63.3)	0.209

Data are shown as number of women (%). * Fisher's exact test for individual 4 (ethnicity) x 2 (yes/no) tables for each row.