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Is a change in the vaginal flora associated with an increased risk of preterm birth?

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KEY WORDS
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Objective: The purpose of this study was to determine if a change in the vaginal flora was associated with an increased risk of preterm birth, and to determine if metronidazole therapy before 32 weeks increased the risk of preterm birth.

Study design: We compared cultures taken at 23 to 26 weeks of gestation with cultures taken at delivery from women enrolled in the Vaginal Infections and Preterm Birth study to analyze the association of changes in the vaginal flora with preterm birth.

Results: Metronidazole therapy before 32 weeks was associated with an increased risk of preterm birth (OR 1.5, 95%CI 1.05-2.1) in an unadjusted model. A change to heavy growth of *Escherichia coli* or *Klebsiella pneumoniae* at delivery was found to be associated with preterm birth (OR 2.4, 95%CI 1.6-3.8). After controlling for race, parity, prepregnancy weight <100 pounds, smoking or drinking during pregnancy, *Trichomonas vaginalis,* bacterial vaginosis, chlamydia, mycoplasmas, group B streptococcus, metronidazole therapy before 32 weeks, vaginal pH >5.0, and an increase in *E coli* or *K pneumoniae*, only prepregnancy weight <100 pounds (adjusted odds ratio [AOR] 2.07, 95%CI 1.01-4.21) and increased *E coli* or *K pneumoniae* in the vagina at delivery (AOR 2.99, 95%CI 1.37-6.53) were found to be significantly associated with preterm birth.

Conclusion: An increase in *E coli* or *K pneumoniae* in the vagina is an independent risk factor for preterm birth. Changes in the vaginal flora may explain the increased risk of preterm birth seen with vaginal clindamycin or oral metronidazole therapy.

Preterm birth is a leading cause of neonatal morbidity and mortality. The cause of most preterm births is not known, but there is a substantial body of evidence that many preterm births are related to asymptomatic or minimally symptomatic infections.[1] Carriage of a wide variety of organisms has been associated with preterm birth.[2-9] We have also shown that the presence of bacterial vaginosis[10] or carriage of *Trichomonas vaginalis*[11] is associated with an increased risk of preterm birth.

However, clinical trials of treatment of specific organisms to prevent preterm birth have been disappointing.[12-17] We have previously shown that treatment of *Ureaplasma* urealyticum,[18] group B beta hemolytic streptococci,[19] or *Chlamydia trachomatis*[20] does not reduce the risk of preterm birth in asymptomatic women. In the parallel clinical trials of metronidazole treatment of bacterial vaginosis[21] and *Trichomonas vaginalis*[22] to

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Table I Pregnancy outcomes and metronidazole before 32 weeks

Outcome	Metronidazole <32 wk	No metronidazole <32 wk	χ^2 unadjusted OR	OR, metronidazole adjusted for *Trichomonas** or BV†	OR, *Trichomonas** or BV† adjusted for metronidazole
Delivery <37 wk	37/229 (16.2%)	1489/13079 (11.4%)	$P = .025$ 1.5 (1.05-2.1)		
Trichomonas +	19/92 (20.7)	220/1524 (14.4)		1.4 (0.97-1.99)*	1.4 (1.2-1.6)*
Trichomonas −	18/134 (13.4)	1227/11,219 (10.9)			
BV +	7/57 (12.3)	256/2011 (12.7)		1.5 (1.06-2.2)†	1.1 (0.99-1.3)†
BV −	30/168 (17.9)	1173/10,528 (11.1)			
Low birth weight	21/228 (9.2%)	992/12,902 (7.7%)	$P = .39$ 1.2 (0.8-1.9)		
Trichomonas +	9/92 (9.8)	173/1507 (11.5)		0.9 (0.59-1.5)*	1.7 (1.4-2.0)*
Trichomonas −	10/133 (7.5)	784/11,064 (6.2)			
BV +	4/56 (7.1)	196/1986 (9.9)		1.1 (0.68-1.8)†	1.4 (1.2-1.6)†
BV −	15/168 (8.9)	743/10,381 (7.2)			
PPROM	11/227 (4.9%)	366/12,465 (2.9%)	$P = .09$ 1.7 (0.9-3.1)		
Trichomonas +	5/90 (5.6)	55/1431 (3.8)		1.5 (0.83-2.9)*	1.4 (1.03-1.8)*
Trichomonas -	6/134 (4.5)	302/10717 (2.8)			
BV +	1/56 (1.8)	62/1899 (3.3)		1.7 (0.9-3.1)†	1.1 (0.83-1.4)†
BV -	10/167 (6.0)	291/1051 (2.9)			

* *Trichomonas*.
† Bacterial vaginosis.

prevent preterm birth, we found that metronidazole therapy of bacterial vaginosis did not reduce the risk of preterm birth. However, in women who carried *T vaginalis* with or without bacterial vaginosis, metronidazole therapy increased the risk of spontaneous preterm birth. It has also been observed that treatment with vaginal clindamycin may increase the risk of preterm birth in women with bacterial vaginosis.[12,13,15,16]

Why is there such a consistent association between genital infections and preterm birth, but antibiotic treatment been so unsuccessful at preventing preterm birth? It is possible that genital infections are not causally related to preterm birth, but that preterm birth and infections are markers for an underlying condition—possibly an altered immune status. Antibiotic treatment might not eradicate upper tract infections, or inflammatory processes might persist after antibiotic therapy eradicates organisms. It is also possible that the infections that have been associated with preterm birth are markers for another infection that is causally associated with preterm birth, or that a change in vaginal flora is associated with both preterm birth and certain infections. The observation that metronidazole therapy increased the risk of preterm birth in asymptomatic women with *T vaginalis* but not bacterial vaginosis suggests that a change in vaginal flora may be associated with *T vaginalis,* preterm birth, and metronidazole therapy.

We formed the hypothesis that changes in the vaginal flora are associated with preterm birth, and that these changes explain the increased risk of preterm birth seen with metronidazole therapy in women who carry *T vaginalis*. To test this hypothesis, we analyzed data collected as part of the Vaginal Infections and Prematurity (VIP) study.

Material and methods

Data collected during the Vaginal Infections and Prematurity (VIP) Study were used. The VIP study and microbiological methods have been described previously.[23] Briefly, we screened low-risk pregnant women from 7 academic centers at 23 to 26 weeks of gestation. Cultures for aerobes, anaerobes, genital mycoplasmas, *C trachomatis*, *T vaginalis,* vaginal pH, and Gram stains of the vagina and cervix were obtained, as well as extensive obstetric, medical, and social history. A random sample of women was screened again at 31 to 33 weeks and another sample at 34 to 36 weeks; 1496 women were screened at 1 of these 2 times. Four hundred eighty-three women who delivered preterm were screened at delivery, and another sample of 2354 was screened at term delivery.

U urealyticum, group B streptococci, *C trachomatis*, *Mycoplasma hominis, Neisseria gonorrhoeae*, and *T vaginalis* were recorded as positive if found in any quantity. Group A *Streptococcus*, group D *Streptococcus*, enterococcus, viridans *Streptococcus*, *Streptococcus aureus*, *E coli, K pneumoniae, Proteus mirabilis,* aerobic lactobacilli, diphtheroids, coagulase negative staphylococci, candida, *B bivius, B disiens, B fragilis,* other bacteroides, *Fusobacterium, Peptococcus, Peptostreptococcus*, anaerobic lactobacilli, and other anaerobic gram-positive rods were recorded if found in streaks 3 or 4 of a culture plate (heavy growth). To determine changes in the vaginal flora, organisms were defined as present (1) or absent (0) at screening culture and delivery cultures. Screening culture values were subtracted from delivery culture values to determine the change in flora. A positive value indicated acquisition of an organism; a negative value indicated loss of an