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ABSTRACTS

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Identification of microbes from sequencing of bacterial rDNA obtained from culture-negative amniotic fluid samples of women in preterm labor

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Introduction: Culture is an insensitive technique to identify microbes in small concentrations or microbes that are difficult to isolate by culture.

Objective: To determine the microbes present in culture-negative but 16S rDNA-positive amniotic fluid (AF) samples.

Study design: Amniocentesis was used to obtain amniotic fluid (AF) from 69 women in preterm labor. AF was cultured on media to isolate bacteria and genital mycoplasmas and examined for pro-inflammatory cytokines. Polymerase chain reaction was used to detect a broad-spectrum bacterial 16S rDNA conserved in bacteria. As previously reported[1], bacterial rDNA was recovered from 15 of 16 culture-positive AF samples, Five of 14 culture-negative but elevated IL-6 samples, and one of 39 culture-negative and low IL-6 samples. Direct sequencing was performed on the DNA of the five culture-negative, elevated IL-6 AF specimens with bacterial rDNA identified.

Results: Microbes recovered from amniotic fluid: (i) two *Lepotrichia sanguinegens*; (ii) one human oral bacterium A33; (iii) one *Fusobacterium nucleatum*; and (iv) one *Ureaplasma urealyticum*.

Conclusion: This technique identified both difficult-to-isolate bacteria and more-common microbes. The technique should be utilized further to identify unique microbes in AF.

REFERENCE

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Screening cultures for group B streptococcus (GBS) – is transport media appropriate?

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Objectives: Neonatal group B streptococcal (GBS) sepsis remains a significant cause of neonatal morbidity and mortality. In the prevention strategy of screening pregnant women for colonization (+ intrapartum chemoprophylaxis), optimal detection of GBS is paramount.

Study design: We compared inoculation directly into GBS enrichment broth (NPC) with delayed inoculation into a transport medium. Clinical isolates of GBS ($n = 104$) were inoculated into Transtube™ (charcoal transport medium swabs) in a concentration of 10 CFU/swab. Swabs were then transferred to NPC enrichment broth at time intervals of 0, 2, 6 and 24 hours. Broths were then incubated for 18–24 hours at 35°C in O_2, after which swabs were transferred to Modified New Granada Medium (NGM) and incubated for a further 18–24 hours at 35°C in O_2. If the characteristic orange-pigmented colonies were observed after this period, the specimen was recorded as positive for GBS.

Results: Overall 95/103 (92.2%) were positive in all tubes at all time intervals and another 3/103(2.9%) were non-haemolytic non pigment forming GBS. Of note 3/103(2.9%) were negative until 2 hours delayed inoculation and 2/103(1.9%) gave inconsistent results, likely due to the low inoculum used in this experiment.

Conclusion: Delayed inoculation gave a similar and acceptable GBS detection rate to direct inoculation; hence Amies charcoal transport medium is an alternative where selective broth cannot be practically and directly inoculated.

Maternal blood group, Rh type and group B streptococcal colonization during pregnancy

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Objectives: Studies from the 1970s and 80s reported associations between maternal blood group, Rh type, and group B streptococcal (GBS) colonization during pregnancy. We sought to confirm or reject these reports using a large database and the current CDC screening protocol.

Study design: Data for maternal blood group, Rh type and GBS colonization was obtained from a computerized obstetric database (OBNET). We evaluated the time period January 1, 1998 through December 31, 2001. Specimens for culture of GBS were obtained by separate swabs of the vaginal introitus and anorectum at 35–37 weeks of gestation. Chi-square analysis was performed on all comparisons, and $p < 0.05$ was considered significant.

Results: Complete data was available for 7652 patients. Of these patients, 1951 (25.5%) were GBS positive, while 5701 (74.5%) were GBS negative. Six thousand, four hundred and twenty-three (83.9%) were Rh positive, while 1229 (16.1%) were Rh negative. A comparison of maternal blood groups ABO and GBS colonization revealed no significant differences. A comparison of Rh type and GBS colonization also revealed no significant differences.

Conclusions: We found no significant association between GBS colonization and maternal blood group or Rh type.

Group B streptococcus vaginal serotype switching among young non-pregnant women

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Objective: To determine the risk factors for group B streptococcus (GBS) vaginal colonizing serotype switching.
Study design: The study was a prospective longitudinal examination of selected genital flora and behavioral characteristics of young, non-pregnant women during four clinical visits over 1 year from 1997–2001. Among 1248 women enrolled, 965 women had three or more study visits. GBS was inoculated into 5% sheep blood agar, *Lactobacillus* was inoculated into Rogosa agar and H_2O_2 identified by an HBT plate. Bacterial vaginosis (BV) was scored using the Nugent system. Switching serotype was defined as being colonized with one vaginal serotype and at a later clinical visit being colonized with a second vaginal serotype. Data were analyzed using STATA® software using interval, time-dependent covariate Cox proportional hazard models.
Results: Among 965 women at the baseline visit, the vaginal frequencies of the five most predominant serotypes were: Ia 90 (10%); Ib 23 (3%); II 36 (4%); III 41 (7%); V 41 (4%); no GBS 674 (72%); and missing 34. Overall 131 (14%) of 965 women and 49% of colonized women switched vaginal serotype. Women switched serotypes equally often among visits, among starting serotypes, and were more likely to switch to Ia, III, or V regardless of starting serotype. African-American race (AA), number of sexual partners, and BV significantly increased the hazard ratio (HR) of switching serotypes. The following HRs and 95% confidence intervals were estimated: AA (HR = 1.9, 1.4–2.8); number of sex partners in the last year – one (HR = 3.8, 1.2–12.5); two (HR = 4.2, 1.3–14.1); three or more (HR = 6.4, 2–21.2); BV (HR = 1.6, 1.1–2.4). Increased number of partners and frequency of sex in the last 4 months elevated the HR.
Conclusions: Switching vaginal GBS serotypes was strongly influenced by sexual activity. The GBS serotypes are all immunologically different, therefore, changing serotypes may give us an insight into the important risk factors for acquiring GBS for the first time in life.This study was supported by The Streptococcal Initiative: NIH/N01-AI-75326.

An investigation for novel virulence determinants of group B streptococcus

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Objectives: Although group B streptococcus (GBS) is an important pathogen of neonates, pregnant women, and non-pregnant adults, little is known about what determines its virulence.
Study design: To investigate this, we performed subtractive DNA hybridization using an isolate from a patient with invasive disease as the tester strain and an isolate from a healthy carrier as the driver strain. These two isolates also differed in their ability to invade CHO cells ($p < 0.004$), and in their pathogenicity for neonatal mice ($p < 0.02$).
Results and Conclusions: Two hundred difference products were cloned and 60% of these were confirmed as tester-specific by hybridization using labelled tester or driver genomic DNA as probes. These were then sequenced and analyzed for similarities to protein sequences in the Genbank database using the BLASTX algorithm. Some of the sequences obtained showed homology to known virulence-associated proteins of GBS, such as the αC protein; others were similar to putative and characterized proteins of other bacteria, such as outer membrane protein precursors, DNA methyltransferase, PBSX repressor and proteins involved in cell secretion. For some sequences no homology was found. These may represent novel virulence factors. We are currently determining the prevalence of these determinants in clinically invasive and non-invasive isolates to elucidate their possible role in the virulence of GBS.

Genetic relatedness of macrolide-resistant serotype versus group B streptococci

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Objectives: An increasing proportion of group B streptococcus (GBS) infections is due to serotype V (GBS-V). Macrolide (erythromycin and clindamycin) resistant GBS has also been reported with increasing frequency. We previously reported a high rate of macrolide resistance among GBS-V. We hypothesize that the simultaneous increase in GBS-V and macrolide resistance is due in part to the emergence of a macrolide resistant GBS-V clone.
Study design: From 1997–1999, 122 neonatal bloodstream isolates of GBS were collected from 48 centers throughout the Western hemisphere. Serotyping, antibiotic susceptibility testing and pulsed field gel electrophoresis (PFGE) were performed on all isolates. A CDC strain, identified previously as the predominant GBS-V strain in the US, was included in the PFGE for comparison.
Results: Only 11 of 122 isolates (9%) were GBS-V, but eight of the 11 were macrolide resistant (representing 40% of all macrolide resistant GBS). Five GBS-V were resistant to both clindamycin and erythromycin, the remaining three to erythromycin alone. Seven of the eight macrolide-resistant GBS-V, isolated from seven geographically diverse US centers (from California to New York), were indistinguishable from the CDC strain by PFGE. The remaining macrolide-resistant GBS-V strain, from a Canadian center, was a closely related subtype. Non-GBS-V macrolide resistant isolates included six different PFGE types that were not related to the GBS-V isolates.
Conclusions: A large proportion of macrolide resistance in GBS is due to a single PFGE type of serotype V, which is also the most common GBS-V PFGE type noted in previous CDC surveillance. Any increase in serotype V may be associated with an increase in macrolide-resistant GBS.

Immunoregulatory gene polymorphisms in women with preterm birth and preterm prelabor rupture of membranes

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Objectives: Altered expression of certain immunoregulators as a result of gene polymorphism(s) may be associated with preterm birth (PTB). Our objective was to investigate differences in the frequencies of immunoregulatory gene polymorphisms in women with PTB, and preterm pre-labor rupture of membranes (PPROM) compared with women with term births. Frequencies of single nucleotide polymorphisms (SNPs) were studied for IL-1α, IL-1 receptor 1, IL-1 receptor antagonist, IL-4, IL-6 (each at one position), IL-1β®, TGFβ-1, FAS, Mannose Binding Lectin (at two positions), and IL-10 and TNFα (at three positions).

Study design: Peripheral blood was collected from 202 Caucasian women with a history of PTB $\leq$ 34 weeks' gestation, and 185 women with term birth(s) and no PTB. DNA was extracted and genotyped by polymerase chain reaction using sequence specific primers.

Results: For women with term births the frequencies of polymorphisms were similar to those previously reported. Overall, no single polymorphism was strongly associated with PTB or PPROM. The raw data showed that TGFβ-1 frequencies for the high producing allele T at -509, were higher in women with PTB $\leq$ 34 weeks' gestation than in those with term birth (0.28 vs. 0.22, OR 1.4, $p = 0.05$). IL-10 frequencies for genotype G/G at -1082 were also increased in women with PTB $\leq$ 34 weeks' gestation (0.3 vs. 0.22, OR 1.6, $p = 0.05$). IL-6 frequencies for G/G at -174 were higher in women with PTB $\leq$ 28 weeks' gestation and PPROM than in those with intact membranes (0.52 vs. 0.26, OR 3.1, $p = 0.07$).

Conclusion: PTB may be influenced by the presence of certain immunoregulatory SNPs.

Amniotic fluid secretory leukocyte protease inhibitor and its relationship to idiopathic preterm labor

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Objective: Secretory leukocyte protease inhibitor (SLPI) may protect epithelial cells from inflammatory injury, and vaginal SLPI (VSLPI) declines in the presence of infection. We examined the concentrations of SLPI in vaginal (VSLPI) and amniotic fluid (AFSLPI) among women in preterm labor to determine whether decreased VSLPI and AFSLPI were associated with vaginal or AF infection, and preterm birth ≤ 34 weeks.

Study design: In total 102 women in preterm labor with intact membranes had AF obtained by amniocentesis for culture, and had interleukin-6 (IL-6) and SLPI quantitated by enzyme-linked immunoassay (EIA). Vaginal fluid was collected for bacterial vaginosis (BV) by gram stain; semi-quantitative anaerobic culture; and SLPI determination. Statistical analysis included nonparametric methods, Pearson's chi-square test, and linear and logistic regression.

Results: VSLPI was detected in 82% (84/102) and AFSLPI in 100% of paired samples. VSLPI and AFSLPI were not correlated with each other. VSLPI was lower with BV ($p = 0.03$). Lower VSLPI and early preterm birth were not related. AFSLPI levels were significantly, inversely, and independently correlated with shorter interval to delivery from time of amniocentesis ($p < 0.01$), controlled for both BV status and AF IL-6 level. Women delivering ≤ 34 weeks had a significantly higher median AFSLPI level than those delivering > 34 weeks (851 ng/mL vs. 557 ng/mL, $p = 0.01$). Logistic regression revealed that higher AFSLPI ($p = 0.03$) and AF IL-6 levels ($p < 0.01$) were independently associated with increased risk of preterm birth.

Conclusions: AFSLPI levels are independently and significantly higher in women at risk for preterm delivery, as are AF IL-6 levels. VSLPI levels are decreased in women with abnormal vaginal flora.

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Tumor necrosis factor-α promoter polymorphism −308 and chorioamnionitis

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Objectives: A single-base polymorphism within the promoter of the gene for tumor necrosis factor-alpha (TNF-α) at position −308 relative to the transcriptional start site of the gene is correlated with differences in constitutive and inducible gene transcription. There are two allelic forms: TNFA1 and TNFA2. The rarer allele, TNFA2, contains a substitution of guanine by adenosine at position −308, and is associated with increased production of TNF-α. The purpose of this study was to assess the relationship between carriage of the TNFA2 allele and clinical chorioamnionitis.

Study Design: In this retrospective cohort study, previously banked DNA from 149 women who had spontaneous labor at 37–42 weeks' gestation was evaluated by polymerase chain reaction (One Lambda, Canoga Park, CA) for polymorphism. The amplified fragments were detected by gel electrophoresis and ethidium bromide staining. Patterns of positive and negative amplification yielded the relevant phenotype. Demographic and clinical information were obtained from a perinatal database. Clinical chorioamnionitis was defined as at least one temperature elevation above 38°C combined with at least two of the following signs; maternal or fetal tachycardia, uterine tenderness greater than expected, foul smelling vaginal discharge, and white blood count more than 18 000.

Results: Clinical chorioamnionitis was present in 18 women (12.1%). Among women who carried TNFA2, the chorioamnionitis rate was 24.4%, compared with 7.4% among women not carrying TNFA2. The relative risk for chorioamnionitis with carriage of TNFA2 was 3.29 ($p = 0.007$). This increased risk was not altered after adjustment for race, type of rupture of membranes, intrauterine pressure catheter use, smoking, and prolonged rupture of membranes.

Conclusions: The TNFA2 allele, which is associated with increased production of TNF-α, is associated with an increased risk of clinical chorioamnionitis, even when accounting for important clinical and microbiologic risk factors.

Serial maternal serum granulocyte colony stimulating factor, not interleukin-8 levels, predicts impending funisitis in preterm premature rupture of membrane patients

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Objective: Preterm premature rupture of membrane (PPROM) patients are managed expectantly until labor or infection is present. We previously reported that maternal serum (MS) interleukin (IL)-6 is elevated at 12–36 hours prior to delivery in PPROM with subsequent funisitis. Our present objective was to determine if MS granulocyte colony stimulating factor (G-CSF) and IL-8 are elevated before clinical infection or labor in PPROM patients with subsequent funisitis.

Study design: All PPROM patients were eligible for this cohort study. After IRB approval, 82 subjects with PPROM consented to daily blood sampling. Aliquots of sera were frozen at –80°C and IL-6 levels determined by ELISA (CC Lab, MD). MS G-CSF and IL-8 levels at 12–36 hours prior to delivery were compared in subjects with and without funisitis. Data were analyzed by Mann–Whitney *U* test (StatView, Cary, NC).

Results: Of the 82 PPROM subjects enrolled, 29 remained undelivered > 48 hours without clinical infection or labor, and had daily samples and pathology. There was no difference in age, race, insurance, parity or prior preterm birth between groups. Subjects were divided into those with ($n = 11$) and without funisitis ($n = 18$). MS G-CSF levels were significantly higher at 12–36 hours prior to delivery in those with funisitis compared with those without (116.4 vs. 64.8 pg/ml, $p = 0.005$). The difference between the 12–36 hours and > 48 hours was calculated for each subject. Subjects with funisitis had a significant rise in MS G-CSF compared to those without funisitis (80.8 vs. 0 pg/ml, $p = 0.002$). Insignificant differences were found when identical analyses were performed with MS IL-8. These same analyses were performed on MS IL-8 and were not significant.

Conclusions: MS G-CSF levels, not IL-8, rise prior to the onset of labor or infection in PPROM patients with subsequent funisitis. MS G-CSF may identify those PPROM patients at risk for both intrauterine and fetal infection. Identifying PPROM patients at increased risk for maternal/fetal infection will improve our ability to manage these patients.

Interleukin-6, interleukin-8, and granulocyte colony stimulating factor are elevated in cord blood of neonates with funisitis, in patients with preterm premature rupture of membranes

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Objective: Preterm premature rupture of membranes (PPROM) is a leading cause of neonatal morbidity and mortality. We previously reported that maternal serum interleukin (IL)-6 is elevated 12–36 hours prior to delivery in PPROM patients with subsequent funisitis. Funisitis, a significant risk factor for neonatal morbidity and mortality, is diagnosed by postpartum examination of the placenta and therefore not available for antepartum management. In the setting of PPROM, our objective was to determine if cord blood IL-6, IL-8, and granulocyte colony stimulating factor (G-CSF) are elevated in neonates with funisitis.

Study design: All PPROM patients were eligible for study enrollment. Eighty-two patients consented to daily maternal blood sampling and cord blood sampling. Aliquots of sera were frozen at −80°C. Cytokine levels were determined by ELISA (Cytokine Core Lab, MD, R&D Systems, MN) and then compared in patients with and without funisitis. A single pathologist performed placental evaluation. Data were analyzed by the Mann–Whitney *U* test (Statview, Cary, NC).

Results: Of the 82 patients enrolled in the study, 31 had cord blood samples and placental pathology. Subjects were divided into those with funisitis ($n = 9$) and those without funisitis ($n = 22$) for comparison. Between the two groups, there was no difference in race, insurance status, or marital status. However, there was a significant difference in maternal age, gestational age at delivery, and neonatal birthweight. Median cord blood IL-6 was significantly higher in those patients with funisitis compared to those without (65.6 vs. 5.3 pg/ml, $p < 0.001$). The median cord blood G-CSF (223.6 vs. 49.9 pg/ml, $p = 0.21$) and IL-8 (29.7 vs. 5.1 pg/ml, $p = 0.44$) were higher in patients with funisitis but were not statistically significant.

Conclusion: Cytokines are elevated in neonatal cord blood in PPROM patients with subsequent funisitis. Measurement of cord blood cytokines at birth may identify those neonates at highest risk of morbidity and mortality.

Cytokine profiles in response to bacterial vaginosis in pregnancy before and after treatment with oral or vaginal metronidazole

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Objective: To compare the levels of cervical cytokines (interleukin (IL)-1β, IL-6, and IL-8) in pregnant women with bacterial vaginosis (BV) before and after treatment with either systemic (oral) or local (intravaginal) metronidazole.
Study design: Seventy-four women were recruited from an antenatal clinic between December 2000 and January 2002. Inclusion criteria included pregnancy ≤ 24 weeks' gestation, BV diagnosed by gram stain, and age ≥ 18 years. Exclusion criteria included the presence of concurrent vaginal or cervical infections or bleeding. Subjects were randomized to receive oral metronidazole 500 mg bid for 7 days or intravaginal metronidazole 0.75% daily for 5 days and scheduled for follow-up 1 month later. Cervical specimens were collected for cytokine analysis and clinical signs of BV were assessed at baseline and follow-up. Statistical analysis was performed to determine whether there were differences between cytokine levels before and after treatment, and between the two groups.
Results: Of the 74 women enrolled, 36 (49%) were randomized to receive oral and 38 (51%) were randomized to receive intravaginal metronidazole. There was no significant difference between rates of gram stain cure between the two groups (72 and 71% respectively, $p = 1$). IL-8 levels did not change significantly following therapy. IL-1β and IL-6 profiles (median, in pg/ml) by treatment group and response to therapy are summarized in the table.

	Cytokine profiles					
	IL-1β			*IL-6*		
Treatment response	*Baseline*	*Return*	*p value*	*Baseline*	*Return*	*p value*
Cure						
oral	4007	1511	< 0.01	2694	1814	0.03
intravaginal	1666	841	0.03	1336	1373	0.12
Failure						
oral	1883	2387	0.37	2574	2216	0.38
intravaginal	3503	3578	0.92	2342	1148	0.57

Conclusions: There is a decrease in cervical IL-1β with cure of BV in pregnancy, irrespective of whether treatment was systemic or topical. This suggests that the increased levels of cervical IL-1β seen in women with BV are derived from the cervix rather than the upper genital tract, since topical therapy should have no effect on the upper genital tract. The lack of change in the cytokine profile among women who fail therapy compared with those who are cured suggests that IL-1β elevations are directly related to abnormal flora in pregnancy. The trends in IL-6 deserve additional study.

Vaginal inflammation and secretory leukocyte protease inhibitor among women with bacterial vaginosis in early pregnancy: response to antibiotic treatment

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Objectives: To compare the concentrations of vaginal interleukin-1β (IL-1β), IL-8 and the defense molecule secretory leukocyte protease inhibitor (SLPI) among pregnant women with and without bacterial vaginosis (BV), and to determine whether treatment of BV alters concentrations of these molecules, in the context of an ongoing randomized treatment trial for BV.

Study design: One hundred and sixty-three pregnant women at < 20 weeks had vaginal fluid collected for gram stain, culture, IL-1β, IL-8 and SLPI measurements by EIA. Fifty-nine BV+ women were randomized to oral or intravaginal metronidazole in an ongoing treatment trial (as yet unblinded), and 41 have had a second vaginal sample collected 6 weeks after treatment. Vaginal IL-1β, IL-8 and SLPI concentrations were compared between BV+ and BV– women by Mann–Whitney U test. Among BV+ women, cytokine and SLPI concentrations were compared before and after treatment with the Wilcoxon signed rank test.

Results: The median (and range) IL-1β, IL-8 and SLPI concentrations are presented in the table.

	Entire cohort			*BV+ only*		
Factor	*BV+ (n = 59)*	*BV– (n = 104)*	*p value*	*Pre-rx (n = 41)*	*Post-rx (n = 41)*	*p value*
Median IL-1β, pg/ml (range)	138 (< 1 = 3810)	24 (< 1–920)	0.001	156 (< 1–3810)	119 (< 1–1626)	0.09
Median IL-8, pg/ml (range)	2308 (23–58 345)	1074 (< 6–38 436)	0.04	3170 (23–46 207)	3472 (164–49 914)	0.52
Median SLPI, ng/ml (range)	97 (15–1599)	162 (< 12–1347)	0.002	97 (19–1599)	179 (24–2000)	0.02

Among BV+ women, African-Americans had lower SLPI concentrations before antibiotic treatment, compared with women of other racial/ethnic backgrounds. This difference did not persist after treatment.

Conclusions: In this interim analysis, BV+ women in early pregnancy have higher vaginal pro-inflammatory cytokine concentrations and lower SLPI concentrations than BV– women. Antibiotic treatment results in an increase in SLPI, however IL-1β and IL-8 concentrations remain higher than among BV– women.

A trial of intravaginal *Lactobacillus crispatus* as an adjunct to metronidazole therapy for treatment of bacterial vaginosis

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Objective: To evaluate whether colonization with a human-derived strain of H_2O_2-producing *Lactobacillus crispatus* plus metronidazole therapy results in higher resolution of bacterial vaginosis (BV) compared with women treated with metronidazole alone.

Study design: Four hundred and twenty-four women having all four clinical signs of BV plus a vaginal gram stain score > 4 (Nugent's criteria) were treated with 2 g of oral metronidazole and randomized in a double-blind manner to receive gelatin capsules containing either 10^8 *L. crispatus* CTV 05 or placebo. Women used the capsules intravaginally twice daily for 3 days monthly, and were scheduled to return at 30, 60 and 90 days. Testing for STDs was performed. The *L. crispatus* CTV 05 strain was identified using a repetitive DNA sequence polymerase chain reaction fingerprinting method. Clinical cure was defined as absence of all clinical signs of BV.

Results: Follow-up data at 30 days was available for 355 (84%) of the women. The CTV 05 strain was identified in 111 (62%) of the 180 women randomized to CTV 05 group at 30 days. The clinical cure at follow-up stratified by the treatment group and colonization is summarized in the table.

	Clinical cure by treatment group				
	Lactobacillus CTV 05				
Follow-up visit	*Colonized*	*Not colonized*	*Placebo*	*p^1 value*	*p^2 value*
30 days	78/111 (70%)	9/69 (13%)	81/172 (47%)	< 0.001	< 0.001
60 days	66/93 (71%)	10/51 (20%)	69/134 (51%)	< 0.001	0.005
90 days	49/74 (66%)	6/51 (12%)	48/114 (42%)	< 0.001	0.002

p^1 comparing CTV 05 colonized with not colonized; p^2 comparing CTV 05 colonized with placebo-treated subjects

Conclusions: Vaginal colonization with an exogenous strain of H_2O_2-producing *Lactobacillus* can be achieved in 60% of women with BV after a single course (twice daily capsule use for 3 days). Further, women who become colonized by the *Lactobacillus* CTV 05 strain were significantly more likely to have resolution of all signs of BV at follow-up. The higher than expected clinical failure for the subset of women randomized to receive *Lactobacillus crispatus* capsules who did not become colonized requires further study. This study was supported by NIH grant U01-AI-47785.

High-level vancomycin-resistant bacteria in *Lactobacillus*-containing probiotic food supplements

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Objective: *Lactobacillus* species have a long history of safe use in food products and are being evaluated for prevention of urogenital and gastrointestinal infections. Many brands of probiotics have been reported to be contaminated with undesirable bacteria including *Enterococcus spp*. Since many women report the use of probiotic strains as alternative therapies for vaginal infections, the objective of this study was to determine whether such products could act as a reservoir for vancomycin-resistant bacteria.

Study design: Eight brands of *Lactobacillus*-containing products were examined for the presence of vancomycin-resistant species of *Lactobacillus* and *Enterococcus*. In addition, 102 recent human and animal isolates were tested for vancomycin susceptibility using the agar dilution method (NCCLS).

Results: One of the eight products contained no viable bacteria. Of the seven remaining products, all had both *Lactobacillus* and *Enterococcus* species, and one had both *Enterococcus* and *Lactobacillus* having a high level vancomycin resistance (> 128 μg/ml, HLVR). Retesting each of the brands confirmed these results. A different lot of the same product was purchased, and high-level vancomycin-resistant *Enterococcus* and *Lactobacillus* were again detected. To eliminate the possibility that all brands contained low or non-detectable levels of vancomycin-resistant microorganisms, samples were plated onto blood agar containing 10 μg/ml vancomycin and examined after 24 and 48 hours of culture. Again, the same product was the only one which yielded growth, and the isolates all demonstrated high level vancomycin resistance. None of the 102 *Enterococcus* clinical isolates carried vancomycin resistance.

Conclusions: *Lactobacillus*-containing probiotics usually contain *Enterococcus* spp, and may contain HLVR organisms. The observation of HLVR in different genera from the same product suggests that the probiotic may be a reservoir for HLVR.

Leukorrhea and bacterial vaginosis as in-office predictors of cervical infection in high-risk women

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Objectives: To evaluate: (i) whether microscopic detection of leukorrhea or bacterial vaginosis identifies patients at high risk for cervical infection with *Chlamydia trachomatis* or *Neisseria gonorrhea*; and (ii) if pregnancy alters the predictive value of those findings.

Study design: Wet mount screening examination of vaginal discharge was performed on consecutive new patients seen at two resident-staffed clinics serving primarily indigent women. Leukorrhea was defined as more than ten white blood cells per high-power field on microscopic examination; Amsel's criteria were used to determine the presence of bacterial vaginosis, with a positive clue-cell test result defined as > 20% of epithelial cells. Cervical *C. trachomatis* and *N. gonorrhea* infection were established by DNA amplification tests.

Results: The study population consisted of 194 women, 118 (61%) of whom were pregnant. Overall, 11% of women had positive cultures for gonorrhea or chlamydia. Both leukorrhea (relative risk (RR) = 123.7; confidence interval (CI) 17.3–884.2) and clue cells (RR = 5.9; CI 2.8–12.3) were associated with positive cervical cultures in a univariate analysis. However, multivariable analysis found that clue cells did not contribute to the predictive value of leukorrhea alone for cervical infection, among both pregnant (RR = 58.7; CI 8.3–413.2) and non-pregnant (RR = 15.7; CI 7.7–32.2) women. Negative predictive values for the screening tests were comparably high (98–100%), independent of pregnancy status, while the positive predictive value of the tests were slightly lower among pregnant women (60%) compared with those who were not pregnant (92%).

Conclusions: Leukorrhea, in the presence or absence of bacterial vaginosis, was strongly associated with cervical infections with *C. trachomatis* or *N. gonorrhea* among both pregnant and non-pregnant patients. In settings where patient follow-up is uncertain, on-site screening tests identify women for whom empiric 'diagnose-and-treat' antibiotic therapy for sexually transmitted diseases may be appropriate.

What predicts STD positivity among female contacts of men with STDs?

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Objectives: To identify risk factors for STD positivity among women reporting sexual exposure to men with STDs.
Study design: Two hundred and twenty-seven non-pregnant women ages 15–30 seeking care at ambulatory sites in Pittsburgh who reported recent sexual contact with a male partner diagnosed with gonorrhea (GC), chlamydia (CT), or nongonococcal urethritis. All women underwent a comprehensive history, physical examination, and microbiologic evaluation of the vagina and cervix. Bacterial vaginosis (BV) was diagnosed by the Nugent criteria on gram-stained vaginal smears, and cervical samples were obtained for culture for GC and PCR for CT.
Results: Among 227 women enrolled, 81 (36%) were infected with either GC or CT. BV was identified in 104 participants (46%). Logistic regression analysis of factors associated with testing positive for GC or CT on univariate analysis is shown in the table.

	n	*Number infected with GC or CT*	*Odds ratio (95% CI)*
Bacterial vaginosis	104	52	3 (1.6, 5.4)
Age < 21	108	46	1.9 (1.1, 3.4)
African-American	134	55	1.7 (0.9, 3.1)
More than two partners in prior 3 months	71	33	1.6 (0.8, 3)

CI, confidence interval

Conclusions: Among female contacts of men with STDs, BV is strongly associated with testing positive for GC or CT. Our study suggests that the presence of BV increases the likelihood of STD acquisition and reinforces the importance of a normal vaginal ecosystem as a defense against cervical infection. This study was supported by NIH grant 5-R01-AI-41624.

Cellular immune responses to common subtypes of HPV increase with dysplastic cervical change

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Objectives: Human papillomavirus (HPV) causes a spectrum of cervical lesions ranging from condyloma to invasive cancer[1]. Immune surveillance is important in host defense against HPV induced pathology[2]. We hypothesize that individuals display a spectrum of cellular immune responses to HPV that can be quantified and correlated with severity of dysplastic transformation.

Study design: Whole blood was obtained from 19 women referred for abnormal screening cytology. Isolated peripheral mononuclear cells from each patient were exposed to pools of synthetic peptides representing the E6/E7 regions of HPV6 and HPV18 and the entire sequence of HPV16 in a stimulation assay developed in our lab. Responses were detected using surface and intracellular cytokine staining and flow cytometry, and results were quantified with FACScan Cyclops software. Dysplasia diagnosis was determined by colposcopy-guided biopsy. A multiple logistic regression model was constructed by stratifying results according to peptide pool and dysplasia grade.

Results: Significant responses occurred in 44% of women with high-grade dysplasia versus 10% of women with low-grade or benign findings. Only the early genes of HPV 6 and 16 induced a significant response with the strongest correlation between response and dysplasia severity observed with peptides spanning HPV16 E1 and E7 (spearman correlation coefficient, SCC = 0.779, $p = 0.0001$).

Conclusions: This study is the most comprehensive measurement of cellular immune responses to HPV 6, 16, and 18 as it relates to cervical dysplasia. The association of positive responses to HPV 16 early genes, particularly E7, in women with severe cervical dysplasia reflects the role of this virus in cervical carcinogenesis. Current strategies of vaccination focus on this peptide given its superior ability to induce an immune response. The implications of these results and the potential diagnostic role of this assay are the subject of future research.

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The effect of long-term depomedroxyprogesterone (DMPA) use in women on vaginal epithelium and immune cells

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Introduction: DMPA use is associated with the acquisition of human immunodeficiency virus (HIV) in humans and simian immunodeficiency virus (SIV) in macaques. Progesterone use in monkeys caused profound thinning of the vaginal epithelium and increased SIV transmission[1]. Previously, we reported that DMPA use for 6 months caused a minor decrease in glycogen-positive vaginal epithelial thickness in women[2].

Objective: To examine the vaginal epithelium for thickness, glycogen content, and subepithelial immune cells before and after 1–4 years of DMPA (150 mg) use.

Study design: Vaginal biopsies were taken prior to and at three-month intervals of DMPA use. Histopathology was assessed and immune cell populations identified by immunocytochemistry. Linear regression for correlated data compared the results of the initial and annual visits for 31 women followed for 1 year, seven for 2 years, eight for 3 years, and seven followed for 4 or more years.

Results: The mean number of epithelial cell layers, the total epithelial thickness, and the glycogen-positive epithelial thickness did not change significantly over time. Intensely stained glycogen-positive thickness increased significantly after 4 years of DMPA use ($p = 0.04$). The mean number of CD4 and CD8 lymphocytes, macrophages, granulocytes and Langerhan's cells did not vary significantly by years of DMPA use.

Vaginal epithelium	*Baseline*	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*p*
Mean number of cell layers	27.3 ± 0.7	28.2 ± 0.9	24.6 ± 0.5	26.5 ± 1.4	23.2 ± 0.7	0.42
Total thickness (mm)	1.04 ± 0.04	1 ± 0.05	0.99 ± 0.09	1.04 ± 0.08	0.87 ± 0.1	0.7
Glycogen-positive thickness (mm)	0.79 ± 0.05	0.76 ± 0.05	0.74 ± 0.07	0.66 ± 0.06	0.67 ± 0.1	0.73
Intensely stained glycogen-positive thickness (mm)	0.37 ± 0.04	0.52 ± 0.05	0.51 ± 0.07	0.46 ± 0.08	0.56 ± 0.1	0.04
Cell population (mean/5hpf)						
CD4+	4 ± 0.8	1.6 ± 0.2	2.1 ± 0.5	5.1 ± 1.1	1.1 ± 0.3	0.21
CD8+	13.3 ± 1.8	13 ± 1.4	14.9 ± 3.0	16.4 ± 2.8	10.6 ± 2.3	0.7
Macrophages	4 ± 0.6	3.2 ± 0.3	3.1 ± 0.5	4.5 ± 0.5	3.3 ± 0.7	0.08
Langerhan's cells	2.5 ± 0.6	0.4 ± 0.2	1.1 ± 0.6	3 ± 1.5	2.6 ± 1	0.09
Granulocytes	1 ± 0.3	0.6 ± 0.1	0.4 ± 0.2	0.6 ± 0.3	0.9 ± 0.4	0.11

Conclusion: Long-term DMPA use does not significantly impact vaginal epithelial thickness or immune cell populations in women. Supported by R01HD 33203, NICHD Contraceptive Development Branch.

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Prevalence of yeast colonization and its value in the prediction of clinical outcomes

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Objectives: To determine the prevalence of yeast colonization among reproductive age females in a prospective longitudinal manner and correlate that colonization with use of yeast medications.

Study design: A cohort of 1248 asymptomatic 18–30 year old women was enrolled with baseline demographic data. Vaginal yeast was detected by agar plate and broth enrichment methods. Follow-up occurred at 4-month intervals (0, 4, 8 and 12 months) for an entire year.

Results: Complete data for all four visits was available for 709 women (57%). Yeast colonization at each visit ranged from 28–33%. Over the year of follow-up, 496 (70%) of the women were colonized by vaginal yeast at one or more visits, but only 26 (3.7%) were colonized at all four visits. Evaluating only agar plate positive women, 267 (38%) were colonized at one or more visits over a year follow-up. The reported rates of pruritis and use of vaginal yeast medications by women is summarized in the table.

		Cumulative rates over 12 months	
Colonization pattern for vaginal yeast	*n*	*Pruritus (%)*	*Yeast medication use (%)*
Persistent positive	26	58	35
Intermittent/transient	470	45	23
Persistent negative	213	37	24
p-value		< 0.01	0.8

Conclusions: Yeast colonization and yeast medication use are common. However, one in four women who were persistently negative for yeast over the year follow-up reported use of yeast medication, similar to the incidence of yeast medication use among women colonized by yeast. The frequent reporting of vaginal medication use by women having no evidence of yeast suggests that misdiagnosis and unnecessary treatment for yeast vulvovaginitis is very common. Supported by The Streptococcal Initiative: NIH/N01-AI 75326.

Treatment of vulvovaginitis due to *Candida glabrata*: efficacy of boric acid and flucytosine

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Objective: To determine optimal therapy for recurrent *Candida glabrata* (CG) vaginitis in women failing azole therapy.

Study design: Retrospective chart review of 141 symptomatic patients with CG infection seen at WSU Vaginitis Clinic ($n = 102$) and in Beersheva, Israel ($n = 39$) between 1992 and 2001.

Results: Of 102 patients in Detroit, 23% were African-American and 76% white; median age was 41 and only five were diabetics; 20% had associated gynecological disease, for example lichen sclerosus; saline microscopy was positive for budding yeast in 89.2%. Boric acid (BA) 600 mg/day for 14 or 21 days achieved cure in 47/73 episodes of vulvovaginitis (VVC) (64.4%). Cure rates marginally improved by addition of maintenance nystatin suppositories. Boric acid achieved similar clinical and mycological response in Israel (70.3%). Topical flucytosine therapy in Detroit achieved cure in 27/30 episodes (90%), all previous boric acid failures. Adverse reactions were rare.

Conclusion: In the absence of prospective data or a randomized controlled trial, this large retrospective study confirms the usefulness of boric acid and high cure rates accompanying flucytosine therapy.

Experimental intrauterine infection with *Prevotella bivia* (Pbiv)

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Objective: Subclinical intrauterine infection is implicated in preterm birth (PTB). We sought to develop a model of chronic intrauterine infection with Pbiv, an anaerobe of the lower genital tract.

Study design: To date, 22 timed pregnant New Zealand White rabbits were inoculated transcervically, 6 cm into each uterine horn on day 21 (70%) gestation with 10^{5-8} cfu of Pbiv ($n = 17$) or saline ($n = 5$). Animals underwent necropsy on days 4, 6, or 7. Cultures were taken from doe and fetus. Amniotic fluid (AF) was assayed for TNF-α by bioassay. Tissue from placenta, uterus, fetal brain and lung were evaluated using histologic inflammation (HI) scores (Davies 2000). Categoric data were compared by the Fisher exact test; continuous data by the Wilcoxon Rank Sum.

Results: Of three animals inoculated with 10^8 cfu/uterine horn, two (67%) developed fever ($T \geq 104°F$) in 24 hours. Because the inoculum was not compatible with chronic, subclinical infection, we then employed 10^{5-6}/horn. Among 14 does with 10^{5-6}cfu inoculum, cultures were positive in uterus in nine (64%), AF in eight (57%), blood in one (7%), and fetus in six (43%). Compared with five saline controls, differences were significant ($p < 0.04$) in AF and uterus. Main outcomes are shown in Figure 1; TNF-α levels in Figure 2. Compared with control animals, those with Pbiv had higher HI scores in the placenta ($p < 0.04$) but scores were very mild. There were no differences in HI scores for decidua or for fetal lung.

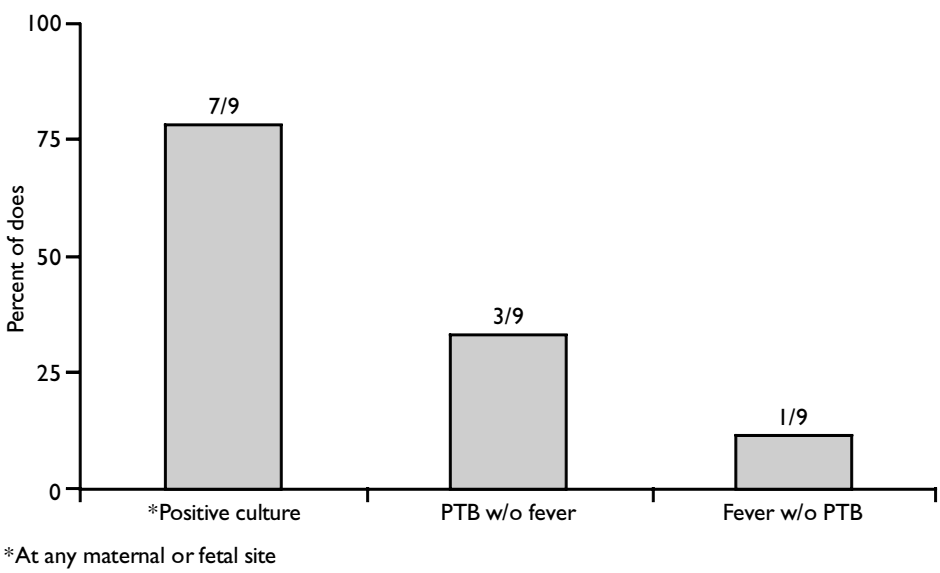

Figure 1 Outcomes in nine does inoculated with 10^{5-6} *P. bivia* and observed for 6–7 days

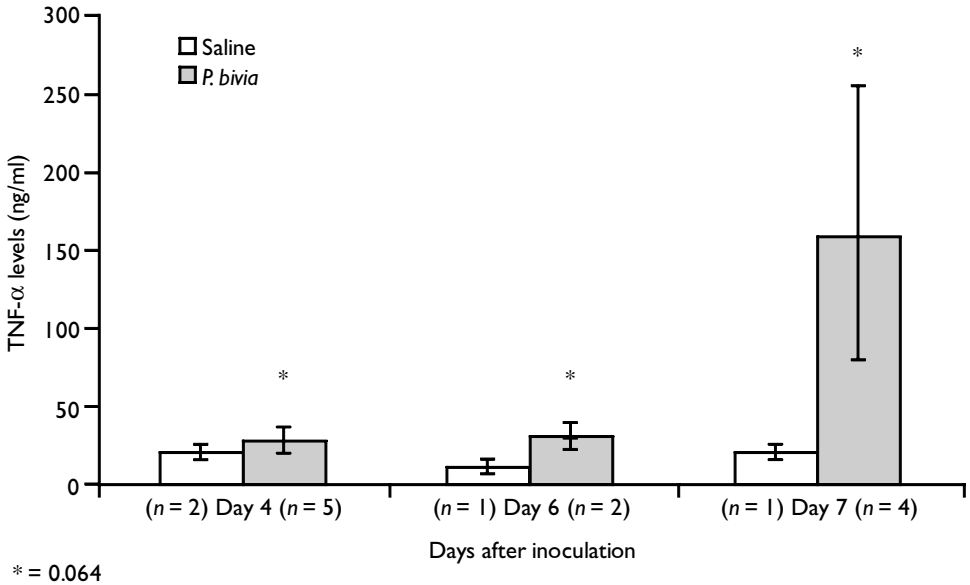

Figure 2 TNF-α levels in AF of rabbits inoculated with *P. bivia* or saline

Conclusions: Inoculation with Pbiv (at 10^{5-6}/horn) leads to chronic, intrauterine and fetal infection accompanied by PTB in three of 14 (21%). This model may serve to explore infection-induced spontaneous PTB in humans. Support: March of Dimes #6-FY99-308.

A rabbit model of chronic anaerobic infection during pregnancy

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Objective: The objective of this study was to develop an animal model of chronic infection with *Mobiluncus curtisii*, a vaginal anaerobe associated with bacterial vaginosis, and *Porphyromonas gingivalis*, an oral anaerobe associated with periodontal disease.

Study design: Surgical steel chambers were aseptically implanted subcutaneously on the dorsal aspect of 12 reproductive-aged New Zealand female rabbits. The animals were then immunized through the chamber with 10^9 cfu heat-killed *M. curtisii* (six) or *P. gingivalis* (six). Twenty-one days later animals were inoculated with 10^9 cfu of live *M. curtisii* or 10^7 live *P. gingivalis*. Adult serum was collected prior to immunization, prior to inoculation with live organism (day 0) and on days 1, 5, 10, and 15. A subset of animals was bred 7 days following immunization with heat-killed organisms; date of live inoculation corresponded to the time of implantation in the rabbit. Animals were sacrificed 15–22 days following inoculation with live organisms. Adult liver, fetal liver and spleen, and placentas were collected, cultured and then flash frozen for PCR. Serum was analyzed for antibody to *M. curtisii* or *P. gingivalis* by immunoblot, and IL-1b, PGF_{2a}, and PGE_2 by ELISA. PCR primers for the 16S rRNA gene were used: *M. curtisii*: 5′GCCAGCCTTCGGGTGGTGT3′ and 5′TAGCCCACCTCACGGTATC3′; *P. gingivalis*: 5′AGGCAGCTTGCCATACTGCG3′ and 5′ACTGTTAGCAACTACCGATGT3′.

Results: All animals tolerated implant placement, and none showed signs of systemic illness. All animals that were bred become pregnant. Minimal increases in serum mediators were noted (see table).

	Pre-immunization	*Pre-infection (day 0)*	*Day 1*	*Day 7*
PGE_2 (pg)	23.6 ± 18.9	32.9 ± 17.1	12.4 ± 6.4	19.6 ± 10.6
PGF_{2a} (pg/ml)	37.5 ± 3.4	43.5 ± 11.1	40.9 ± 24.4	41.2 ± 10.6
IL-1β (pg/ml)	0.78 ± 0.96	1.4 ± 1.6	1.1 ± 1.4	1.5 ± 1.9

Anti-*M. curtisii* IgG and *P. gingivalis* were detected in all adult rabbits following immunization

Conclusion: The lack of significant systemic inflammation but demonstration of a systemic humoral response in the pregnant dam makes this a unique model to study the effects of chronic anaerobic infection during pregnancy.
Supported by NIH grant HD01441.

Allelic variation in the interleukin-1 receptor antagonist (IL-1ra) gene influences pregnancy outcome and circulating HIV concentrations

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Objectives: IL-1ra is a major down-regulator of inflammation. The gene coding for IL-1ra is polymorphic and individuals possessing allele 2 (IL1RN*2) generally are less able to limit pro-inflammatory immune activity than are individuals with other alleles. We hypothesized that possession of IL1RN*2 might be beneficial for immune defense against infection but detrimental to pregnancy outcome.

Study design: Subjects included 86 HIV-seropositive women and 82 pregnant Hispanic women and their babies. DNA was extracted from buccal or vaginal cells and analyzed for IL-1ra alleles by polymerase chain reaction. HIV-1 RNA in plasma were determined by the Amplicor Monitor Assay and circulating CD4 levels were determined by flow cytometry.

Results: Among the HIV-infected women, 28% had one or two copies of IL1RN*2, while 57% were homozygous for IL1RN*1. There was no relation between IL-1ra genotypes and CD4 levels, indicating that all women were at similar stages of their disease. However, women homozygous for IL1RN*2 had a median of 469 copies/ml of circulating HIV RNA as compared with 2050 for IL1RN*1/IL1RN*2 heterozygotes and 7900 for IL1RN*1 homozygotes ($p = 0.04$). Among the pregnant women, 39% of mothers and 39% of babies were IL1RN*2 positive. There was no relation between maternal carriage of this allele and pregnancy outcome. However, 26.4% of mothers whose fetuses had the IL1RN*2 allele had a term birth as opposed to 57.9% with a preterm birth and 70% with preterm premature rupture of membranes ($p = 0.02$).

Conclusions: Possession of IL1RN*2 may increase immune defense against HIV infection but fetal carriage of this allele may increase susceptibility to preterm birth.

Morbidity of spontaneous and elective abortions in an HIV-infected indigent population

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Objective: There is increased morbidity associated with surgical procedures and childbirth in the HIV infected woman. However there is limited literature on abortion complications in these women. Our objective was to determine the morbidity associated with spontaneous and elective abortions in an indigent HIV-infected population.

Study design: Retrospective chart and database review of all HIV-infected women whose pregnancy terminated in either elective or spontaneous abortion, requiring surgical intervention, from January 1, 1993 to December 31, 2000. Demographic characteristics, early pregnancy information, HIV status including CD4 count, viral load and medication usage, and abortion complications were reviewed.

Results: Sixty-three HIV-infected women were identified who underwent a surgical procedure for abortion in the first half of pregnancy. Twenty-four (38%) women underwent D&C for spontaneous or missed abortion, 36 (57%) had an elective procedure (including two women who underwent hysterectomy for cervical dysplasia) and three (5%) had an exploratory laparoscopy for an ectopic pregnancy. Nineteen (30%) women had a BTL performed at the time of the surgical procedure. The mean age of the study cohort was 26.2 $\pm$ 5.8. The majority of women were African-American [40 (63%)] or Hispanic [14 (22%)]. The mean CD4 count was 473.8 $\pm$ 305.7; 11 (17%) of these women had a CD4 count below 200. The mean viral load was 25 159 $\pm$ 46 637 copies/ml. Surgical complications included infection in six (10%) women, retained placenta requiring repeat curettage in two and uterine perforation, alveolar hemorrhage and postoperative hemorrhage each in one woman. The overall complication rate was 17%. Of the six women with infectious complications, three had AIDS and only one was receiving antiretroviral therapy.

Conclusions: This study revealed that nearly one in five women undergoing a surgical procedure for termination or spontaneous abortion suffered significant morbidity. The observed infectious complication rate of 10% is ten-fold higher than that found in our HIV-negative population (< 1%).

State laws regarding prenatal syphilis screening in the United States

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Objective: To assess the frequency and pattern of state laws or regulations regarding prenatal syphilis serologic screening in the United States in 2001.

Study design: Fifty states and the District of Columbia were surveyed for existing laws and regulations regarding serologic screening for syphilis during pregnancy and at delivery. Information was obtained through a combination of Internet searches of state legislative web sites and correspondence with health departments. Testing was categorized according to the presence, frequency, and timing of screening requirements. Testing was compared with 2000 state rates of syphilis in women and newborns, states that had syphilis high morbidity areas, and national 2000 and 2010 objectives for rates of syphilis.

Results: Forty-six states (90%) have laws regarding antenatal syphilis screening. Thirty-four of the 46 statutes (76%) mandate one prenatal test, usually at the first prenatal visit or early in pregnancy. Twelve laws (26%) include third trimester testing for all or high-risk women. Three states also require maternal or cord blood screening at delivery. Presence of high morbidity areas, incidence of early syphilis in women and rates of congenital syphilis are associated with increasing frequency of antepartum syphilis screening.

Conclusion: Only 90% of states have statutes requiring antepartum syphilis screening, and there is variation in the content about the number and timing of tests. States with a heavy burden of infectious syphilis in women tend to require more prenatal testing.

Infecting serovars of *Chlamydia trachomatis* during 1988–96

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Objective: To evaluate relationships between age, serovar type, and temporal trends in females with *Chlamydia trachomatis* (Ct) infections over a 9-year period.

Methods: All females ($n = 7096$) presenting to Seattle King County Health Department Sexually Transmitted Disease clinics with first time Ct infection between 1988 and 1996 were included. We used a multiple regression model to compare age, date and Ct serovar type, determined by low-passage microtiter plate culture method with immunotyping utilizing 17 serovar-specific monoclonal antibodies, from these cultures.

Results: Serovar types E (32%) and F (18%) were the most prevalent and B (1.4%) and I (0.5%) the least prevalent. Over the 9-year period among Ct positive females, the percentage of infections which were serovar types F and G increased ($p = 0.007$, 0.009), serovar types I and K decreased ($p < 0.001$, $p = 0.008$) and serovars B, D, D-, E, H, Ia, J remained stable. The percentage of infections in girls aged 10–15 increased ($p < 0.001$), and more commonly were B class or mixed serovar types, with fewer C class serovar infections ($p < 0.001$).

Conclusions: In this population, major serovar types appeared stable over time. Greater fluctuation occurred in minor serovars, and age was associated with serovar type. The stability of major serovar types support the potential utility of a Ct vaccine.

HPV infection and abnormal cytology in a high-risk adolescent population

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Background: Susceptibility to HPV and its complications, such as cervical dysplasia, may be increased when initial exposure occurs at a young age.

Objective: To determine prevalence of abnormal cytology and HPV, and risk factors for HPV in adolescents.

Methods: Sexually active adolescent females (12–19 years) attending a primary care clinic were enrolled. Behavioral data were obtained by interviews. Exfoliated cervical cells were collected and placed in PreservCyt media for ThinPrep Pap test (Cytyc Corp). Material remaining was tested for HPV using PCR and typed using a reverse line blot assay (Roche Diagnostics).

Results: The study population ($n = 313$) was predominantly African-American (96%), median age 16.9 years (range, 12.8–19.9), with recent onset of sexual activity (2.3 median years). The median number of sex partners was four (range 1–50) with maximum age difference of partners ranging from 1–43 years (median 3). Overall, 20% had ASCUS, 15% LSIL, and 1% HSIL. HPV prevalence was 64% (201). Among those with HPV, 101 (50%) had multiple types (range 2–7 types). Adolescents with abnormal cytology had higher prevalence of HPV (54.4% in normal, 66.7% in ASCUS and 91% in LSIL, $p = 0.001$) and higher prevalence of multiple types (41% in normal, 45% in ASCUS, 74% in LSIL, $p = 0.002$). HPV was associated with marijuana use, current douching, sex abuse, greater number of sex partners, greater years of sexual activity, and age difference of sex partner. In multivariate analyses, number of sex partners (2–3, OR = 2.9 [95% CI 1.4–6.2]; 4–7, OR = 4.1 [95% CI 2–8.7]; ≥ 8, OR = 6.5 [95% CI 2.6–16]), current douching (OR = 2.2 [1.3–3.8]), and age difference of partner(s) (≥ 1.5 years OR = 2.2 [1.3–3.7]) remained significantly associated with HPV.

Conclusions: HPV infection, infection with multiple types, and abnormal cytology were common in this population. In addition to previously described risk factors, douching was a significant risk factor for HPV.

Implications for programs: Understanding of HPV epidemiology will be important for programs as HPV prevention interventions develop.

Implications for research: Prospective follow-up of this population is needed to evaluate factors associated with HPV incidence and persistence.

Measurable learning objectives: By the end of the session, participants should be able to describe risk factors for HPV infection in adolescents.

Vulvovaginal trichosporonosis: a case series to determine the pathogenic role of *Trichosporon* species

P. Makela, J. D. Sobel and D. Leaman

Objective: A case series to determine the pathogenic role of *Trichosporon* spp. in vulvovaginal infections.
Study design: Isolation of *Trichosporon* spp. from vaginal secretions is a rare event with no available data as to its pathogenic role. We performed a retrospective chart review of patients seen in the W.S.U. Vaginitis Clinic to identify patients from whom *Trichosporon* spp. were isolated.
Results: Between 1986 and 2001, 13 patients had positive vaginal cultures for *Trichosporon* spp. Notably 11/13 (85%) of patients were African-American with a mean age of 32 years. All 13 vaginal isolates were *T.inkin*. *Candida albicans* was present in mixed cultures with *T.inkin* in 3/13 vaginal isolates and consecutively in two patients. In only one of six symptomatic patients could a clear pathogenic (cause-effect) role be established because of co-existence of concomitant pathogens: *C.albicans* (three), HSV (one), *T.vaginalis* (one). This patient resolved symptoms and positive cultures with boric acid therapy. Microscopy was rarely yeast positive and could not determine a pathogenic role. In general positive vaginal cultures were accompanied by low colony counts. *In vitro* susceptibility tests revealed resistance to flucytosine and susceptibility to all topical and oral azoles. Notably eight of 11 patients had simultaneous or recent bacterial vaginosis and in one concomitant trichomonas was present.
Conclusion: *T.inkin* selectively is occasionally found in vulvovaginal cultures and usually is a non-pathogen. Transient colonization tended to occur in women with major perturbations in vaginal flora (bacterial vaginosis and trichomonas) with increased pH. Pathogenic consequences of *Trichosporon* spp. colonization appear extremely rare.

Neurocysticercosis complicating pregnancy

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Objective: Neurocysticercosis is a well-known cause of seizure disorder in women from endemic areas. However, neurocysticercosis complicating pregnancy is poorly understood. Our objective was to review the presenting symptoms of neurocysticercosis in pregnant women and to evaluate diagnostic modalities and treatment options.

Study design: Retrospective chart review of pregnant women with neurocysticercosis diagnosed peripartum at a large indigent hospital. Data regarding patient demographics, travel/residence history, presentation, diagnosis, treatment, pregnancy outcome, and long-term follow-up were recorded.

Results: Fifteen women were identified. The mean age was 24.1 ± 5.8 years. Seven (48%) were nulliparous. All women were Hispanic, and 80% were born in or had traveled to Mexico. The most common presentation was seizures, occurring in 80% of patients. Five women (3%) presented with headache, and one each with syncope, nausea/vomiting, dizziness, and papilledema. Nine (60%) were diagnosed during pregnancy, two (13%) women prior to pregnancy, and four (27%) postpartum (within 5 days of delivery). The diagnosis was made by either CT or MRI. Six women had serum antibody titers; all were non-reactive. In addition, eight patients had electroencephalograms (EEG) performed; all were normal. Forty-three percent of the women had a single lesion on CT/MRI, while 57% had multiple lesions. Nine patients (60%) had calcified lesions, and five (33%) had edema surrounding a lesion. Eleven women (73%) received anti-helminthic therapy, five (33%) were administered steroids, and six (40%) were placed on anti-epileptic medications. Two women underwent surgical management. There were no adverse neonatal outcomes. Long-term follow-up ranging from 3 months to 3 years was available in five women; 80% of these women showed improvement in imaging studies, and one remained unchanged.

Conclusions: Neurocysticercosis should be considered in any pregnant patient presenting with unexplained seizures. Neuroradiographic imaging studies are invaluable in the diagnosis, with little utility in serum testing or EEG. The optimal treatment regimen has yet to be determined.

Production of a bacteriocin-like inhibitor by *Enterococcus faecium* strain 62-6 antagonistic to the growth of vaginal lactobacilli: potential significance to the development of bacterial vaginosis

M. C. Kelly and V. Pybus

Objectives: To determine whether bacteria with the potential of invading and/or establishing themselves as part of the vaginal microflora are able to produce bacteriocin-like inhibitors antagonistic to the growth of vaginal lactobacilli.

Study design: The vaginal syndrome bacterial vaginosis (BV) is characterized by an alteration in the vaginal microflora such that *Lactobacillus* populations that are generally dominant in healthy women decline in concentration and are replaced by *Gardnerella vaginalis*, obligate anaerobes, and genital mycoplasmas. At present, mechanisms which could account for the decline in *Lactobacillus* populations are not well understood. The production of bacteriocins antagonistic to the growth of vaginal lactobacilli is one unexplored mechanism which could account for this. Seventy-two vaginal isolates of streptococci/enterococci were tested for bacteriocin production against 32 strains of vaginal lactobacilli, using the deferred antagonism technique on blood agar.

Results: *Enterococcus faecium* strain 62-6 exhibited strong, reproducible inhibitory activity against vaginal lactobacilli. Results from characterization experiments suggested that the inhibitor(s) has: a spectrum of inhibitory activity limited to Gram-positive bacteria, an essential proteinacous component being sensitive to pepsin but not trypsin, α-chymotrypsin or proteinase K, is retained on a membrane filter of pore size 0.22 μm, is heat stable (100°C for 30 minutes), and is stable following exposure to acid conditions (pH 4–7 for 30 minutes). The observed inhibitory effect against the vaginal lactobacilli was shown to be independent of blood-breakdown products, acid or H_2O_2 production.

Conclusions: These properties are consistent with the production of a bacteriocin-like substance by *Enterococcus faecium* 62-6. If the strain 62-6 inhibitor is a true bacteriocin and the producer strain is able to invade and/or establish itself in the vagina, it may be one mechanism contributing to the decline of the lactobacilli thus potentially paving the way for the establishment of a BV-associated microflora.

Ion mobility spectrometry (IMS) for diagnostic assessment in bacterial vaginosis

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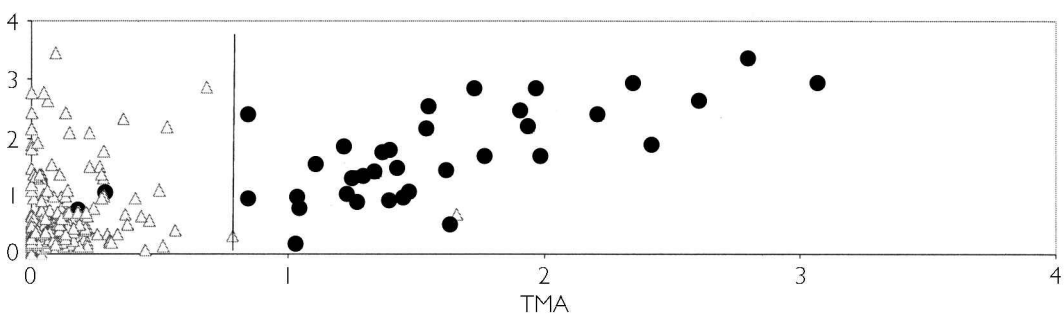
Objective: Determination of trimethylamine (TMA), putrescine, and cadaverine levels in vaginal fluid by IMS has shown to be of value in the diagnosis of bacterial vaginosis (BV).

Study design: This new method was used in our vaginitis clinic for diagnosis and follow-up of patients, and compared with the traditional Amsel test. During the trial period 293 samples corresponding to 190 patients were examined. Patients diagnosed clinically as BV positive were treated and followed-up. At every visit a vaginal discharge sample was tested by IMS as well.

Results: TMA was identified by IMS in 35 of 37 samples, diagnosed as BV positive (dots in the graph). Two samples were false negative, with high pH, positive whiff test but very low TMA levels, excluding BV. Triangles in the graph represent samples diagnosed as BV negative. One otherwise healthy patient, examined at four visits with clinically negative results, showed TMA once (1/256 false positive). Our results represent a false positive index of 0.4%, a false negative index of 5.4% (positive predictive value of 94.6%, negative predictive value of 99.6%, and a diagnostic accuracy of 94.2%).

Conclusions: The results obtained with this diagnostic method show a very high accuracy. Furthermore, the level of TMA becomes an index of severity of the infection and very low levels of TMA in vaginal discharge, even in presence of high pH, positive whiff test and low percentage of clue cells excludes a diagnosis of BV. The IMS diagnostic procedure is technically very simple and rapid, requiring no more than 2 minutes of time and no special technical skills.



Assessing vaginal fluid sialidase in women with bacterial vaginosis

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Objective: Bacterial vaginosis (BV) is a common cause of abnormal vaginal micro-ecology, which is associated with increased vaginal fluid sialidase (neurominidase) activity. We assessed test performance of BV Blue™, a FDA-approved, 'point of care' of colorgenic test of neuramidase activity as a means to diagnose BV in unselected, healthy women.

Study design: One hundred and eighteen women who presented for routine gynecologic or obstetric care in Birmingham, AL or Denver, CO were systematically evaluated for vaginal fluid sialidase activity using BV Blue™ (Gryphus Diagnostics, Birmingham, AL) and Nugent's Gram stain score (7–10, BV), as well as Amsell's and other test criteria.

Results: BV Blue™ was easy-to-perform and gave rapid, unambiguous findings. Baysian performance characteristics were calculated using Nugent's score 7–10 as 'gold standard'.

	Sensitivity	*Specificity*	*PPV*	*NPV*
BV Blue™	90%	97%	90%	97%
Clinical criteria	58%	95%	82%	86%
pH	90%	66%	48%	95%

PPV, positive predictive value; NPV, negative predictive value

Conclusions: BV Blue™ is an easy-to-perform, unambiguous 'point of care' test for sialidase that can be performed in an office or local laboratory. In these populations, BV Blue™ performed comparably to Gram stain criteria or clinical criteria for diagnosis of BV. In specified populations, BV Blue™ sialidase testing may prove superior to clinically inconvenient Gram stain scoring or more subjective clinical testing/screening.

Vaginal fluid concentration of sialidase (neuronminidase): correlation with common vaginal conditions

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Background: Measurement of vaginal fluid sialidase (neurominidase) is a potentially useful means of diagnosis of bacterial vaginosis (BV) or other causes of altered vaginal micro-ecology.
Objective: Measure and compare bacterial sialidase activity in unselected women with common vaginal micro-ecologic conditions.
Study design: One hundred and eighteen (118) unselected gynecologic and obstetrical patients in two study sites (Birmingham, AL and Denver, CO) were evaluated for vaginal fluid neurominidase activity characterized by colometric text (BV Blue™, Gryphus Diagnostics, Birmingham, AL). Histories were obtained for vaginal symptoms as well as possible interfering substances including blood, menses, semen, contraceptive substances. Multiple microbial tests were performed: aerobic and anaerobic semi-quantitative cultures, Nugent's scoring of Gram stain, pH, amine testing, as well as clinical and wet prep microscopic examinations.
Results: No evidence of interference with vaginal substances was noted. Mean vaginal fluid sialidase activities are shown in the table.

	Healthy	*BV*	*Yeast*	*Trichomoniasis*
Units x	2.7	12.3	3.7	1.99
95% CI	2.2–3.2	8.1–16.6	2.6–4.8	0.6–3.4

Conclusions: (i) In this multi-center study, some level of neurominidase activity was found even in normal/healthy subjects; (ii) BV was associated with distinctively increased sialidase activity; and (iii) these findings should be confirmed in larger trials.

Clinical aspects of infections with virus-bearing and virus-negative *Trichomonas vaginalis* in women

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Objectives: To identify the presence of a previously described double-stranded RNA virus in *Trichomonas vaginalis* (TV) organisms cultured from minority women, and to correlate the presence or absence of virus with clinical symptomatology.

Study design: African- and Mexican-American women with STDs were enrolled in long-term follow-up of a randomized trial of a behavioral-cognitive intervention to reduce STD recurrence. At annual screening visits and interval visits triggered by pregnancy or symptoms, each woman underwent testing for STDs including a culture for TV utilizing the In-Pouch system. At annual visits extensive symptom data was acquired as well (more limited data was acquired at interval visits). Testing TV for dsRNA virus was performed by RNA extraction, followed by gel electrophoresis utilizing positive and negative controls.

Results: To date, 137 positive TV cultures have been obtained from 120 women. Thus far, dsRNA virus has been present in 56 isolates (57% of those tested) and absent in 42 isolates (43%). Ten isolates died prior to testing and 29 are awaiting electrophoresis. Overall, the dsRNA virus was present in 41% of TV isolates from African-American women as compared to 63% from Mexican-American women ($p = 0.067$). Rates of dsRNA virus presence did not differ by age in this cohort. Full symptom and virus data was available for 65 women (two-thirds of the tested isolates). There were no differences detected in occurrence or severity of vaginal discharge, dyspareunia, abdominal pain, or urinary symptoms based on presence or absence of virus. There was also no correlation noted between virus presence and the presence of other STD infections. Additionally, TV isolates from two male partners have been tested thus far, both with dsRNA virus absent which matched their female partner's isolate.

Conclusions: Presence of dsRNA virus is common among minority women with TV, but does not seem to correlate with type or severity of clinical symptoms.

Bacterial vaginosis in a high-risk population is not associated with a shortened cervical length

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Objective: It is well established that both shortened cervical length (CL) and bacterial vaginosis (BV) are independently associated with an increased risk of preterm birth. We sought to investigate whether CL is affected by vaginal flora.

Study design: Forty-five women at high risk for preterm birth ranging from 15.4–34 weeks' gestational age (mean 23.5 weeks) were enrolled in a prospective cohort study. Criteria for enrollment included high-risk status secondary to a prior history of preterm birth, multiple gestation, or uterine anomalies. Women with cerclage were excluded from this analysis. Transvaginal cervical ultrasound was performed at the initial study visit, along with collection of vaginal swabs for BV gram stain. Serial transvaginal ultrasounds were performed on a weekly to biweekly basis for the remainder of gestation. Multivariable linear regression, Fisher's exact test, and the Mann–Whitney *U* Test were employed as appropriate to evaluate the relationship between CL and BV.

Results: The overall prevalence of BV in this population was 8.9% (4/45), while 22% (10/45) had an intermediate gram stain. CL on initial visit as well as minimal CL overall for the entire pregnancy were recorded. Neither CL at time of initial sampling for BV, nor minimal CL over serial examinations were independently associated with BV status. Minimal CL was predicted significantly by initial CL ($p = 0.004$), but not by dynamic cervical change at a single visit, multiple gestation, or BV status.

Conclusion: In this high-risk population with a low prevalence of BV, there was no independent association of CL and abnormal vaginal flora.

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Analysis of bacterial vaginosis and PTB: effects of timing of diagnosis and treatment and antimicrobial choices, and study population attributes

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Background: Bacterial vaginosis (BV) and other abnormal genitourinary tract microbial conditions are associated with adverse pregnancies as well as non-pregnancy-associated reproductive outcomes. Inconsistency of study findings requires explanation prior to planning of new investigations.
Objective: Analyze published controlled trials for: (i) design attributes including timing of diagnosis and treatment and outcome(s) selection; (ii) antimicrobial treatment selection and routes of administration; and (iii) likely causes and/or population attributable risks (PARs) of prematurity or low birth weight (LBW) in studied populations.
Study design: Analyses focused on: (i) correlation or non-correlation of intrauterine microbes associated with adverse pregnancy outcomes and selection of study antimicrobials; (ii) timing, including early versus late pregnancy enrollment and treatment; (iii) non-antimicrobial effects of study specified antibiotics, including anti-virulence, anti-inflammatory, and host defense enhancing antimicrobial attributes in fetal and maternal tissues; (iv) pharmacokinetic and sub-MIC/sub-MBC attributes of study antibiotics; and (v) evaluation of study populations focusing on attributable causes of PTB/LBW and likelihood/power calculations for demonstrating study outcomes.
Conclusion: This analysis suggests that significant benefits of screening and treating for BV are more likely accrue to both fetus/perinate and mother in these settings: (i) prior history of prematurity/LBW; (ii) early pregnancy diagnosis and treatment; (iii) oral (systemic) treatment with antimicrobial matching intrauterine microbes including genital mycoplasmas; (iv) use of macrolide/clindamycin antimicrobials with demonstrated anti-virulence factor and inflammation stabilizing effects as well as intracellular decidual and trophoblast concentration; (v) treatment in populations in which PTB/LBW is more likely to be caused by infection/inflammation; and (vi) when prevention of extreme prematurity/VLBW is a study outcome. Future clinical trials should be designed focusing on: (i) patient selection; (ii) timing of diagnosis and treatment; and (iii) choice of specific antimicrobial regimens likely to show benefit.

Risk factors for neonatal infectious morbidity in pregnancies complicated by preterm premature rupture of membranes (PPROM)

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Objective: To characterize risk factors for neonatal infectious morbidity in pregnancies complicated by preterm premature rupture of membranes (PPROM).

Study design: We reviewed maternal and neonatal outcomes of all women with confirmed PPROM ≥ 24 weeks that resulted in delivery < 37 weeks at our institution from August 1998 through August 2000. Multifetal gestation, fetal anomalies, and mothers with hypertension were excluded from analysis. Standard PPROM management included the administration of antibiotics, betamethasone for those pregnancies < 32 weeks, and expectant management with labor induction at ≥ 34 weeks' gestation. The primary study outcome was a composite neonatal infectious morbidity rate (defined as the presence of any of the following: sepsis, pneumonia, or meningitis). Neonates with infectious complications were compared to those who did not develop infectious complications. Statistical analyses included the Students' *t*-test, Fisher exact test, and logistic regression analysis where appropriate.

Results: From August 1998 to August 2000, 430 PPROM cases were identified among the 6003 deliveries that occurred (7.2%). Mean gestational age of PPROM for the study cohort was 32.4 ± 3.8 weeks with mean delivery gestational age of 32.9 ± 3.4 weeks and average length of latency 3.3 ± 6.8 days. Overall composite neonatal infectious morbidity rate was 8.4% (36/430) for the study cohort. Individual neonatal infectious morbidities evaluated included sepsis (7.4%, 30/430), meningitis (1.6%, 7/430), and pneumonia (0.5%, 2/430). Gestational age at time of PPROM (27.9 ± 3.6 vs. 32.8 ± 3.5 weeks, $p < 0.0001$), delivery gestational age (28.6 ± 3.5 vs. 33.3 ± 3.1 weeks, $p < 0.0001$), and birthweight (1188 ± 601 vs. 2154 ± 715 g, $p < 0.0001$) were significantly lower among those pregnancies that resulted in an infant with neonatal infectious complications compared with pregnancies that did not result in neonatal infectious morbidity. The incidence of maternal chorioamnionitis was significantly higher among those pregnancies that resulted in an infant with neonatal infectious complications (38.9%) compared with pregnancies that did not result in neonatal infectious morbidity (10.7%, $p < 0.0001$). In a multiple logistic regression model that included gestational age at delivery, maternal chorioamnionitis, birthweight, and latency, both chorioamnionitis ($p = 0.0009$) and delivery gestational age ($p = 0.004$) remained significantly associated with neonatal infectious morbidity.

Conclusion: Neonatal infectious complications in pregnancies complicated by PPROM are significantly associated with maternal chorioamnionitis and with early delivery gestational age.

Dangerous aerobic Gram-negative rods: a complication of prophylactic antibiotics for preterm premature rupture of membranes (PPROM)

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Expectant management of PPROM ≤ 34 weeks' gestation has become routine, with most centers using broad-spectrum antibiotics (ABTX) to prolong the latency period.

Objective: We report three cases of significant maternal morbidity and mortality due to infection with resistant organisms, following ABTX treatment of PPROM.

Results: *Patient 1*: PPROM of Twin B at 23.9 weeks' gestation. Treated with 2 days of ampicillin/sulbactam (A/S) and 5 days of amoxicillin/clavulanate. Developed chorioamnionitis at 25 weeks. Baby B expired DOL 1 with *Escherichia coli* sepsis, resistant to A/S. On POD 4, mother developed cystitis and severe abdominal wall cellulitis with A/S-resistant *E. coli*, necessitating two extensive operative debridements and ABTX for 14 days.

Patient 2: PPROM at 25.6 weeks' gestation. Given IV clindamycin due to PCN allergy. Three days following PPROM, became hypotensive, neutropenic, and IUFD diagnosed. Postpartum course complicated by septic shock, DIC, ATN, and pancreatitis. *Klebsiella pneumoniae* isolated from maternal blood.

Patient 3: PPROM at 27.1 weeks' gestation. Given A/S for 5 days. Eight days following PPROM, became febrile, hypotensive, and IUFD diagnosed. Course complicated by septic shock and DIC. Three hours following hysterectomy to control bleeding, suffered a cardio-pulmonary arrest. *E. coli* resistant to A/S isolated from maternal blood and placenta.

Conclusions: Although expectant management of PPROM with administration of antibiotics has resulted in improved neonatal outcomes, significant maternal morbidity and even mortality may occur as a result of the development of serious maternal infections. Organisms resistant to the antibiotics administered can be expected to play a major role in these infections.

Does a maternal history of vesicoureteral reflux increase the risk for pyelonephritis in pregnancy?

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Objective: To evaluate the incidence of pyelonephritis among pregnant women with a history of vesicoureteral reflux (VUR) and those without VUR.

Study design: With IRB approval, we queried our obstetric database to identify a control cohort of women with no history of VUR who were cared for in our obstetric practice from 1993–1999. For comparison, a cohort of pregnant women with a history of VUR who received prenatal care and delivered at our institution from 1978–2002 were identified through our institutional patient database. The primary study outcome evaluated was the incidence of pyelonephritis in pregnancy. Additional data collected included maternal age, parity, delivery gestational age, the incidence of cystitis, urinary tract culture results, type of urinary tract surgery, and the use of antibiotic prophylaxis. Statistical analysis included the Students' *t*-test and chi-square analysis.

Results: Between 1993 and 1999, 12 481 pregnant women with no history of VUR were identified. From 1978–2002, 83 pregnant women with a history of VUR were identified. The incidence of pyelonephritis complicating pregnancy was significantly higher among the women with a history of VUR (14.5%, 12/83) as compared with the control cohort of women without VUR (0.7%, 82/12 481) ($p < 0.0001$). Among those women with pyelonephritis, *Escherichia coli* was the predominant causative pathogen identified in both women with and without VUR; however, *E. coli* was found significantly less frequently among women with a history of VUR (58%) when compared to women without VUR, where *E. coli* was found in more than 90% of cases of pyelonephritis ($p < 0.0001$).

Conclusions: Women with a history of VUR are more susceptible to developing pyelonephritis in pregnancy when compared to women without VUR. The organisms responsible for pyelonephritis in women with VUR differ from the pathogens found in women without VUR.

Infectious morbidities in pregnancies complicated by maternal diabetes

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Objectives: To assess the incidence of infectious complications of labor and delivery in pregnancies with gestational diabetes mellitus (GDM) or pre-gestational diabetes (PGDM) as compared to nondiabetics.

Study design: All deliveries between February 1999 and December 2001 at or beyond 34 weeks' gestation were analyzed after exclusion of elective Cesarean sections. Active management of labor protocol was utilized. Induction of labor was carried out with misoprostol, cervidil or oxytocin based on Bishop score. The risk of chorioamnionitis was assessed for diabetic pregnancies controlled for type of labor, mode of delivery, macrosomia and labor abnormalities. Other outcomes measures included endometritis and wound infections. Descriptive statistics, logistic regression and chi-square tests were used.

Results: To date, 5719 women have been analyzed (701 diabetics, 5018 nondiabetics). The chorioamnionitis rate for women with diabetes was 8% compared with 7.7% for non-diabetics. Regarding potential confounding factors, the diabetic pregnancies had higher rates of induction (44.8 vs. 19.2%, $p < 0.001$), Cesarean section (20.5 vs. 13.0%, $p < 0.0001$) and labor abnormalities requiring Cesarean section (14.1 vs. 8.5%, $p < 0.05$), whereas non-diabetics were more likely to receive augmentation (36.2 vs. 26.7%, $p < 0.001$). Rates of multiparity (68.1 vs. 59.8%), macrosomia (8.6 vs. 9.4%) and operative vaginal deliveries (8 vs. 9.4%) did not differ. Logistic regression analysis controlling for potential confounding factors (mode of delivery, labor type, nulliparity and macrosomia), showed that maternal diabetes remained unassociated with risk of chorioamnionitis. All confounding factors analyzed were significantly associated with risk of chorioamnionitis.

Conclusions: Pregnancy complicated by diabetes mellitus (either GDM or PGDM) was not associated with a higher risk for chorioamnionitis, despite a high rate of induction, labor abnormalities and Cesarean section.

Results of a phase III randomized, double-blind study of ertapenem versus piperacillin/tazobactam for acute pelvic infection in women

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Objective: Ertapenem (ETP), Invanz™ is a novel and structurally unique, once daily parenteral carbapenem with aerobic and anaerobic spectrum. This study compared ETP (1 g IV qd) with piperacillin/tazobactam (P/T) (3.375 g IV q6h) in women with acute pelvic infection.

Methods: Eligible diseases were obstetric and gynecologic/post-op infections. The primary efficacy endpoint was clinical response in clinically evaluable patients at 2–4 weeks post-treatment. The clinical modified ITT (MITT) population (i.e., patients. with minimal disease def. and outcome) was also analyzed.

Results: Of 412 women randomized, 402 met clinical MITT criteria, and 316 were clinically evaluable. Success rates (adjusted for baseline strata; obstetric versus gynecologic infections) shown below met predefined criteria for equivalence of ETP.

Population	*n*	*ETP*	*n*	*P/T*	*Difference (95% CI)*
Clin Eval	163	93.9%	153	91.5%	2.4 (–4.1, 8.8)
MITT	211	85.9%	191	88%	–2.1 (–9.2, 5)

The treatment groups had similar demographics, disease severity and duration of treatment. The most common infection was endomyometritis (about 75%). Of clinically evaluable patients, 80% were microbiologically evaluable and around 60% had polymicrobial infection *E. coli* and anaerobes were the most common pathogens. Of the 23 failures (ETP = 10 and P/T = 13): seven versus seven had persistent/worsening infection, one versus four had infection requiring surgery, and two versus two had wound infection, respectively. Common drug-related adverse experiences (AEs) (infused vein complications, diarrhea, increased PLT, ALT, AST) were seen at similar rates in both groups. Drug-related AEs considered serious or interrupting treatment were uncommon in both groups.

Conclusion: This study demonstrated equivalent efficacy of ETP versus P/T in the treatment of acute pelvic infections, with the benefit of once a day dosing. ETP was generally well tolerated in this study.

Patterns of azithromycin use in pregnancy

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Objective: Azithromycin is a macrolide antibiotic with a broad-antimicrobial spectrum, extensive deep tissue penetration, and a prolonged duration of action. Increasingly, azithromycin has been used in pregnancy for a variety of indications including the treatment of community acquired pneumonia and chlamydia cervicitis. We sought to evaluate the safety, the patterns and indications for azithromycin use during pregnancy in women presenting to our institution for prenatal care.

Study design: We reviewed our obstetric database from January 1998 to January 2002 to identify all women receiving the antibiotic azithromycin during pregnancy. Medical records were reviewed to collect information regarding maternal demographic data, trimester of azithromycin treatment, and indication for treatment. Statistical analysis was performed using the chi-square analysis.

Results: Over the study interval, 1467 women (8.3%) received antepartum treatment with azithromycin among the 17 752 women cared for in our system over the same time period. Antepartum use of azithromycin was significantly more common in the third trimester (≥ 28 weeks' gestation; 836/1467, 57%) compared with the first (≤ 12 weeks' gestation; 143/1467, 9.8%: $p < 0.0001$) and second trimester (12–27 weeks' gestation; 488/1467, 33.3%: $p < 0.0001$). Use of azithromycin in the second trimester was also significantly more common than utilization in the first trimester ($p < 0.0001$). Primary indications for the antepartum use of azithromycin included the treatment of community acquired pneumonia, presumed bronchitis, chlamydia cervicitis, and non-specific cervicitis.

Conclusion: Azithromycin is a commonly used antibiotic in our obstetric practice, with the greatest use noted in the second and third trimesters of pregnancy. While generally thought to be relatively safe when used in pregnancy, further data are needed to evaluate the overall safety of azithromycin in pregnancy given its prevalent use.

HSV-2 serological testing in pregnancy, combined with suppressive antiviral therapy for seropositive women, is a cost-effective strategy for reducing the incidence of neonatal herpes in the USA

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Objectives: Genital herpes simplex virus (HSV) infection in pregnant women is associated with a risk of neonatal herpes (NH), with most cases born to women who are unaware they are infected. Cesarean delivery is not 100% effective for preventing vertical transmission of HSV. This analysis investigated the cost-effectiveness of providing a serological type-specific HSV-2 test to all women unaware of their HSV status at week 15 of pregnancy, and offering seropositive women suppressive antiviral therapy from week 36 until delivery. Incremental cost-effectiveness was estimated over current care, where HSV testing is not standard practice and antiviral therapy is offered to a minority with known genital herpes.

Study design: A decision analytical model was constructed and populated using costs, clinical outcomes and probabilities from the literature and/or expert opinion. Costs were inflated to 2001 values. The cost per case of NH was estimated using an average lifetime cost, including expenditure on health care, special education and long term institutional care, weighted by NH outcome severity. The average number of life years (LY), and quality adjusted life years (QALYs), lost per case of NH were estimated from the literature. Sensitivity analysis was used to test the impact of uncertainty around model inputs.

Results: Introducing a serological testing and antiviral suppression program avoids 14 cases of NH and 176 Cesarean deliveries per 100 000 women, at an incremental cost per LY gained of $24 566. The cost per QALY gained $17 126, falls well within the widely accepted $50 000 cost-effectiveness threshold. Cost per QALY estimates remained below this threshold in all one-way sensitivity analyses.

Conclusions: Introducing HSV-2 testing for pregnant women without a prior genital herpes diagnosis, and offering antiviral suppression to those testing positive, is cost-effective in the USA.

Defense mechanisms against GBS infection in pregnant rats involves nitric oxide

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Objectives: Group B streptococcus infection is associated with significant risks for fetomaternal complications. The mechanism(s) responsible for GBS infection of the fetus including septicemia, meningitis and high risk of mortality is not understood. In this study, we investigated the hypothesis that inhibition of nitric oxide (NO) synthesis would increase GBS morbidity.

Study design: Experimental pregnant rat intrauterine infection model was used to evaluate the severity of GBS infection in the presence and absence of NO inhibitor, nitro-L-arginine methyl ester (L-NAME). Cervical intrauterine infection with 200 μl /suspension of GBS (10^6–10^7 cfu/ml) was induced on day 18 of pregnancy and rats were sacrificed 24 hours later. Colony forming units (cfu) counts in the uterus, placenta, fetus, and kidneys were measured. Weight of placentas and fetuses were also recorded.

Results: Preliminary results showed that in rats with GBS infection, only 50% of animals became infected after 24 hours and at very low rate of 10^{1-2} cfu/g. However, all rats with L-NAME treatment were GBS positive, with infection rate of 10^3–10^8 cfu/g. All organs examined were GBS positive with the highest rate of infection (10^9/g) noted in placentas of L-NAME-treated animals. Both control and L-NAME only groups without GBS inoculation had no evidence of infection. Significant decrease in fetal weights was also noted in the L-NAME + GBS group compared with GBS or L-NAME alone groups.

Conclusions: We propose that NO, especially in the placenta, could be a significant regulator of pregnant rat immune defenses against GBS infection.