“VICTOR BABES” UNIVERSITY OF MEDICINE AND PHARMACY TIMISOARA

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DISCIPLINE OF FAMILY MEDICINE

BRATOSIN FELIX

DOCTORAL THESIS

THE INVOLVEMENT OF UROGENITAL TRACT INFECTIONS IN PREMATURITY

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T i m i s o a r a

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STUDY 1: COMPARATIVE ANALYSIS OF MICROBIAL SPECIES AND MULTIDRUG RESISTANCE PATTERNS ASSOCIATED WITH LOWER URINARY TRACT INFECTIONS IN PRETERM AND FULL-TERM BIRTHS.

BACKGROUND

Preterm birth, occurring before 37 weeks of pregnancy, is a major cause of neonatal and infant health issues, with urinary tract infections (UTIs) being a significant risk factor, especially when associated with Group B Streptococcus. UTIs, affecting around 8% of pregnancies, range from asymptomatic bacteriuria to severe infections such as pyelonephritis, and are linked with an increased risk of preterm and early term births. This highlights the importance of addressing UTIs as a common perinatal complication.

UTIs are a global public health concern, with around 150 million people affected each year, mostly due to uropathogenic Escherichia coli. The complexity of UTI microbial landscape is shaped by various factors including host physiology and the role of the microbiome in the pathogenesis of UTIs. Traditional diagnostic methods like urine culture and microscopy, despite being standard, are challenged by high rates of false results and the evolving diagnostic criteria, underscoring the need for more advanced techniques such as next-generation sequencing for a deeper understanding of microbial diversity in UTIs.

The emergence of multidrug-resistant organisms has made the management of UTIs more complex, emphasizing the necessity for personalized antimicrobial treatments. This situation calls for a detailed examination of microbial species and resistance patterns in UTIs, particularly in the context of preterm births, to better understand the bacterial species associated with preterm birth and their antibiotic resistance patterns, aiming for more effective treatment strategies.

SUMMARY OF FINDINGS

In a study analyzing the impact of urinary tract infections (UTIs) on pregnancy outcomes, significant differences were observed between pregnant women experiencing preterm and full-term births, particularly in their background characteristics and infection markers. The mean ages of the preterm and full-term groups were 27.5 and 28.3 years, respectively, with BMI and smoking rates showing no significant differences between the two groups. Parity and trimester of infection were similarly nondescript in distinguishing preterm from full-term births, emphasizing the subtle nature of UTI impacts on pregnancy outcomes.

Laboratory data revealed significant disparities in markers of infection and inflammation. The preterm group exhibited a higher median white blood cell (WBC) count of 12.3 versus 9.1 in the full-term group, indicating a more pronounced infection presence. Neutrophil counts were also notably higher in the preterm group (7.8 compared to 6.1), alongside a significant elevation in C-reactive protein levels, which were 18 in the preterm group versus 7 in the full-term group. These markers underscored the heightened inflammatory response associated with preterm births. Urine cultures from the preterm group were more likely to contain multiple bacteria types, indicating a broader infection spectrum.

Microbial analysis further detailed the infection landscape. Escherichia coli was prevalent among both preterm (65.9%) and full-term (58.8%) groups, while Klebsiella spp. and Enterobacter spp. presented significant variances in occurrence between preterm and full-term births. Gram-positive bacteria, notably Group B Streptococcus (GBS), were significantly more prevalent in preterm births (17.5%) compared to full-term births (1.6%), marking a stark difference in microbial profiles that could influence birth outcomes.

The study also uncovered significant associations between microbial resistance patterns and preterm births. Extended-spectrum beta-lactamases (ESBL) producing organisms were found in 19.8% of the preterm group versus only 4.4% of the full-term group, with vancomycin-resistant Enterococci (VRE) and carbapenem-resistant Enterobacteriaceae

(CRE) also more prevalent in preterm cases. This indicates a concerning link between antibiotic resistance and the likelihood of preterm birth.

Risk factor analysis provided compelling insights. The presence of Group B Streptococcus infection more than doubled the odds of preterm birth with an odds ratio (OR) of 2.5. Enterobacter spp. infections and the presence of multidrug resistance were also significant predictors of preterm birth, with ORs of 1.8 and 3.2, respectively. These findings highlight the critical impact of specific bacterial infections and their resistance patterns on the risk of preterm birth, underscoring the importance of targeted interventions and treatment strategies to mitigate these risks.

Figure 1 – Evidence of MDR organisms isolated from urine cultures.

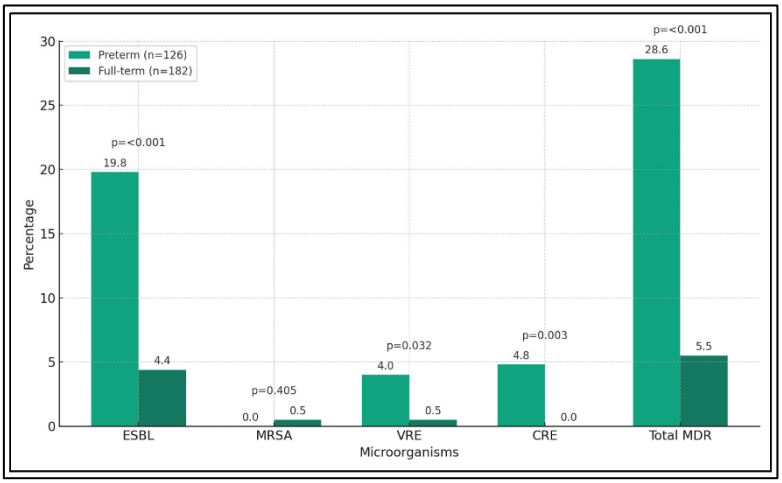
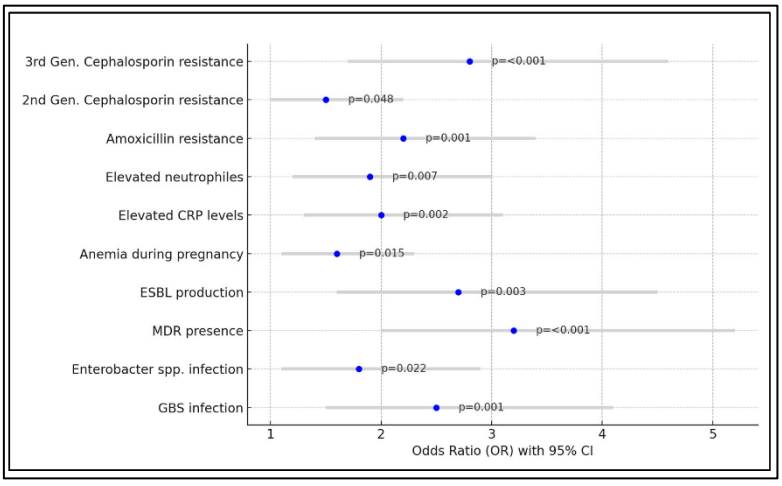


Figure 2 – Risk factor analysis for preterm birth.



CONCLUSIONS

This study identified significant variances in the microbial flora and multidrug resistance patterns between UTIs in preterm and full-term births, highlighting the complex interplay between microbial resistance and pregnancy outcomes. The increased incidence of multidrug-resistant organisms, particularly ESBL producers and carbapenem-resistant Enterobacteriaceae in preterm births, underscores the urgency of developing targeted antimicrobial strategies. The association of specific bacteria, like Group B Streptococcus and Enterobacter spp., with preterm deliveries further emphasizes the need for precise microbial identification. Future research should focus on exploring novel antimicrobial agents and personalized treatment approaches.

STUDY 2: ANALYSIS OF VAGINAL MICROBIOTA VARIATIONS IN THE THIRD TRIMESTER OF PREGNANCY AND THEIR CORRELATION WITH PRETERM BIRTH: A CASE-CONTROL STUDY.

BACKGROUND

Preterm birth, occurring before 37 weeks of gestation, is a major cause of neonatal morbidity and mortality globally, with little change in occurrence rates despite medical advancements. Emerging studies highlight the significant impact of vaginal microbiota on maternal and neonatal health, emphasizing the association between microbial imbalance and adverse pregnancy outcomes such as preterm birth. The composition of the vaginal microbiome, which comprises a diverse bacterial ecosystem, is subject to variation among women and is influenced by genetic, environmental, and lifestyle factors throughout pregnancy.

Recent research has provided a clearer picture of the vaginal microbiota, identifying specific bacteria that are prevalent in healthy pregnancies compared to those linked with negative outcomes. The presence of Lactobacillus species is often associated with a healthy vaginal environment, offering protection by inhibiting harmful pathogens. In contrast, increased microbial diversity and certain anaerobic bacteria like Gardnerella and Ureaplasma are associated with inflammation and a higher risk of adverse birth outcomes.

The interaction between the vaginal microbiota and the host's immune system plays a pivotal role in maintaining a pregnancy. A balanced microbiome supports a tolerant immune environment essential for pregnancy, whereas dysbiosis may disturb this balance, leading to pro-inflammatory responses and increasing the risk of preterm birth. Despite advancements in metagenomics enhancing our understanding of the vaginal microbiome's role in pregnancy, there remains a need for further investigation into how microbial communities influence preterm birth risks and the development of interventions to prevent such outcomes.

SUMMARY OF FINDINGS

The study analyzed 89 women with preterm deliveries and 106 with full-term deliveries, revealing no significant difference in mean age between the preterm (28.6 years) and full-term (29.1 years) groups. Lifestyle factors displayed notable differences; 13.5% of women in the preterm group smoked during pregnancy compared to 7.5% in the full-term group, and alcohol use was reported at 5.6% versus 0.9%, respectively. Medical history showed a higher, yet not statistically significant, incidence of urinary tract infections in the preterm group (24.7%) compared to the full-term group (14.2%).

Laboratory results highlighted a significant elevation in white blood cell count in the preterm group with a median of $10.2 \times 10^3/mm^3$ versus $7.6 \times 10^3/mm^3$ in the full-term group (p-value = 0.009). Neutrophil counts also differed significantly, with preterm births showing a median count of $7.2 \times 10^3/mm^3$ compared to $5.1 \times 10^3/mm^3$ in full-term births ($p < 0.001$). However, other parameters such as lymphocyte counts, platelet counts, and hemoglobin levels showed no significant difference between groups.

Gestational weight and birth type statistics were singificantly different; only 52.8% of preterm newborns weighed over 2500g compared to 84.9% in the full-term group. The distribution of gestational age at birth was markedly different, with 7.9% of preterm births occurring before 28 weeks. Cesarean section rates were significantly higher in the preterm group (65.2%) compared to the full-term group (18.9%).

Microbiota analysis revealed a higher median vaginal pH in the preterm group (5.6) versus the full-term group (4.4), and the prevalence of bacterial vaginosis was significantly higher in the preterm group (29.2%) compared to the full-term group (12.3%). Furthermore, the presence of specific microorganisms like Candida spp. and Gardnerella vaginalis was significantly associated with preterm delivery, with Candida spp. present in 24.7% of the preterm group versus 9.4% in the full-term group ($p = 0.004$) and Gardnerella vaginalis in

25.8% versus 12.3%, respectively (p = 0.015). The odds ratios for preterm birth were significantly increased by the presence of Candida spp. (OR = 1.84, p = 0.018) and Gardnerella vaginalis (OR = 2.29, p = 0.003), emphasizing the critical role of vaginal microbiota in pregnancy outcomes.

Figure 3 – Vaginal smear results by preterm and full-term births.

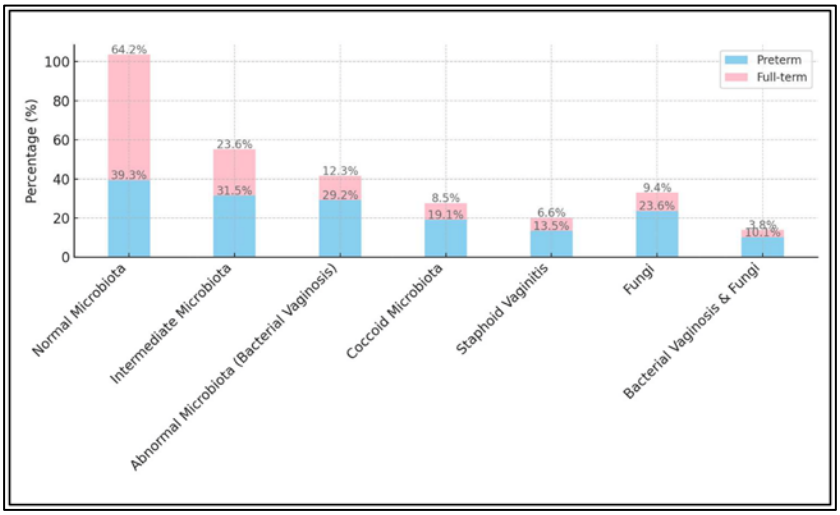
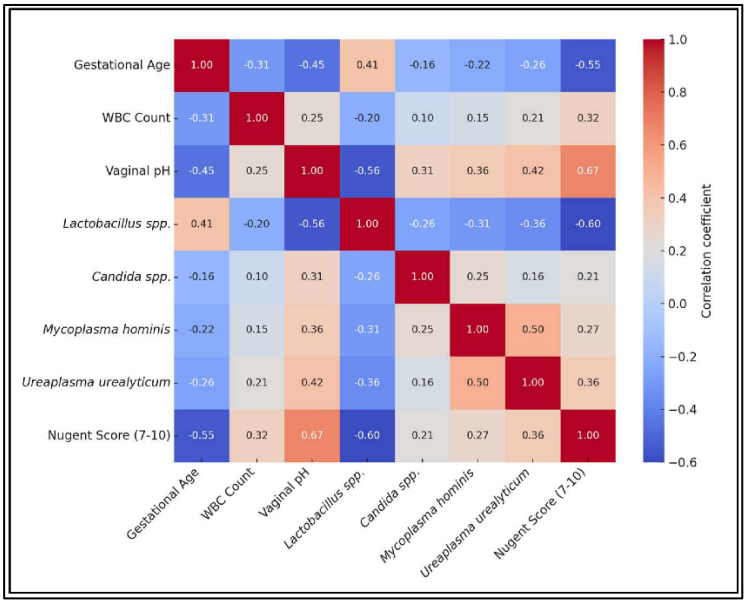


Figure 4 – Correlation matrix.



CONCLUSIONS

This research concludes that significant correlations exist between the vaginal microbiota composition and the risk of preterm birth. Elevated white blood cell and neutrophil counts increased vaginal pH, and the presence of specific bacteria, such as Candida spp., Gardnerella vaginalis, Mycoplasma hominis, and Ureaplasma urealyticum, are strongly associated with higher PTB rates. Conversely, a higher proportion of Lactobacillus spp. appears to confer a protective effect against PTB. These insights emphasize the potential of microbiota-focused interventions as strategies for PTB risk reduction. However, understanding the complex interplay between microbial dynamics, host immune response, and PTB is crucial for developing effective prevention and treatment methods. This study highlights the need for further comprehensive research to fully understand these relationships and develop targeted interventions to improve pregnancy outcomes.

STUDY 3: IMPACT OF GENITAL INFECTIONS AND ANTIBIOTIC USE ON INCIDENCE OF PRETERM BIRTH: A RETROSPECTIVE OBSERVATIONAL STUDY.

BACKGROUND

Preterm birth, defined as delivery before 37 weeks of gestation, is a major public health issue, with around 15 million babies born prematurely each year, accounting for an average of 10% of all live births worldwide. This condition is a leading cause of neonatal mortality and long-term morbidity, significantly affecting families and healthcare systems. Various factors, including demographic, genetic, environmental, and especially infectious agents, play roles in the onset of preterm labor, with genital infections in pregnant women being identified as crucial disruptors of normal gestation.

Genital infections, caused by bacterial, viral, and fungal pathogens, can ascend from the lower genital tract, leading to inflammation in the uterus, amniotic fluid, and affecting the fetus. This inflammation triggers a cascade of events, including the release of pro-inflammatory cytokines and prostaglandins, causing cervical ripening, uterine contractions, and possibly premature rupture of membranes. Organisms such as Group B Streptococcus and Trichomonas vaginalis, associated with bacterial vaginosis, are frequently implicated in these processes.

The management of genital infections during pregnancy involves a delicate balance between eliminating the infection and ensuring the safety of both the mother and fetus. The choice, timing, and duration of antibiotic treatment are crucial, considering the potential for antibiotic resistance. Despite the recognition of the link between genital infections and preterm birth, gaps remain in our understanding of the mechanisms involved and the effectiveness of various antibiotic treatments. This highlights the need for ongoing research to develop precise strategies for preventing and managing genital infections in pregnant women, taking into account antibiotic stewardship to combat resistance.

SUMMARY OF FINDINGS

Within a group of 165 pregnant women studied for the impact of genital infections on birth outcomes, 71 experienced preterm births while 94 delivered at term. The demographics revealed no significant age difference between groups, with preterm mothers averaging 27.3 years and full-term mothers 28.1 years. Obesity rates were comparable across groups, but smoking was more prevalent in the preterm group (18.3%) versus the full-term group (11.7%).

The medical history highlighted a stark contrast in the prevalence of sexually transmitted diseases (STDs) between groups, with 25.4% in the preterm group versus 3.2% in the full-term group, marking a statistically significant difference ($p < 0.001$). Genital herpes was also significantly more common among preterm births (8.5% vs. 1.1%, $p = 0.019$).

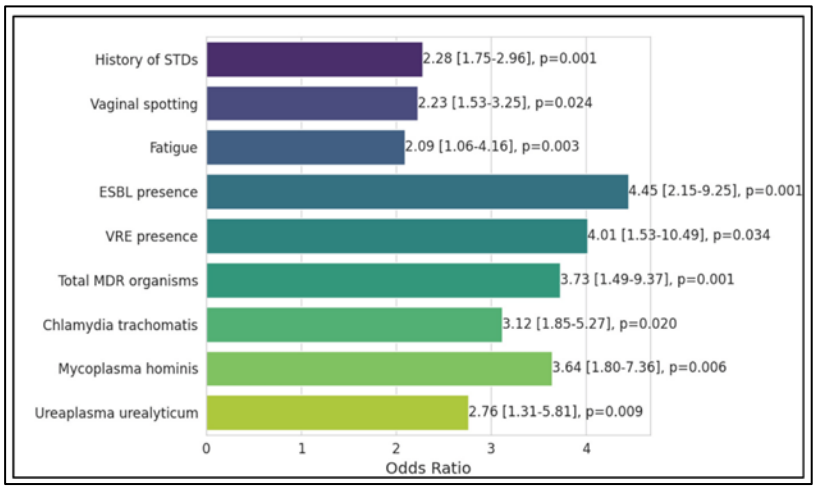
Symptom analysis showed a higher occurrence of fever (81.7% vs. 25.5%, $p < 0.001$) and pelvic pain (50.7% vs. 21.3%, $p < 0.001$) in the preterm group. Additionally, vaginal spotting and fatigue were more prevalent in the preterm group, with 40.8% experiencing spotting (vs. 24.5% in the full-term group, $p = 0.024$) and 59.2% reporting fatigue (vs. 36.2% in the full-term group, $p = 0.003$).

Neonatal outcomes were notably different, particularly in gestational weight and age categories. Preterm infants were more likely to be in lower weight categories, with 2.8% weighing 500–999 g and 8.5% weighing 1000–1499 g, absent in the full-term group. Cesarean rates were higher in the preterm group (56.3% vs. 20.2% in the full-term group), and vaginal pH was significantly higher in the preterm group, indicating a difference in vaginal flora between groups.

Pathogens such as Chlamydia trachomatis (12.7% vs. 3.2%, $p = 0.020$) and Mycoplasma hominis (16.9% vs. 4.3%, $p = 0.006$) were significantly more common in the preterm group. This highlights the impact of specific genital infections on the risk of preterm

birth, suggesting the need for targeted interventions and improved management of genital infections during pregnancy to mitigate these risks.

Figure 5 – Significant risk factors associated with preterm birth.



CONCLUSIONS

In conclusion, this study elucidates the intricate relationship among genital infections, antibiotic use, and the risk of preterm birth. Our findings underscore the significance of several key factors in influencing pregnancy outcomes. Notably, the presence of sexually transmitted diseases, particularly Chlamydia trachomatis and Mycoplasma hominis, emerged as substantial risk factors for preterm birth. Genital herpes, fever during pregnancy, vaginal spotting, and fatigue were also identified as significant contributors to the increased odds of preterm birth. Furthermore, this study revealed a striking association between the presence of MDR organisms/ESBLs/VRE and the risk of preterm birth. These findings emphasize the critical importance of early detection, appropriate antibiotic therapy, and vigilant management of genital infections during pregnancy to mitigate the risk of preterm birth and improve maternal and neonatal outcomes. Further research is warranted to explore the mechanisms underlying these associations and to develop targeted interventions for at-risk populations.