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Frequency of Aerobic Microorganisms Causing Vaginal Infections in Third Trimester of Pregnancy at Lady Reading Hospital Peshawar

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ABSTRACT

Background: Pregnancy is a time when vaginal infections are a major concern as they can lead to preterm birth and premature rupture of membranes. Managing these risks requires identifying the microbial pathogens responsible. Further exploration is required to assess the prevalence and associations of aerobic microorganisms (Escherichia coli, Group B Streptococcus, Enterococcus faecalis, Klebsiella pneumoniae, and Methicillin sensitive Staphylococcus aureus) during the third trimester of pregnancy in order to improve pregnancy outcomes. **Objective:** To determine the frequency of aerobic microorganisms causing vaginal infections in the third trimester of pregnancy at Lady Reading Hospital Peshawar. **Study Design:** Cross-sectional observational study. **Duration and Place of Study:** The study was conducted from December 2020 to June 2021 at the Obstetrics and Gynaecology Department, Lady Reading Hospital, Peshawar. **Methodology:** A total of 164 pregnant women, aged 18-40 years, with singleton pregnancies over 28 weeks gestation, and presenting with vaginal infections lasting more than five days were enrolled. Clinical assessments were performed, and high vaginal swabs were collected for microbiological analysis. The presence of pathogens was determined through laboratory cultures. **Results:** The mean age of participants was 27.66 ± 2.94 years, with a predominant middle-class socioeconomic status (75%). E. coli was identified in 34.8% of cases, followed by Group B Streptococcus (19.5%), Enterococcus faecalis (17.1%), Methicillin-sensitive Staphylococcus aureus (18.3%), and Klebsiella pneumoniae (9.1%). Statistically significant associations were observed between E. coli prevalence and lower parity ($p = 0.039$). **Conclusion:** This study highlights the high prevalence of E. coli, Group B Streptococcus, and Klebsiella pneumoniae as significant pathogens in vaginal infections during pregnancy.

INTRODUCTION

Vaginal infection is one of the common health disorders that is seen by numerous women at some point or another.[1] The infection is caused by an imbalance of the healthy bacterial flora of the vagina, mostly ruled by lactobacilli.[2] When this balance is interrupted, other bacteria, fungi, or viruses can grow, leading to infection. Such types of infection lead to symptoms such as itchiness, abnormal discharge, as well as painful urination.[3]

The body undergoes various changes physiologically throughout pregnancy, some of them including changes in the hormones, making females more susceptible to vaginal infection.[4] Higher levels of estrogen levels throughout pregnancy provide an environment within the vagina that is favorable for overgrowth of some causative organisms.[5] The relaxed nature of the cervix is also favorable for the passage of microbes upwards into the uterus, thus increasing the risk of infection.[6] In pregnancy, vaginal infection has crucial consequences, some of them including preterm labor, low birth weight, as well as neonatal infection.[7]

Vaginal infection is caused by various microorganisms, of which the commonest are bacteria, fungal, and viral types.[8] Bacterial vaginosis results from an overgrowth of certain types of bacteria, while overgrowth of Candida species results in causing yeast infection.[9] STIs such as chlamydia, gonorrhea, and trichomoniasis are causatives of vaginal infection as well.[10] Besides such pathogens, viral infections like herpes simplex virus can also affect the vagina.

Aerobically grown bacteria play a very important role in infection of vagina, especially at the third trimester of pregnancy.[11] The oxygen dependent these bacteria are there regularly; these bacteria are present in small number as part of the vaginal flora.[12] Normally the aerobic bacteria like Escherichia coli, Streptococcus, and

Staphylococcus aureus do exist but they are out competed and not the causative agents of infection when the vaginal flora is disrupt. Aerobic bacterial infection at pregnancy complicate the pregnancy i.e. preterm labour, chorioamnionitis or urinary tract infection.[13] Immediate treatment with antibiotics in combination with the proper adherence to antiseptic practices will not harm the mother or child and can manage these types of infection.[14]

Tansarli GS et al.[15] identified Escherichia coli as the most prevalent pathogen in pregnant women, affecting 34.8% of cases. Other frequently encountered pathogens included Group-B Streptococcus (22.7%), Enterococcus faecalis (18.2%), and Klebsiella pneumoniae (12.1%), all of which were associated with vaginal infections during the third trimester of pregnancy.

Similarly, a study conducted by Verma I et al. [16] reported that the incidence of aerobic bacterial infections in pregnant women during the third trimester was 21.79%. Among these infections, Enterococcus was found in 41.1% of cases, followed by E. coli (23.5%) and Methicillin-sensitive Staphylococcus aureus (23.5%).

In Khyber Pakhtunkhwa, the study of aerobic microorganisms responsible for causing vaginal infections in the third trimester of pregnancy is of interest, given the region's specific issues with healthcare and demographics. In KPK, where healthcare services may not be available to certain areas, understanding the prevalent pathogens can allow more targeted and better prevention and treatment strategies for pregnant women. Reducing the incidence of complications such as preterm birth, low birth weight and maternal morbidity is a priority in the region, and identifying these microorganisms is vital in achieving this goal. The findings from this research can be used to develop local health care policy and improve the maternal and neonatal health outcomes in KPK by generating early diagnosis, timely treatment, and awareness among both urban and rural populations.

METHODOLOGY

This cross-sectional study was conducted from December 2020 to June 2021 at the Obstetrics and Gynaecology Department of Lady Reading Hospital in Peshawar. A total of 164 pregnant women were included in the study, with the sample size determined using a 95% confidence level and a 5% margin of error, based on the expected prevalence of Klebsiella pneumoniae (12.1%) causing vaginal infections.[15]

A non-probability consecutive sampling technique was used to select participants who met the inclusion criteria. These criteria included women aged 18 to 40 years, with a singleton pregnancy confirmed by ultrasound, a gestational age of over 28 weeks according to the last menstrual period, any parity, and vaginal infections lasting more than five days as defined by symptoms such as abnormal vaginal discharge, dysuria, lower abdominal pain (VAS>3), vaginal dyspareunia, vulvo-vaginal itching, and foul-smelling discharge. Exclusion criteria involved women who had unprotected sexual intercourse within the past 72 hours or had taken antibiotics or antifungal medications within the last 10 days.

Upon obtaining approval from the CPSP and the hospital's ethical committee, participants who met the inclusion criteria were enrolled through the Emergency/OPD and admitted to the Obstetrics and Gynaecology Department. A written informed consent was obtained from each participant. Comprehensive obstetric and medical histories were taken, and clinical examinations were performed. After the general and obstetric assessments, a sterile Cusco's speculum was gently inserted, and high vaginal swabs were collected by a trained 4th-year trainee in a private setting to maintain privacy. The collected samples were sent to the hospital's laboratory for microbiological analysis to minimize bias.

The data collected included variables such as age, gestational age, parity, socioeconomic status (classified into poor, middle, or rich based on husband's monthly income), and the identification of aerobic microorganisms causing vaginal infections. The microorganisms of interest were Escherichia coli, Group-B Streptococcus, Enterococcus faecalis, Klebsiella pneumoniae, and Methicillin-sensitive Staphylococcus aureus. The presence of these microorganisms was determined by the presence of specific growth characteristics on laboratory culture media, such as the appearance of shiny white colonies for E. coli and gray to whitish-gray colonies with a weak zone of beta-hemolysis for Group-B Streptococcus, among others. This information was recorded using a pre-designed proforma. To reduce confounding factors and biases, the exclusion criteria were rigorously enforced.

The data analysis was performed using SPSS version 22. Descriptive statistics, including means and standard deviations, were computed for continuous variables such as age, gestational age, parity, and the duration of infection. Frequencies and percentages were used for categorical variables, including socioeconomic status and aerobic microorganisms. Stratification of aerobic microorganisms was performed based on age, gestational age, parity, socioeconomic status, and infection duration. Post-stratification analysis using the chi-square test was conducted, and a p-value of ≤ 0.05 was considered statistically significant.

RESULTS

In Table-I, patient demographics are presented with mean values and standard deviations for continuous variables: the mean age is 27.664 ± 2.94 years, mean gestational age is 31.268 ± 1.51 weeks, mean parity is 1.073 ± 1.39, and the mean duration of infection is 9.097 ± 1.89 days. The socioeconomic status of participants was predominantly middle class, with 75% classified as middle, 14.6% as poor, and 10.4% as rich.

Table I

Patient Demographics

Demographics		Mean ± SD
Age (years)		27.664±2.94
Gestational age (weeks)		31.268±1.51
Parity		1.073±1.39
Duration of infection (days)		9.097±1.89
Socioeconomic Status	Poor n (%)	24 (14.6%)
	Middle n (%)	123 (75%)
	Rich n (%)	17 (10.4

Figure 1

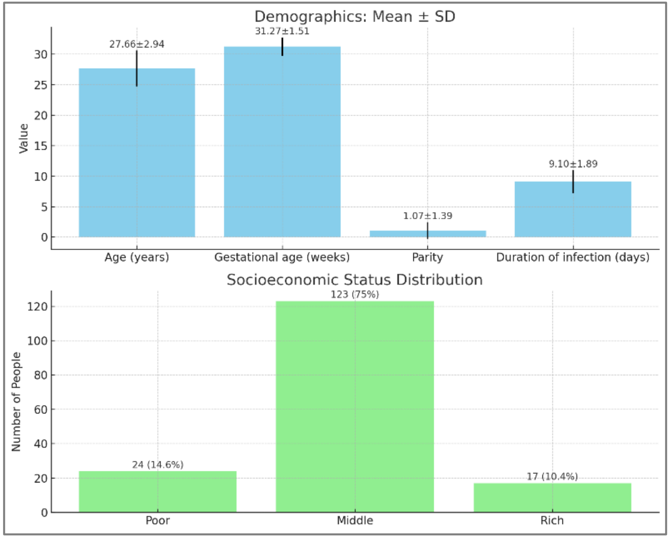


Table-II lists the aerobic microorganisms responsible for vaginal infections during the third trimester, with E. coli identified in 34.8% of cases, followed by Group B Streptococcus (19.5%), Enterococcus faecalis (17.1%), Methicillin-sensitive Staphylococcus aureus (18.3%), and Klebsiella pneumoniae (9.1%).

Table II

Aerobic microorganisms causing vaginal infections in the third trimester of pregnancy

Aerobic microorganisms	Frequency	% age
E coli	57	34.8%
Group B Streptococcus	32	19.5%
Enterococcus faecalis	28	17.1%
Methicillin Sensitive Staph aureus	30	18.3%
Klebsiella pneumoniae	15	9.1%

Table-III explores the association between aerobic microorganisms and demographic factors. For E. coli, significant associations were observed with parity (p = 0.039), where lower parity (0-2) had a higher prevalence (38.2%) compared to higher parity (>2, 17.9%). There were no significant associations with age, gestational age, socioeconomic status, or duration of infection (p > 0.05). Group B Streptococcus showed no significant associations with age, gestational age, parity, socioeconomic status, or duration of infection (p > 0.05). Similarly, Enterococcus faecalis exhibited no significant associations with these demographic factors, except for parity, where higher parity (>2) showed a higher prevalence (25%) than lower parity (15.4%), but this was not statistically significant (p = 0.221). Klebsiella pneumoniae had no significant associations with age, gestational age, parity, socioeconomic status, or duration of infection (p > 0.05). For Methicillin-sensitive Staphylococcus aureus, no significant associations were found with age, gestational age, parity, socioeconomic status, or duration of infection (p > 0.05).

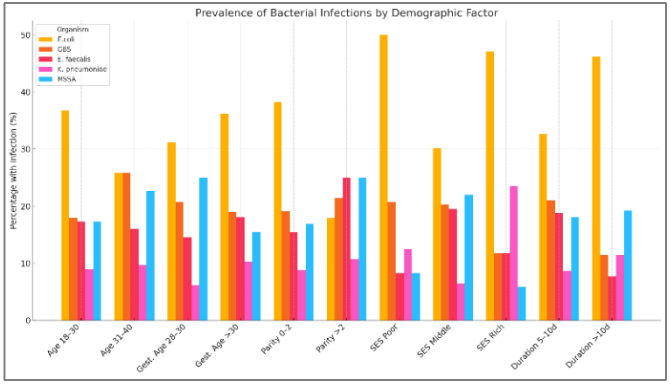
Table III

Association of Aerobic microorganisms with Demographic Factors

Demographic Factors		E-coli		p-value
		Yes n(%)	No n(%)	
Age (years)	18-30	49 (36.8%)	84 (63.2%)	0.245
	31-40	8 (25.8%)	23 (74.2%)	
Gestational Age (weeks)	28-30	15 (31.2%)	33 (68.8%)	0.544
	>30	42 (36.2%)	74 (63.8%)	
Parity	0-2	52 (38.2%)	84 (61.8%)	0.039
	>2	5 (17.9%)	23 (82.1%)	
Socioeconomic Status	Poor	12 (50%)	12 (50%)	0.092
	Middle	37 (30.1%)	86 (69.9%)	
	Rich	8 (47.1%)	9 (52.9%)	
Duration of infection (days)	10-May	45 (32.6%)	93 (67.4%)	0.183
	>10	12 (46.2%)	14 (53.8%)	
Demographic Factors		**Group B Streptococcus**		**p-value**
		Yes n(%)	**No n(%)**	
Age (years)	18-30	24 (18%)	109 (82%)	0.326
	31-40	8 (25.8%)	23 (74.2%)	
Gestational Age (weeks)	28-30	10 (20.8%)	38 (79.2%)	0.784
	>30	22 (19%)	94 (81%)	
Parity	0-2	26 (19.1%)	110 (80.9%)	0.779
	>2	6 (21.4%)	22 (78.6%)	
Socioeconomic Status	Poor	5 (20.8%)	19 (79.2%)	0.695
	Middle	25 (20.3%)	98 (79.7%)	
	Rich	2 (11.8%)	15 (88.2%)	
Duration of infection (days)	10-May	29 (21%)	109 (79%)	0.263
	>10	3 (11.5%)	23 (88.5%)	
Demographic Factors		**Enterococcus faecalis**		**p-value**

		Yes n(%)	No n(%)	
Age (years)	18-30	23 (17.3%)	110 (82.7%)	0.877
	31-40	5 (16.1%)	26 (83.9%)	
Gestational Age (weeks)	28-30	7 (14.6%)	41 (85.4%)	0.588
	>30	21 (18.1%)	95 (81.9%)	
Parity	0-2	21 (15.4%)	115 (84.6%)	0.221
	>2	7 (25%)	21 (75%)	
Socioeconomic Status	Poor	2 (8.3%)	22 (91.7%)	0.341
	Middle	24 (19.5%)	99 (80.5%)	
	Rich	2 (11.8%)	15 (88.2%)	
Duration of infection (days)	10-May	26 (18.8%)	112 (81.2%)	0.166
	>10	2 (7.7%)	24 (92.3%)	
Demographic Factors		**Klebsiella pneumoniae**		**p-value**
		Yes n(%)	**No n(%)**	
Age (years)	18-30	12 (9%)	121 (91%)	0.909
	31-40	3 (9.7%)	28 (90.3%)	
Gestational Age (weeks)	28-30	3 (6.2%)	45 (93.8%)	0.408
	>30	12 (10.3%)	104 (89.7%)	
Parity	0-2	12 (8.8%)	124 (91.2%)	0.752
	>2	3 (10.7%)	25 (89.3%)	
Socioeconomic Status	Poor	3 (12.5%)	21 (87.5%)	0.061
	Middle	8 (6.5%)	115 (93.5%)	
	Rich	4 (23.5%)	13 (76.5%)	
Duration of infection (days)	10-May	12 (8.7%)	126 (91.3%)	0.645
	>10	3 (11.5%)	23 (88.5%)	
Demographic Factors		**Methicillin Sensitive Staph aureus**		**p-value**
		Yes n(%)	**No n(%)**	
Age (years)	18-30	23 (17.3%)	110 (82.7%)	0.493
	31-40	7 (22.6%)	24 (77.4%)	
Gestational Age (weeks)	28-30	12 (25%)	36 (75%)	0.153
	>30	18 (15.5%)	98 (84.5%)	
Parity	0-2	23 (16.9%)	113 (83.1%)	0.313
	>2	7 (25%)	21 (75%)	
Socioeconomic Status	Poor	2 (8.3%)	22 (91.7%)	0.108
	Middle	27 (22%)	96 (78%)	
	Rich	1 (5.9%)	16 (94.1%)	
Duration of infection (days)	10-May	25 (18.1%)	113 (81.9%)	0.893
	>10	5 (19.2%)	21 (80.8%)	

Figure 2



DISCUSSION

The results indicate that E. coli were the most predominant pathogens but other microorganisms including Group B Streptococcus, Enterococcus faecalis, Methicillin susceptible Staphylococcus aureus and Klebsiella pneumoniae were also identified. The strong association of E. coli with parity (higher prevalence in the 0 to 2 parity group) could be due to the increased susceptibility of women with fewer pregnancies to these infections; perhaps because of less fully developed immune response, or because of prior exposure to certain pathogens during childbirth. The lack of strong associations with other demographic factors including age or socioeconomic status suggests that E. coli prevalence is not so largely affected by these factors, and the pathogen may be more related to physiological than to external socioeconomic conditions.

The demographic factors did not show any significant associations with Group B Streptococcus or Enterococcus faecalis, indicating that these microorganisms were less reliant on individual demographic factors and might be opportunistic pathogens prevalent to pregnant women at all age groups and parity level.

Particularly, Methicillin sensitive Staphylococcus aureus prevalence was elevated among participants with higher parity, possibly due to increased exposures to infections in women with more pregnancies as well as persistence of pathogens in healthcare settings. This reinforces the lack of significant associations with gestational age and duration of infection as these microorganisms are not as dependent on these parameters and are prevalent throughout demographics.

The demographic characteristics of our participants, including mean age (27.664 ± 2.94 years), gestational age (31.268 ± 1.51 weeks), parity (1.073 ± 1.39), and infection duration (9.097 ± 1.89 days), align with studies in similar settings, such as Elshabrawy et al. [17] who also observed similar maternal characteristics in their case-control study on preterm premature rupture of membranes (PPROM). Both studies highlight the commonality of BV and AV as significant risk factors in pregnancy.

The prevalence of E. coli, the most common pathogen in our study (34.8%), is in line with findings from Ma et al. [18] where *E. coli* was also identified as a major pathogen in AV. Our results showed a higher prevalence of E. coli in the 18-30 age group (36.8%), compared to Yalew et al. [19] who found a similar trend with higher infection rates among younger women but with slightly different statistical associations. Similarly, Elshabrawy et al. [17] identified the role of E. coli in BV and AV and emphasized its link to adverse pregnancy outcomes. However, the lack of significant associations between age and E. coli infection in our study (p = 0.245) contrasts with studies like Yalew et al. [19] where such associations were more pronounced, indicating that regional factors or study design differences may explain this discrepancy.

Our findings also showed a statistically significant association between E. coli infection and parity (p = 0.039), similar to findings by Yalew et al. [19] where BV was more common in women with lower parity. This finding emphasizes the importance of parity as a risk factor for vaginal infections, a pattern that Elshabrawy et al. [17] also reported in their case-control study, linking BV and AV with preterm birth.

Group B Streptococcus (GBS) was another prevalent microorganism in our study (19.5%), with no significant association with age, parity, or socioeconomic status, consistent with findings from Yalew et al. [19] where GBS prevalence was also widespread, but with limited statistical associations with demographic factors. These consistent findings underscore the ubiquitous nature of GBS in pregnant women, which may be less influenced by individual demographics, as suggested by both Yalew et al. [19] and Ma et al. [18]

For Enterococcus faecalis, our study observed a higher prevalence among those with higher parity (25%), but this was not statistically significant (p = 0.221), aligning with the findings from Elshabrawy et al. [17] where no significant association was found between Enterococcus and demographic factors. However, studies like Oh et al. [20] which investigated the vaginal microbiota in pregnant women with AV, showed a stronger association between dysbiosis and specific pathogens like *Gardnerella* and *Megasphaera*, potentially indicating that other anaerobic bacteria may contribute more to adverse pregnancy outcomes than Enterococcus faecalis.

In terms of Klebsiella pneumoniae, our study's findings were similar to Son et al. [21] where Klebsiella pneumoniae was significantly associated with preterm delivery in the second trimester, particularly in women with abnormal vaginal colonization. However, our study found a marginally significant trend (p = 0.061) with a higher prevalence in the rich socioeconomic group, which was not observed in Son et al. [21] who found no such correlation with socioeconomic status. This difference could be attributed to regional variations in healthcare access, socioeconomic status, and the distribution of pathogens.

Lastly, no statistically significant relationship between age, parity, or socioeconomic status with Methicillin sensitive Staphylococcus aureus (MSSA) as reported also by Yalew et al. [19] who also found no statistical significance in S.aureus. This indicates S. aureus as a normal pathogen that is not as much affected by demographic factors as other microorganism like E. coli or Klebsiella pneumoniae which do show trend in certain groups.

The findings of this study show valuable insight into the prevalence of aerobic microorganisms and their relation with unfavorable pregnancy outcome in a pregnant population. We find that during pregnancy, E. coli, Group B Streptococcus and Klebsiella pneumoniae are all prevalent pathogens that contribute to the occurrence of vaginal infections and are related to preterm birth and other complications. Although these factors are not strongly correlated in some cases, this indicates that such relationships are complex and that more detailed study of the underlying factors and putative interventions is needed.

This study has several limitations. One major aspect is a study done in one center, so that the generalizability of the results to a community may be a limitation. While the sample size is sufficient, it is restricted to a specific geographic region and may not reflect pregnant women in all regions. Furthermore, the cross sectional characteristics of the study do not allow us to infer causality of microorganisms identified with pregnancy outcomes. These findings need to be confirmed further in other multicenter longitudinal studies to understand them better and to develop more effective interventions.

CONCLUSION

The results of our study suggest that pregnant women are colonized with aerobic microorganisms, particularly E. coli, Group B Streptococcus and Klebsiella pneumoniae, and pregnant women thus have an increased risk for developing preterm birth and premature rupture of membranes. The findings indicate the importance of the effect of vaginal infection on determining pregnancy outcomes and the need of timely assessment and appropriate treatment.

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Author's Contribution

The authors have played crucial roles in the creation of this manuscript, as outlined below. Dr. Qandil Sattar spearheaded the development of the study, wrote the initial draft of the article, and facilitated the collection of hospital data.

Dr. Wajeeha Syed provided significant input in shaping the article, contributed to the study's conceptual framework, and conducted the analysis and interpretation of the data.

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