Isolation and Identification of Microorganisms from High Vaginal Swab of Pregnant Women visiting Paropakar Maternity and Women's Hospital, Kathmandu, Nepal

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ABSTRACT

Objectives: The primary objective was to isolate and identify the bacteria present in high vaginal swabs (HVS) of pregnant women and assess their antibiotic susceptibility patterns.

Methods: A cross-sectional study was conducted between January and June 2024, involving 300 HVS samples collected from pregnant women attending Paropakar Maternity and Women's Hospital (PMWH). The samples were cultured on Mac Conkey and Blood agar plates. Antibiotic susceptibility test of the identified bacterial colonies was carried out by Kirby Bauer disc diffusion method.

Results: Of the 300 samples, 79 tested positive for bacterial growth, revealing a diverse range of organisms, with *Escherichia coli* (29.11%), *Citrobacter koseri* (22.79%), *Proteus mirabilis* (20.25%), and *Klebsiella pneumoniae* (17.72%) as the predominant species. The highest incidence of bacterial presence was observed in the 21-25 age group (35.44%).

Conclusion: This study highlights the concerning prevalence of bacteria and their antibiotic resistance in HVS from pregnant women, underscoring the need for improved infection control measures and targeted antibiotic strategies.

Keywords: Bacterial Vaginosis, high vaginal swab, maternal health, bacterial load, pregnant women.

INTRODUCTION

Vagina has a dynamic ecosystem composed of vaginal flora which contains a diverse collection of microorganisms. It also maintains the acidic vaginal pH and provides protection against a diversity of pathogens (Islam et.al, 2009). Vaginal health is particularly crucial during pregnancy, as changes in the vaginal microbiome can significantly impact both maternal and fetal outcomes. The vaginal environment undergoes various physiological changes during pregnancy, influenced by hormonal fluctuations that affect the balance of microbial communities (Amin et al., 2023). Predominantly, the healthy vaginal microbiome is dominated by Lactobacillus species, which play a vital role in maintaining an acidic environment through the production of lactic acid (Condori et al., 2022). Disruptions in the balance of these microorganisms can lead to various health issues, emphasizing the importance of understanding vaginal microbiology (Ravel et al., 2021). The identification and isolation of microorganisms from high vaginal swabs (HVS) of pregnant women are used to detect infections that could lead to complications such as preterm labor, miscarriage, low birth weight and neonatal infections (Borges et al., 2021).

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However, it may lead to vaginal dysbiosis, which is linked to conditions such as bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and increased susceptibility to sexually transmitted infections (STIs) including HIV (Han et al., 2021). BV occurs when there is an overgrowth of harmful bacteria, particularly a decrease in the beneficial Lactobacillus bacteria (Central for Disease Control and Prevention, 2020). Escherichia coli and Klebsiella species are frequently isolated from high vaginal swabs, highlighting their significant presence in vaginal infections (Machado et al., 2015).

Studies have reported that there can be a chance of overlap of BV and aerobic vaginitis, leading to a mixed condition, but whether one condition can evolve into the other has not yet been determined (Eliza et.al, 2018). The importance of maintaining a balanced vaginal microbiota cannot be overstated, especially during pregnancy. Ongoing research continues to shed light on the complex interactions within the vaginal microbiome and their impact during pregnancy.

METHODS

Study design and setting

This descriptive and prospective study on the isolation and characterization of bacteria in high vaginal swabs (HVS) from pregnant women was conducted at Paropakar Maternity and Women's Hospital, Kathmandu, Nepal, from January 2024 to June 2024.

Inclusion and exclusion criteria

All clinical samples collected from pregnant women presenting at the hospital were included in the study. Additionally, samples from women who provided informed consent were prioritized.

If the samples were not properly labeled, had insufficient volume excluded. Repeated samples from the same patients were also excluded.

Sample collection

A total of 300 HVS samples were collected from consenting pregnant women. Sterile cotton swabs were used to obtain samples from the posterior fornix of the vagina, transported in sterile containers with transport medium, and processed under sterile conditions (Centers for Disease Control and Prevention, 2020).

Sample processing

All clinical specimens received in the microbiology laboratory were handled with strict aseptic techniques to prevent contamination. Upon receiving each specimen was inoculated onto MacConkey agar and blood agar plates using the quadrant streaking method. Then the inoculated MacConkey agar plates were incubated aerobically at 37°C for 24 hours and inoculated blood agar plates were incubated in CO_2 rich conditions at 37°C for 24 hours. To ensure accurate identification, colonies that grew on the primary plates were subcultured onto nutrient agar. The colony morphology, hemolytic properties on blood agar, colour change on MacConkey agar, and other observable traits, were meticulously noted. Following the observation of cultural characteristics, Gram's staining & biochemical tests were performed for the identification of the isolates.

Antibiotic susceptibility testing

Antibiotic susceptibility testing of isolates was conducted by using the Kirby-Bauer disk diffusion method on MHA according to CLSI guideline 2020. Antibiotic-impregnated discs including Cephalexin, Cefixime, Amikacin, Cotrimoxazole, Piperacillin-tazobactam, Ciprofloxacin, Meropenem, and Ampicillin were used for AST. The MHA plates were incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones around each disc were measured and compared to standardized interpretive charts to determine the susceptibility, intermediate or resistance of the bacterial strains to each antibiotic (Manandhar & Sharma, 2018).

Data analysis

Data were entered into MS Excel spread sheets and analyzed.

Ethical approval

Ethical clearance was obtained from the Institutional Review Committee (IRC) of Paropakar Maternity and Women's Hospital (PMWH), Ref No. 64/884, ensuring adherence to ethical standards, including informed consent and confidentiality.

RESULTS

Out of the 300 high vaginal samples analyzed, 79 were found to be positive for bacterial growth, representing 26.33% of the total. The remaining 221 samples, accounting for 73.67%, showed no bacterial growth (Figure 1).

The study revealed a diverse array of organisms, with significant prevalence of *Escherichia coli* (29.11%), *Citrobacter koseri* (22.79%), *Proteus mirabilis* (20.25%), and *Klebsiella pneumoniae* (17.72%). Additionally, organisms included *Klebsiella oxytoca* (5.06%), *Citrobacter freundii* (2.53%), *Enterobacter cloacae* (1.27%) and *Acinetobacter baumannii* (1.27%) were comparatively few (Table 1). These results shed light on the varied bacterial landscape within the sample population. Bacterial culture and AST on different media plates are shown in Figure 2.

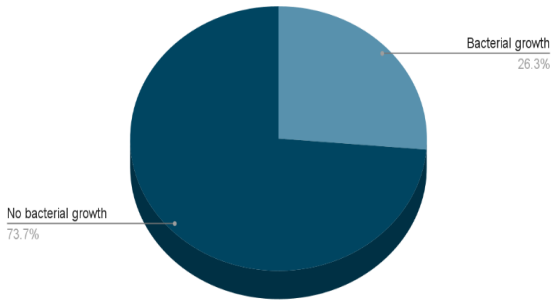

Figure 1: Bacterial growth pattern in total sample population

The study also revealed that the highest incidence of positive microbial cases was among women aged 21 to 25, who accounted for 35.44% of the total positive cases. This was followed by women aged 25 to 30, comprising 29.11% of the positive cases. Additionally, women in the age groups 15 to 20 and 31 to 35 each represented 16.45% of the positive cases. The lowest incidence was observed in women aged 36 to 40, who made up 2.53% of the positive cases (Figure 3).

Table 1: Distribution and frequency of bacterial isolates in the clinical specimens

S. N.	Isolated organism	No. of positive isolates form HVS	Positive isolates from HVS (%)
1	*Escherichia coli*	23	29.11
2	*Citrobacter koseri*	18	22.79
3	*Proteus mirabilis*	16	20.25
4	*Klebsiella pneumoniae*	14	17.72
5	*Klebsiella oxytoca*	4	5.06
6	*Citrobacter freundii*	2	2.53
7	*Enterobacter cloacae*	1	1.27
8	*Acinetobacter baumannii*	1	1.27
	Total	**79**	**100**

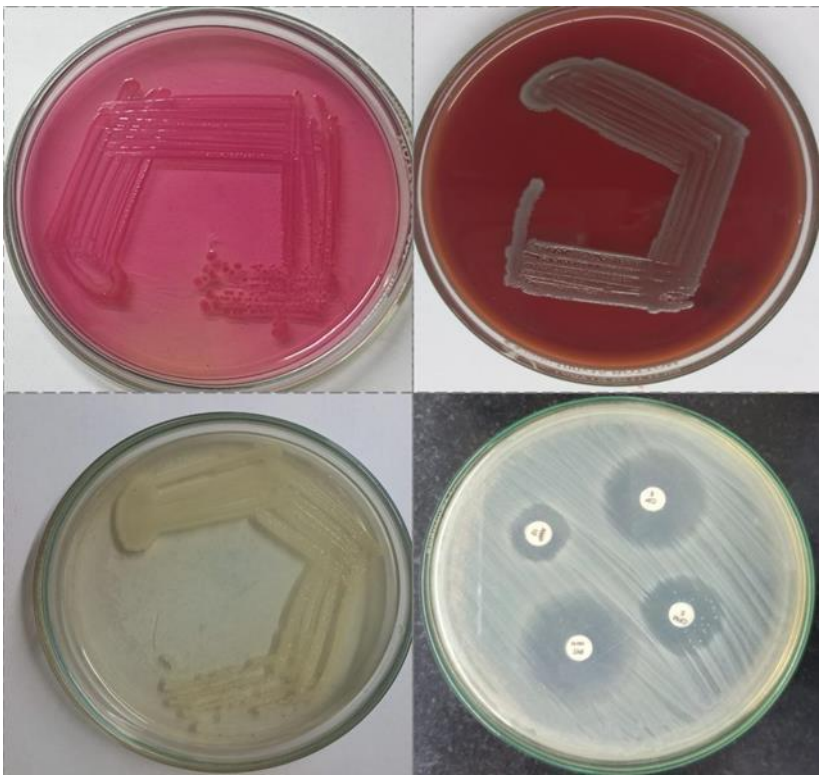

Figure 2: Bacterial culture showing different bacteria different media, MacConkey agar (upper left), blood agar (upper right) and nutrient agar (lower left) and antibiotic susceptibility test on MHA media (lower right).

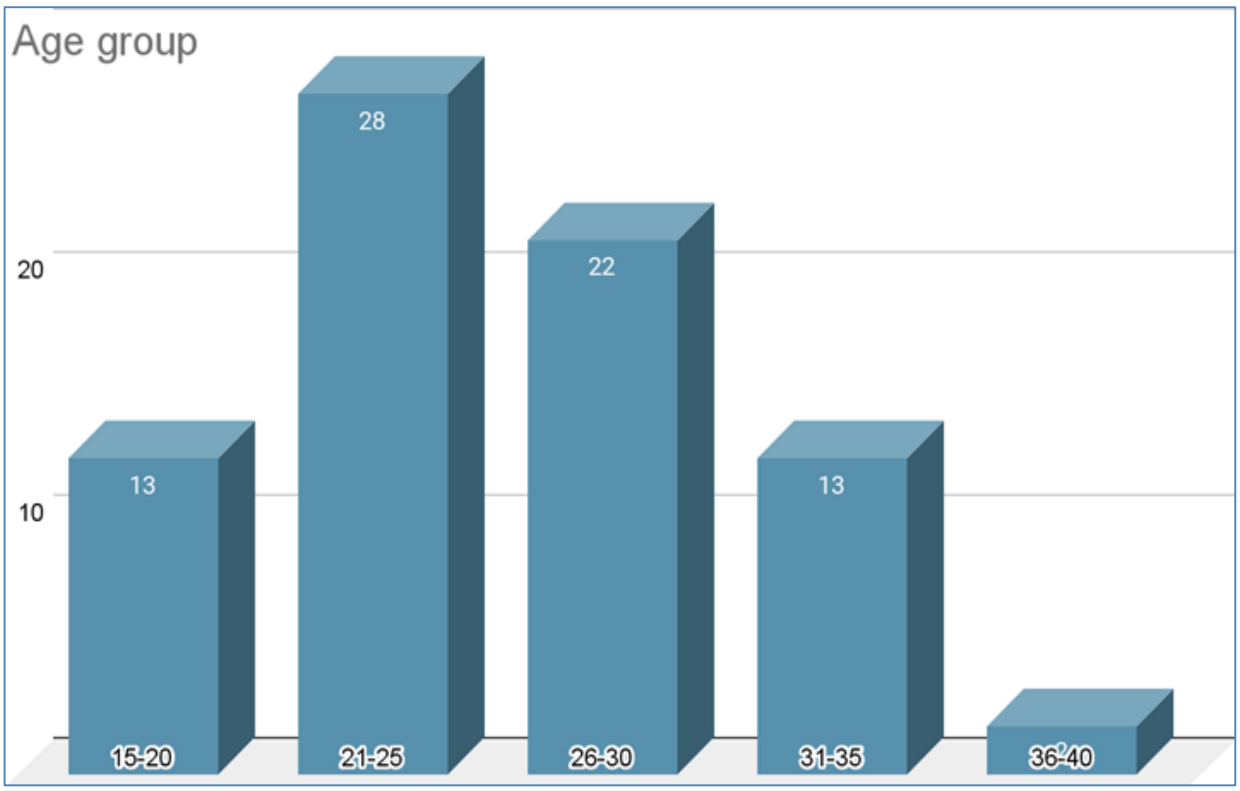

Figure 3: Distribution of bacterial isolates according to the age group of patients.

Table 2 shows the antibiotic resustance pattern of the isolated bacteria. *Proteus mirabilis* showed complete sensitivity to Meropenem and Amikacin (100%). It exhibited 81.25% sensitivity to Ciprofloxacin, Piperacillin-Tazobactam, and Cotrimoxazole. The isolates were 50% resistant to Ampicillin and Cephalexin, and 62.5% were resistant to Cefixime.

Escherichia coli demonstrated complete sensitivity to Meropenem and Amikacin and showed 56.52% sensitivity to Cephalexin. *E. coli* of 95.65% isolates were sensitive to Cefotetan and Piperacillin-Tazobactam. All *E. coli* were resistant to Ampicillin, while 56.52% were resistant to Cefixime, and 47.82% to Cotrimoxazole.

All *Klebsiella pneumoniae* isolates were sensitive to Meropenem and Amikacin with 92.85% sensitivity to Piperacillin-Tazobactam. Ciprofloxacin and Cotrimoxazole were found to be sensitive with 68.28% isolates. Resistance to Ampicillin was 100%, while 92.85% were resistant to Cefixime, and 57.14% were resistant to Cephalexin.

Citrobacter koseri isolates were 100% sensitive to Meropenem and Amikacin and 94.44% sensitive to Ciprofloxacin. However, they showed high resistance to Ampicillin and Cephalexin (83.33%), Piperacillin-Tazobactam (94.44%), Cotrimoxazole (61.11%), and Cefixime (55.55%).

Klebsiella oxytoca showed 100% sensitivity to Meropenem and Piperacillin-Tazobactam, 75% sensitivity to Amikacin and Ciprofloxacin, but complete resistance to Ampicillin and Cefixime. Cephalexin and Cotrimoxazole were found to be resistant with 50 % of the isolates.

Citrobacter freundii showed sensitivity to Ciprofloxacin, Cotrimoxazole, Amikacin, and Meropenem. However, it exhibited resistance to Ampicillin, Cephalexin, Piperacillin-Tazobactam, and Cefixime.

Enterobacter cloacae exhibited resistance to Ampicillin, Cotrimoxazole, Cefixime, and Cephalexin, while showing sensitivity to Ciprofloxacin, Amikacin, Piperacillin-Tazobactam, and Meropenem.

Similarly, *Acinetobacter baumannii* demonstrated resistance to Ampicillin, Cephalexin, Piperacillin-Tazobactam, and Cotrimoxazole, but was sensitive to Ciprofloxacin, Amikacin, Meropenem and Cefixime.

Table 2: Antibiotic resistance pattern of the isolated bacteria

Bacterial isolates (n)	CN	CFM	AMP	COT	PIT	CIP	AK	MRP
P. mirabilis (6)	50%	62.5%	100%	18.75%	18.75%	18.75%	0%	0%
E. coli (23)	43.48%	56.52%	100%	47.82%	4.35%	4.35%	0%	0%
K. pneumoniae (14)	57.14%	92.85%	100%	35.72%	7.15%	35.72%	0%	0%
C. koseri (18)	83.33%	55.55%	100%	61.11%	94.44%	5.56%	0%	0%
K. oxytoca (4)	50%	100%	100%	50%	100%	25%	25%	0%
C. freundii (2)	100%	100%	100%	0%	100%	0%	0%	0%
E. cloacae (1)	100%	100%	100%	100%	0%	0%	0%	0%
A. baumanii (1)	100%	100%	100%	100%	100%	0%	0%	0%

CN= Cephalexin; CFM= Cefixime; AMP= Ampicillin; COT= Co-trimoxazole; PIT= Piperacillin-tazobactem; CIP= Citrofloxacin; AK= Amikacin; MRP= Meropenem

DISCUSSION

In this study, 79 out of 300 samples (26.33%) tested positive for bacterial growth, with the remaining 221 samples (73.67%) showing no bacterial growth. This highlights a diverse bacterial landscape among the pregnant women sampled.

The predominant organisms identified were *Escherichia coli* (29.11%), *Citrobacter koseri* (22.79%), *Proteus mirabilis* (20.25%), *Klebsiella pneumoniae* (17.72%). Other microorganisms included, *Klebsiella oxytoca* (5.06%), *Citrobacter freundii* (2.53%), *Enterobacter cloacae* (1.27%), *Acinetobacter baumannii* (1.27%). Comparing these results with a study from Uganda, where was the *E. coli* predominant isolate, shows a similar trend but with notable differences in the presence of *Citrobacter koseri* in this study (Lutambi et al., 2023). Additionally, research in India also found *E. coli* and *Klebsiella pneumoniae* as leading pathogens in HVS samples, corroborating oufindings regarding the prevalence of *E. coli* (Singh et al., 2021).

Age-based analysis of this study reveals that the highest incidence of bacterial growth in high vaginal swabs (HVS) was observed in women aged 21 to 25 years, followed by those aged 26 to 30 years. These findings are consistent with research conducted in other regions, which also identified young, sexually active women as having a higher susceptibility to infections.

The resistance patterns observed in this study highlight the need for ongoing surveillance of antibiotic susceptibility in clinical settings. The significant resistance to Ampicillin across multiple species suggests that its use in this population should be reconsidered, as its efficacy is increasingly limited. Conversely, the consistent sensitivity to Meropenem and Amikacin suggests these antibiotics remain reliable options for treating infections in pregnant women. Careful antibiotic selection is crucial, particularly given the observed resistance to commonly used antibiotics like Ampicillin and Cefixime. This approach will help ensure effective treatment and prevent the further development of resistance, emphasizing the importance of tailored therapy based on current susceptibility data.

These findings underscore the importance of enhancing infection control measures within the hospital to prevent the spread of these resistant organisms. Given the high patient load, strict adherence to infection control practices is paramount. Additionally, this data also highlights the need for further research on the prevalence and resistance patterns of bacteria, especially considering the limited studies on HVS in Nepal. Educational programs aimed at increasing awareness among pregnant women about maintaining vaginal health, recognizing symptoms of infections, and seeking timely medical care are crucial. Lastly, investing in advanced laboratory equipment and staff training is essential for improving the accuracy and efficiency of bacterial identification and susceptibility testing, thereby contributing to better maternal health outcomes.

Conclusion

This study highlights the concerning prevalence of bacteria and their antibiotic resistance in HVS from pregnant women, underscoring the need for improved infection control measures and targeted antibiotic strategies.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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