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Prevalence of bacterial vaginal infections in asymptomatic diabetic women

Lakshmi K*[1], Chitralekha S[1] , Illamani V[1], Menezes GA[1]

1. Department of Microbiology, Sree Balaji Medical College & Hospital, Chromepet, Chennai

Abstract:

Vaginal infection is a common disease of women but Diabetic women are more often affected. Around menopause, there is decrease in estrogen production with thinning and inactivity of the vaginal epithelium along with increase in pH. An estrogen deficient vagina as well as the immunocompromised status due to diabetes can lead to growth of abnormal flora which may in turn lead to infections. The aim of the study is to compare the prevalence of asymptomatic vaginal infections among the diabetic and non diabetic women. Type 2 diabetic women and non diabetic women of the age group 40 – 70 years were randomly selected from the patients attending a tertiary care hospital in Chennai, who were asymptomatic of vaginal infections. High vaginal swab specimens were collected from them and processed immediately in the microbiology laboratory and the micro organisms identified and their antibiotic susceptibility noted. Fifty diabetic and fifty non diabetic women were enrolled in this study. The prevalence of pathogenic organisms like *Escherichia coli* (18%), *Staphylococcus aureus* (12%), *Candida* (18%) in diabetic women was found to be higher than that reported in non-diabetic women (12 %, 6%, and 14% respectively). Diabetic women are more prone for vaginal infections. Pathogenic bacteria are also found as frequently as the *Candida* sp. in the diabetic women. So, the use of empirical antifungal therapy without taking high vaginal swab may not be appropriate for all cases of vaginal infections.

Key words: Vaginal infections, Diabetes, Asymptomatic.

*Corresponding Author: *Dr. Lakshmi K, Assistant Professor, Dept. of Microbiology, Sree Balaji Medical College & Hospital, Chennai. Email: laksh45@gmail.com

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Introduction

Vagina is equipped with physiological defence mechanisms to prevent colonization by pathogenic microorganisms. The factors which influence the growth of organisms in the vagina include pH, glycogen content, vascularity and hormonal status. Type 2 Diabetes is a very important cause for increase in the occurrence of vaginal infections in the perimenopausal age group. Poor glycemic control in diabetics is thought to result in impaired action of polymorphonuclear leucocytes resulting in decrease in the resistance causing opportunistic infections.

The two most common causes of vaginitis are bacterial vaginitis and *Candida*

vaginitis. Bacterial vaginitis is caused by growth of pathogenic bacteria in the vagina along with decrease in Lactobacilli [1,2]. *Candida* vaginitis is caused by *Candida* sp, characterized by inflammation of the female genitalia.

In diabetic women, vulvovaginitis is often treated with antifungal agents on the assumption that the causative organism is mainly *Candida albicans*. But it is not only *Candida* sp, some bacteria and other organisms may also cause the infection. To our knowledge, there has been no study which demonstrates an association between diabetes and the risk of bacterial vaginal infections. Therefore it was determined to analyse the prevalence of bacterial vaginal infections in asymptomatic type 2 diabetic women and compare the results with that of non diabetic women of similar category. The evaluation of the asymptomatic vaginal infections may help in assessment of the nature of infection, need for proper investigation and appropriate management of vaginitis in such patients.

Materials and methods

This cross sectional study was done at a tertiary care hospital in Chennai, from August 2010 to May 2011. Fifty diabetic and fifty non diabetic women were enrolled in the study of the age group 40 – 70 years who were randomly selected from the patients attending the hospital. Those who were symptomatic of vaginal infection and those with the history of usage of antimicrobial and antifungal therapy in the last 14 days were excluded from the study. The study was approved by the institutional ethical committee and an informed consent was obtained from all the participants. Patients were assessed using a questionnaire.

During the visit the women were put into the lithotomy position and a clean bi-valve speculum was inserted into the vagina. After proper inspection of the vagina, 3 high vaginal swab samples were collected from lateral or posterior wall of the vagina. The swabs were transported immediately to the microbiology laboratory and processed. The pH of the vaginal discharge was assessed using standard Litmus paper test. Direct smear Gram staining and wet mount examinations were done under microscope. Swabs were cultured onto Nutrient Agar, Blood Agar, Mac Conkey agar and Columbia Agar plates and incubated aerobically at 37˚C for 24 - 48 hrs. Another swab was inoculated in Chocolate agar, Columbia agar and Bile Esculin agar and incubated anaerobically. After 24 hours, the plates were examined for the presence of growth and the degree of growth was noted as profuse, moderate or scanty. Preliminary identification of organism was made by colony morphology using hand lens. In case of mixed growth, the relative degree of growth of each species was noted. Then the growth was subjected to Gram stain, hanging drop, oxidase, catalase tests and other biochemical tests and sugar fermentation tests. If no growth occurred, the plates were examined after further incubation for another 24 hours before reporting as No growth. After identification of the microorganism, Antibiotic susceptibility testing of the bacterial isolates were done by Kirby Bauer Disc diffusion technique using Mueller Hinton agar plates and the susceptibility or resistance of the isolates were recorded.

Statistical analysis: The clinical and laboratory data thus obtained were computerised and analysed using the statistical package of the Microsoft Office Excel 2007 Enterprise Edition. Data were analysed by SPSS statistical software and p value of <0.05 was considered significant. Mean values are reported as mean ± standard deviation.

Table - 1
Age distribution of the cases

Age in years	Study group	Control group	Total
40 – 50	21	27	48
50 – 60	19	16	35
60 – 70	10	7	17

Table – 2
Distribution of diabetic women according to the duration of diabetes

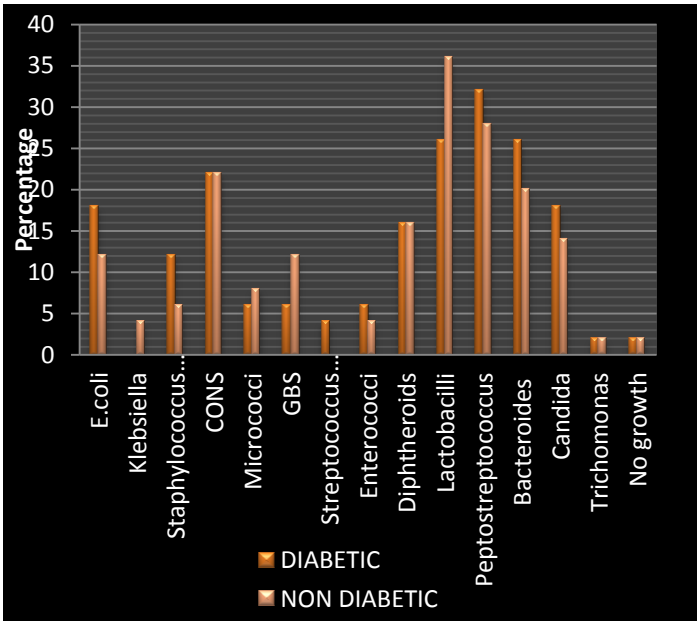
Duration of diabetes	Pre menopause	Post menopause	Total
0 – 5 yrs	11 (57.9 %)	10 (32.3 %)	21 (42%)
5 – 10 yrs	8 (42.1 %)	13 (41.9 %)	21(42%)
>10 yrs	-	8 (25.8 %)	8 (16%)

Table – 3
Microorganisms isolated from culture

The microorganisms isolated were Bacteria, *Candida* spp. and Trichomonas spp. Majority of the bacteria in the vagina of both diabetic and non-diabetic women were found to be anaerobes. In addition to Lactobacilli, *Peptostreptococcus* sp. and *Bacteroides* sp. were the anaerobes reported.

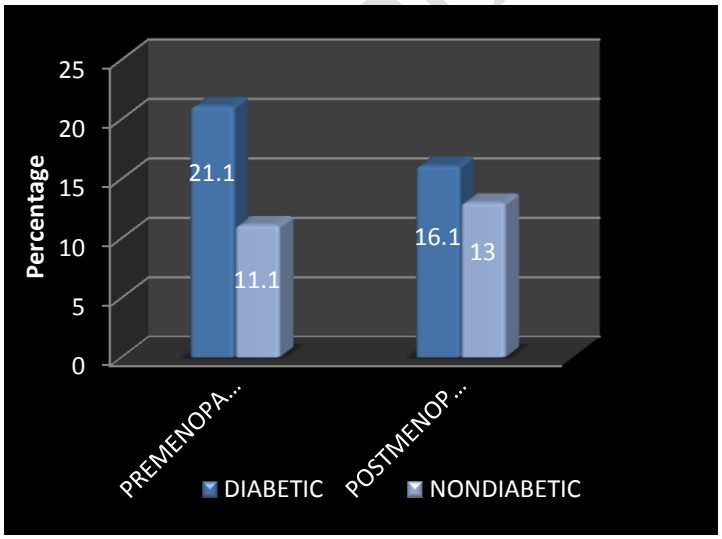
Organisms	Diabetic women (n=50)	Non diabetic women (n=50)	Total (n=100)
E.coli	9(18%)	6(12%)	15(15 %)
***Klebsiella* sp.**	0	2(4%)	2(2%)
Staphylococcus aureus	6(12%)	3(6%)	9(9%)
Coagulase negative *Staphylococcus aureus*	11(22%)	11(22%)	22(22%)
Micrococci	3(6%)	4(8%)	7(7%)
Group B *Streptococcus*	3(6%)	6(12%)	9(9%)
Streptococcus viridians	2(4%)	0	2(2%)
Enterococci	3(6%)	2(4%)	5(5%)
Diphtheroids	8(16%)	11(22%)	19(19%)
Lactobacilli	13(26%)	18(36%)	31(31%)
Peptostreptococcus	16(32%)	14(28%)	30(30%)
***Bacteroides* sp.**	13(26%)	10(20%)	23(23%)
***Candida* sp.**	9(18%)	7(14%)	16(16%)
***Trichomonas* sp.**	1(2%)	1(2%)	2(2%)
No growth	1(2%)	1(2%)	2(2%)

Graph 1: Comparison of the prevalence of microorganisms among the diabetic and non-diabetic women

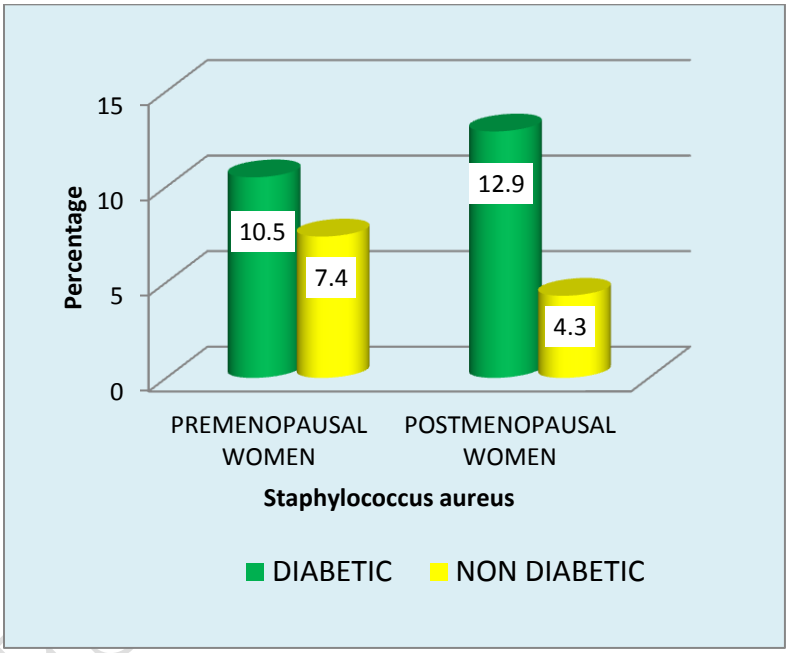


E. coli, *Staphylococcus aureus*, *Peptostreptococcus*, *Bacteroides* sp. and *Candida* sp. were found to be more prevalent in diabetic women than the non-diabetic women. Lactobacilli, Group B Streptococci, Micrococci were more prevalent in non-diabetic women.

Graph 2: Chart showing the difference in the prevalence of *Escherichia coli* among the diabetic and non-diabetic women:



Graph 3: Prevalence of *Staphylococcus aureus* among the diabetic and non-diabetic women



Results and Discussion

The vaginal flora plays a major role in the occurrence and prevention of any vaginal infection in women, especially with diabetes. Our study demonstrates the prevalence of normal vaginal flora as well as the potential pathogens, in essentially healthy, asymptomatic sample of diabetic women and compares it with non-diabetic women of similar category.

The microorganisms isolated in our study were predominantly bacteria along with *Candida* sp. and *Trichomonas* sp. The bacterial isolates were *E. coli*, *Klebsiella* sp., *Staphylococcus aureus*, Coagulase negative *Staphylococcus*, Micrococci, Group B Streptococci, *Streptococcus viridans*, *Enterococcus* sp., Diphtheroids, Lactobacilli, Peptostreptococci, *Bacteroides* sp. Hiller [3] reported similar spectrum of microflora in the vagina of pregnant women. The increased prevalence of pathogenic organisms in the diabetic women in our study show that the Diabetic women are significantly more prone to developing vaginitis (both bacterial and fungal) than non-diabetic women in correlation with the study by Rahman T *et al*. [4].

Table – 7 antibiogram
Antibiotic sensitivity for gram positive organisms

Isolates	P	Am	Ac	Co	Cu	Az	Cf	Of	T	Ox
***S. aureus* (n=9)**	9 (100%)	2 (22.2%)	6 (66.7%)	2 (22.2%)	8 (88.9%)	8 (88.9%)	2 (22.2%)	1 (11.1)	7 (77.8)	6 (66.7)
CoNS (n=22)	11 (50)	15 (68.1)	20 (90.9)	19 (86.3)	20 (90.9)	20 (90.9)	14 (63.6)	15 (68.1)	18 (81.8)	22 (100)
Micrococci (n=7)	2 (28.5)	2 (28.5)	4 (57.1)	5 (71.4)	4 (57.1)	5 (71.4)	3 (42.8)	2 (28.5)	6 (85.7)	7 (100)
Group B *Streptococcus* (n=9)	5 (55)	6 (66)	8 (88)	6 (66)	2 (22)	3 (33)	5 (55)	6 (66)	4 (44)	6 (66)
***Streptococcus viridians* (n=2)**	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	1 (50)	1 (50)	2 (100)	2 (100)
Enterococci (n=5)	3 (60)	3 (60)	4 (80)	4 (80)	4 (80)	5 (100)	4 (80)	4 (80)	4 (80)	3 (60)
Diphtheroids (n=19)	15 (78.9)	16 (84.2)	18 (94.7)	17 (89.4)	18 (94.7)	18 (94.7)	15 (78.9)	16 (84.2)	18 (94.7)	16 (84.2)

P- penicillin, Am- Amoxycillin, Ac- Amoxycillin/Clavulanic acid, Co- cotrimoxazole, Cu- cefuroxime, Az- azithromycin, Cf- ciprofloxacin, Of- ofloxacin, T- tetracycline, Ox- oxacillin. CoNS- Coagulase negative *S. aureus*

Table - 8
Antibiotic sensitivity pattern for gram negative isolates

Three of the *Staphylococcus aureus* isolates were found to be Oxacillin resistant (Methicillin Resistant Staphylococcus Aureus - MRSA). MRSA strains were found to be susceptible to vancomycin. The Gram negative isolates were found to be highly sensitive to nitrofurantoin, amikacin, and gentamicin.

Isolates	Nf	Na	Nf	G	Ak	Az	Ce	Cn	Ca	Cfx	Cf	Cu
E.coli (n =15)	0	0	15 (100%)	10 (66.7%)	15 (100%)	10 (66.7%)	8 (53.3%)	8 (53.3%)	10 (66.7%)	6 (40%)	2 (13.3%)	4 (26.7%)
Klebsiella (n=2)	2 (100%)	0	2 (100%)	2 (100%)	2 (100%)	0	2 (100%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	0

Nf- norfloxacin, Na- nalidixic acid, Nf- nitrofurantoin, G- gentamicin, Ak- amikacin, Az- aztreonam, Ce- cefotaxime, Cn- ceftriaxone, Ca- ceftazidime, Cfx- cefixime, Cf- ciprofloxacin, Cu- cefuroxime

Like the earlier study [4], *Escherichia coli* were found to be the most pathogenic bacteria (15%) isolated from culture. The rate of colonization of *E. coli* was found to be inversely associated with the presence of lactobacilli in our study which is in agreement with the existing literatures [5,6]. This suggests that lactobacillus plays an important role in the preventing invasion of uropathogens and subsequent development of urinary

tract infection (UTI). Diabetic women have been found to have higher prevalence of *E. coli* than non-diabetic women in accordance with the study by Wendy [6]. Similar to the previous studies [7,8,9], we also found that the diabetic women with recent history of UTI were at high risk of *E. coli* vaginal colonization. This may be because of the fact that type 1 fimbriated *E. coli* adheres in significantly higher numbers to the uroepithelial cell of diabetic women than the non-diabetic women, as demonstrated by Geerlings SE [10].

Klebsiella were seen in only two of the non-diabetic women. *Klebsiella* sp. was surprisingly absent in the diabetic women. *S. aureus* was found to be more prevalent in diabetic women (12%) when compared to non-diabetic (6%) women. Group B *Streptococcus* (GBS) was isolated in 9% of the women. A range of 5 to 40% of vaginal carriers had been found in different studies due to difference in the sample sites and the various cultural methods employed [11,12,13]. It is found to be significant during pregnancy and in neonatal infection. Its pathogenicity in diabetes is not clearly known, which require further extensive studies in future. The prevalence of coagulase negative *Staphylococcus* which is considered as the skin commensal is found to be similar in both the diabetic and the non-diabetic women. *Streptococcus viridans* was found in 4% diabetic women (p value = 0.065).

Lactobacilli, Peptostreptococci, Bacteroides, were the anaerobes isolated in our study which is similar to the study by Aggarwal in 2003 [14], who also found only the above mentioned anaerobic species in asymptomatic group of women. Our data demonstrate that the diabetic women have much lower prevalence of vaginal lactobacilli than healthy non diabetic women. The anaerobic Gram positive cocci (*Peptostreptococcus* sp.) and Gram negative rods (*Bacteroides* sp.) were the most frequent anaerobic microorganisms recovered from the women next to Lactobacilli. The prevalence of these anaerobes was comparatively more in diabetic women than non-diabetic women.

Candida sp. was found in 16% of the women in our study which similar to the study by O. Grigoriou [15]. Peer AK *et al* [16] isolated *Candida species* in 25% of asymptomatic diabetic women. *Candida* sp. was found to be significantly more in diabetic women than non-diabetic control group in agreement with the study by Goswami *et al* [17]. However, in the present study *Candida* sp. has been reported more than that reported by Fischer et al (3%) [18].

Cauci *et al* [19] have suggested that the Nugent scoring system may not be adequate for evaluation of vaginal flora in women >40 years old, because no lactobacilli or BV-associated microorganisms are detected in many cases. Hence, the usual scoring methods of bacterial vaginosis are not followed in our study.

Conclusion

Our present study shows that the Diabetic women are at higher risk of vaginal infections. The finding of increased colonization of *E. coli* in addition to *Candida* sp. in asymptomatic diabetic women provides insight into the pathophysiology of the increased risk of UTI in them and the bacterial cause of any vaginitis should not be forgotten in future. In conclusion, both pathogenic bacteria and *Candida* sp. are found to be more prevalent in diabetic women. So, in diabetic women with genital symptoms, an attempt at diagnosis should be made prior to commencement of therapy. The practice of initiating antifungal treatment for any vaginal infection in diabetic women without taking high vaginal swabs should be reviewed. The use of empirical antifungal therapy alone may not be appropriate for some cases and consideration should be given for the use of appropriate antimicrobial agents along with antifungal drugs.

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