



Original article

Micro-organisms associated with cervico-vaginal discharges: A cross-sectional study

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ABSTRACT

Introduction: Regional establishment of microbiological profile helps in management of vaginal infections. The present study aims to profile the microorganisms associated with cervico vaginal discharges in females from low socio economic semi urban centres of a developing country causing STI syndrome. **Materials and methods:** Total 150 patients with complaint of vaginal discharges were studied. Cervico-vaginal secretions were obtained from females coming to the peripheral hospitals and Urban Health Centre, in Mumbai. Two high vaginal swabs were obtained from all the women. Gram's staining, Pap stain, wet mount, amine test, pH and culture were done. Blood sugar level, anti HIV, HBsAg and RPR tests were performed. Descriptive statistics was used with mean (SD) for quantitative variables and number for categorical variables. Chi-square test for association was used to assess the significance of differences in the proportion of categorical variables between different groups. **Results:** Of the 150 participants, 90% were found to be infected of which 47.4% had aerobic micro-organisms; *Candida* species (19.3%). Tests for HIV, HBsAg or VDRL were negative. No association was obtained between the levels of blood sugar and micro-organisms grown. Statistically significant ($P < 0.0001$) difference was observed in the proportion of samples positive for *Trichomonas* and *Candida* species. 48% were detected to have bacterial vaginosis by Amsel's criteria while 24% and 30.6% were detected by Hay/Ison gradation and Pap smear respectively. **Conclusion:** This study indicates the need for further assessment of the trends that might be associated with vaginal infections and hence the modalities for their prevention.

KEYWORDS: Microbiological profile, criteria, vaginal secretions.

INTRODUCTION

One of the common complaints of women visiting general practitioners is vaginal discharge. An estimate of 5-10 million visits to out-patient clinics per year worldwide has been attributed to vaginal discharge [1]. Accurate diagnoses of etiology for vaginal discharge are important to individualize the treatment. Although provisional diagnoses depend on the clinical profile of the vaginal discharge, a definitive diagnosis can be attained only after laboratory examinations that include microscopy and culture and sensitivity [2]. The importance of accurate diagnoses stems from the fact that vaginal discharge predisposes to pelvic inflammatory diseases, infertility, endometriosis, cuff cellulitis, urethral syndrome, pregnancy loss, preterm labour, increased susceptibility to sexually transmitted infections (STI) and to be associated with low birth weight and preterm birth [3].

Few risk factors such as diabetes mellitus and use of drugs like immune suppressants, broad spectrum antibiotics and pregnancy have been identified to predispose infectious vaginal discharge [4]. Other than pathological states, vaginal discharge can also be seen physiologically in women during following states: pre-pubertal, during menstruation, pregnancy or using agents like oral contraceptive pills and intra-uterine devices [5]. Of the above mentioned etiologies, bacterial vaginosis (BV) is the most common cause of abnormal vaginal discharge among women of childbearing age and is associated with STI including human immunodeficiency virus (HIV) infections and adverse birth outcomes [6].

Clinically, Amsel's criteria (having three items) are used for diagnosing BV and are considered as gold standard. Recently, it has been observed that the diagnostic accuracy of two criteria. (pH and vaginal discharge) is similar to the

three (pH, vaginal discharge and presence of clue cells) thus improving the clinical utility of the criteria even in areas with limited resources [7]. The causes, microbiological profile and sensitivity of the isolated micro-organisms from the vaginal secretions vary from one region to the other. Mayo researchers conducted the first direct assessment uterine microbiome study published in *Genome Medicine*.

The study attempted to discover whether there is a microbiome component in the malignancy of tumors and if its appearance in patients diagnosed with the disease is distinguishable from that of patients without malignancy. The study is investigating the possibility of using vaginal swabs as early detection tools for endometrial cancer [8].

Hence the present study is an attempt to assess the microbiological profile and their antimicrobial sensitivity from vaginal secretions. Additionally, we also attempted to compare potassium hydroxide mount, wet mount, Gram stain, PAP stain and culture and sensitivity of the isolated micro-organisms and also the diagnostic accuracy of Amsel's criteria amongst the patients with BV.

MATERIALS AND METHODS

Study ethics:

The study was commenced after obtaining approval from the institutional ethics committee and informed consent from the study participants between January 2012 and December 2014. Appropriate institutional approvals were taken for recruiting patients from Dr. R.N. Cooper Municipal General Hospital and Malawani Urban Health Centre in Mumbai.

Study population:

Women with complaints of vaginal discharge with or without symptoms were included in this study. Those who were not married, sexually inactive or had menstruation at the time of sampling were excluded from the study.

Study procedure:

Two high vaginal swabs were obtained from each of the eligible participants. Both were taken in the same pattern, one of the swabs was kept in 3 ml of sterile modified Stuart's transport medium to preserve the viability of facultative organisms such as *Neisseria gonorrhoeae* and other pathogens. The other swab was kept in 3 ml of 0.9% normal saline for microscopical examination and Gram's staining. All the vaginal swabs (except a wet preparation that was examined immediately) were delivered to the laboratory within 24 hours of collection. Gram's staining, Pap stain [9], wet mounting of the film, Amine test [10] and pH determination [11] were carried out.

The collected vaginal swabs were cultured on superimposed blood agar, Columbia human blood bilayer agar with added *Gardnerella vaginalis* selective supplement (HBT agar), MacConkey agar, Sabouraud agar and Modified Thayer Martin medium. The inoculated culture media were incubated at 37°C for 24-48 hrs. Modified Thayer-Martin medium and HBT agar were incubated in anaerobic Jar with

Anaero Hi Gas Pack (HiMedia Laboratories, Pvt. Ltd, Mumbai, India) to provide an increased CO₂ tension of 5-10% for optimum growth of *Neisseria gonorrhoeae* and *Gardnerella vaginalis*.

The isolated organisms were diagnosed by colonial morphology and characteristics of the culture. The purified isolates were inoculated in bio chemicals to identify them using Bergey's Manual [12] and *Candida* species were identified using CHROM agar and germ tube method as described by Sheppard *et al* [13]. Antibiotic sensitivity test was done using antibiotic discs on Muller Hinton agar by Kirby-Bauer method using Clinical and Laboratory Standards Institute guidelines [14]. Additionally, for the diagnosis of bacterial vaginosis, both Amsel's criteria and Hay/Ison gradation were compared.

Also blood was collected for random blood sugar level and tests for HIV and hepatitis B surface antigen (HBsAg) in addition to the rapid plasma reagin (RPR).

Statistical analysis:

Descriptive statistics was used with mean (SD) for quantitative variables and number (percentages) for categorical variables. Chi-square test for association was used to assess the significance of differences in the proportion of categorical variables between different groups. With an expected proportion of prevalence of infections by micro-organisms to an extent of 90%, 5% precision and 5% type 1 error; sample size was calculated with Dunlop's formula [15] to be 140 and so was rounded off to 150 in the present study.

RESULTS

Demographic details:

Socioeconomic status

All the women who participated in this study were asked about their literacy, personal hygiene and socioeconomic status. Almost all i.e. 96.7% of patients having complaint of vaginal discharge were under low socioeconomic status. Illiteracy rate was higher i.e. 68%. Poor hygiene was found in 88.7% of cases. (Table 1)

A total of 150 participants were recruited in the present study of which 135 (90%) were found to be infectious in terms of growing one or the other micro-organism. Slightly more than half [54%, 81] of the study participants had complaints of lower abdominal pain, 64 (42.7%) patients had burning micturition and 57 (38%) had itching. (Table 2) Also, in a large majority of the cases (133/150, 88.7%) a poor hygiene was observed. In 90(60.0%) cases thick white homogeneous vaginal discharge was found followed by thin white in 31 (20.7%), curdy white 16 (10.6%), yellowish thin 12 (8%) and greyish white homogeneous discharge 1 (0.7%). Of the total 135 women, 77 (57.3%) belonged to the age group of 17-30 years followed by 40 (29.6%) between 31 and 40 years of age. (Table 3)

Table 1: Classification of patients according to their socioeconomic status, literacy and hygiene.

Status	No of cases	Percentages
Literate	48	32.0
Illiterate	102	68.0
Good hygiene	17	11.3
Poor hygiene	133	88.7
High socioeconomic status	5	3.3
Low socioeconomic status	145	96.7

Table 2: Number of percentages of other findings on P/V

Other findings	Number of cases		Percentages%	
	Yes	No	Yes	No
Genital sores	0	150	0	100
Itching	57	93	38.0	62.0
Urinary burning and frequency	64	86	42.7	57.3
Pain in lower abdomen	81	69	54.0	46.0

Table 3: The prevalence of pathogens causing vaginal discharge in different age groups

Type of infections	17-30	31-40	41-50	>50	Total
BV	0	1	2	0	3
Candidiasis	14	8	4	0	26
Trichomoniasis	2	2	0	0	4
Aerobic infection	37	20	4	3	64
Mixed infections	24	9	5	0	38
Total	77	40	15	3	135

(Chi-Square test=16.99, df =12, P=0.155) non-significant

Table 4: Diagnoses of the patients with infective vaginal discharge

Causative agent	Number	Percentage%
Aerobic vaginitis	64	42.7
Candidiasis	26	17.3
Bacterial vaginosis	3	2.0
Trichomoniasis	4	2.7
Aerobic vaginitis and candida	25	16.7
Aerobic vaginitis and BV	4	2.7
Aerobic vaginitis and Trichomoniasis	5	3.3
Candida and trichomonas	2	1.3
Aerobic vaginitis, Candida and Trichomoniasis	2	1.3
No infection	15	10.0
Total	150	100

Table 5: Microbiological profile of the specimens

Pathogenic organisms	Numbers	Percentage
<i>Staphylococcus aureus</i>	28	18.7
<i>Staphylococcus epidermidis</i>	16	10.7
<i>Candida</i> spp	55	36.7
<i>Klebsiella</i> spp	20	13.3
<i>Streptococcus</i> spp	21	14.0
<i>Escherichia coli</i>	11	7.3
<i>Gardnerella vaginalis</i>	6	4.0
<i>Pseudomonas aeruginosa</i>	2	1.3
<i>Listeria monocytogenes</i>	2	1.3
Total	161	107.3%

*Total number of micro-organisms grown may exceed 150 as some participants had growth of multiple micro-organisms

Diagnoses in patients with vaginal discharge:

Excluding the 15 participants who had non-infective vaginal discharge, in the remaining, nearly one-half (64/135, 47.4%) of the study participants predominantly grown aerobic micro-organisms followed by *Candida* species (26/135, 19.3%). Table 4 depicts the diagnostic profile of study participants. No statistically significant differences were obtained between the diagnoses and the age group.

Microbiological profile of the infective vaginal discharges:

Although 135 participants were diagnosed to have infectious vaginitis, 161 micro-organisms were grown in total. Table 5 depicts the micro-organisms grown in the culture. A total of 55/161 (34.1%) of the isolated organisms were *Candida* species. A total of 59 participants had clue cells suggestive of *Gardnarella vaginalis* in the Gram stain of which only six (9.2%) had actually grown the micro-organism in the

culture. However, in all these six cases, a high pH ranging between 7 and 8 was observed. Amongst the *Streptococcus* species (n=21), six (28.6%) each Group B *Streptococci* and Group D non *Enterococci*, 5 (23.8%) Group D *Enterococci* and two (9.5%) each *Streptococcus viridans* and Group C, F and G *Streptococci*. Similarly, amongst the *Candida* spps, *Candida albicans* was the most common (22/55, 40%) followed by *Candida glabrata* (20, 36.4%), *Candida dubliniensis* (7, 12.7%) and *Candida krusei* (6, 10.9%). Of the 22 *Candida albicans* positive specimens, 19 (86.4%) were positive in germ tube test. Interestingly, Candidal growth was also associated with other pathogenic micro-organisms such as *Staphylococcus aureus* (51.7%) and *Klebsiella pneumoniae* (31%). Table 6 compares the antibiotic sensitivity pattern of various isolated organisms. The sensitivity of the individual *Candida* species to fluconazole was as follows: *Candida albicans* (59.1%), *Candida glabrata* (5%), *Candida dubliniensis* (28.6%) and *Candida krusei* (16.7%).

Table 6: Antibiotic sensitivity pattern (percentage of isolated micro-organisms)

Antibiotics	<i>S. aureus</i>	<i>S.epidermidis</i>	<i>Streptococcus pp</i>	<i>E.coli</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Listeria</i>
Gentamicin	21(75%)	13(81.3%)	17(80.9%)	10(90.9%)	18(90%)	2(100%)	1(50%)
Ciprofloxacin	19(67.9%)	9(56.3%)	17(80.9%)	5(45.5%)	15(75%)	1(50%)	2(100%)
Ceftriaxone	27(96.4%)	16(100%)	21(100%)	11(100%)	20(100%)	2(100%)	12(100%)
Ceftazidime	25(89.3%)	16(100%)	16(76.2%)	11(100%)	19(95%)	2(100%)	0%
Cefotaxime	26(92.8%)	12(75%)	18(85.7%)	11(100%)	16(80%)	2(100%)	1(50%)
Ofloxacin	24(85.7%)	12(75%)	14(66.7%)	8(72.7%)	15(75%)	2(100%)	2(100%)
Erythromycin	22(78.6%)	9(56.3%)	15(71.4%)	9(81.8%)	3(15%)	2(100%)	0%
Lomefloxacin	21(75%)	9(56.3%)	17(80.9%)	9(81.8%)	19(95%)	2(100%)	1(50%)
Imipenem	-	-	-	11(100%)	16(80%)	2(100%)	0%
Cefixime	13(46.4%)	13(81.3%)	18(85.7%)	10(90.9%)	17(85%)	2(100%)	0%
Azithromycin	19(67.9%)	11(68.7%)	14(66.7%)	1(9.1%)	8(40%)	0%	2(100%)
Doxycycline	18(64.3%)	11(68.7%)	13(61.9%)	11(100%)	20(100%)	2(100%)	2(100%)
Amoxicillin	24(85.7%)	13(81.3%)	17(80.9%)	-	-	-	-
Amoxicillin and clavulanate	21(75%)	15(93.7%)	15(71.4%)	-	-	-	-
Methicillin	22(78.6%)	16(100%)	21(100%)	-	-	-	-
Co-trimoxazole	26(92.8%)	12(75%)	9(42.8%)	9(81.8%)	20(100%)	2(100%)	0%

Blood and serological tests:

In all the recruited women in the study, none of the tests for HIV, HBsAg or RPR was positive and no association was obtained between the levels of blood sugar and micro-organisms grown.

Profiles of detection methods for *Trichomonas* and *Candida* species:

A statistically significant ($P < 0.0001$) difference was observed in the proportion of samples that were found to be positive for *Trichomonas* and *Candida* species individually as listed in Table 7.

Comparison of detection methods for bacterial vaginosis:

In the total 150 study participants, 48% (72) were detected to have bacterial vaginosis by Amsel's criteria while 24% (36) and 30.6% (46) were detected by Hay/Ison gradation and Pap smear respectively. Among four Amsel's criteria

pH more than 4.5 was found in 69 cases of patients having BV and 62 women without BV. 61 women with BV had white homogenous vaginal discharge and 59 women without BV. A positive whiff test i.e. amine test was positive in 35 cases of women having BV and only 1 case found positive in women without BV. Clue cells were present in 55 cases of women having BV and only 3 cases without BV. (Table 8)

Comparison of Pap smear and culture report of vaginal secretions:

Table 9 lists the correlation of infectivity between Pap smear and culture of vaginal secretions. The tests of diagnostic accuracies have also shown that Pap smear has a poor sensitivity, specificity, positive and negative predictive values in comparison to the gold standard culture and sensitivity reports.

Table 7: Results of detection methods for *Trichomonas* and *Candida* species

Laboratory procedure	<i>Trichomonas</i> infection	<i>Candida</i> infection
	Number (%)	Number (%)
Direct microscopy of wet mount/KOH mount	9 (6)	50 (33.3)
Gram's stained smear	-	47 (31.3)
PAP stained smear	13 (8.7)	16 (10.7)
Culture	-	55 (36.7)

(Chi-Square test=45.49, df =3, P=<0.001) significant, n=150

Table 8: Association of Amsel's criteria and bacterial vaginosis

Diagnostic criteria	Women with bacterial vaginosis	Women without bacterial vaginosis
pH > 4.5	69	62
Clue cells	55	3
Amine Test	35	1
White homogenous discharge	61	59

(Chi-Square test=57.53, df =3, P=<0.001) significant

Table 9: Agreement of Pap smear with culture report of vaginal secretions

Investigation method	Infections	Non infections
	Number (%)	Number (%)
PAP smear	70 (46.7)	80 (53.3)
Culture	131 (87.3)	19 (12.7)
Tests of diagnostic accuracy [n (95% confidence interval)]*		
Sensitivity	34.8 (28.3, 41.9)	
Specificity	19.2 (12.2, 28.6)	
Positive predictive value	46.7 (38.5, 55)	
Negative predictive value	12.7 (8, 19.3)	

(The values mentioned under the tests of diagnostic accuracy are in percentage that shows the extent of agreement between Pap smear with culture report of vaginal secretions.)

DISCUSSION

We undertook the present study in 150 women presenting with the complaints of vaginal discharge in two tertiary care hospitals in Mumbai, India. Highlights of our observation were that 90% of the vaginal secretions were infective, 88.7% of the participants had poor hygiene, 60% had thick whitish homogeneous discharge and 57.3% of the participants were belonging to reproductive age group. Of the 90% who grew micro-organisms, 47.4% were aerobic bacteria (the most common being *Staphylococcus aureus*) and 34.1% were *Candida* species. Nearly 40% (22/55) of the *Candida* species were *Candida albicans* of which only 59.1% were sensitive to fluconazole. The sensitivity of the isolated aerobic bacteria for various antimicrobial agents ranged between 40 and 90% except for *Pseudomonas aeruginosa* (absolutely resistant to azithromycin) and *Listeria monocytogenes* (absolute resistance was observed for ceftazidime, erythromycin, imipenem, ceftriaxone and cotrimoxazole).

For the diagnosis of *Candida*, both direct microscopy and wet mount are well in agreement with the culture results while for diagnosis of BV, clue cells and amine test were observed to be more conforming to Amsel's criteria.

Various authors have assessed the microbiological profile of the vaginal secretions and the results obtained from these

studies are fairly similar to the present study. Women of reproductive age were found to be more susceptible to develop vaginal discharge as also with practices quite likely to them being more sexually active than other groups. Similarly, women with poor literacy & with poor hygiene were found to be at risk of developing infective vaginal secretions emphasizing the need for creating more awareness amongst general public. We found that a few of the isolated micro-organisms were highly resistant to available broad spectrum antimicrobials. This might be due to indiscriminate rampant use of broad spectrum antimicrobial agents for treating non-bacterial infections and trivial bacterial infections in human beings. This finding from the present study emphasizes the need to implement measures for containing antimicrobial resistance.

In addition to the scientific validity, a method for laboratory diagnosis has to also consider the complexity, cost, and the frequency of specimens which could not be interpreted. Although polymerase chain reaction and various other novel tests have evolved in diagnosing BV, still the above mentioned factors do not favour using these as screening methods especially in resource-limited regions. The most commonly used methods to diagnose BV include either Amsel or Nugent criteria or scores. With regard to the

agreement of the individual items of Amsel's criteria, we observed that the presence of clue cells and the amine test are more specific. A similar finding was observed by Modak *et al* [16] where the sensitivity of Amsel criteria was observed to be 66.7% in diagnosing BV and the presence of clue cells had 100% sensitivity followed by pH (81.33% sensitivity).

Similarly Minou *et al* [17] did a cross-sectional study in 69 women and found out that both vaginal pH and clue cell were the criteria with the highest sensitivity (83%) and specificity (84%), respectively. Contrastingly, Chaijareenont *et al* [18] found that vaginal pH had a lower sensitivity than amine test (58.9% versus 97.3%). All these studies are limited in sample size and a specific regional population. More robust tightly controlled studies are the need of the hour to assess the diagnostic accuracy of individual items of Amsel's criteria. On the other hand, Nugent's score is being debated to be more sensitive and reproducible in the diagnosis of BV than Amsel's criteria. More studies comparing both the scoring systems for their diagnostic accuracy is needed before firm recommendation.

The association between HIV seropositivity and candidiasis has been reported in a number of studies [19, 20, and 21]. However in the present study none of the women who were suffering from candidiasis tested positive for HIV. In fact, none of the women in the present study were seropositive for HIV. Although there is documented evidence of positive correlation between diabetes and candidiasis [22, 23, 24, 25, 26] the present study did not find any case associated with high blood sugar level. Hence association of diabetes with candida infection could not be established.

The study is limited in not having assessed the past use of any antimicrobial agents, especially the broad spectrum ones. Follow up of the study participants was not done and so their response to the prescribed antimicrobial agents and time taken for their recovery from the current infectious condition could not be assessed in the present study.

CONCLUSION

The association of organisms with various vaginal pathologies is documented. The present study aimed at documenting the types of microorganisms in the vaginal discharges of the target population. Aerobic vaginitis was the dominant infection in these patients. The microbiological profile indicated a preponderance of candida infections followed by infection with *S.aureus*. Significant differences have been discovered in the vaginal microbiome related to ethnicity. Larger studies are therefore essential to arrive at generalizations regarding the vaginal microbiome, particularly in the Indian population.

Competing interest: The authors declare that they have no competing interests.

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