

# A microbiological study of vaginal discharge in women attending a Malaysian gynaecological clinic

Y. M. Cheong, MBBS, MSc, MRCPath\*

Y. P. Ng, MBBS, Dip. Ven\*

C. S. Chin, BSc. (Hons)\*

J. Thambu, MBBS, AM, FICS, FACS, FRCOG†

\* *Bacteriology Division*

*Institute for Medical Research, Kuala Lumpur*

† *Obstetrics & Gynaecology Department, General Hospital, Kuala Lumpur*

## Summary

Vaginal discharge is a common complaint of women attending gynaecological clinics. The purpose of this study was to compare the occurrence of commonly implicated microorganisms in vaginal discharge amongst women with or without the complaint, attending a gynaecological and family planning clinic. The association of *Gardnerella vaginalis* with bacterial vaginosis was also studied. It was found that there were no significant differences between the cases and controls in the isolation rate of *Gardnerella vaginalis*, *Torulopsis glabrata*, *Ureaplasma urealyticum*, *Mycoplasma* ssp and Group B streptococcus ( $p > 0.05$ ). Only the isolation rate of *Candida albicans* was significantly higher in the cases than controls ( $p < 0.01$ ). However there was a significant association of *G. vaginalis* with bacterial vaginosis.

**Key Words:** Vaginal discharge, bacterial vaginosis.

## Introduction

Vaginal discharge is one of the most common complaints of women attending gynaecological clinics. The causes can be infectious or non-infectious in nature, and it can be a result of inflammation or infection anywhere along the genital tract. Many micro organisms have been implicated as the cause of vaginal discharge. The etiological role of *Candida albicans* and *Trichomonas vaginalis* are well documented and accepted<sup>1-4</sup>. However, the causative role of organisms like *Gardnerella vaginalis*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Torulopsis glabrata* which are frequently isolated from the vagina has been much debated<sup>5-10</sup>.

The main objectives of this study are to determine the prevalence of these micro-organisms in the vagina of two groups of women, one with a complaint of vaginal discharge and the other without, to see if there is any difference between them; and to evaluate the significance of *Gardnerella vaginalis* in cases of bacterial vaginosis.

## Methods

### Study population

Women between the ages of 15 and 51 years who attended the gynaecological and family planning clinics at the General Hospital, Kuala Lumpur from April 1987 to August 1988, were examined by one of the authors. As the symptom of vaginal discharge can be rather subjective, a case of vaginal

discharge was defined for the purpose of this study as one who had a complaint of vaginal discharge plus on examination was confirmed to have an abnormal discharge based on the amount, colour and consistency of the discharge and the presence or absence of vaginal inflammation. A control case was one with no complaint of discharge and no sign of abnormal discharge and vaginitis on examination.

#### Clinical examination

From each case, a brief sexual and menstrual history was taken together with a description of the discharge and any symptoms relating to the genital tract.

A vaginal examination was performed and the following tests were done at the bedside:-

- a) measuring the pH of the vaginal secretion using litmus paper with a pH range of 4.0 to 7.0.
- b) amine test, which was done by adding 10% potassium hydroxide to secretions smeared on a glass slide.
- c) wet mount for *Trichomonas vaginalis*, yeast cells and fungal filaments.

High vaginal swabs were also taken for gram stain and culture of *Candida albicans* and other yeasts, *Gardnerella vaginalis*, *Ureaplasma urealyticum*, *Mycoplasma* spp and other bacteria. Endocervical swabs were taken for culture of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

A case of bacterial vaginosis was defined as one with a thin, grey, homogenous and adherent discharge, vaginal secretion of pH > 4.5, presence of clue cells and a positive amine test<sup>11</sup>.

#### Laboratory methods

All the specimens were plated directly onto the respective media at the bedside except for the isolation of *Chlamydia trachomatis* which was sent in a transport medium held in ice to the laboratory for processing within 24 hours.

Gram stain of the discharge was done for the presence of pus and clue cells.

For the isolation of *Gardnerella vaginalis*, bilayer human blood agar made selective with gentamicin (4 mg/l), nalidixic acid (3 mg/l) and amphotericin B (2 mg/l) was used. It was identified by the following criteria:-

- a) colonies producing diffuse beta-haemolysis on human blood agar
- b) no greening on chocolate agar
- c) gram variable coccobacilli
- d) negative oxidase & catalase tests
- e) positive starch hydrolysis test
- f) positive hippurate hydrolysis test
- g) sensitive to 50 ug metronidazole and resistant to 1000 ug sulphadiazine.

For the isolation of *Ureaplasma urealyticum* and other *Mycoplasma* spp. A7 agar and Boston Broth media were used<sup>12-13</sup>. The identification of these organisms was based on its typical appearance on A7 agar and colour change in Boston Broth. The mycoplasmas were not speciated.

The modified-Thayer Martin was used for the isolation of *Neisseria gonorrhoeae* and its identification was based on gram stain, oxidase test and sugar utilisation tests.

Sabouraud's medium was used for the isolation of *Candida albicans* and other yeast. The identification was based on morphology on slide culture using cornmeal with Tween 80 agar and sugar fermentation and assimilation tests.

Cycloheximide treated McCoy cells were used to isolate *Chlamydia trachomatis* and immunofluorescence staining with monoclonal antibodies was used to identify it.

The vaginal and endocervical swabs were also plated on a plain blood agar medium for the isolation of other bacteria and their identification were according to conventional bacteriological methods.

#### Statistical methods

Statistical analysis was performed with chi-square tests and the Fisher's exact test.

#### Results

A total of 455 women were studied, of which 384 met with our definition of a case with vaginal discharge and normal controls. Of these 384 women, 32 had received antibiotics fourteen days prior to the clinic visit, leaving 352 cases and controls to be analysed. All the women were not menstruating at the time of examination. 164 were classified as cases with vaginal discharge while 188 were normal controls. The age and marital status of the two groups in the study population were comparable and are shown in Table I.

Table I  
Age and marital status of the study population

	Cases n = 164	Control n = 188
Age		
Mean (years)	32.4	33.7
Range (years)	17 - 51	21 - 49
Marital status		
Single	4 (2.4)	2 (1.1)
Married	160 (97.6)	186 (98.9)

Table II shows the isolation rate of the organisms from the cases and controls. Using the chi-square test for significance, there were no significant differences between the cases and controls in the isolation rates of *T. glabrata*, *G. vaginalis*, *U. urealyticum*, *Mycoplasma* spp and Group B streptococcus ( $p > 0.05$ ). Only the isolation rate of *C. albicans* was significantly higher in the cases than controls ( $p < 0.01$ ). Test of significance was not done with the *T. vaginalis*, *N. gonorrhoeae* and *C. trachomatis* as the numbers were too small and their role in vaginal discharge was well established.

Based on the 4 criteria listed earlier, 10 cases of bacterial vaginoses were identified in the group of women with discharge and none in the control group. The association of *G. vaginalis* amongst the women with and without bacterial vaginosis is shown in Table III. Eight out of 10 (80%) of the bacterial

Table II  
Organisms isolated from women with discharge (cases) and controls

	Case (n = 164)		Control (n = 188)	
	No.	%	No.	%
<i>Candida albicans</i>	41	25	17	9
<i>Torulopsis glabrata</i>	10	6.1	10	5.3
Other yeasts	6	3.6	8	4.3
<i>Trichomonas vaginalis</i>	8	4.9	0	0
<i>Neisseria gonorrhoeae</i>	2	1.2	1	0.5
<i>Chlamydia trachomatis</i>	3	1.8	0	0
<i>Gardnerella vaginalis</i>	25	15.2	25	13.2
<i>Ureaplasma urealyticum</i>	77	47.0	72	38.2
Group B strep	18	11.0	27	14.4
<i>Mycoplasma spp</i>	11	6.7	5	2.7
None of the above isolated	41	25	70	37.2

Table III  
Association of *Gardnerella vaginalis* in patients with and without bacterial vaginosis

	<i>G. vaginalis</i>		Total
	Present	Absent	
Bacterial vaginosis			
Present	8	2	10
Absent	42	300	342
Total	50	302	352

$p < 0.01$   
Fisher's exact test

vaginosis cases yielded *G. vaginalis* on culture against 42 of 342 (12%) without bacterial vaginosis. This association by the Fisher's exact test was very strong ( $p < 0.0001$ ). Therefore we proceeded to assess the value of isolation of *G. vaginalis* in the diagnosis of bacterial vaginosis by calculating the predictive values of a positive or negative culture. Of 164 women with vaginal discharge, 8 out of 25 positive isolates were cases of bacterial vaginosis, giving a positive predictive value of 32% while the negative predictive value was 99%.

Positive predictive value =  $\frac{\text{no. of positive isolates in women with BV}}{\text{total no of positive isolates}}$

Negative predictive value =  $\frac{\text{no. of negative isolates in women without BV}}{\text{total no of negative isolates}}$

## Discussion

The microbiological examination of vaginal discharge in the laboratory has been an area of much controversy as a number of organisms have now been implicated to cause infections in the genital tract. *G. vaginalis*, anaerobes of the genus *Bacteroides* and curved motile anaerobes of the genus *Mobiluncus* have been associated with the clinical condition of bacterial vaginosis and clinicians are requesting for the isolation of such bacteria to support the diagnosis<sup>1,14</sup>. In this study, we did not include any anaerobic cultures, but we examined the role of *G. vaginalis* in bacterial vaginosis by studying its prevalence in the asymptomatic population as well as those with vaginal discharge; and its association with bacterial vaginosis as defined earlier. We found that there was no significant difference between its occurrence in the two groups of population as in other studies but there was a significant association between the presence of *G. vaginalis* and bacterial vaginosis<sup>7,15</sup>.

However, when we calculated the predictive values, we found that the negative predictive value was much higher than the positive predictive value. Therefore the isolation of *G. vaginalis* should not be an important criterion in the diagnosis of bacterial vaginosis as a positive isolation can also occur in an asymptomatic individual and has a low positive predictive value. For practical purposes, the four criteria mentioned earlier should be sufficient for the diagnosis of bacterial vaginosis.

*Ureaplasma urealyticum* was the most frequently isolated organism in this study. However there was no significant difference in the isolation rates of the organism between the two groups. Although it has not been established to cause vaginal infections in women, it is an accepted cause of non-gonococcal urethritis (NGU) in men and has also been linked to spontaneous recurrent abortion and perinatal infections<sup>16,17</sup>. In a study by Arya and Pratt, they found that resolution of symptoms and signs in the male partners was achieved only after treatment to eliminate the *Ureaplasma urealyticum* organism from both partners<sup>18</sup>. Therefore the high prevalence of this organism in the women population here suggests that this could be an important reservoir for the failure of treatment in men with recurrent NGU or even perinatal infections and further studies would be required to look into these aspects.

The next most commonly encountered group of organisms was the yeasts. Except for *C. albicans* ( $p < 0.01$ ) there was no significant difference between the two groups for the isolation of *T. glabrata* and other yeasts. The clinical and mycological observations on vulvovaginal candidiasis will be discussed in another paper.

In this study, 13% of the two groups of women examined, carried Group B streptococci in the vagina and endocervix. Although this organism has been implicated in pelvic inflammatory diseases, urinary tract infections and even cervicitis and urethritis, its presence in the vagina is usually considered as part of the normal flora and a potential source of neonatal infections in late pregnancy<sup>19,20,21</sup>. The colonisation rate obtained is comparable to those obtained in another study and a local study which varied from 5 to 23.3% in different groups of women<sup>22,23</sup>.

*Trichomonas vaginalis* was found in only 5% of the cases, while *N. gonorrhoeae* was found in 1.5% of the cases and 0.5% of the controls; and *C. trachomatis* in 1.8% of the cases. Therefore from this study 25% of the vaginal discharge cases studied were probably due to *C. albicans*, followed by *T. vaginalis*

(5%), *C. trachomatis* (1.8%) and *N. gonorrhoeae* (1.2%) and 10 cases of bacterial vaginosis (6%), leaving 61% of whom with isolates of doubtful significance or none of the isolates listed in Table I.

In conclusion, this study has reaffirmed that it is not practical and cost effective to routinely culture for organisms like *G. vaginalis*, yeasts other than *C. albicans*, mycoplasmas and group B streptococci in the investigation of the causative agents in vaginal discharge as these organisms also occur in the same frequency in the normal healthy women.

### Acknowledgements

We thank Dr. M. Jegathesan, Director, Institute for Medical Research for permission to publish this paper; Keah Lee Moy, Koh Yin Tee and Lee Poh Chun for technical assistance and Norangizan Abdul Hamid for typing this manuscript.

### References

1. Hurley R, and Lonvois J. *Candida* vaginitis. *Postgrad Med J* 1979; 55 : 645-7.
2. Goldacre MJ, Milne LTR, Watt B, London N, and Vessey MP. Prevalence of yeasts and fungi other than *Candida albicans* in vagina of normal women. *B J Obstet Gynaecol* 1981; 88 : 596-600.
3. Russell RE, and Wilson ME. Vaginal trichomoniasis. *Am. J. Obstet Gynaecol* 1942; 44 : 292-5.
4. Bard DS. *Trichomonas vaginalis* (urogenital trichomoniasis) In Monif GRG (ed.) : Infectious Diseases in Obstetrics and Gynaecology, 2nd ed. Har-Row, Philadelphia, 1982 : 286-300.
5. Hill LVH. Anaerobes and *Gardnerella vaginalis* in non-specific vaginitis. *Genitourin Med* 1985; 61 : 114-9.
6. Gardner HL, and Dukes CD : *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "non specific vaginitis". *Am. J. Obstet Gynaecol* 1955; 69 : 962-76.
7. McCormack WM, Hayes CM, Rosner B, Evard JR, Creckett VA, Alpert S, and Zinner SH. Vaginal colonisation with *Corynebacterium vaginale* (*Haemophilus vaginalis*) *J. Infect Dis* 1977. 136 : 740-5.
8. Taylor E, Blackwell AL, Barlow D, and Phillips I. *Gardnerella vaginalis*, anaerobes, vaginal discharge. *Lancet* 1982; i : 1376-9.
9. McCormack WM, Braun P, Lee Y-H, Klein JO, and Kass EH. The Genital Mycoplasmas. *New Eng J of Med* 1973; 288 : 2 : 78-89.
10. Grimley M, Wright LD and Jennings AE. *Torulopsis glabrata* infection in man. *Am. J. Clin Pathol* 1975; 43 : 216-23.
11. Amsel R, Totten PA, and Spiegel CA et al. Non-specific vaginitis : diagnostic criteria and microbiologic and epidemiologic associations. *Am J Med* 1983 : 74 : 14-22.
12. Shepard MC and Lunceford CD. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (Human T Mycoplasma) in primary cultures of clinical material. *J Clin Microbiol* 1976; 3 (6) : 613-25.
13. Leland DS, Lapworth MA, Jones RB and French MLV. Comparative evaluation of media for isolation of *Ureaplasma urealyticum* and genital *Mycoplasma species*. *J Clin Microbiol* 1982; 16(4) : 709-14.
14. Spiegel CA, Eachenbach D, Amsel R, Holmes KK. Curves anaerobic bacteria in bacterial (non-specific) vaginosis nad their response to antimicrobial therapy. *J Infect Dis* 1983; 148 : 817-22.
15. Kelsey MC, Mann GK, Bangham AM, and Milnthorpe. Non-specific (anaerobic) vaginitis : relevance of clinical and laboratory studies in a practice population. *J Royal College of General Practitioners*. 1987; 37, 56-58.
16. Taylor-Robinson D. Mycoplasma infections of the human urogenital tract with particular reference to non-gonococcal urethritis. *Ann Microbiol (Paris)* 1984; 135A : 129-34.
17. Embree AE, Krause VW, Embil JA and MacDonald S. Placental infection with *Mycoplasma hominis* and *Ureaplasma urealyticum* : Clinical correlation. *Obstet Gynecol* 1980, 56(4) : 475-81.

18. Arya OP and Piatt BC. Persistent urethritis due to *Ureaplasma urealyticum* in conjugal or stable partnerships, *Genitourin Med*, 1986; 62 : 329–32.
19. Pass MA, Gray BM, and Dillon HC Jim. Puerperal and perinatal infections with group B streptococci *Am J Obstet Gynecol* 1982; 143 : 147–52.
20. Buttigie G. Cervicitis and urethritis caused by group B streptococcus : case report. *Genitourin Med* 1985; 61 : 343–4.
21. Anthony BF and Okada DM. The emergence of group B streptococci infections of the newborn infant. *Annu Rev Med* 1977; 28 : 355–69.
22. Embil JA, Martin TR, Hausen NH, MacDonald SW, and Manuel FR. Group B beta haemolytic in the female genital tract: A study of four clinic populations. *Br J Obstet Gynaecol* 1978; 85 : 783-6.
23. Parasakthi N and Ngeow YF. Group B Streptococci – Incidence and Clinical Significance of isolates from the genital tract. *Proceedings of the XVI Singapore-Malaysian Congress of Medicine. The Academy of Medicine Singapore*. 1982; 16 : 442–4.