# Prevalence of Gardnerella vaginalis in the Urinary Tract

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Received 19 October 1987/Accepted 26 February 1988

Midstream urine samples from 106 patients presenting to the Casualty Department of The Royal Melbourne Hospital with frequency or dysuria were cultured for Gardnerella vaginalis and conventional uropathogens. Urine samples collected via an open-end catheter from 70 healthy pregnant women were examined similarly. Midstream urine and other samples, including the seminal fluids and swabs of the mouths, throats, rectums, and vaginas of 33 healthy subjects, were cultured for G. vaginalis. Another 15 female patients with proven G. vaginalis bacteriuria were given a bladder washout localization test to determine the site of infection in the urinary tract. G. vaginalis in counts greater than 10<sup>3</sup> CFU/ml was recovered from the midstream urine of 27 of 106 patients (25%), 7 of whom also harbored conventional pathogens in counts greater than 10<sup>4</sup> CFU/ml. Another 11 patients with cultures negative for G. vaginalis yielded greater than 10<sup>4</sup> CFU of conventional pathogens per ml. G. vaginalis was cultured ( $>10^3$  CFU/ml) from catheter samples of 19 of 70 healthy pregnant women (27%), 6 of whom also harbored greater than  $10^3$  CFU of conventional uropathogens per ml. Two women yielded growths of conventional pathogens only. Midstream urine samples from 13 of 13 healthy males were free of G. vaginalis, whereas 5 of 20 healthy nonpregnant females yielded greater than  $10^3$  CFU of G. vaginalis per ml from midstream urine samples. G. vaginalis was recovered from 4 of 12 semen samples and from urethral samples from four of seven males and four of eight females. All four culture-positive females also harbored G. vaginalis in their vaginas. There was no evidence of oral or rectal carriage of G. vaginalis in 15 healthy subjects. Localization studies with 15 female patients having underlying renal disease showed that 11 patients harbored G. vaginalis in their kidneys. The results suggest that colonization or infection of the bladder and upper urinary tract by G. vaginalis is very largely a phenomenon of females, with the highest frequency in pregnant women. The prevalence of G. vaginalis in the urinary tracts of healthy females is similar to that of symptomatic subjects. However, G. vaginalis in counts greater than 10<sup>5</sup> CFU/ml is more likely to be associated with urinary tract symptoms. In males, this bacterial species infects the genital tract rather than the urinary tract.

Gardnerella vaginalis has been implicated as the etiologic agent in bacterial vaginosis by numerous investigators. However, the possible importance of this bacterial species in the urinary tract has attracted comparatively little attention. We have recovered G. vaginalis from the bladder aspiration urine of patients with reflux nephropathy and from subjects with acute symptoms of urinary tract infection, (3, 6, 13). Other researchers have associated G. vaginalis with hemorrhagic cystitis (1), chronic pyelonephritis (12), and symptomatic bacteriuria (16). Since the natural history of G. vaginalis in healthy populations was unknown, the significance of these findings was unclear. The present study examined the prevalence of this bacterial species in the urinary tracts of symptomatic and asymptomatic subjects. Carriage of G. vaginalis in other body sites of asymptomatic individuals was also investigated.

### **MATERIALS AND METHODS**

**Study group.** Four groups of subjects were studied. Group 1 consisted of 106 patients (94 females and 12 males) presenting consecutively at the Casualty Department with frequency or dysuria. Group 2 consisted of 15 female patients selected for localization studies. These patients were referred for consultation for a range of urinary tract diseases and were selected on the basis of *G. vaginalis*-positive cultures of bladder aspiration urine. Five patients had mesangial proliferative glomerulonephritis; three had focal, segmental hyalinosis and sclerosis; three had polycystic

kidneys; one had reflux nephropathy; two had preeclampsia; and one had undiagnosed abdominal pain. Group 3 consisted of 70 healthy pregnant women attending an antenatal clinic of The Royal Women's Hospital. Group 4 consisted of 33 healthy laboratory staff and medical students (20 nonpregnant females and 13 males).

**Collection of MSU. (i) Females.** The patient was instructed to hold the labia minora apart and swab the periurethral area with sterile saline. After allowing the first 200 ml of urine to drain, a 20-ml sample (midstream urine [MSU] sample) was collected.

(ii) Males. The subject was asked to retract the prepuce, if present, and swab the urinary opening with sterile saline. A 20-ml urine sample was collected as described for females.

**Collection of bladder urine samples by using an open-ended catheter.** The subject was asked to separate the labia so that the urethral meatus was visible. The periurethral area was swabbed with sterile saline, and a 20-F end-hole catheter was introduced into the bladder. The first 200 ml of urine was allowed to drain into a graduated kidney dish, following which a 20-ml sample (midcatheter specimen) was collected. The catheter was removed when the urine flow had ceased.

**Bladder washout localization test.** Individuals on a normal fluid diet were asked to undergo the localization test (6) with a full bladder. A Foley catheter was passed into the bladder, and after 200 ml of urine was removed, a bladder sample was collected. The bladder was then drained and refilled with sterile saline until the patient experienced moderate discomfort, whereupon the bladder was emptied. This procedure was repeated until 2 liters of saline had been used. A sample

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of washout fluid was collected early during the final emptying of the bladder. After the bladder was drained of saline, samples of urine were collected from the freely draining catheter during four 10-min intervals.

**Culture media.** Modified colistin-nalidixic acid (CNA) agar medium consisted of Columbia agar-CNA (4.4 g/100 ml; GIBCO Laboratories, Grand Island, N.Y.), Proteose Peptone no. 3 (1 g/100 ml; Difco Laboratories, Detroit, Mich.), metronidazole (2,500  $\mu$ g/ml), and citrated human blood (5%).

Horse blood agar medium consisted of Columbia agar (4.25 g/100 ml; BBL Microbiology Systems, Cockeysville, Md.), vitamin K (0.5  $\mu$ g/ml), hemin (5 mg/liter), vitamin B<sub>6</sub> (0.5%), and 4% defibrinated horse blood.

To cysteine lactose electrolyte-deficient agar medium (GIBCO), acid fuchsin (0.1 g/liter) was added.

Urine culture. Urine samples were cultured for G. vaginalis by plating 20  $\mu$ l of undiluted urine and 20  $\mu$ l of a 10<sup>-2</sup> saline dilution of urine on modified CNA agar medium for colony counts. Cultures were incubated anaerobically for 48 h at 37°C. For the recovery of other uropathogens, samples were plated on horse blood agar and cysteine lactose electrolyte-deficient agar medium and incubated for 18 h in air plus 5% CO<sub>2</sub>.

Semen culture. Samples of seminal fluid were cultured for G. vaginalis by spreading 20  $\mu$ l over the surface of CNA agar medium. Plates were incubated anaerobically for 48 h.

**Rectal, vaginal, and oral swab cultures.** Mucus samples were streaked on modified CNA agar medium for *G. vaginalis* determination. The plates were incubated as described above.

Identification of microorganisms. G. vaginalis was identified on the basis of production of diffuse beta-hemolysis on CNA agar medium, a Gram stain showing small gramnegative to gram-variable bacilli, and a negative catalase reaction. Other microbial isolates were identified by using a standard scheme (4).

# RESULTS

Patients with frequency and dysuria. Twenty-eight patients (all female) yielded greater than  $10^2$  CFU of *G. vaginalis* per ml from MSU samples (Table 1). Of these, 27 had counts greater than the threshold for bacteriuria ( $10^3$  CFU/ml) and 17 had counts greater than  $10^5$  CFU/ml. Seven *G. vaginalis*-positive patients also harbored greater than  $10^4$  CFU of conventional uropathogens per ml, namely *Escherichia coli* (n = 3), *Staphylococcus saprophyticus* (n = 1), *Proteus mirabilis* (n = 1), *Klebsiella pneumoniae* (n = 1), and *Streptococcus faecalis* (n = 1). Another 11 patients (8 females and 3 males) yielded conventional pathogens only.

**Pregnant women.** Bladder urine samples, collected via an open-end catheter from 70 healthy pregnant women, were

 TABLE 1. Frequency of isolation of G. vaginalis and other bacteria from MSU samples of 106 patients

Culture (CFU/ml)	No. (%) of patients	
	Female $(n = 94)$	Male (n = 12)
$\overline{G}$ . vaginalis (>10 <sup>2</sup> )	28 (30)	0
G. vaginalis $(>10^3)$	27 (29)	0
G. vaginalis (10 <sup>5</sup> )	17 (18)	0
Conventional uropathogens (10 <sup>4</sup> )	18 (19)	3
G. vaginalis $(>10^3)$ + uropathogens $(>10^4)$	7 (7)	0

 

 TABLE 2. Frequency of isolation of G. vaginalis and other bacteria from catheter urine samples of 70 healthy pregnant women

Culture (CFU/ml)	No. (%) of subjects
<b>G</b> . vaginalis (>10 <sup>2</sup> )	
G. vaginalis $(>10^3)$	19 (27)
G. vaginalis $(>10^5)$	4 (6)
Conventional pathogens $(>10^3)$ G. vaginalis $(>10^3)$ + conventional	
uropathogens (>10 <sup>3</sup> )	6 (9)

cultured for G. vaginalis and conventional uropathogens. G. vaginalis was cultured (> $10^2$  CFU/ml) from 26 women (37%; Table 2). Nineteen (27%) had counts greater than the threshold for bacteriuria, and four had greater than  $10^5$  CFU/ml. In six women, G. vaginalis was accompanied by greater than  $10^3$  CFU of conventional uropathogens per ml. Another two women had conventional uropathogens only.

**Control Subjects.** (i) MSU. MSU samples from 13 healthy males and 20 healthy, nonpregnant females were cultured for *G. vaginalis*. Thirteen males were culture negative, whereas five females had greater than  $10^3$  CFU/ml and one had a count greater than  $10^5$  CFU/ml.

(ii) Semen. Seminal fluid samples, collected from 12 healthy volunteers after masturbation, was cultured as described above. G. vaginalis was recovered from four samples (33%). Colony counts were in the range of  $10^2$  to  $10^3$  CFU/ml.

(iii) Other body sites. Eight healthy females and seven healthy males were studied for the presence of *G. vaginalis* in different body sites. A sample of the urethral urine (the first 10 ml of voided urine) and swabs of the mouth, throat, rectum, and vagina were cultured. *G. vaginalis* was recovered from the vaginas of six females, four of whom also harbored *G. vaginalis* in their urethras. Of seven males, four yielded positive cultures from their urethras. *G. vaginalis* was not recovered from swabs of the mouth, throat, or rectum of any of the 15 subjects.

Level of colonization of urinary tract. A procedure to determine the site of infection was performed in 15 females with G. vaginalis-positive, needle-aspirated bladder samples. Subjects whose counts of G. vaginalis in postwashout urine were greater than 10 times that of the bladder washout sample were considered to harbor G. vaginalis in the kidneys. Individuals with lower postwashout urine counts were considered to have bladder involvement only. Table 3 shows the localization test results for two patients. Patient 1 was judged to have upper tract involvement, and patient 2 was judged to have lower tract involvement. By these criteria, 11

 TABLE 3. Results of localization tests from two representative patients by using the bladder washout method

Sample	Colony count (CFU/ml)		
	Patient 1	Patient 2	
Bladder urine	$1.5 \times 10^{6}$	$1.2 \times 10^{4}$	
Saline washout fluid	100	200	
Postwashout urine			
0–10 min	$1.5 \times 10^{4}$	<50	
10–20 min	$6.6 \times 10^{4}$	<50	
20–30 min	$2.6 \times 10^{5}$	<50	
30–40 min	$3.6 \times 10^{5}$	<50	

subjects harbored G. vaginalis in the kidneys. The remainder (four subjects) had bladder colonization.

# DISCUSSION

Thirty-one years ago, H. L. Gardner and C. D. Dukes implicated G. vaginalis as the etiological agent in bacterial vaginosis. Since then, numerous studies have confirmed the link between G. vaginalis and this syndrome. Although anatomical proximity should allow easy transfer of G. vagi*nalis* from the genital tract to the urinary tract, to date only a few investigators have examined the role of G. vaginalis in urinary tract infections and renal disease. All of these researchers have recovered G. vaginalis from urine samples, although the clinical significance of these findings is unclear, since the natural history of G. vaginalis in healthy populations is unknown. The present study examined the prevalence of this bacterial species in the urinary tracts of symptomatic and asymptomatic subjects. Carriage of G. vaginalis in other body sites of 15 asymptomatic individuals was checked also.

When the frequency of bacteriuria is documented, it is important to consider the detection threshold and the positive predictive value of the results obtained. Colony counts from bladder aspiration urine have a positive predictive value of 100% for any detection threshold. It follows that use of the lowest practicable threshold provides the highest sensitivity without loss of specificity. A low threshold (10<sup>3</sup> CFU/ml) was selected in this study to maximize carriage rates in target cohorts. Obviously, however, use of a low threshold with catheter urine and MSU samples results in decreased specificity, since bacteria may be expected to enter the urine below the bladder during sample collection. Previous studies (6, 16, 18) have shown that G. vaginalis rarely occurs in bladder aspiration urine in counts less than  $10^3$  CFU/ml. In addition, patients with greater than  $10^3$ CFU/ml in urine collected via an end-hole catheter invariably had positive cultures of aspirated urine (16). We therefore conclude that counts greater than 10<sup>3</sup> CFU/ml from an open-ended catheter sample have a high predictive value for bacteriuria and that lower counts are likely to indicate contamination. Interpretation of MSU cultures is less certain, since the positive predictive value of such colony counts is unknown. It is reasonable to conclude, however, that counts less than 10<sup>3</sup> CFU/ml probably reflect contamination of MSU samples.

In the present study, by using a threshold detection count of 10<sup>3</sup> CFU/ml, G. vaginalis was cultured from urine samples of 27% of the healthy pregnant females tested and 25% of the healthy, nonpregnant females tested. A high rate of colonization of the urinary tract during pregnancy has previously been reported by McFadyen and Eykyn (15), who recovered G. vaginalis from bladder aspiration urine samples of 159 of 1,000 healthy pregnant women. McDowall et al. (14) subsequently confirmed this pattern of recovery in pregnancy by showing that 18% of a group of healthy pregnant women harbored G. vaginalis in their bladders. Interestingly, these researchers found that 58% of pregnant women with underlying renal disease harbored G. vaginalis in their bladders. Savige et al. (19) studied bacteriuria due to Ureaplasma urealyticum and G. vaginalis in women with preeclampsia and in healthy pregnant women. These workers found that G. vaginalis was regularly present in bladder aspiration urine samples of 22% of pregnant women tested, regardless of whether they were preeclamptic. It is possible that the greater prevalence of G. vaginalis in the urinary tracts of healthy pregnant women is estrogen dependent, since it is known that increased levels of estrogen are present in the urine during pregnancy. Incidently, a greater prevalence of G. vaginalis in the vaginal fluid of healthy pregnant women, compared with that of nonpregnant subjects, has also been reported (11). In subjects with greater numbers of G. vaginalis in the genital tract, a shift of G. vaginalis from the genital tract to the urinary tract would be expected, hence giving an increased number in the urinary tract as well.

The present study further showed that G. vaginalis alone was recovered from the urine of 13 of 70 healthy pregnant women (19%), whereas other bacterial species were the sole organisms isolated from only 2 of 70 women (3%). Kincaid-Smith and Bullen (7) and Asscher (2) reported asymptomatic bacteriuria due to aerobic bacteria in 4 to 7% of healthy pregnant subjects. It is evident that asymptomatic bacteriuria, involving fastidious bacteria such as G. vaginalis, is considerably more prevalent in this population than that due to conventional urinary pathogens. The significance of this finding is unclear at present, although it is possible that this is a benign condition in some individuals. However, the findings of McDowall et al. (14) suggest that there may be an association between G. vaginalis and renal disease in pregnancy.

To investigate further the role of G. vaginalis in urinary tract disease, MSU samples from 106 patients with frequency and dysuria were examined. G. vaginalis alone, in counts greater than 10<sup>5</sup> CFU/ml, was recovered much more frequently from these subjects than any other bacterial species. It is known that up to 50% of women with acute urinary tract infection fail to yield a conventional uropathogen on standard culture (9). This study indicates that fastidious bacteria, such as G. vaginalis, could account for some of the discrepancy between symptoms and negative laboratory findings. Anecdotal evidence supporting the above hypothesis comes from Abercrombie et al. (1), who reported their findings for a patient with severe hemorrhagic cystitis. Multiple MSU samples for conventional uropathogens yielded no growth. However, when a bladder urine sample was examined for fastidious organisms, a pure growth of G. vaginalis was isolated. McDonald et al. (13) reported the recovery of G. vaginalis, frequently accompanied by U. urealyticum, from the bladder urine of 7 of 101 patients with acute symptoms of urinary tract infection. Loulergue et al. (12) isolated G. vaginalis from urine obtained directly from the obstructed renal calyx of a female patient with chronic pyelonephritis, whose routine preoperative urine culture was negative for any other organism. Petit and Mouton (17) recovered G. vaginalis from urine samples of 14 of 23 symptomatic kidney transplant patients. Wilson and Barratt (21) recently reported a case of G. vaginalis septicemia with severe endotoxic shock following G. vaginalis urinary tract infection in a previously fit man.

The ability of G. vaginalis to colonize the upper urinary tract was studied in 15 female patients with proven renal disease and bacteriological evidence of G. vaginalis bacteriuria. Of 15 patients, 11 harbored G. vaginalis in the kidney. The possibility that G. vaginalis is part of the normal flora in the kidney seems unlikely. More probably, it would appear that G. vaginalis may colonize damaged kidneys or that this microorganism may have a disease-causing potential at this level of the urinary tract.

The present study showed that *G. vaginalis* was isolated more frequently from the genital tracts of healthy females than from those of healthy males. This finding is in accord with the findings of Leighton (10) and Totten et al. (20), demonstrating that G. vaginalis was recoverable from the vaginal fluids of 12 to 68% of asymptomatic women tested, and the findings of Kinghorn et al. (8) and Dawson et al. (5), who reported prevalences of 7.1 and 11.4% in the seminal fluids of healthy men. These findings suggest that the vagina may provide more favorable conditions for the growth of G. vaginalis than does the prostate. Prostatic fluid from uninfected males contains a high concentration of antibacterial zinc salts. Furthermore, adhesion to host cells is an important initial step in the host-parasite interaction leading to colonization. The fact that G. vaginalis adheres to vaginal squamous epithelial cells suggests that this organism might have a role in lower genital tract infection. Indeed, numerous researchers have implicated G. vaginalis as the etiologic agent in bacterial vaginosis. Fairly and Birch (6) have also demonstrated the ability of G. vaginalis to adhere to squamous epithelial cells from the trigone region of the bladder in patients with urinary tract disease. However, the basal lining of the prostate comprises only columnar or cuboidal epithelial cells, and there is no evidence that G. vaginalis readily attaches to these cells. These factors might account for the greater prevalence of G. vaginalis in the genital tract of females than in that of males. A similar sex-related difference was noted in the recovery of G. vaginalis from the urinary tract. MSU samples from 5 of 20 healthy females yielded G. vaginalis. In contrast, all 13 males were culture negative. This result is in agreement with the findings of Fairley and Birch (6) and Murphy et al. (16), who reported the recovery of G. vaginalis from the bladder urine of 2 of 35 and 5 of 30 healthy females, respectively. Since the studies showed that G. vaginalis was recoverable from both genital and urinary tracts, the transfer of organisms probably occurred between the genital and urinary tracts. The finding that a greater prevalence was observed in the genital tract than in the urinary tract suggests that the transfer of G. vaginalis is from the genital tract to the urinary tract.

There was no evidence that G. vaginalis was carried in the rectum of any of the seven healthy males and eight healthy females studied, although six of the females harbored G. vaginalis in their vaginas. This result is in accord with the finding of Dawson et al (5), who showed that G. vaginalis was not present in the rectum of 430 men. The present study also showed that G. vaginalis was not carried in the oral cavities of 15 healthy subjects. The fastidious growth requirements of G. vaginalis might account for the negative findings from these body sites.

The present study has shown clearly that colonization or infection of the bladder and upper urinary tract by *G. vaginalis* is very largely a phenomenon of females. In the symptomatic group, *G. vaginalis* counts greater than  $10^5$  CFU/ml were obtained from 17 of 28 culture-positive samples, whereas in the healthy group, a count greater than  $10^5$  CFU/ml was recovered from only 1 of 5 culture-positive samples. A similar observation was also noted for the healthy pregnant group, with *G. vaginalis* counts exceeding  $10^5$  CFU/ml in only 4 of 26 culture-positive samples. It can be concluded that the prevalence of *G. vaginalis* in the urinary tracts of healthy females is similar to that in the urinary tracts of symptomatic subjects. However, *G. vaginalis* in counts greater than  $10^5$  CFU/ml is more likely to be associated with urinary tract symptoms.

### ACKNOWLEDGMENTS

We acknowledge the cooperation of the staff and students from the Department of Nephrology and the Department of Microbiology of The Royal Melbourne Hospital. We thank Merry Geare for assistance in the preparation of the manuscript.

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