Infectious Diseases in Obstetrics and Gynecology 6:52–56 (1998)

Female Genital Tract Bacterial Coisolates With *Candida albicans* in Patients Without Clinical Vaginitis

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ABSTRACT

Objective: In vitro, *Candida albicans* has demonstrated the ability to inhibit replication of selected bacteria. Little information exists on the impact of *C. albicans* on the vaginal bacterial flora in vivo. The purpose of this study is to identify the coexisting bacterial flora when *C. albicans* is isolated from vaginal cultures submitted to a hospital-based testing facility for reasons other than vulvovaginitis.

Methodology: All specimens (240) received from ambulatory care clinics over a six-month period were cultured for aerobic and anaerobic bacteria and *Candida* species. Those specimens submitted for cervicitis, vaginitis, or vaginal discharge and those from which yeasts other than *C. albicans* were isolated were eliminated. To control for sample biases, a subgroup composed of all pregnant women for whom cultures were done as screening procedures was similarly studied. Chi-square analyses, comparing the prevalence of individual bacteria isolated with and without the presence of *C. albicans*, were done for all study populations using SPSS for Windows software (1994).

Results: Two hundred and forty consecutive specimens were bacteriologically analyzed. Of the 220 vaginal samples used in the study, *C. albicans* was isolated in 44 instances (20%). Neither the presence of the lactobacilli nor the presence of *Gardnerella vaginalis* markedly influenced the isolation rate of *C. albicans*. The group B streptococci had a greater probability of coisolation when *C. albicans* was present (27.3% verses 16%), but this was not statistically significant ($P < 0.8$). Dissociation between the presence of *C. albicans* and the coisolation of *Peptostreptococcus* species and anaerobic gram-positive cocci and/or bacilli was noted ($P < 0.0819$). while the incidence of gram-positive aerobic bacilli was reduced in the presence of *C. albicans* (30/176 [17.1%] versus 6/44 [13.6%]), this reduced incidence was not statistically significant. Isolation data of the subgroup of pregnant women supported these observations.

Conclusion: Within the limitations of the study, statistically, the data suggests that an inverse relationship exists between the presence of *C. albicans* and recovery of *Peptostreptococcus* and anaerobic gram-positive cocci and bacilli. Infect. Dis. Obstet. Gynecol. 6:52–56, 1998.

KEY WORDS

Candida albicans; vaginal bacterial flora; bacterial coisolates

Candida albicans is a common constituent of the vaginal flora of the female genital tract.[1–4] To achieve disease status, a fungus must be in its tissue invasive form (pseudohyphae) and obtain a quantitate representation within the vaginal flora equalling or exceeding 10^6 colony-forming units per milliliter of vaginal fluid.[5] In vitro, *C. albicans* has demonstrated the ability to inhibit replication of selected bacteria.[6] Little information exists on the impact, if any, of *C. albicans* on the vaginal bacterial flora in vivo. When characterization of the bacteria flora concomitantly present with *C. albicans* has been done, these analyses have occurred in patients with clinically overt vulvovaginal candidiasis.[1,5]

The purpose of this paper is to identify the co-

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Clinical Study

Received 5 December 1997
Accepted 19 March 1998

TABLE 1. Clinical setting from which vaginal specimens were obtained[a]

Indication	Number of specimens derived
Abdominal pain	75
Pregnancy (ruling out group B streptococcus)	67
Sexually transmitted disease evaluation	19
Cervicitis/vaginal discharge	13
No diagnosis	28
Pelvic pain	7
Fever/sepsis	5
Hemorrhage	6
Rape	5
Urinary tract infection	5
Abortion	4
Other	4

[a]More than one indication may be listed.

existing bacterial flora when *C. albicans* is isolated from vaginal cultures submitted to a hospital-based facility for reasons other than vulvovaginitis.

MATERIALS AND METHODS

Patient Population

The patient population was made up of women attending the ambulatory care facilities of Resurrection Medical Center. The clinical indications for the vaginal specimens obtained are listed in Table 1. All cultures submitted for endometritis, cervicitis or vaginal discharge, or vaginal itching/burning were excluded from analysis. All culture data derived from pregnant women was analyzed separately.

Specimen Handling

All specimens received over a six-month period were cultured for aerobic and anaerobic bacteria and *Candida* species. These specimens were received primarily from ambulatory care clinics. The specimens had been obtained with Culturette II swabs. Gram stains were performed on all cases, and the swabs were cultured for aerobes on colistin-nalidixic acid agar, chocolate blood agar and eosin-methylene blue agar; and for anaerobes on phenylethyl alcohol agar, Centers for Disease Control and Prevention (CDC) anaerobic blood agar, kanamycin-vancomycin agar, and enriched thioglycollate medium (media were obtained from BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD). Aerobic media were incubated at 35°C and analyzed after 24 hours, while anaerobic media were incubated in 70% nitrogen and 30% carbon dioxide at 35°C and analyzed after 48 hours.

Additional work-up of aerobic cultures involved interpretation of gram stains, hemolysis patterns, and colony characteristics. These findings plus catalase (0.1% hydrogen peroxide) and coagulase (Staphaurex, Murex Diagnostics, Daford, England) test results were used in the Vitek System (Bio-Merieux, Hazelwood, MO) for identification and antibiotic sensitivities.

Additional work-up of anaerobic cultures required confirmation of anaerobic bacteria by inoculating the suspected anaerobic organism onto blood agar and anaerobic CDC media. After overnight incubation in aerobic and anaerobic conditions, respectively, organisms, which grew on the latter medium but not the former, were worked up as anaerobes. Gram stains, colony morphology, and the Rapid ANA II System (Innovative Diagnostic Systems Inc., Norcross, GA) contributed to the identification of these organisms.

STATISTICAL ANALYSES

Chi-square analysis compared the prevalence of individual bacteria in both subgroups of pregnant women. No continuity correction for sample size was necessary. Power analysis showed a 75% and a 57% power to detect differences of sizes observed using a one-tail test at the $P < 0.05$ significance level. Statistical analysis were done using the statistical software package SPSS.

RESULTS

Two hundred and forty consecutive specimens were bacteriologically analyzed. Thirteen were eliminated because of the diagnosis of cervicitis (two) or vaginitis/vaginal discharge (11). Seven other cultures were removed from the analyses because of the presence of other vaginal yeast (*Candida glabrata* [six] and *Saccharomyces cerevisiae* [one]). Of the 220 remaining vaginal samples, *C. albicans* was isolated in 44 instances (20%). The distribution of bacteria derived from these vaginal samples relative to the presence or absence of *C. albicans* is presented in Table 2. The group B strep-

TABLE 2. Distribution of bacteria within vaginal samples relative to the presence or absence of *Candida albicans*

Bacteria	Absence of *Candida albicans* (N = 176)	Presence of *Candida albicans* (N = 44)
Aerobic bacteria		
Lactobacilli	80	22
Gardnerella vaginalis	74	17
Staphylococcus		
coagulase-negative staphylococci	95	19
S. aureus	7	1
S. saprophyticus (micrococcus)	1	—
Streptococci	3	—
groups A, F, G	1	
group B	28	12
group D (nonenterococcus)	2	—
Alpha hemolytic	20	2
Gamma hemolytic	6	2
Enterococcus	66	16
Diphtheroids	24	5
Aerobic gram-positive bacilli	30	6
Enterobacteriaceae	55	17
Escherichia coli	(40)	(13)
Klebsiella pneumoniae	(4)	(2)
Klebsiella oxytoca	(2)	—
Enterobacter cloacae	(2)	—
Enterobacter aerogenes	(2)	—
Ptoteus mirabilis	(4)	(1)
Haemophilus influenzae		(1)
Haemophilus parainfluenzae	(1)	—
Neisseria gonorrhoeae	1	—
Neisseria sicca	1	—
Alcaligenes	—	1
Peptostreptococcus	17*	—*
species	(4)	
P. anaerobius	(7)	
tetradians	(2)	
magnus	(2)	
asaccharolyticus	(2)	
Anaerobic gram-positive cocci	7*	1*
Anaerobic gram-positive bacilli	7*	—*
Propionibacterium	1	—
Bifidobacterium	—	—
Clostridium	2	—
Prevotella	18	7
species	(1)	(0)
bivius	(13)	(7)
melaninogenica	(4)	(0)
Bacteroides	29	9
species	(7)	—
corporis	(4)	(1)
intermedius	(2)	(2)
uniformis	(2)	—
magnus	(4)	(2)
fragilis	(6)	(2)
disiens	(2)	(2)
vulgatus	(1)	—
thetaiotaomicron	(1)	—
Fusobacterium	2	—
Veillonella	1	—
Anaerobic gram-negative bacilli	—	1
Mobiluncus	—	1

tococci had a greater probability of coisolation when *C. albicans* was present (27.3%), but this was not statistically significant ($P < 0.8$). While the overall percentage of anaerobic bacteria isolated between the two groups was not statistically different, dissociation between the presence of *C. albicans* and the coisolation of the *Peptostreptococcus* and aerobic gram-positive cocci and/or bacilli was noted ($P < 0.0819$). Similarly, the incidence of gram-positive aerobic bacilli was reduced in the presence of *C. albicans* (30/176 [17.1%] versus 6/44 [13.6%]), but this reduced incidence was not statistically significant.

Neither the presence of the lactobacilli nor the presence of *Gardnerella vaginalis* markedly influenced the isolation rate of *C. albicans*. Fewer bacteria were isolated in the presence of *C. albicans*, when compared with the absence of *C. albicans*, but the difference was not statistically significant (137/44, or 3.11 isolates per specimen with *C. albicans*, versus 576/176, or 3.27 isolates per specimen without *C. albicans*). In the subgroup of pregnant women, the corresponding figures were 167/45, or 3.71 isolates per specimen with *C. albicans*, versus 46/13, or 3.54 isolates per specimen without *C. albicans*.

The principal subgroup (N = 67) within the study sample was composed of pregnant women. The main reason for taking these cultures was to rule out the presence of group B stretococci and *Neisseria gonorrhoeae*. *Candida glabrata* was isolated from two of these specimens, and they were discarded from the study. Seven additional specimens were deleted from the study based on a clinical diagnosis suggesting vaginitis. Of the remaining 58 vaginal specimens derived from pregnant women, *C. albicans* was identified in 13 (22.4%). The relationships previously described for the nonpregnant women were sustained in the analyses applied to this subgroup. The incidence of isolation of group B streptococci was 12% (7/58) in the absence of *C. albicans* versus 30.8% (4/13) in the presence of *C. albicans*. The incidences of recovery of *Peptostreptococcus* and anaerobic gram-positive bacteria among the pregnant patients were 20% (9/45) in the absence of *C. albicans* versus 7.7% (1/13) in the presence of *C. albicans*. The distribution of bacteria among the pregnant women relative to the presence or absence of *C. albicans* is listed in Table 3.

TABLE 3. Distribution of bacteria within vaginal samples derived from pregnant women relative to the presence of *Candida albicans*

Bacteria	Absence of *Candida albicans* N = 45	Presence of *Candida albicans* N = 13
Aerobic bacteria		
Lactobacilli	20	7
Gardnerella vaginalis	21	8
Coagulate-negative staphylococci	24	7
Staphylococcus aureus	2	—
Micrococcus	1	—
Streptococci		
groups A, G, F	1	—
group B	7 (15.6%)	4 (30.8%)
group D	2	1
Alpha hemolytic	5	—
Gamma hemolytic	3	—
Enterococcus	21	6
Diphtheroids	8	1
Aerobic gram-positive bacilli	16	—
Enterobacteriaceae	12	2
Escherichia coli	8	1
Klebsiella pneumoniae	—	1
Enterobacter cloacae	1	—
Enterobacter aerogenes	1	—
Proteus mirabilis	3	—
Haemophilus influenzae	—	1
Acinetobacter	1	—
Neisseria gonorrhoeae	1	—
Peptostreptococcus	4	
tetradians	(1)	
P. anaerobics	(1)	
asaccharolyticus	(1)	
species	(1)	
Anaerobic gram-positive cocci	2	1
Anaerobic gram-positive bacilli	3	
Prevotella	7	4
bivius	5	4
melaninogenica	1	—
species	1	—
Bacteroides	6	2
fragilis	1	1
uniformis	1	1
disiens	2	
Species	1	
corporis	2	
Mobiluncus	—	1
Anaerobic gram-negative bacilli	—	1

DISCUSSION

To date, quantitative studies of the bacterial flora of the female genital tract are, unfortunately, limited in both numbers and scope of analyses. Their primary value has been identifying gross interrelationships between bacteria. Because of the intricacies of interbacterial and interspecies regulation, a true understanding of governing mechanisms requires quantitative as well as qualitative data and observation in a significant number of patient groups with a variety of divergent disease processes. Without quantitative studies, the significance of the presence of *C. albicans* is speculative.

While numerically superior to most published studies, the current study suffers from a number of defects, namely, the lack of quantitative microbiology for each isolate and the use of prospectively identified study populations constituted by diverging clinical status. The clinical indications received with the specimens might not have adequately characterized the actual reasons for culture submission. Fortunately, the samples derived from pregnant women, which were submitted primarily for prenatal indications, provided a subset against which observation derived from the general population could be compared. An additional shortcoming of this study is the technique used to obtain clinical specimens and the handling of specimens. The specimens were suboptimally handled for recovery of anaerobic bacteria specimens prior to reaching the biological test facility. Those isolates achieved probably represent bacteria present in high numbers in terms of colony-forming units per gram of vaginal fluid.

Microbiological studies of the bacterial flora in cases of vulvovaginal candidiasis have been severely limited by their limited scope and by the lack of quantitative data. The issues of interspecies regulation by *C. albicans* (whether through bacteriocins, hydrogen peroxide or other bactericidal proteins, or enzymes) have largely been neglected.[6–8] Confronted with a paucity of data, the prohibitive costs of obtaining such data, and the small likelihood that the required funding will materialize, one is forced to deal with fragmentary observation.

The overall decrease in the nubmer of coisolates achieved when *C. albicans* is present observed in this study is consistent with the observations in the literature. When disease due to *C. albicans* is present, the number of colony-forming units per milliliter of vaginal fluid is probably increased to a point where *C. albicans* can probably exert a maximal selective inhibitory effect. This study focused on cases where vulvovaginal candidiasis was not clinically present and asked whether at nondisease levels there was any disparity in the vaginal flora when *C. albicans* was present and when it was not.

Statistically, the current study suggests that an inverse relationship exists between the presence of *C. albicans* and recovery of *Peptostreptococcus* and anaerobic gram-positive cocci and bacilli.

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