Colonization of Candida Albicans in Vagina, Rectum, and Mouth

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To better understand the frequency of appearance, the density of growth, and the most common sites in which female patients harbor Candida albicans, a study was initiated of all patients receiving a pelvic examination for any reason at a solo family practice office. From February 1980 to November 1981, 341 pelvic examinations were accompanied by cultures and colony counts of the vagina, rectum, and mouth. A semiquantitative method adapted to Microstix-Candida (Ames Company) was utilized. Only 39 percent of all examinations had negative cultures in all three sites. Twenty-three percent of the positive cultures for C albicans were found from the vagina, 41 percent from the rectum, and 34 percent from the mouth. Incidence of colonization in any site did not vary significantly from 16 to 75 years of age. Negative rectal colonization was associated with lower vaginal colony counts and less frequent vaginal symptomatology. Relatively high vaginal colony count was associated with symptomatic vaginal candidiasis.

Vaginitis ranks 23rd in ambulatory encounters with office-based general and family physicians.[1] Candida albicans is the most frequent single pathogen causing vaginitis.[2-5] The alimentary tract is a common reservoir for this potential pathogen.[6-11] Miles et al[6] in 1977 concluded that once the gastrointestinal tract was colonized, it would probably remain so for life. Warnock and colleagues[12] in 1979 found that the vagina, rectum, and mouth in each studied subject generally grew identical strains of C albicans from these orifices, strongly implicating autoinoculation. The recent advent of systemic treatment of C albicans with ketoconazole,[4,13-15] although not yet proved to rid the gastrointestinal tract of C albicans, gives further impetus to look to the alimentary tract as a reservoir for chronic reinfections of the vaginal tract.

It would be helpful to determine the incidence of vaginal, rectal, and oral Candida colonization found among private family practice patients to better understand the multiple locations of this potential pathogen. Awareness of the incidence of gastrointestinal and vaginal colonization should

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aid the physician in the intelligent management of this common and highly annoying disease of the vulvovaginal tract. A semiquantitative measurement of yeast density in these orifices should add useful information.

This study included women between the ages of 16 and 93 years. Distribution was equal above and below 50 years of age. Since most studies are limited to women whose age is less than 50 years, an analysis of Candida colonization in the elderly with comparison to younger age groups should also be helpful to the family physician.

Culturing for vaginal C albicans is important to make a specific diagnosis that will help ensure primary treatment success. The time-honored potassium hydroxide (KOH) preparation is reliable only when positive, but it misses at least 40 percent of positively colonized sites.[5,16-18] The wet smear and the Gram-stained dry smear also offer unacceptable lack of sensitivity for establishing the presence of Candida.[5,16-18] An acceptable culture method should be simple, inexpensive, and reliably read by the physician or his staff. Microstix-Candida answers these criteria.

Methods

The study population was derived from a private solo family practice of approximately 2,000 active patients (those seen within the preceding three years). The office is located in a light-business, residential area of Roanoke, Virginia, a city of about 100,000 population. Most patients are from urban and suburban areas; approximately 15 percent come from adjacent rural areas. From February 3, 1980, through November 20, 1981, every patient 16 years or older receiving a pelvic examination for any reason was routinely tested for C albicans growth by culturing the vagina, rectum, and mouth (358 examinations). Seventeen examinations were excluded because of incomplete information. The remaining 341 examinations were included (less than 10 percent were repeat examinations). Seven patients were black; the remainder were white. Age range was 16 to 93 years. There was fairly even distribution within 10-year age groups from 16 to 75 years, the smallest number (35) appearing in the 16- to 25-year group and the largest number (80) appearing in the 56- to 65-year group. Only seven patients were 76 to 85 years old, and only three were 86 to 95 years old.

The culture medium used was modified Nickerson agar, distributed by Ames Company under the trade name of Microstix-Candida. A medium-impregnated fibrous pad, 1-cm square, occupies the end of a plastic strip, measuring 1 × 8 cm. The pad was rehydrated with two drops of sterile normal saline immediately before being inoculated by vaginal, rectal, or oral swab. The strip then was placed into a see-through plastic bag having an airtight seal and incubated at 35°C for 24 hours. C albicans colonies were identified and counted, utilizing their distinctive ability to reduce the colorless bismuth sulfite contained in the medium to black or very dark brown bismuth sulfide, thus staining the culture at each colony site.[5,19]

Swabs for culture were obtained from the vagina by dipping a dry cotton-applicator stick tip into the posterior fornix, then rubbing the tip against each side of the vagina exposed by a sterile bivalve speculum. The rectum was swabbed with a saline-moistened cotton applicator tip. The mouth and tongue were swabbed by a single dry cotton applicator tip. The following strict routine for inoculating the Microstix pad was followed: Each swab was immediately rolled upon the pad, then swirled 20 times on the pad while being rotated between the fingers; during this inoculation, an assistant placed two drops of normal saline onto the pad through the cotton applicator tip.

Following incubation of 24 hours, a single observer counted through the plastic bag the number of black or dark-brown colonies on the medium pad. The actual count of colonies was recorded when their number ranged from 0 to 50. Owing to inaccuracy in higher counts, a symbol of +50 was assigned to counts greater than 50.

To ensure reliability of a single-observer colony count, a random sampling of 57 patients' cultures was recounted by a second and third observer independently, and the deviation of counts statistically analyzed by interrater correlation. Similarly, in a series of 45 consecutive patients, four separate vaginal swabs for culture were obtained, two from the vaginal pool and two from the vaginal walls, avoiding the vaginal pool. The variation of colony count was again statistically analyzed for reliability by intraclass correlation. Interrater correlation

Table 1. Incidence by Site of Positive Cultures of C albicans or C tropicalis Comparing Results of Present Study with Those of Hilton and Warnock[8]

Site(s) of Infection	Bertholf and Stafford No. (%)	Hilton and Warnock[8] No. (%)
Vagina	24 (7.0)	15 (5.0)
Vagina and mouth	5 (1.5)	8 (2.7)
Vagina and rectum	26 (7.6)	23 (7.7)
Vagina, rectum, and mouth	23 (6.8)	36 (12.0)
Rectum	42 (12.3)	12 (4.0)
Rectum and mouth	48 (14.1)	42 (14.0)
Mouth	40 (11.7)	55 (18.3)
No infection	133 (39.0)	109 (36.3)
Totals	341 (100.0)	300 (100.0)

by three observers was 0.84; intraclass correlation of four replicated cultures was 0.91. Both single observations and single cultures were highly reliable.

Results

All patients were categorized according to (1) site of colonization (vagina, rectum, mouth) either alone or in combination, (2) 10-year age groups, 16 through 95 years old, (3) presence or absence of vaginal symptoms, and (4) positive vaginal colonization, separating those with fewer than 30 vaginal colonies from those with 30 or more vaginal colonies.

Table 1 compares the positive isolates (by site) of this study with those of Hilton and Warnock.[8]

Sixty-one percent of all patients had colonization of vagina, rectum, or mouth, alone or in combination. There was no significant change in incidence of colonization of any orifice associated with increasing age to 75 years. Groups of individuals aged over 75 years included too few patients for meaningful results.

Twenty-three percent of all patients had vaginal colonization alone or in combination with rectum or mouth or both. Grouping the patients into 10-year age spans from 16 to 75 years showed a range of 18 percent (for those 26 to 35 years old) to 29 percent (for those 46 to 55 years old) having positive vaginal colonization.

Forty-one percent of all patients had rectal colonization alone or in combination with vagina or mouth or both. Grouped patients ranged from 34 percent (for those aged 16 to 25 years and those 26 to 35 years) to 54 percent (for those aged 46 to 55 years) having positive rectal colonization.

Thirty-four percent of all patients had mouth colonization alone or in combination with vagina or rectum or both. Grouped patients ranged from 23 percent (for those aged 16 to 25 years) to 45 percent (for those aged 46 to 55 years) having positive oral colonization.

When the vagina alone was colonized (24 patients), the median colony count was four. Nine patients were symptomatic. Four of these symptomatic patients were among 18 patients growing fewer than 30 colonies. The other five symptomatic patients were among six patients growing 30 or more colonies.

When the vagina and mouth were colonized together (five patients) the median vaginal colony count was two. One patient was symptomatic and was included in the four patients growing fewer than 30 vaginal colonies. The one patient of this group growing 30 or more colonies was asymptomatic.

When vagina and rectum were colonized to-

gether (26 patients) the median vaginal colony count was +50. Seventeen patients were symptomatic. Four of these symptomatic patients were among ten patients growing fewer than 30 vaginal colonies. The other 13 symptomatic patients were among 16 patients growing 30 or more vaginal colonies.

When vagina, rectum, and mouth were colonized together (23 patients), the median number of vaginal colonies was 50. Thirteen patients were symptomatic. Three of these symptomatic patients were among nine patients growing fewer than 30 vaginal colonies. The other 10 symptomatic patients were among 14 patients growing 30 or more vaginal colonies.

Discussion

Culturing of C albicans

C albicans and C tropicalis are reported by Nickerson[19] and confirmed by others[5,16,18] to cause very dark brown to jet-black discoloration of the glycine, dextrose, yeast extract, bismuth sulfite medium (Nickerson agar) at their sites of colony growth. C tropicalis is rarely found in human cultures (1 percent or less),[5,8] and it is not an important cause of vaginitis.[20] Other Candida species cause less intense dark staining, and other yeasts produce either colorless or much lighter-colored stains at colony sites.[19] Hilton and Warnock[8] chose to use Sabouraud's glucose peptone medium, which has no special staining characteristics. Identification of C albicans cultured on Sabouraud's medium requires subcultures, observation of germ-tube production in serum, filament formation in corn meal agar, sugar assimilation patterns, and fermentation characteristics. Using this multiple-procedure technique did not, however, produce significantly different data. The lack of specificity of Nickerson medium in differentiating C albicans from C tropicalis is not a significant shortcoming when dealing with human cultures. Table 1 shows a striking similarity in the prevalence of growth sites between two distant populations; the one in this study, located in the Roanoke Valley of Virginia, and the other in Bristol, England. This similarity existed despite the different culture media used. Only the rectum group and the vagina-rectum-mouth combination group displayed a major variance in incidence. The number of patients found to be free of C albicans at all sites differed by less than 3 percent. It would appear that the 1-cm square pad of Microstix-Candida is adequately sensitive and specific to C albicans to be a reliable single culture method in the human subject and is ideally suited for use in the office of the family physician. This conclusion agrees with the experience of Weissberg[5] and Sian et al.[21] They found, respectively, 96 percent and 97 percent accuracy of their own interpretation of Nickerson medium results when compared with results supplied by mycological laboratories.

Distribution of C albicans

Twenty-three percent of the positive cultures for C albicans were found from the vagina, 41 percent from the rectum, and 34 percent from the mouth. Thus, female patients beyond menarche who visit the family physician are likely to be harboring C albicans in the vagina, rectum, or mouth (61 percent of all women in this study).

Candida colonization of vagina or rectum or mouth (regardless of presence or absence of combinations) varied insignificantly with age of the patient (from 16 to 75 years). It appears that in the population studied, menopause imparted no immunity to Candida colonization.

Significance of Colony Density

It is generally accepted that there is little significance to colony density as it pertains to the commensal (saprophytic) vs the pathologic (parasitic) state of C albicans in the vagina. In 1977 Oriel[22] stated, "there is little evidence that patients with vaginal candidosis yield higher concentrations of Candida than those without clinical disease." This observation has become so widely accepted as

correct by researchers that the literature on vaginal candidiasis of the past five years has practically failed to consider colony density at all. To again test the possibility that colony density may be associated with pathogenicity, the data of this study were analyzed comparing colony counts with vaginal symptomatology.

Such a comparison relies upon an accurate assessment of vaginal colonization. Despite the obvious lack of exact quantitation of vaginal contents used in this study, a reproducible number of vaginal colonies was possible. That four separate vaginal cultures produced an intraclass reliability of 0.91 may reflect that the counted range from 0 to 50 colonies represented relatively sparse density of vaginal colonization. The great variation of colony counts was absorbed in the +50 group.

Patients having vaginal colonies numbering fewer than 30 were compared with patients having vaginal colonies numbering 30 or more because of the greater concentration of symptomatic women in the 30 or more group. Forty-one patients with vaginal colonization had fewer than 30 vaginal colonies of C albicans. Twelve of these (29 percent) complained of vaginal symptoms, and 29 were symptom-free. Thirty-seven patients grew 30 or more vaginal colonies. Twenty-eight (76 percent) complained of vaginal symptoms, and nine were symptom-free.

The proportion test was used to test the hypothesis that the proportion of symptomatic and asymptomatic women having 30 or more vaginal colonies was equal. The results of this test indicated that symptomatic women (76 percent, or 28/37) were significantly ($P < 0.01$) more likely to have 30 or more colonies than asymptomatic women (24 percent, or 9/37).

A review of the records of the 12 symptomatic patients noted above who grew fewer than 30 vaginal colonies revealed that 6 had concurrent atrophic vaginitis, 4 had concurrent vaginitis caused by a Gardnerella vaginalis infection, and 1 had concurrent gonorrhea. Thus, 11 of the 12 had other pathology that may have accounted for their vaginal symptoms.

A review of the records of the 28 symptomatic patients noted above who grew 30 or more vaginal colonies revealed none to have other pathology to account for their vaginal symptoms.

All nine of the symptom-free patients who were among the 30 or more colony group grew more than 50 vaginal colonies. A review of these nine patients' records revealed that six had chronic factors predisposing to Candida growth. Three of the six were receiving long-term parenteral estrogen therapy, two more had diabetes mellitus, and one patient was taking oral contraceptives. This last patient had been found to have asymptomatic heavy vaginal colony growth on yearly examinations in 1978 and 1979 and had received treatment at the time of each visit. Therefore, when she was again found to have heavy asymptomatic colonization in 1980, she was not treated. She appeared within the time of this study in 1981 with colony counts of +50 in the vagina, 12 in the rectum, and 2 in the mouth, still having acquired no vaginal symptoms.

These data appeared to support (1) an increased probability of finding other causes of vaginal symptoms when vaginal colony count was below 30, (2) an increased probability that symptomatic vaginal candidiasis would yield higher colony counts, and (3) a tendency that women with asymptomatic high vaginal counts were chronically exposed to factors predisposing to Candida growth. This last observation suggests that an acquired tolerance to Candida may exist. One must remember, however, that the colony counts are at best semiquantitative and that controlled studies are needed to verify or nullify these impressions.

Association of Vaginal to Rectal Colonization

A lower median vaginal colony count was found when rectal colonization was absent (vagina-positive, rectum-negative, mouth-negative, vaginal median colony count equaled 4; vagina-positive, rectum-negative, mouth-positive, vaginal median colony count equaled 2). A higher median vaginal colony count was found when rectal colonization accompanied vaginal colonization (vagina-positive, rectum-positive, mouth-negative, vaginal median colony count was greater than 50; vagina-positive, rectum-positive, mouth-positive, vaginal median colony count equaled 50).

Of the vagina-positive, rectum-negative patients, 10 of 29 (34 percent) had vaginal symptoms.

When rectal colonization accompanied vaginal growth, 30 of 49 (61 percent) patients had vaginal symptoms. Using the proportion test, this difference is significant ($P = 0.014$). Freedom from rectal colonization appeared to be associated with less dense vaginal colonization and more freedom from vaginal symptoms.

In light of these findings, it may be to the patients' best interest to avoid rectal inoculation with vaginal contents. The time-honored technique by health professionals of following a digital-vaginal examination with a digital-rectal examination without change of glove is suspect in inoculating C albicans into a possibly Candida-free rectum. In a similar vein, Neumann[23] has warned against possibly causing "iatrogenic gonorrhea" of the rectum by following a digital-vaginal examination of a known or unknown carrier of vaginal (cervical) Neisseria gonorrhoeae with a digital-rectal examination without change of examining glove. As in the case of rectal gonorrhea, rectal candidiasis is more difficult to eradicate than is the corresponding vaginal infection. The association of positive rectal C albicans colonization with higher vaginal colony counts and greater vaginal symptomatology does not imply cause and effect, but a change of gloves between digital-vaginal and digital-rectal examinations would seem a wise precaution until the safety of no change can be established.

Conclusions

Vaginal culture using Microstix-Candida is simple and reliable for C albicans and is recommended to the family physician for routine use in the patient with vulvovaginal symptoms.

Over 50 percent of patients beyond menarche who visit the family physician are likely to harbor C albicans in the vagina, rectum, or mouth. Incidence of vaginal, rectal, and oral colonization with C albicans remains fairly constant throughout life after menarche.

Lower vaginal colony density and less frequent vaginal symptomatology tend to accompany negative rectal colonization. Low vaginal colony counts should raise suspicion that C albicans may not be the cause of vaginal symptomatology.

Acknowledgments

Nancy Thomas, LPN, assisted in the observations recorded here, and Charles Houston, PhD, Director of Institutional Research, Virginia Western Community College, Roanoke, Virginia, provided the statistical analyses. Ames Company, a Division of Miles Laboratories, furnished the Microstix-Candida for this study.

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