Can the diagnosis of recurrent vulvovaginal candidosis be improved by use of vaginal lavage samples and cultures on chromogenic agar?

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Can the diagnosis of recurrent vulvovaginal candidosis be improved by use of vaginal lavage samples and cultures on chromogenic agar?

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Objective: To investigate if introital and vaginal flushing samples inoculated on chromogenic agar could increase the recovery rate and rapid identification of *Candida* and non-*albicans* species, as compared to culture of posterior vaginal fornix samples on Sabouraud agar and speciation of isolates by biochemical tests.

Methods: Samples from the introitus and the posterior vaginal fornix and vaginal lavage samples were collected from 91 women with a history suggestive of recurrent vulvovaginal candidosis (RVVC), and with a suspected new attack of the condition. The specimens were cultured on Sabouraud and CHROMagar®. Speciation of yeast isolates was made on the chromogenic agar by API 32C® kits and by an atomized system (Vitek®).

Results: Forty-six (51%) women were positive for *Candida* from one or more of the samples. The introital cultures were positive in 43 (47%) women, both on Sabouraud and chromogenic agar. From the posterior vaginal fomix, 42 (46%) women were positive on the Sabouraud and 43 (47%) on chromogenic agar cultures, while the vaginal lavage cultures yielded *Candida* on those two media in 40 (44%) and 41 (45%) cases, respectively. *Candida albicans* was the most frequent species recovered, from 40 (87%) cases, followed by *C. krusei* in 4 (9%), *C. glabrata* in 2 (4%), and *C. parapsilosis* in one case. There was only one woman who had a mixed yeast infection, by *C. albicans* and *C.* krusei. There was only one discrepancy in the speciation as demonstrated by mean of chromogenic agar and API 32C kit.

Conclusions: Neither cultures of introital nor of vaginal lavage samples increases the detection rate of *Candida* in RVVC cases as compared to cultures of posterior vaginal fornix samples. Use of chromogenic agar is a convenient and reliable means to detect colonization by *Candida* and differentiate between *C. albicans* and non-*albicans* species.

Key words: VULVOVAGINAL CANDIDOSIS; *CANDIDA ALBICANS;* NON-ALBICANS SPECIES; VAGINAL LAVAGE; CHROMOGENIC AGAR

The most common means to diagnose vulvovaginal *Candida* infections is to study a wet smear of vaginal secretion that has been treated by potassium hydroxide (KOH) for *Candida* morphotypes, and/or to culture samples collected with a cotton-tipped swab from the posterior vaginal fornix. The specimens can either be sent to a laboratory for culture and speciation of *Candida* isolates or plated on Sabouraud agar directly at the clinic[1–3].

Although the vast majority of strains isolated from the genital tract in recurrent vulvovaginal

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candidosis (RVVC) cases belongs to *C. albicans*[4], it may be essential to identify instances of colonization with non-*albicans* species. Non-*albicans* strains have a natural greater resistance to the antifungal drugs most commonly used to treat vulvovaginal candidosis (VVC), i.e. various azole drugs[5,6]. By the use of chromogenic agar[7,8] it is possible to directly identify a number of non-*albicans* species of *Candida* by mere inspection of the plates, i.e. *C. albicans*, *C. tropicalis*, and *C. krusei*, according to the package insert[7]. Koehler and co-workers[9] reported, however, that as many as eight *Candida* species could be identified on chromogenic agar. The species identities on chromogenic agar correspond well to those obtained by fermentation and assimilation tests[10]. Apart from use of samples collected by cotton-tipped swabs, analyses of vaginal flushing samples have been proved of value in the diagnosis of genital pathogens[11].

A large proportion of women suspected on the basis of history and clinical examination prove negative for *Candida* on culture of vaginal posterior fornix samples. One hypothesis is that analysis of vaginal lavage samples would increase the chance of detecting only a low number of *Candida* organisms in the vagina compared to the most commonly applied sampling technique, which involves just touching the vaginal mucosa with a swab. This latter procedure may not leave enough time for adsorption of yeast organisms by the swab. Even if rubbing the mucosa, many organisms may remain attached to the swab material after inoculation of culture media, resulting in a negative culture.

The present study explored the possibility of increasing the detection rate of *Candida* in women with a history suggestive of RVVC and who did attend with symptoms and signs that were consistent with a new attack of the condition. Analyses of introital swab and vaginal lavage samples were made. Furthermore, the value of chromogenic agar for culture of such specimens was investigated.

SUBJECTS AND METHODS

The 91 women recruited to the study had all attended the outpatient department of the Maternity Hospital 3, Kiev, because of genital symptoms and a history suggestive of RVVC. They all had the diagnosis of a genital *Candida* infection by a physician at least four times during the previous year on the basis of history, clinical examination and at least one positive microscopic test for *Candida* morphotypes, in methylene-blue-stained smears prepared from posterior vaginal fornix samples. The patients presented with symptoms, such as vulvovaginal pruritis, irritation, unpleasant odor and dyspareunia, and/or with dysuria and burning at micturition. They had signs such as one or more of edema, erythema, fissures and caseous discharge.

The vaginal introitus was rubbed with a sterile, cotton-tipped swab moistened with physiological saline, which was plated on Sabouraud dextrose agar (Beckton Dickinson®, Baltimore, USA)[3] and chromogenic agar (CHROMagar®, Paris)[9], like swab samples from the posterior vaginal fornix. The order of the inoculation on the two media was changed for each consecutive case studied. Thereafter, vaginal lavage fluid was obtained by flushing the vagina with 3 ml of sterile physiological saline and by regurgitating the fluid with the aid of a sterile syringe. Of the mixture, 0.1 ml was inoculated on each of a Sabouraud and chromogenic agar plate.

Isolated strains of *Candida* were frozen at −70°C in fetal calf serum for later speciation, which was done with the aid of API 32C® test kits and an automized identification system, Vitek® (BioMérieux, Marcy-l'Etoile, France). Chromogenic agar plates were read according to the criteria described by Pfaller and co-workers[10].

The study was approved by the ethic committee of the Maternity Hospital. Written consents were obtained from the patients.

RESULTS

Table 1 shows the results of cultures made from swab samples from the introitus and posterior vaginal fornix and vaginal lavage samples, inoculated on Sabouraud and chromogenic agar. The recovery rate from the introitus was the same as that from the posterior vaginal fornix. The vaginal lavage samples missed two *Candida*-positive cases otherwise diagnosed by the swab samples.

C. albicans was the most frequent isolate, recovered in 40 (87%) cases, followed by *C. krusei* detected in 4 (9%) cases, *C. glabrata* in two and

Table I Results of swab sample cultures from the introitus and the posterior vaginal fornix and of vaginal lavage samples from 91 patients with a history and current symptoms of recurrent vulvovaginal candidosis grown on Sabouraud and chromogenic agar

	Number of positive Candida cultures		
Sample type	*Sabouraud agar* (n = 91)	*Chromogenic agar* (n = 91)	p-*value*
Introital swab	43 (47%)	43 (47%)	1
Posterior vaginal fornix swab	42 (46%)	43 (47%)	1
Vaginal lavage samples	40 (44%)	41 (45%)	1

C. parapsilosis in another case. There was only one case of mixed *Candida* infection, i.e. by *C. albicans* and *C. krusei*. The isolation rate on chromogenic agar was almost the same as on Sabouraud agar: the difference was 1.4% in favor of chromogenic agar.

A discrepancy in the result of speciation by means of chromogenic agar versus API 32C kits was only found in two of the 46 *Candida*-positive cases. In these two patients, *C. albicans* was identified by the API 32C kit, while *C. glabrata* was indicated to grow on the chromogenic agar. In another case, a mixed infection by *C. albicans* and *C. krusei* was found on the chromogenic agar, while only growth of *C. albicans* was identified by the API C32 and Vitek identification methods.

DISCUSSION

The diagnosis of genital *Candida* infections, in cases with a history of RVVC who attend with symptoms and signs suggestive of a new attack, fails more often than generally realized – even if both microscopic and culture studies are made. Thus the presence of *Candida* cannot be confirmed. Specialists in gynecology often misdiagnose the condition if not based on patient-close or central laboratory test results. In a previous study by Ledger[12], the presence of *Candida* in the genital tract could only be confirmed in half of cases given the diagnoses of VVC or RVVC based on history, clinical examination and patient-close microscopy of vaginal secretion. In our study, half the women also proved to be culture-negative for *Candida* in spite of a 'typical' RVVC case history and findings at the clinical examination. In another study, *Candida* was identified by vaginal cultures in only 28% of 554 women with symptoms consistent with 'candidal vaginitis'[13]. Thus, there are reasons to believe that a current genital *Candida* infection is commonly overdiagnosed in women diagnosed with RVVC. In fact, those women may suffer from other vaginal flora changes, such as those seen in bacterial vaginosis (BV)[14]. Women may switch between BV and VVC[15].

At least 10–15% of all women of reproductive age are colonized in the vagina by *Candida*, i.e. are carriers without any occurrence of either VVC or RVVC[4]. This colonization rate adds to the difficulty in diagnosing RVVC exclusively on the basis of genital yeast cultures, i.e. to differentiate carriers from cases with a true vaginal mucosal and/or introital invasion by yeast.

In our study, the sensitivity of chromogenic agar compared to Sabouraud dextrose agar for detection of *Candida* did not differ (p = 1). This is in accordance with the findings by Beighton and co-workers[8]. Nor did we find the use of vaginal lavage samples to positively influence the recovery rate of *Candida*.

The use of chromogenic agar has one disadvantage compared to Sabouraud agar, namely its rather high cost. On the other hand, chromogenic agar can identify most of the species occurring in the female genital tract. Consequently other expensive identification tests may be avoided. The use of an incubation temperature below 30°C does not allow speciation of *Candida* strains[9,16] on chromogenic agar. Thus, chromogenic agar cannot be used at doctors' offices for speciation if no incubator is available.

C. albicans, *C. krusei* and *C. tropicalis* have been indicated to represent more than 99% of all yeast isolates from the human vagina[7]. In an Italian study of symptomatic women with a positive *Candida* culture, *C. glabrata* was the next most common species found, identified in 1207 of 3351 cases. *C. krusei* and *C. tropicalis* were found in 404 and 290 cases, respectively[17]. In our study, *C. glabrata* was found in only two cases.

The possibility of rapidly identifying the species of *Candida* by which a women is colonized

increases the possibility of obtaining successful cure. Women infected with non-*albicans* species may not respond to azole drugs, to which such species are naturally resistant or have a low sensitivity[18]. In that respect chromogenic agar serves a diagnostic purpose in RVVC cases.

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