The Epidemiology of Mycotic Vulvovaginitis and the use of Antifungal Agents in Suspected Mycotic Vulvovaginitis and its Implications for Clinical Practice

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ABSTRACT

Data in the Caribbean documenting the speciation of yeast associated with vulvovaginitis are lacking. The widespread use of antibiotics and increased availability of antimycotic agents, both prescribed and over-the-counter, predisposes both to a change in the epidemiologic patterns and the possible development of secondary resistance among previously susceptible yeast. This study was conducted to evaluate the aetiologic agents associated with mycotic vulvovaginitis and to review the appropriateness of prescribed antifungal therapy. Of 134 positive isolates, the most frequent yeast isolate was C albicans *accounting for 78%,* C tropicalis *10%,* Prototheca wickerhamii (P wickerhamii) *5%,* C glabrata 4%, Cryptococcus albidus (C albidus) *2% and* C lusitaniae *(1%) were also isolated. Of the positive cases, 75% were treated with antifungals, 17% with antibiotics and 8% were not treated. The azole group was the most frequently prescribed antifungal (71%). Of cases with negative yeast cultures, 83% were treated with antifungals. The presence of non-albicans Candida species and other opportunistic fungi is an important finding and combined with the pattern of therapy, represents a major challenge for future empirical therapeutic and prophylactic strategies in the treatment of mycotic vulvovaginitis.*

Epidemiología de la Vulvovaginitis Micótica y el uso de Agentes Antifungales Ante la Sospecha de Vulvovaginitis Micótica, y sus Implicaciones en la Práctica Clínica

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RESUMEN

La región del Caribe carece de datos que documenten la especiación de la levadura asociada con la vulvovaginitis. El uso extendido de antibióticos y la mayor disponibilidad de agentes antimicóticos – tanto los adquiridos mediante prescripción facultativa como los que pueden comprarse sin receta médica – predisponen por un lado a un cambio en los patrones epidemiológicos, y por otro al posible desarrollo de resistencia secundaria en la levadura previamente susceptible. Este estudio se llevó a cabo con el fin de evaluar los agentes etiológicos asociados con la vulvovaginitis micótica y examinar cuán adecuada resulta la terapia antifúngica prescrita. De 134 aislados positivos, el aislado de levadura más frecuente fue el C albicans *responsable del 78%. También fueron aislados* C tropicalis *10%,* Prototheca wickerhamii (P wickerhamii) *5%,* C glabrata *4%,* Cryptococcus albidus (C albidus) *2% y* C lusitaniae *(1%). El 75% de los casos positivos fueron tratados con antifúngicos, el 17% con antibióticos, en tanto que un 8% no recibió tratamiento alguno. Los medicamentos antifungosos de la familia azol (71%) fueron los más frecuentemente prescritos. El 83% de los casos con cultivos de levadura negativos, fue tratado con antifúngicos. La presencia de especies de Candida no albicans y otros hongos oportunistas, constituye un hallazgo importante, y en combinación con el modelo de terapia, representa un desafío de importancia considerable para las futuras estrategias empíricas – tanto terapéuticas como profilácticas – en el tratamiento de la vulvovaginitis micótica.*

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INTRODUCTION

Speciation of yeast associated with vulvovaginitis has been documented worldwide with frequencies of distribution of non-albicans Candida species varying in relation to the geographic location. *Candida albicans (C albicans)* is reported

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to be the most frequently isolated species in all regions accounting for 45% to 90% of cases (1, 2). In recent years, a change in epidemiological trends has been observed in some countries, with vaginal candidiasis showing a higher frequency of recurrence and a significant increase in infections being associated with non-albicans species (3). Once correctly diagnosed, most cases of vaginitis due to Candida are uncomplicated in their treatment. Unfortunately, increasing numbers of complicated cases now occur requiring additional microbiological study and longer duration of therapy with selected antifungal agents (3). The inappropriate use of antifungal drugs and the introduction of over-the-counter antimycotics in countries worldwide predispose to the development of antifungal resistance (4). In addition to acquired resistance, antifungal resistance may be primary or innate, as is typified by some non-albicans species, such as *C lusitaniae* and *C krusei* (5). To date, there is no documentation of the frequency of occurrence of non-albicans species associated with vulvovaginitis in Jamaica. This study documents the frequency of occurrence of non-albicans species and describes the trends in antifungal use in the treatment of mycotic vulvovaginitis in patients seen at the University Hospital of the West Indies (UHWI) and discusses implications for clinical practice.

PATIENTS AND METHODS

A prospective study was conducted over a six-month period at the UHWI, with the approval of the University Hospital of the West Indies/University of the West Indies ethical committee. Written consent was received from all participants enrolled in the study. All patients attending the Obstetrics and Gynaecology Outpatient Clinics with a clinical diagnosis of vulvovaginitis were enrolled in the study.

A clinical diagnosis is commonly based on the presence of one or more of the following symptoms and/or signs (6, 7): vulval itching, vaginal discharge, dyspareunia or dysuria, localized or generalized erythema of the vulva, vagina, labia majora and perineum, fissuring of the vagina and/or oedema, white and cheese-like vaginal discharge, watery or purulent discharge, odourless discharge and presence of satellite lesions.

The criteria for laboratory diagnosis of mycotic vulvovaginitis were based on the following (7, 8): microscopic findings of hyphae and budding yeast, a positive germ tube test for *Candida albicans,* a positive yeast culture and a positive biochemical yeast assimilation test using the Remel RapID Yeast Plus System®. A definitive diagnosis of mycotic vulvovaginitis was made when both the clinical vulvovaginitis and laboratory criteria were satisfied.

For the study, the required specimens were a high vaginal and an endocervical specimen collected using a separate dry cotton tip swab turned through 360 degrees. Each swab was then placed in a separate bottle of Stuart's transport medium and transported to the laboratory at room temperature within 24 hours of collection. The laboratory investigations were performed in the Microbiology Department at the The University of the West Indies (UWI) according to recommended guidelines (8).

Gram stains and potassium hydroxide preparations were performed directly on specimens for observation of yeast and hyphae. Cultures for fungi were done using Saborauds Dextrose and Mycobiotic Agar media. The inoculated media were incubated at 30°C and observed every two to three days for fungal growth. All yeast isolates were examined for the production of pseudohyphae by the Germ Tube test. Speciation of yeast was done using the commercially prepared assay for assimilation, The Remel RapID Yeast Plus System®.

RESULTS

A total of 422 patients were enrolled in the study. Observed ages ranged from 15 to 50 years, with the majority of cases occurring between the ages 20 to 35 years. Specimens were received from 354 (84%) of the 422 enrolled cases. Yeast isolates were confirmed in 134 (32%) cases. Frequencies of yeast isolated included, 104 (78%) *C albicans,* 14(10%) *C tropicalis,* 7 (5%) *Prototheca wickerhamii (P wickerhamii)*, 6 (4%) *C glabrata,* 2 (2%) *Cryptococcus albidus, (C albidus)* and 1(1%) *C lusitaniae.* Of the 134 positive cases, treatment prescribed was: antifungals : 100 (75%); antibiotics : 23 (17%) and no treatment : 11 (8%).

Azole antifungals were used in 95 (71%) cases, polyenes in a single (0.7%) case and cicloproxolamine in 4 (3%) cases. Of the 95 cases treated with azoles, imidazoles were used in 87 (92%) cases and the triazoles in 11 (12%). A negative yeast culture was obtained in 220 (62%) of the cases of which 182 (83%) were treated, 119 (54%) with antifungal and 63 (29%) with antibiotics whilst 38 (17%) received no treatment. Empirical treatment in yeast culture positive cases are shown in the Table. Of note, 56% of cases of *C albicans* were treated with imidazoles (clotrimazole-Mycelex, Lotrim, Gyne-Lotrim, Clotriderm, Myoril and Canesten and ketokonazole-Nizoral) 4%, with triazoles (fluconazole-Diflucan and itraconazole-Sporonox); combined imidazoles and triazoles 3%; imidazoles and antibiotics 7%; triazoles and antibiotics 1%; cicloprox olamine (Batrafen) 2%; polyenes (Nystatin) 1% and antibiotics alone 16%.

DISCUSSION

In this study, we observed that *C albicans* (78%) was the most frequent aetiologic agent associated with mycotic vulvovaginitis, with non-albicans species accounting for 15%. This finding is in keeping with other studies worldwide, in which the frequency of *C albicans* varies between 45% to 90%. Among the non-albicans species, *C tropicalis* (10%) was more frequently isolated than *C glabrata* (4%). *C lusitaniae,* uncommonly associated with vulvovaginitis, was isolated in one (1%) case. Other unusual findings of this study include the isolation of the opportunistic pathogens *C albidus* and *P wickerhamii,* not commonly isolated in vulvovaginitis.

Table: Treatment in culture positive yeast cases

Specie	Treatment								Total
	Imidazole (%)	Imidazole and Triazole (%)	Imidazole and Antibiotic (%)	Triazole (%)	Triazole and Antibiotic (%)	Antibiotic (%)	Cicloprox olamine (%)	Polyene (%)	(%)
C albicans	61(56)	3 (3)	7 (7)	5 (4)	1 (1)	16 (16)	3 (2)	1(1)	97 (100)
C tropicalis	6 (50)	0	0	1 (8)	0	4 (34)	1 (8)	0	12 (100)
C glabrata	3 (50)	0	1 (16)	0	0	2 (34)	0	0	6 (100)
C lusitaniae	1 (100)	0	0	0	0	0	0	0	1 (100)
P wickerhamii	3 (50)	0	1 (16.6)	0	1 (16.6)	1 (16.6)	0	0	6 (99.8)
C albidus	1 (100)	0	0	0	0	0	0	0	1 (100)
Total	**75 (61)**	**3 (2.4)**	**9 (7)**	**6 (5)**	**2 (1.6)**	**23 (19)**	**4 (3.2)**	**1 (0.8)**	**123 (100)**

C = *Candida,* P = *Prototheca,* C *albidus* = *Cryptococcus albidus*

The fact that *C tropicalis* was more frequently isolated than *C glabrata* has been observed in other Latin American countries, but is reversed in the United States of America and Canada, where C glabrata occurs more frequently than *C tropicalis* (1, 9).

Results of this study showed that the azole antifungals were prescribed most frequently in patients with Candida vaginitis, with the older imidazoles (ketoconazole, clotrimazole) being used more commonly than the triazoles (itraconazole and fluconazole). Of concern is the wide variety of agents used in the treatment of the most common isolate, *C albicans.* It is well known that Candida specie vary in their susceptibility to antifungal agents, with secondary or acquired resistance of *C albicans* and shifts in flora away from *C albicans* towards the less susceptible non-albicans species being associated with multiple courses of azole therapy. The resistance of Candida species to the older azoles, such as ketoconazole, occurs in a pattern that appears to parallel resistance to itraconazole. Currently, the most significant form of azole resistance is that seen between Candida and fluconazole, with estimated frequencies varying up to 23% (7, 9,). Oral fluconazole represents the only systemic therapy that the Centers for Disease Control and Prevention (CDC) recommends for the management of uncomplicated vulvovaginal candiasis. Vaginal triazole resistant *C albicans* isolates are considered to be rare, with reported cases being associated with refractory vulvovaginal candidiasis and a history of azole self-medication. A Belgian hospital-based study of 612 women attending obstetrics-gynaecology outpatient clinics found the prevalence of fluconazole-resistant *C albicans* isolates to be 23%. The authors commented that when compared with intravaginal azole antifungals, oral azole therapy is considered to achieve comparable or marginally higher therapeutic cure rates for vulvovaginal candidiasis. They also advised that the appropriateness of prescribing the triazoles for vulvovaginal candidiasis should be within the recommended guidelines with particular attention to the dosage and duration of the drug and the potential for drug resistance (7). Cross-resistance to other azole antifungal agents is common but not universal and is not associated with cross-resistance to polyene antibiotics, such as amphotericin B and nystatin (9). Unlike the azole drugs that exert their effect by inhibition of fungal cytochrome P450 enzymes, the polyene antifungals act by binding to ergosterol, in the fungal cell membrane. Resistance to the polyene antifungals remains an uncommon event among Candida isolates. The polyenes still have reliable activity against most of the Candida species, except *C lusitaniae,* which is often intrinsically resistant. In addition, reduced susceptibility appears common among isolates of *C glabrata* (9, 10). The susceptibility of Candida species to Cicloproxolamine has been shown to be similar to that of the imidazoles (11).

The emergence of non-albicans Candida species is also clearly a concern. Prototheca, a genus of achlorophyllic algae, is a rare cause of opportunistic infections in humans. Polyene antifungals remain the most effective drugs for eradicating Prototheca infections. Azole antifungals may also be used with more localized infections, itraconazole being the most effective drug of this class (12). Of the three Prototheca isolates (66.6%) two were appropriately empirically treated, one with an imidazole, and the other with a triazole. The other was in the group inappropriately empirically treated with antibiotics.

Recently, the number of reports of the isolation of non-neoformans Cryptococcus species from clinical specimens and opportunistic infection by this species has been increasing. This basidiomycetous yeast is known to be sensitive to the polyene antifungals and to vary in resistance to azoles such as fluconazole and itraconazole. In view of this possible resistance, the use of polyene antifungals for the treatment of *C albidus* associated vaginitis in this study would have been the more appropriate drug of choice (13).

Antifungal therapy was inappropriately prescribed in 54% of culture negative cases. Such unnecessary use of anti-

fungal therapy contributes to the development of antifungal resistance and the emergence of infections associated with non-*C albicans* yeast and other opportunistic fungi.

Mycotic vulvovaginitis, which is largely treated empirically, presents both a diagnostic and therapeutic challenge. Inappropriate management may result in the progression to complicated vaginitis, enhance the development of antifungal resistance or the emergence of uncommon species. In this context, the role of the laboratory in the identification and speciation of aetiological pathogens is essential to the avoidance of pitfalls of empiricism and the identification of developing trends in species distribution.

In summary, this study has demonstrated the need to emphasize the importance of accurate diagnosis and appropriate use of antimycotic agents in patients presenting with the common complaint of vulvovaginitis/vaginal discharge. There is also need for further study to identify resistance patterns of yeast.

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