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Original Research Article

Prevalence and identification of *Candida* sp. in pregnant women using VITEK-2

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ABSTRACT

Background: *Candida* sp. is seen in several areas of body such as, mouth, groin area including vagina and digestive tract as thrush or gastroenteritis. The slide-culture technique and the VITEK-2 automated system were used for species-identification of the fungus; nonetheless, a gold standard or any first identification method would have inherent errors in arriving at a correct identification of a microorganism at species level.
Methods: Morphological fungal criteria were ascertained with germ tubes, glucose agar, sugar fermentation and sugar assimilation tests *Candida* from vaginal swabs and other clinical samples of 85 infected pregnant women with diabetes, by growing swab lots on Sabouraud's Dextrose Agar (SDA) plates, the slide culture technique and the VITEK-2 automated system.
Results: Of 85 patients, 122 isolates in SDA culture were determined as 7 Candida sp. with number of isolates of each species, as follows: 47 *C. albicans*, 9 *C. famata*, 11 *C. glabrata*, 13 *C. guilliermondii*, 8 *C. krusei*, 3 *C. parapsilosis* and 37 *C. tropicalis* from vaginal swabs. From 60 vaginal swabs, 46 urine samples and 12 throat swabs it was seen that C. albicans was most prevalent. However, withVITEK-2, 201 fungal strains were identified; Candida sp. was isolated in all samples: 59 *C. albicans*, 19 *C. famata*, 21 *C. glabrata*, 23 *C. guilliermondii*,18 *C. krusei*, 13 *C. parapsilosis* and 48 *C. tropicalis*.
Conclusions: The most prevalent species among the isolated fungi was *C. albicans*, causing VC in diabetic pregnant women.

Keywords: Candidiasis, *C. albicans*, Pregnant women, VITEK-2

INTRODUCTION

Vaginal infections occur by many types of pathogens such as, yeast or other fungi, bacteria (bacterial vaginosis) and the protozoan *Trichomonas vaginalis* (trichomoniasis vaginitis). Thrush or candidiasis caused by the fungus, *Candida* sp. is seen in several areas of the body, mouth as thrush, vagina and the digestive tract as gastroenteritis.[1] Common causatives of vaginal candidiasis (VC) or vaginitis are *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. krusei*, *C. pseudotropicalis*, *C. tropicalis*, and *C. dubliniensis.*[2] VC is marked with a white discharge resembling cottage cheese, soreness, dyspareunia with irritation from itching, unpleasant odour and burning with urination; at different ages, it is experienced by about 75% women at least once in lifetime.[3,4] It is a frequent companion of pregnancy, which gets complicated jeopardizing health of mother and child.[5,6] Women with gestational diabetes are more likely to develop VC and vulvo-vaginal candidiasis (VVC), because of elevated sugar levels encouraging overgrowth of fungi.[7,8] Furthermore, *Candida* sp. are

normally present as minor constituents in microbial flora at soft body parts and groin, but the outgrowth of a species disrupts the balance of microorganisms in the genital area and invades the vaginal tract, causing the disease. Identifications of *Candida* sp. of infected pregnant women with diabetes were evaluated by slide culture technique (SCT) with Sabouraud's Dextrose Agar (SDA) and in parallel by a VITEK-2 automated system.

It is anticipated that this study would help the designing of suitable antifungal therapy in a typical teaching hospital in a sub-tropical zone, as fungal infections cause staggering diseases basically and several complications are triggered at pregnancy stage; certain antifungal are contraindicated at pregnancy.[9,10] This study gives has a comparative account of a tedious, traditional and inexpensive method, the slide culture technique with the advanced, accurate and expensive method of diagnosis, the VITEK-*2* automated system. Obviously, later is the gold standard method and the first method has inherent errors in arriving at the correctness of identification of fungi up to species level.

METHODS

In one year from July 2015, at the outpatient department of O and G Department of the hospital,85 pregnant women presented with the complain of white discharge with an unpleasant odour and burning sensation while urination. Prima facie, they reported having diabetes, and the diabetic affliction criterion was ascertained by random blood sugar level higher than 110 mg/dl. Clinical samples as vaginal and throat swabs as well as, urine samples with corollary patient information were collected. Clinical samples were grown on plates with *SDA* medium (Hi-media) for fungi and were identified with the SCT.[9] Additionally, morphological fungal criteria were ascertained with germ tube, glucose agar, sugar fermentation and sugar assimilation tests.[11]

Formats of VITEK-2 are focused in clinical microbiology laboratories for automation and specificity in identification, quickly even for larger sample sizes. The used reagent cards had 64 wells with individual test substrates for each sample. An optically clear film was present on both sides of the card, which allowed for the appropriate level of oxygen transmission while maintaining a sealed vessel preventing contact with the organism-substrate admixtures during growth of fungal strains from clinical samples. Each card had a pre-inserted transfer tube suitable for inoculation. Cards had bar codes with pertinent information on product type, lot number and a unique identifier that can be linked to the sample either before or after loading the card onto the system.[12]

RESULTS

Of the total 125 women, 85 were diabetic and the rest 40 were non-diabetic pregnant women. By surveillance the diabetic women were mostly infected with fungi. Of 85 diabetic pregnant women, 122 isolates in SDA culture of *Candida* sp. as 7 species were determined with number of isolates of each species, as follows: 47 *C. albicans,* 9 *C. famata*, 11 *C. glabrata*, 13 *C. guilliermondii,* 8 *C. krusei*, 3 *C. parapsilosis* and 37 *C. tropicalis* from vaginal swabs or clinical. Fungal infections were identified in 60 vaginal swabs, 46 urine samples and 12 throat swabs. However, with VITEK-2, 201 fungal strains were identified; *Candida* sp. was isolated in all samples, as 59, *C. albicans*, 19 *C. famata*, 21 *C. glabrata*, 23 *C. guilliermondii,* 18 *C. krusei*, 13 *C. parapsilosis* and 48 *C. tropicalis* were identified (Figure 1).

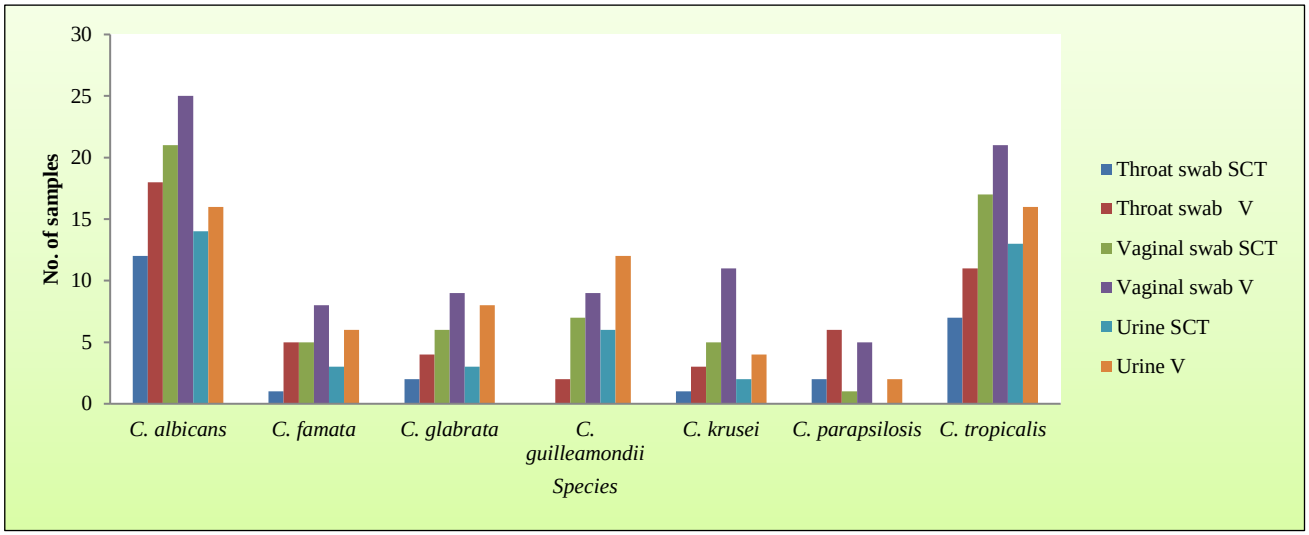

Figure 1: Frequency of *Candida* sp. isolated from diabetic pregnant women by conventional method from three types of clinical samples, throat swabs, vaginal swabs and urine, identified with comparison to slide culture technique (labelled as SCT) and automated VITEK-2 system (labelled as V).

DISCUSSION

During pregnancy, vagina is more sensitive and more often picks up opportunistic infections. Moreover, the high incidence of vaginitis in pregnant women is related to levels of estrogens, which is considered as the primary factor for infection; and VVC was more prevalent with gestational diabetic women.[13] Increased glucose levels in genital tissues enhance yeast adhesion and growth, which often in pregnant women with whom may not have started gestational diabetes, even. Thus, the prevalence of *Candida* infection is higher in diabetic pregnant women than healthy pregnant women, because of the increased levels of glucose, favouring the growth of *Candida* sp.[14] Vaginal epithelial cells bind to *Candida* with greater propensity in diabetic patients as there are increased numbers of intermediate vaginal epithelial cells in diabetic pregnant women compared to non-pregnant women; this enhances the adherence of *C. albicans.*[15] Thus in diabetic pregnant women, the rate of infection is higher in comparison to non-diabetic pregnant women, which explains the highest prevalence of *Candida* sp. cultured from three different body sites as observed in present study. Indeed, underlying mechanisms of pregnancy induced *Candida* colonization are complex. During pregnancy, levels of both progesterone and estrogen hormones are elevated. Progesterone has the suppressive effect on the anti-*Candida* activity of neutrophils, while estrogen level had been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *C. albicans;* and estrogen decreases immunoglobins in vaginal secretions resulting in increased vulnerability of pregnant women to VC.[6]

Furthermore, the efficacy of the VITEK-2 system and the conventional method for identification of *Candida* sp. after culturing of clinical samples were compared herein. The VITEK-2 system is routinely regarded as the more efficient method over the conventional culture method in the identification of microbes. However, the involved hardware with the VITEK-2 system does have stored data of unlimited microbes up to species level ordinarily, which can only be identified. In the VITEK system, substrates measure several metabolic activities such as, acidification, alkalinization, enzyme hydrolysis and growth in the presence of inhibitory substances, such as antibiotics/antifungals. There are currently 4 reagent cards available for the identification of different organism classes as follows: GN - Gram-negative fermenting and non-fermenting bacilli, GP - Gram-positive cocci and non-spore-forming bacilli, YST - yeasts and fungi, BCL - Gram-positive spore-forming bacilli, limiting the arena of microbial identifications.[12] Thus, this system has the limitation in identification of microbes.

CONCLUSION

In this study too, the *Candida* carriage rate was higher in diabetic pregnant women than non-diabetic pregnant women. Thus, as expected, the automated VITEK system was more efficient over the conventional method for the fungus *Candida* for species identification, with results in a shorter period of time as well as, specificity. A high occurrence of *Candida* sp. among diabetic pregnant women was recorded with *C. albicans* as the most prevalent species among the isolates. There was a high prevalence of VC among pregnant women.

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