Vol 5|Issue 4| 2015 |345-349.

International Journal
of
Pharmacy Review & Research
www.ijprr.com

e-ISSN: 2248 – 9207
Print ISSN: 2248 – 9193

OCCURRENCE AND ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDURIA ISOLATES AMONG DIABETIC PATIENTS

R. Ponmudi, C. Uma*, P. Sivagurunathan, and S. Aruljothi

Division of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu, India.

ABSTRACT

Diabetic patients are considered as the most vulnerable group for various infections; high level of sugar in blood and urine invites most pathogen to reside within the body and thus cause infection. Urinary Tract Infection (UTI) in diabetic patients if not dealt with caution will result in morbidity due to kidney failure. In the present study, the prevalence of *Candida* species as the causative agent for UTI and antifungal susceptibility pattern of the *Candida* isolates were assessed. About 24% of significant candiduria was observed from urine samples collected from diabetic patients. *Candida* isolates were identified up to species level by using conventional methods. Among the isolates obtained, *Candida albicans* was predominant (57%), followed by *C. glabrata* (28.6%) and *C. tropicalis* (14.2%). All the isolates were sensitive to all the antifungal agents used in the study.

Key words: *Candida albicans, C. glabrata, C. tropicalis,* UTI.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome characterized by an inappropriate elevation of blood glucose as a result of relative or absolute lack of insulin. It has a long term effect on genito urinary system and diabetics are more prone to UTI, particularly to upper UTI [1]. The clinical manifestation of UTIs depend on the portion of the urinary tract involved, the etiologic organisms, the severity of the infection and the patient's ability to mount an immune response to it [2]. It has been estimated that globally symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments and 100,000 hospitalizations annually [3].

UTIs are a frequent problem worldwide which are caused by microbial invasion to different tissues of the urinary tract [4,5]. Fungal infection of the kidneys and urinary tract occur most commonly as part of systemic fungal infections in patients with underlying immunodeficiency, focal UTI obstructive lesions, or as a result of indwelling catheters. Majority of fungal infection of the kidney and bladder result from *Candida albicans* followed by *C. glabrata* and *C. tropicalis*, antibiotic therapy, diabetes, urinary tract pathology and malignancy have been considered. They also remain the major cause of death among these patients.

There has been increase in the prevalence of *Candida* species causing urinary tract infections in diabetic patients. The application of urinary tract drainage devices can also trigger the infection [6]. Candiduria refers to the presence of *Candida* species in urine. It is an increasingly common finding in hospitalized patients [7,8]. *Candida* growth in urine is enhanced when urinary levels of glucose exceed 150 mg/dl. The candiduria is a marker for hematogenous seeding in the kidney. Candiduria most likely reflects colonization or infection of the lower urinary tract or the collecting systems of the kidneys. Most patients with candiduria are asymptomatic, and the yeasts merely represent colonization [9]. Infected patients may have dysuria, frequency and suprapubic discomfort, while others have no symptoms. The clinical characteristics of fungal kidney infections depend on whether the disease is present acutely or insidiously. Acute infection of the kidney microabscesses in the cortex and medulla [9,10].

The data pertaining to fungal infection of the UT with special reference to diabetic cases is very scarce; hence the present investigation was performed to evaluate the prevalence of candiduria among diabetic patients and also to study the antifungal sensitivity of the *Candida* isolates.

*Corresponding Author **C. Uma** E mail: umasaravanan1@gmail.com

METERIALS AND METHODS

Patient Inclusion Criteria

The study was conducted at the laboratory of the Division of Microbiology, Faculty of Science between December 2013 and March 2014. Both male and female diabetic patients with complaints of UTI were included in the present study.

Collection of Samples

Each diabetic patient was provided with sterile capped, wide–mouth container of 20 ml capacity and instructed about the Mid–Stream Urine (MSU) collection by following aseptic procedure. All the samples were immediately transferred to laboratory and processed within 1 hr.

Preparation of Wet Mount

This test is used to selectively screen the candiduria urine samples. A drop of urine was placed on a clean glass slide, and then a cover-slip was placed carefully as to prevent air-bubble formation. The slide was observed under microscope to detect the presence of pus cells, red blood cells, bacteria, yeast and fungal elements. The samples that showed positive for yeast cells under microscopic observation alone were taken for further analysis.

CULTURING OF SPECIMENS

Culturing onto SDA

About 0.1 ml of eligible urine sample was swabbed on the surface of chloramphenicol supplemented SDA agar, and then the inoculated plates were incubated at 37^0C for 24–72 hours. After incubation, the plates were examined for the presence of typical yeast colonies. The samples that yielded 10^4 CFU/ml were considered as significant for candiduria cases.

IDENTIFICATION METHODS

Cultural Characteristics

Culturing onto HiChrom agar

The moist, creamy white colonies on SDA plates were transferred to HiChrom agar media to determine speciation of *Candida* isolates. The inoculated plates were incubated aerobically at 37^0C for 24–72 hrs. Based on the colour of the colony the species differentiation was done.

MORPHOLOGICAL CHARACTERISTICS

Gram staining

The Candida isolates were subjected to gram's staining as per method described by Duguid (1996) [11].

Germ tube test

This test is used to differentiate *C. albicans* from other yeast species. The yeast culture was suspended in 1 ml of sterile human serum and then incubated at 37^0C for 2–3 hrs. A small drop of this mixture was placed on a slide with lactophenol cotton blue and observed under the microscope.

Chlamydospore formation test

For the test, corn meal agar was inoculated with the yeast culture and incubated at 20^0C for 24–48 hrs. The presence of chlamydospores were examined under microscope.

Sugar Fermentation Test

The fermentation media was prepared by adding 2% of different sugars (Sucrose, glucose, maltose, galactose and lactose) with bremocresol blue as an indicator. Durham's tubes were placed in an inverted position to the fermentation broth to detect the liberation of gases. The change in colour from purple to yellow, collection of air bubbles in Durham's tubes indicate acid and gas production respectively.

Sugar Assimilation Test

The ability of the isolates to grow in various sugar containing media was tested. The yeast isolates were inoculated onto carbohydrate assimilation media added with 2% of different sugars viz., Sucrose, glucose, maltose, lactose, trehalose and raffinose then incubated at 30^0C for 2–4 days.

Antifungal Susceptibility Test

The susceptibility of the *Candida* isolates towards antifungal antibiotics was performed by disc diffusion method. For the test, SDA plates were prepared and the yeast cultures were swabbed over the surface of the plates. The antifungal antibiotic disc like Flucytosine, Amphotericin B, Ketaconazole, Voriconazole, Fluconazole and Nystatin were placed at equal distance and then the plates were incubated at 37^0C for 24–48 hrs. The zone of inhibition formed after incubation was measured in millimeter and results were interpreted by comparing the values with standard chart.

RESULTS AND DISCUSSION

Diabetics are particularly prone to fungal UTI [12,13]. Diabetic females have higher vaginal and periurethral *Candida* colonization rates. Diabetics are at risk because of impairment of the phagocytic and fungicidal activity of neutrophils associated with insulin deficiency; however, the dominant predisposing factors to candiduria are increased instrumentation, urinary stasis, and obstruction to autonomic neuropathy [14].

UTI is the most common type of nosocomial infection [15] and 10–15% of UTIs are caused by *Candida* species [16]. The surveillance data from the U.S. National Nosocomial Infections Surveillance system (NNIS) reported *C. albicans* to be the fourth most common pathogen in UTI. Although the majority of infections are caused by *C. albicans, C. glabrata* is emerging as a nosocomial pathogen with a predilection for the urinary tract [17]. Urinary catheters have been held responsible as a cause for 80% of hospital urinary tract infections [12].

Majority of the fungal UTIs are caused by *Candida* species; hence the present study was under taken to highlight the presence of *Candida* in urine and possibility of infection of UTI among diabetic individuals.

Candiduria brings morbidity and mortality if they are not properly diagnosed and treated [18]. In our present investigation, about 58 urine samples were collected from diabetic patients suffering from signs of UTI. Out of 58 samples, 33 (57%) obtained from women and 25 (43.1%) from male patients. The significant candiduria were observed in 14 (24.1%) urine samples (Table 1). Eckstein and Kass (1982) [19] pointed out that the presence of yeast in urine is the clue for the diagnosis. In our study, 14 samples gave positive results for yeast cells in urine. In patients without indwelling catheters, but proven renal infection, colony counts as low as 10^4 CFU/ml [20] were found. The frequency of UT infection was high with diabetes that might be due to uncontrolled blood glucose level and subsequent presence of higher amounts of glucose in urine.

Yismaw *et al*. (2013) [21] studied the prevalence of candiduria in diabetic patients who were attending Gonder University Hospital, Ethiopia. The overall prevalence of significant candiduria in both symptomtic and asymptomatic diabetic patients were 8.3% (38 isolates of *Candida* obtained from 35 urine samples). The most common species were *C. albicans* (42.0%), *C. glabrata* (34.2%) and *C. tropicalis* (15.8%). Significant candiduria was strongly associated with female patients. Herein, 30.3% of the women and 16% men gave positive results for candiduria. These results are in agreement with Yismaw *et al*. (2013) [21]. Behzadi *et al*. (2010) [22] who conducted experiments about the UTIs associated with *C. albicans* and revealed that about 6.8% of patients infected with *C. albicans*. The remaining 93.2% of UTI were related to bacterial pathogens.

In the present study, 14 isolates were identified and confirmed to the species level by conducting various experiments like germ-tube test, chlamydospore formation, sugar assimilation and sugar fermentation test (Table 3). Presumptive diagnosis was done by culturing the isolates in HiChrom agar (Table 2). The medium contain chromogenic substrates which are cleaved by enzymes produced by certain *Candida* species [21].

Febre *et al*. (1999) [23] analyzed the incidence of nosocomial candiduria associated with indwelling urinary catheters and to assess microbiological characteristics of the yeast. The presence of yeast was observed in 18.6% of urine specimens from patients with indwelling catheters. Ahmadzadeh *et al*. (2011) [10] described a seven month old male infant infected with fungal UT candidiasis and the urine culture yielded more than $3x10^4$ *Candida albicans* and the fungi were sensitive to Fluconazole. Yashwant *et al*. (2013) determined about the prevalence of candiduria and antifungal susceptibility in a tertiary care hospital of Mangalore. A total of 66 (2.27%) *Candida* species were isolated from 2900 urine samples. Among them, non-*albicans Candida* species were predominant (69.7%) compared to *C. albicans* (30.3%). The *Candida* isolates were more susceptible to Amphotericin B (91%) and Flucytosine (82%) compared to Voriconazole (72.72%) and Fluconazole (66.66%). Our results are contradictory with the findings of Yashwant *et al*. (2013). Among the isolates obtained, *C. albicans* was predominate 57% followed by *C. glabrata* (28%) and *C. tropicalis*(14.2%) (Table 4). Other non *Candida albicans* also to some extent can causes UT infection in diabetic cases. Antifungal susceptibility testing of the yeast isolates was also done, where all the isolates *viz., C. albicans*, *C. glabrata* and *C.tropicalis* were sensitive to all the antifungal antibiotics used in the study (Table 5).

Table 1. List of significant candiduria samples

S.NO	Number of positive candiduria sample		Total[%]
	Female	Male	
1	10(30.3)	4(16%)	14(24.1%)

Table 2. Colony morphology of *Candida* isolates on HiChrom agar

S. no	Isolate no	Colony morphology
1	Us1	Light blue to green
2	Us2	Dark mauve
3	Us3	Light blue to green
4	Us4	Dark blue to metallic blue green
5	Us5	Light blue to green
6	Us6	Dark mauve
7	Us7	Light blue to green
8	Us8	Dark blue to metallic blue green
9	Us9	Light blue to green
10	Us10	Dark mauve
11	Us11	Dark mauve
12	Us12	Dark mauve
13	Us13	Light blue to green
14	Us14	Light blue to green

Table 3. Identification of *Candida* isolates

S. no	Name of the isolates	Gram staining	Germ tube test	Chlamydospore production	Sugar assimilation test						Sugar fermentation test				
					Glucose	Maltose	Lactose	Trehalose	Raffinose	Sucrose	Glucose	Maltose	Galactose	Sucrose	Lactose
1	Us1	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
2	Us2	+ve	_	+	+	+	_	+	_	_	+	_	_	_	_
3	Us3	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
4	Us4	+ve	_	+	+	+	_	+	_	+	+	+	+	+	_
5	Us5	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
6	Us6	+ve	_	+	+	+	_	+	_	_	+	_	_	_	_
7	Us7	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
8	Us8	+ve	_	+	+	+	_	+	_	+	+	+	+	+	_
9	Us9	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
10	Us10	+ve	_	+	+	+	_	+	_	_	+	_	_	_	_
11	Us11	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
12	Us12	+ve	_	+	+	+	_	+	_	_	+	_	_	_	_
13	Us13	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_
14	Us14	+ve	+	+	+	+	_	+	_	+	+	+	_	_	_

Us1, Us3, Us5, Us7, Us9, Us11, Us13, Us14}– *C.albicans*.
Us2, Us6, Us10, Us12}– *C. glabrata*.
Us4, Us8}– *C. tropicalis*.

Table 4. Occurrence of *Candida* species

S. no	*Candida* species	No. of isolates obtained	%
1	*C. albicans*	8	57
2	*C. glabrata*	4	28
3	*C. tropicalis*	2	14.2

Table 5 Antifungal susceptibility pattern of *Candida* isolates

S. no	Name of isolates	Fluconazole	Voriconazole	Flucystosine	Amphotericin B	Ketoconezole	Nystain
1	Us1	S	S	S	S	S	S
2	Us2	S	S	S	S	S	S
3	Us3	S	S	S	S	S	S
4	Us4	S	S	S	S	S	S
5	Us5	S	S	S	S	S	S
6	Us6	S	S	S	S	S	S
7	Us7	S	S	S	S	S	S
8	Us8	S	S	S	S	S	S
9	Us9	S	S	S	S	S	S
10	Us10	S	S	S	S	S	S
11	Us11	S	S	S	S	S	S
12	Us12	S	S	S	S	S	S
13	Us13	S	S	S	S	S	S
14	Us14	S	S	S	S	S	S

Us1, Us3, Us5, Us7, Us9, Us11, Us13, Us14}– *C. albicans*.
Us2, Us6, Us10, Us12}– *C. glabrata*.
Us4, Us8}– *C. tropicalis*.

CONCLUSION

In recent years there has been increasing trends in *Candida* infection of UT among diabetic patients because of higher glucose levels in the urine. The fungal infection of UT becoming dominant as like bacterial infection but it is unnoticed most of the times due to lack of awareness and diagnostic problem associated with it. Many researchers and physicians have little look over the fungal infections involving UT. Due to less attention to fungal UTI, the research about the same is quite essential to overcome the diseases and consequences due to this critical pathogen. These alarming results clearly point out that surveillance about fungal UTI is a must among diabetic cases; therefore the 'opt' treatment should be set up at the earliest to save the life of this sensitive group.

REFERENCES

1. Patterson JE, Andriole VT. Bacterial urinary tract infection in diabetics. *Infect Dis Clin North Am*, 3, 1997, 735–750.

2. Foxman B, Brown P. Epidemiology of urinary tract infection: transmission and risk factors, incidence and costs. *Infect Dis Clin North Am*, 17(2), 2003, 227–241.
3. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. *Clin Infect Dis*, 38, 2004, 150–158.
4. Andrade SS, Sader HS, Jones RN. Increased resistance to first line agents among bacterial pathogens isolated from urinary tract infection in Latin America; Time for local guidelines? *Mem1 Oswaldo Cruz*, 101, 2006, 741–748.
5. Obiogbolu CH, Okonko IO, Anyamere CO. Incidence of Urinary Tract infection (UTIs) among pregnant women in Akwa metropolis, Southeastern Nigeria. *Sci Res Essay*, 4, 2009, 820–824.
6. Kauffman CA, Bradley SF, Ross SC, Weber DR. Hepatosplenic candidiasis: successful treatment with fluconazole. *Am J Med*, 91, 1991, 137–141.
7. Belazi M, Velegraki A, Fleva A, Candidal overgrowth in diabetic patients: potential predisposing factors, Mycoses, 48(3), 2005, 192–196.
8. Wise J, Fungal infections of the urinary tract, In Walsh PC, Retik AB, Stamey TA, Vaughan ED, Jr, editors, Campbell's Urology, 6th ed. Philadelphia: Saunder, 1992, 928–950.
9. Herberg J, Pahari A, Walters S, Levin M. Infectious diseases and the kidney, In: Avener ED, Harmon WE, Niaudet P Yoshikawa N, editors, 6th ed., Berlin: Springer-Verag, 2009, 1267–1268.
10. Ahmadzadeh AR, Valavi E, Shamsizadeh A, Zarei Mahmoudabadi, A., Hydari M, Ahmadzadeh A. Fungal urinary tract infection in an infant with posterior urethral valves. *Jundishapur J Microbiol*, 4 (1), 2011, 71–76.
11. Duguid JP, Staining methods, In: Collee, JG, Fraser AG, Marmion BP, Simmons A, editors, Mackie & McCartney Practical Medical Microbiology, 14th ed. Edinburgh: Churchill Livingstone, 1996, 793–812.
12. Kauffman CA, Vazquez JA, Sobel JD, Prospective multicenter surveillance study of funguria in hospitalized patients, The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group, Clin Infect Dis, 30, 2000, 14–18.
13. Platt R, Polk BT. Murdock B, Risk factors for nosocomial urinary tract infection. *Am J Epidemiol*, 124,1986, 977.
14. Sobel JD, Vazquez JA, Fungal infections of the urinary tract. *World J Urol*, 17(6), 1999, 410–414.
15. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Med*, 1991, 72S–75S.
16. Amer FA, Mohtady HA, el-Behedy IM, Khalil S, el- Hendy YA, el- Gindy EA, Salem HE. Bacteria of nosocomial urinary tract infections at a university hospital in Egypt: identification and associated risk factors. *Infect. Control Hosp. Epidemiol*, 25, 2004, 895–897.
17. Harris AD, Castro J, Sheppard DC, Carmeli Y, Samore MH. Risk factors for nosocomial candiduria due to *Candida glabrata* and *Candida albicans*. *Clin Infect Dis*, 29, 1999, 926–928.
18. Manjunath GN, Prakash R, Vamseedhar A, Kiran S. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore. *Int J Biol Med Res*, 2(2), 2011, 504–507.
19. Eckstein CW, Kass EJ, Anuria in a newborn secondary to bilateral ureteropelvic fungus balls, J Uro, 127, 1982, 109–110.
20. Wise GJ, Goldberg P, Kozinn PJ, Genitourinary candidasis, Diagnosis and treatment, J Urol, 116, 1976, 778–780.
21. Yismaw G, Asrat D, Woldeamanuel Y, Unakal C. Prevalence of candiduria in Diabetic patients attending Gondar University Hospital, Gondar, Ethiopia. *Iranin J Kidney Diseases*, **7**(2), 2013.
22. Behzadi P, Behzadi E, Yazdanbod H, *et al*. A survey on urinary tract infections associated with the three most common uropathogenic bacteria. *Maedica*, 5, 2010, 111–115.
23. Febre N, Silva V, Medeiros EA, Wey SB, Colombo AL, and Fischman O. Microbiological characteristics of yeasts isolated from urinary tracts of intensive care unit patients undergoing urinary catheterization. *J Clin Microbiol*, 37(5), 1999, 1584–1586.
24. Yashwant K, Shivani S, Anshu S, Kavaratty RM. Antibiogram and characterization of resistance markers among *Escherichia coli* isolates from urinary tract infections. *J Infect Dev Ctries*, **7**(7), 2013, 513–519.