Species identification and antifungal susceptibility of Candida isolated from urine specimens in a tertiary care hospital

Anju Sebastian[1,*], Archana S[2]

[1]Assistant Professor, [2]Lecturer, Dept. of Microbiology, Government Medical College, Ernakulam, Kochi, Kerala, India

***Corresponding Author:**
Email: anjusunoj1@gmail.com

Abstract

Introduction and Objectives: Urinary tract infections caused by Candida have become frequent as a result of increased use of broad spectrum antibiotics, corticosteroids, immunosuppressive agents, bladder catheters and increasing cases of diabetes mellitus. The morbidity and mortality caused by non-albicans Candida (NAC) species are increasing. A prospective study was done to study the incidence of non-albicans Candida in patients with urinary tract infection.

Materials and Methods: The speciation of Candida isolated from urine samples of patients was done using conventional yeast identification protocols and chrome agar. Antifungal susceptibility testing was done by the disc diffusion method to amphotericin B, nystatin and azoles.

Results: Candiduria was diagnosed in 1.3% of patients with urinary tract infection. Six species of Candida were isolated, which include C.albicans (36.8%), C. tropicalis (22.9%), C. krusei (13.6%), C. parapsilosis (13.6%), C. guilliermondii (5.2%) and C. kefyr (7.9%). All Candida albicans isolates were sensitive to Amphotericin B and nystatin. Ketoconazole was the next effective drug (75%), followed by clotrimazole (71.42%), fluconazole (70.6%) and itraconazole (62.5%). Amongst the non-albicans Candida species, Nystatin was the most sensitive drug (89%), followed by Amphotericin B (64%), fluconazole (62.2%), itraconazole (58.8%), clotrimazole (58.8%) and ketoconazole (47%).

Conclusion: Urine samples yielded more of non-albicans Candida. Among the non-albicans Candida, Candida tropicalis was the predominant isolate. Antifungal resistance was more in non-albicans Candida than in Candida albicans. Hence there is need for speciation and susceptibility testing of Candida species to optimize the selection of antifungal agents to provide the best possible patient care.

Keyword: Candiduria, antifungal susceptibility testing, Candida speciation, Chrome agar, urinary tract infection, Non albicans Candida.

Introduction

Fungal diseases have gained clinical importance mainly due to advances in medical technologies and interventional procedures. The emergence of Human immunodeficiency (HIV) virus has also increased the incidence of fungal diseases.

Candida is an yeast like fungus and Candida albicans was considered as the major species associated with disease. It mainly causes secondary infections in patients who are immunosuppressed and very rarely a primary disease. Extensive use of antimicrobial agents and chemotherapeutic agents used in cancer therapy has worsened the situation. The certainty of yeasts being the causation of infection includes isolation of yeasts from blood and other sterile body fluids, isolation from patients who are immunosuppressed and repeated isolation from multiple clinical samples.

The incidence of urinary tract infections caused by Candida have increased as a result of prolonged and indiscriminate use of broad spectrum antibiotics, steroids, immunosuppressive agents, use of indwelling catheters and increasing cases of diabetes mellitus.[1]

In the 1980s, over 80% of Candida isolated in the laboratory was Candida albicans. More recently, the morbidity and mortality caused by Candida species other than C. albicans are increasing.[1] The clinical manifestation caused by non-albicans Candida (NAC) species is indistinguishable from those caused by C. albicans but they differ in their susceptibility to antifungal agents.[2] Inappropriate empirical therapy has contributed to the emergence of drug-resistant Candida species, which poses a major challenge for the clinicians. Hence, it is important that yeasts isolated from clinical specimens should be identified up to the species level. Antifungal susceptibility testing has also become increasingly important in order to optimize the selection of antifungal agents so as to provide the best possible patient care.

In this study, the incidence of Candida albicans and non- albicans Candida in patients with urinary tract infection were evaluated. Candida isolated from urine was speciated using conventional biochemical tests. We also evaluated the performance of commercially available chromogenic Candida speciation media, chrome agar. Antifungal susceptibility of the isolates was determined.

Materials and Methods

A prospective study was done in the Microbiology Laboratory of a tertiary care hospital from January to October 2017. 38 isolates of Candida from patients with significant urine counts were included. Contamination was differentiated from infection by obtaining a second urine sample. Only when the second specimen showed the growth of Candida, further mycological workup was done.

The specimens were processed for the isolation of Candida species as per standard yeast identification protocols. Gram staining was done from the specimen. Urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) medium and incubated at 37°C for 24-48 hours. Germ tube test was done from the colonies. Sugar fermentation and sugar assimilation test was performed on the isolates for species identification. Simultaneously the Candida species were inoculated on chrome agar medium and incubated at 37°C for 24-48 hours and the species were identified by type and colour of the colonies as per manufacturer's instructions.

Sugar Assimilation Test: A tube of **s**aline containing the yeast isolated from the clinical specimen was incubated at room temperature for about 24 hours to exhaust the carbohydrate reserves. Yeast extract agar was prepared. A lawn culture of the pre-incubated broth was made on the Yeast extract agar plate. Discs of 6mm diameter were punched out from what man no. 1 filter paper and sterilized in hot air oven. The discs were applied onto the lawn culture of the isolate and a drop of 20% sugar solution was added to each disc. The plates were incubated for 24-72 hours. The sugars tested were glucose, lactose, sucrose, galactose, maltose, melibiose, dulcitol, raffinose, cellobiose and trehalose.

Sugar Fermentation Test: The isolates were incubated overnight in nutrient broth. The suspension was then inoculated into individual sugar tubes and incubated at 37°C for 10 days. The sugars used for the study were dextrose, lactose, sucrose, galactose and maltose.

Antifungal Susceptibility Testing: Antifungal susceptibility of all the isolates were performed by the Kirby Bauer disc diffusion method on Mueller-Hinton agar supplemented with 2% glucose and 0.5 ug/ml methylene blue dye. Inoculum was prepared in 5 ml of normal saline to match the turbidity with 0.5 Mc Farland standard. Lawn culture of inoculum was made on Mueller Hinton agar and then antifungal susceptibility of the isolates for fluconazole, ketoconazole, itraconazole, clotrimazole, amphotericin B and nystatin was done. These plates were incubated at 37°C for 24 hours. The diameters of the inhibition zone were measured and interpreted. C. albicans (ATCC 90028) was used as the control strain.

Results

In the present study, candiduria was diagnosed in 1.3% of the patients with urinary tract infection. Candiduria was more common in females (73.7%). Urinary catheterization followed by the use of broad spectrum antibiotics and diabetes mellitus were the major risk factors in these patients.

Among 38 Candida species recovered from urine, 14 (36.8%) were Candida albicans and the rest (63.2%) were Candida species other than Candida albicans. Among the non-albicans Candida, C. tropicalis (22.9%) was the commonest followed by C. krusei and C. parapsilosis (13.6%). The other species isolated were C. guilliermondii (5.2%) and C. kefyr (7.9%).

A mixed growth of two Candida species, C.albicans and C.tropicalis was obtained from one urine sample. There was an agreement in identification using conventional biochemical tests and by chrome agar method in all strains.

All the Candida albicans isolated were sensitive to Amphotericin B and Nystatin. Ketoconazole was the next effective drug (75%), followed by clotrimazole (71.42%), fluconazole (70.6%) and itraconazole (62.5%). Amongst the non-albicans Candida species, nystatin was the most sensitive drug (89%), followed by Amphotericin B (64%). Sensitivity to azoles for non- albicans Candida was fluconazole (62.2%), itraconazole (58.8%), clotrimazole (58.8%) and ketoconazole (47%). Among the non-albicans Candida species, the highest level of resistance to antifungal agents was shown by C guilliermondii.

Discussion

It has been reported that 11 to 52% of nosocomial urinary tract infections are caused by Candida spp.[3-5] Candida species is the fifth most common nosocomial urinary pathogen in India.[6] Candiduria is seen in association with disseminated candidiasis, diabetes mellitus, pregnancy, long term use of broad spectrum antibiotics and following the use of catheters. The risk to develop candiduria increases by twelve fold after urinary catheterization, six fold after the use of broad spectrum antibiotics and urinary tract abnormalities, four fold following abdominal surgeries, two fold in the presence of diabetes mellitus and one fold in association with corticosteroid administration.[7] Some clinicians consider the presence of Candida species in urine samples as harmless colonization. However, candiduria is an important risk factor for invasive candidiasis which results in morbidity and mortality. Hence, species level identification of Candida and their antifungal susceptibility testing is necessary.

In the recent years, isolation of non- albicans Candida from clinical samples are on the rise and these are more resistant to antifungal agents as compared to C.albicans. Therefore, identification of Candida to species level has a direct impact on choice of empirical antifungal treatment. Also there may be place to place variation in the species isolated which makes it important that we have data on the distribution of Candida species in different geographic regions. In a study conducted by Falagar et al, the highest proportion of Candida albicans was found in North and Central Europe and USA. Non- albicans species were more common in South America, Asia and South Europe. C.glabrata was commonly isolated in USA and North and Central Europe, C. parapsilosis in South America, South Europe and South Asia.[8] In most studies conducted in various parts of India, Candida albicans

continues to be the predominant species isolated. Candida tropicalis has been reported to be the most predominant species among the non-albicans.[9] In our study also, C. tropicalis was the most commonly isolated non-albicans species. In a study conducted by Sumitra Devi et al, Candida albicans (52%) was the predominant isolate followed by Candida tropicalis (25%), C. krusei (17%) and C. glabrata (7%).[10] In another study conducted by Roopa et al, 50.7% isolates were C. albicans, 28.6% were C.tropicals, C.krusei (13.9%), C.glabrata (5.8%), C. guilliermondii (0.7%).[11]

For differentiation between species of Candida, the conventional methods available such as Germ tube test, chlamydospore formation, sugar fermentation and assimilation tests are laborious and time consuming. Chrome agar is a rapid method for detection and identification of Candida from mixed culture and provides results in 24-48 hours. The use of chrome agar facilitates speciation of Candida even in resource poor settings.

Disc diffusion method was employed for antibiotic sensitivity testing, as it is simple and hence can be used for preliminary screening. But to confirm resistance of any strain, broth dilution test should be used. The antifungal susceptibility pattern showed non-albicans Candida are more resistant to antifungals compared to C.albicans. The susceptibility pattern in most Indian studies show a high susceptibility to Amphotericin B and Nystatin for Candida isolates and azoles show more drug resistance.[12,13] The extended prophylactic use of azoles like fluconazole would be a probable reason for high resistance to azoles.[13]

Conclusion

Candiduria is an important nosocomial infection. The shift towards non-albicans Candida as the causative agent of urinary tract infections is of utmost concern since NAC species are more resistant to antifungal agents compared to C. albicans. Therefore species identification of Candida isolated from clinical samples along with their antifungal susceptibility pattern can help in the proper treatment and management of candiduria.

References

1. Jose NV, Mudhigeti N, Asir J, Chandrakesan SD. Detection of virulence factors and phenotypic characterization of Candida isolates from clinical specimens. *J Curr Res Sci Med*. 2015;1(1):27-31.
2. Deorukhkar SC, Saini S. Laboratory approach for diagnosis of candidiasis through ages. *Int J Curr Microbiol App Sci*. 2014;3(1):206-18.
3. Richards MJ, Edwards JR, Culver DH. Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Control Hosp Epidemiol. 2000 Aug;21:510-5.
4. Brindha S, Jayashree M, Singhi S. Study of nosocomial urinary tract infections in a paediatric intensive care unit. *J Trop Pediatr*. 2011;57:357-62.
5. Rivett A, Perry J, Cohen J. Urinary candidiasis: a prospective study in hospital patients. Urol Res 1986;14:183-6.
6. Prasad KN, Agarwal J, Dixit AK, Tiwari DP, Dhole TN, Ayyagiri A. Role of yeasts as nosocomial pathogens and their susceptibility to Fluconazole and Amphotericin. *Ind J of Med Res*. 1999;110:11-17.
7. Bukhary ZA. Candiduria: A review of clinical significance and management. *Saudi J Kidney Dis Transpl*. 2008;19:350–60.
8. Falagar ME, Roussos N, Vardakar KZ. Relative frequency of albicans and various non-albicans Candida species among candidemia isolates from inpatients in various parts of the world. *Int J of Inf Dis*. Nov 2010;14(11):954-66.
9. Yesudhason BL, Mohanram K. Candida tropicalis as a predominant isolate from clinical specimens and its antifungal susceptibility pattern in a tertiary care hospital in Southern India. *J Clin Diagn Res*. 2015 Jul;9(7):14-16.
10. Sumitra Devi L, Maheshwari M. Speciation of Candida species isolated from clinical specimens by using chrome agar and conventional methods. *Int J of Sci Res Pub*. Mar 2014;4(3).
11. Roopa C, Biradar S. Roopa C, Biradar S. Isolation of *Candida* and its speciation in various samples in a tertiary care hospital in North Karnataka, India. *Int. J. Curr. Microbiol.* App. Sci. 2015;4(9):996-1000.
12. Saranya S, Moorthy K, Malar SS, Punitha T, Vinodhini R, Bhuvaneswari M. Prevalence and antifungal susceptibility pattern of Candida albicans from low socioeconomic group. *Int J Pharm Pharm Sci*. 2014;6(2):158-62.
13. Roy RC, Sharma GD, Bruman SR, Chanda S. Trend of Candida infection and antifungal resistance in a tertiary care hospital of North East India. Afr. *J. Microbiol. Res;* Jun 2013;7(29):3112-16.