ORIGINAL ARTICLE

Microbiology Section

The incidence of **Candiduria** in an **ICU –** A study

SEEMA BOSE, ATINDRA KRISHNA GHOSH, REKHA BARAPATRE

ABSTRACT

Due to the increased use of indwelling drainage devices, the incidence of candiduria has been increasing steadily. Candida isolates from the urine of catheterised patients were identified by the germ tube test, by checking for chlamydospore formation on corn meal agar and by the sugar fermentation test. Simultaneously, the identification of the candida isolates was performed by using CHROM agar candida medium and the Hi candida identification kit. Out of 59 urethral catheterized patients, 21 (35.59%) were positive for the growth of yeasts. Candida albicans was the commonest isolate, followed by Candida dubliniensis, Candida glabrata, Candida tropicalis and Candida parapsilosis. The rate of the infection was directly proportional to the number of days during which the catheter was present in a patient.

Key Words: Candiduria, Indwelling catheter, Non albicans candida, CHROM agar

INTRODUCTION

Candiduria is defined as the presence of yeast cells in urine. Due to the increased use of indwelling devices, the incidence of candiduria has been increasing dramatically. The property of adhesion of microorganisms has long been considered as a virulence factor, causing catheters and indwelling medical device associated infections. Colonization and device biofilm formation may occur within 3 days of catheterization [1].

With short term catheterization (upto 7 days), 10- 50% patients develop infections, whereas in long term catheterization (>28 days), usually all patients develop urinary tract infection. The risk of catheter associated infections increases by approximately 10% for each day [2].

The microorganisms are introduced into the urethra while (a) the catheter is inserted and (b) through the sheath of the exudates surrounding the catheter, or (c) they travel intraluminally from the tube or the collection bag. Besides Candida albicans, the incidence of candiduria which is caused by nonalbicans candida has been increasing steadily. It is also necessary to identify the isolates of candida upto the species level, as some of them have an innate resistance to antifungals [3].

This study was undertaken to find out the incidence of candiduria in the catheterized patients of an ICU of a rural tertiary care hospital which is situated in Maharashtra and for the identification of the candida isolates upto the species level by using various phenotypic methods, because it was necessary for timely antifungal therapy.

MATERIALS AND METHODS

The duration of the study period was six months. 59 urine samples from urethral catheterized patients were processed in the microbiology laboratory. Direct microscopy was done from all the samples. Culturing was done on blood agar and MacConkey's agar and these plates were incubated at 37^{o}C for 24 hours. Two Sabouraud's dextrose agar slants were inoculated and incubated at 37^{o}C and 30^{o}C for 7 days. The isolates were identified as candida species by Gram's staining [4].

Candida albicans was identified by the germ tube test, by checking for chlamydospore formation on cornmeal agar and by the sugar fermentation test [5].

Simultaneously, the identification of the candida isolates was performed by using Hi CHROM candida agar medium and the Hi Candida identification kit which was obtained from Hi Media Pvt Ltd, India.

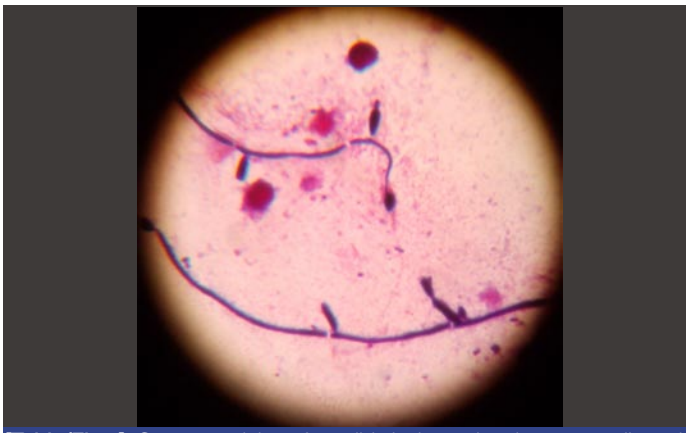
The CHROM agar medium was prepared as per the manufacturer's instructions and all the 21 candida isolates were inoculated separately. The inoculated petri dishes were incubated at 30^{o}C for 48 hours. All the 21 candida isolates were also tested with a Hi Candida identification system. The strips which were provided with the kit were inoculated as per the manufacturer's instructions and these were incubated at 22^{o}C for 48 hours.

Additional tests for the identification of Candida dubliniensis were done by subculturing Candida albicans and Candida dubliniensis on Sabouraud's dextrose agar slants and by incubating the slants at 45^{o}C for 48 to 72 hours [6].

ATCC 10231 Candida albicans was included in this study as a control strain.

RESULTS

All the 21 candida isolates were initially identified by Gram's staining [Table/Fig 1].



[Table/Fig 1]: Gram_s staining of candida isolates showing yeast cells and pseudohyphae

In our study, the rate of infection was directly proportional to the number of days during which the catheter was present in the patient [Table/Fig 2].

Duration in days	No. of isolates	Candida albicans	Candida dubliniensis	Candida glabarata	Candida tropicalis	Candida parapsilosis
<3	3	2	-	-	-	1
3–7	7	4	2	1	-	-
>7	11	6	2	1	2	-
Total	21	12	4	2	2	1
Percentage (%)		57.14	19.04	9.52	9.52	4.76

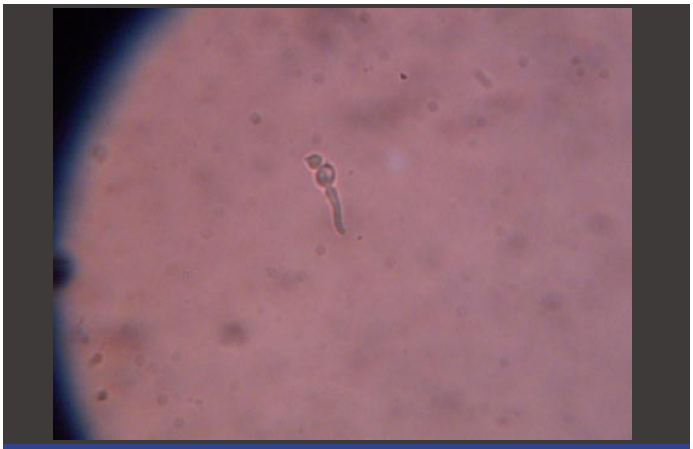
[Table/Fig 2]: Number of various candida species isolates from urine samples in relation to duration of catheter in place (n=21).

The number of the various candida species which were isolated from the urine samples of catheterized patients who were admitted to the ICU, were as follows: 12(57.14%) Candida albicans, 4(19.04%) Candida dubliniensis, 2(9.52%) Candida glabrata, 2(9.52%) Candida tropicalis and 1(4.76%) Candida parapsilosis. The total number of candida isolates was 21(35.59%) [Table/Fig 2].
Out of the 21 candida isolates, 9(42.85%) were non albicans candida [Table/Fig 3].

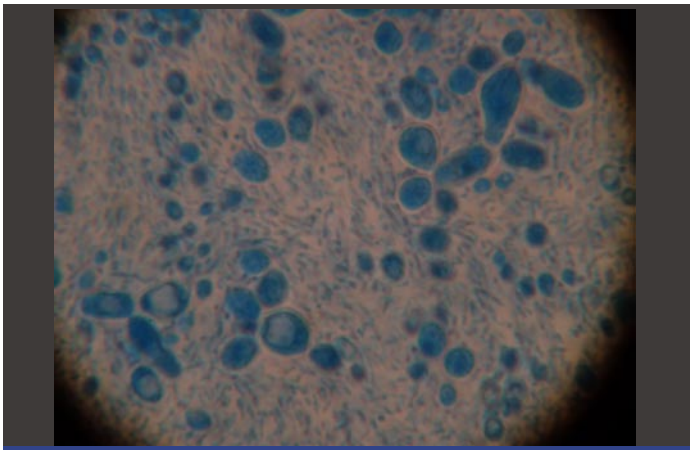
	Candida albicans	Nonalbicans Candida
Number	12	9
Percentage	57.14	42.85

[Table/Fig 3]: Incidence of Candida albicans and non-albicans candida from urine samples of catheterized patients (n = 21).

Candida albicans was identified by the germ tube test [Table/Fig 4] the chlamydospore formation test [Table/Fig 5] and the sugar fermentation test.



[Table/Fig 4]: Germ tube test, showing yeast cells with germ tubes.

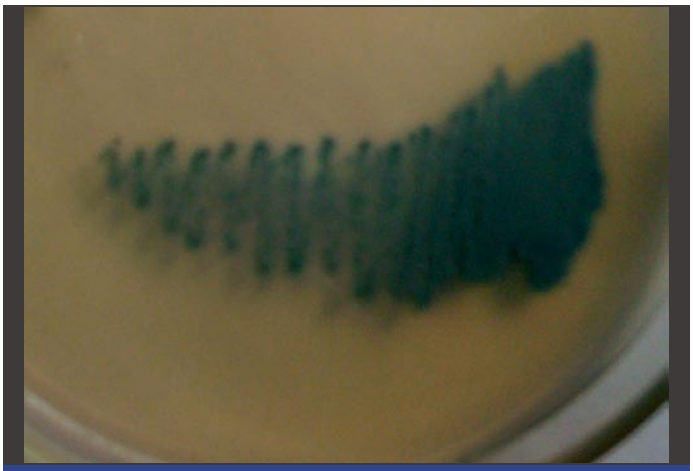


[Table/Fig 5]: Chlamydospores formation of Candida albicans

On the Hi CHROM candida agar medium, Candida albicans formed light green colonies [Table/Fig 6] Candida glabrata formed pink coloured colonies [Table/Fig 7] Candida dubliniensis showed dark green, fuzzy growth [Table/Fig 8] Candida tropicalis formed blue coloured colonies [Table/Fig 9] and Candida parapsilosis formed cream coloured colonies [7] [Table/Fig 10].

[Table/Fig 6]: Growth of Candida albicans (light green) on CHROM agar

[Table/Fig 7]: Growth of Candida glabrata (pink) on CHROM agar



[Table/Fig 8]: Growth of Candida dubliniensis (dark green) on CHROM agar

[Table/Fig 9]: Growth of Candida tropicalis (blue) on CHROM agar

[Table/Fig 10]: Growth of Candida parapsilosis (cream) on CHROM agar

A mixed growth of Candida isolates were identified as Candida albicans and Candida dubliniensis and these were seen as light green and dark green colonies [Table/Fig 11].

[Table/Fig 11]: Mixed growth of Candida albicans and Candida dubliniensis on CHROM agar

All the 21 candida isolates were correctly identified by the Hi Candida identification kit [Table/Fig 12].

[Table/Fig 12]: Hi candida identification kit, showing reactions of Candida glabrata

DISCUSSION

With the use of indwelling medical devices in the ICU, a significant rise in the incidence of Candida albicans and nonalbicans candida infections were reported from various regions [8] Kojic EM et al [9] reported that medical device induced infections contributed about half of all the nosocomial infections and that 10% of such infections were due to the candida species.

The gold standard for yeast identification is the use of molecular diagnostic techniques. However, these sophisticated techniques are expensive and require technical expertise [10].

The identification of the candida species by the conventional methods requires 3 to 5 days or even longer [11].

The routine identification of candida upto the species level depends upon easy to perform, rapid screening methods. In our study, out of 59 clinical samples, 21(35.59%) showed the growth of candida species. The total number of Candida albicans infections was 12(57.14%), followed by 4(19.04%) of Candida dubliniensis, 2(9.52%) of Candida glabrata, 2(9.52%) of Candida tropicalis and 1(4.76%) of Candida parapsilosis.

Out of the 21 candida isolates, 9(42.85%) were non albicans candida. [Table/Fig 3] In our study , the highest number of nonalbicans candida infections were caused by Candida dubliniensis (19.04%). Price MF et al [12] observed that other than Candida albicans, Can- dida tropicalis, Candida krusei and Candida glabrata were the ma- jor isolates which were found in most of the institutions.

Candida dubliniensis and Candida albicans share many morphological and physiological characteristics. This close similarity may cause the misidentification of the isolates of Candida dubliniensis as Candida albicans [5].

The CHROM agar candida medium contains enzymatic substrates which are linked to chromogenic substances which react with different enzymes that are produced by the candida species, thus leading to colour variation in the colonies [13].

Two of our Candida dubliniensis isolates did not show dark green colour on this medium, which were later confirmed by no growth at 45ºC [14].

In one sample, there was a mixed growth of Candida albicans and Candida dubliniensis. On CHROM agar, it was very easy to identify them, as they formed colonies of different colours [Table/Fig 11]. Kathrin et al [15] found that the CHROM agar candida was insufficient for detecting Candida dubliniensis. However, Bernal S et al [16] found this medium to be quite useful for the identification of the Candida species.

The Hi Candida identification kit is a standardized colourimetric identification system, utilizing 12 conventional biochemical tests. The tests are based on the principles of pH change and substrate utilization. In our study, all the candida isolates were correctly identified by this system. P Umabala et al [17] used the fungichrom system for yeast identification, which was also based on the assimilation of carbohydrates and the hydrolysis of chromogenic substrates. She reported it to be quite satisfactory.

CONCLUSION

With the use of indwelling medical devices, candida infections are on the rise. The infection rate is directly proportional to the number of days during which the catheter was present in the patient. Along with Candida albicans, non albicans candida species are also playing a major role in hospital acquired infections. Hi CHROM candida agar is a very useful medium for the identification of mixed candidal infections.

The Hi Candida identification system is a fairly accurate method for the identification of medically important candida species. It is necessary to identify the candida isolates upto the species level, as some of them have innate resistance to the commonly used antifungals.

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