ORIGINAL ARTICLE

COMPARISON OF TWO METHODS OR DETECTION OF SECRETED ASPARTYL PROTEINASE IN URINARY ISOLATES OF CANDIDA SPECIES

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ABSTRACT

Context: Secreted aspartyl proteinase (SAP) is considered to be an important virulence factor in the Candida species. Its production in candidiuria patients is not well studied. Recently there has been increased incidence of urinary tract infection due to Candida species.

Aim: In our study, we have compared the performance of two media for detection of secreted aspartyl proteinase i.e. bovine serum agar (BSA) and bovine haemoglobin agar BHA).

Methods and Material: **F**rom January 2009 to January 2010 a total of 150 urine samples, which were positive for budding yeast cells on gram stain, were included in the study. Bovine serum albumins (Himedia), bovine hemoglobin agar (Himedia) are used as a sole source of nitrogen. Bovine haemoglobin agar and Bovine serum albumin is prepared. Yeast cells freshly grown on saborouds dextrose agar for 48 hours were used for inoculation. After inoculation on both BSA and BHA, they are incubated for 7 days at 370 C. But zone of clarification is looked for every 2, 4, 6 and 7 day and noted diameter of clarification.

Results: Out of 150 candida species isolated from clinical urine samples 86.6% were C. tropicalis, followed by C. albicans (11.3%), C. glabrata (1.33%) and C. dubliniensis (0.6%). SAP detection using Bovine serum agar was 29.3% where as using bovine haemoglobin agar was 18.6%.

Conclusions: So we conclude that the bovine serum agar is far superior to bovine haemoglobin agar for detection of SAP.

Key words: Candida Species, SAP, Bovine serum agar, Bovine haemoglobin agar, virulence factor.

INTRODUCTION

Over the last two decades, there has been increased in the number of infection caused by Candida species that can be easily attributed to the increased use of antibiotics and catheters.[1] Even though clinical significance of candiduria is unclear, it accounts for 10-15% of urinary tract infections.[2] Although many factors have been suggested to be virulence attributes for C. albicans such as hyphal formation, surface recognition molecules, phenotypic switching etc., extracellular hydrolytic enzyme production was considered to be the most important one.[3] Extracellular hydrolytic enzyme secreted by many fungi primarily function to provide nutrients for the cells. Where as pathogenic yeast cells have adapted this property to fulfil the specialized function during the infective process in the host.[4] Limited data exist in relevance to the role of SAP in urinary isolates of candida species more so in case of non C. albicans. There have been studies documenting the presence of family of genes (eg.sap1 sapt1 and sapp genes) for the secretion of secreted aspartyl proteinase in C. albicans, C. tropicalis and C. parapsilosis.[5,6] Information on the extracellular proteolysis of other medically important Candida species (C. guilliermondii, C. stellatoidea, C. glabrata, C. krusei, C. kefyr) is limited[7,8].It has been also documented that deletion of SAP gene has resulted in attenuated virulence in candida species[9]. In developing countries like India molecular technique still remains at large to research laboratories, so there is a need for simple tool for use in day today laboratory for identifying SAP production.

As the virulence attributes of SAP is already proved beyond doubt by many studies,[3, 4, 9, 10] aim of the present study was to determine SAP production in urinary isolates of candida species and to compare two media for better laboratory diagnosis.

SUBJECTS AND METHODS

Source of clinical specimen and isolates: A pilot study was conducted in a tertiary care teaching hospital from January 2009 to January 2010. A total of 150 urine

samples, which were positive for budding yeast cells on gram stain, were included in the study. These samples were received in our diagnostic microbiology laboratory.

Ethical clearance was obtained from the hospital ethical committee.

Materials: Bovine serum albumins (Himedia), bovine hemoglobin agar (Himedia) are used as a sole source of nitrogen. Agar (difco), yeast extract agar (sigma).

Medium: Bovine haemoglobin agar is prepared as described by Staib.[11] Test plates for the assessment of SAP production contained agar (Himedia Ltd.), yeast carbon base (YCB; Himedia), bovine hemoglobin (Sigma), and bromophenol blue (Lachema). The plates were prepared as follows: the agar and YCB were suspended in water, so that the final concentrations were 4.5 and 1.2%, respectively. After sterilization, the suspension was cooled to 55°C and maintained at that temperature with a water bath. Dissolved hemoglobin was filter sterilized and added to the agar-YCB up to a final concentration of 0.08%. Then, bromophenol blue was added to a concentration of 0.02 ppm in the medium (the stock solution consisted of 1.6 g of bromophenol blue/100 ml of 50% ethanol). The suspension was mixed, and its pH was adjusted to 4 or 4.5. A steam-sterilizable pH electrode was used for the pH measurements. Then, the suspension was immediately poured into the plates. Each 90-mm test plate contained approximately 17 ml of the suspension.

Bovine serum albumin is prepared by adding yeast carbon base [himedia], 1.17%; yeast extract [Himedia], 0.01%; BSA [BDH], 0.2%, as a measure of Sap activity. The medium was adjusted to pH 5, sterilized by filtration, and added to the stock solution of autoclaved (2%) agar. Sap activity was scored as follows: when no visible clarification of the agar around the colony was present, 1+ when a visible clear zone (1 to 2 mm in diameter) was observed, and 2+ when agar clarification largely exceeded (by 3 to 5 mm) the margin of colony

Method: yeast cells freshly grown on saborouds dextrose agar for 48 hours were used for inoculation. After inoculation on both BSA and BHA, they are incubated for 7 days at 370 C. But zone of clarification is looked for every 2, 4, 6 and 7 day and noted diameter of clarification.

RESULTS

Out of the 150 Candida species isolated from clinical urine samples, 83.3% were C. tropicalis followed by C.albicans (11.3%), C. glabrata (1.3%) and C. dubliniensis (0.6%). Our study showed a high rate of detection of SAP (29.3%) production by BSA agar compared to BHA agar (18.6%). In present study, even though candida tropicalis showed high number SAP producing strains it is clearly attributed to the high rate of isolation of C. tropicalis from patient sample. Where as C. albicans (88.23%) still remains predominant SAP producing strain in comparision to C. tropicalis (21.54% BSA & 8.46% BHA)

Table 1: SAP detection results of 150 urinary isolates of *Candida* using Bovine serum albumin agar

	Zone size (mm)	*C.tropicalis* (n=130)	*C.albicans* (n=17)	**C.glabrata* (*n*=2)	*C. dublinensis* (n =1)	Total
Highly proteolytic (2+)	3-5	11 (8.46%)	11 (64.7%)		1	23 (15.3%)
Appreciable proteolysis (1+)	1-2	17 (13.08%)	4 (23.53%)			21 (14%)
Non proteolysis (-)	≤1	102 (78.46%)	2 (11.76%)	2 (100%)		106 (70.7%)

* Used as negative control as C. glabrata is not known to produce SAP, n= total no. of strains isolated

Table 2: SAP detection results of 150 urinary isolates of *Candida* Using Bovine hemoglobin agar

	Zone size (mm)	*C.tropicalis* (n=130)	*C.albicans* (n=17)	**C.glabrata* (*n*=2)	*C. dublinensis* (n =1)	Total
Highly proteolytic (2+)	3-5	5 (3.85%)	11 (64.7%)	-	1 (100%)	17 (11.3%)
Appreciable proteolysis (1+)	1-2	6 (4.61%)	4 (23.53%)	-	-	11 (7.3%)
Non proteolysis	≤1	119 (91.54%)	2 (11.76%)	2 (100%)	-	122 (81.3%)

* Used as negative control as C. glabrata is not known to produce SAP, n= total no. of strains isolated

DISCUSSION

In this study we have found the emergence of non C. albicans as a major cause of Candiduria but when compared for the production of SAP as virulence marker, C. albicans still stands out as major pathogen. Other Indian studies have also documented a change in the trend from C. albicans to non-C. albicans [12,13]. Even though there are number of publication showing the presence of genes responsible for SAP production, our study was concentrated on demonstration of simple method for detection of SAP in urinary isolates of candida species.[14, 4] SAP production has been demonstrated by many methods like by demonstration of SAP gene [9,4], spectrophotometric analysis SAP [15], in vivo demonstration of SAP production[16]. But there are no much studies to demonstrate the SAP detection by BSA and BHA and also no comparison studies to see which one is more efficacious.

CONCLUSION

In our study we have successfully compared the BSA and BHA agar for detection of SAP and BSA proved to be more sensitive than BHA. In present era of increased prevalence of candidurea due many immunosuppressing conditions, BSA can act as simple screening tool for detection of SAP. Thus we conclude saying that further studies are required with SAP inhibitors to improve the overall sensitivity and specificity of the test.

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