IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 5 Ser. 7 (May. 2019), PP 57-60
www.iosrjournals.org

Hypertonic Xylose Agar Medium – A Novel Medium to Differentiate *Candida dubliniensis*from*Candida albicans*

Dr.LahariCh [1], Dr RajyalakshmiGunti[2]*, Dr.K.R.L.SuryaKirani[3]
[1](Post graduate, Department of Microbiology,Rangaraya medical college, Kakinada, Andhra Pradesh, India)[2](Associate Professor, Department of Microbiology,Rangaraya medical college, Kakinada, Andhra Pradesh, India)[3](Professor and HOD, Department of Microbiology, Rangaraya medical college, Kakinada, Andhra Pradesh, India)
Corresponding Author:Dr RajyalakshmiGunti

Abstract:*Candida albicans is an opportunistic pathogen which causes candidiasis and candidemia. Other non-albicans Candida species also cause these. All Non-albicans except C.dubliniensis can be differentiated by Germ tube test. C.dubliniensis shares many phenotypic similarities with C. albicans including Germ tube test leading to misidentification. Several identification techniques have been developed for differentiation.The present study was undertaken to evaluate the use of in house hypertonic xylose agar medium to differentiate between C. albicans and C. dubliniensis in a clinical setting. Around 124 samples received in the dept. of microbiology showed Candida isolation. Candida isolates were grown on Sabourauds Dextrose agar, to which Germ tube testing was carried out. Positive germ tube isolates were inoculated onto Hypertonic Xylose agar medium and incubated for 48 hrs at 28^0C. Positive germ tube isolates were also tested for sensitivity towards Fluconazole(25µg), Ketoconazole(10µg) and Miconazole(30µg)(Hi media discs) using Muller Hilton agar supplemented with glucose and Methylene blue. Of all 124 Candida isolates, 50 were Germ tube positive and in these 42(84%) were identified as Candida albicans and 8(16%) as Candida dubliniensis. 97% of all Candida albicans isolates and 85% of all Candida dubliniensis were sensitive to Fluconazole and Ketoconazole. Around 95% of all isolates were resistant to Miconazole. Despite other phenotypic methods, this method is cheaper. It can be observed that HXAM is phenotypic method and can be used in average Mycological settings.*

Key words:*Candida albicans,Candida dubliniensis, Germ tube, Hypertonic Xylose Agar Medium, Phenotypic differentiation*

Date of Submission: 03-05-2019 Date of acceptance: 17-05-2019

I. Introduction

Fungal infections kill more than 1.5 million people and affect more than billion people across the world. Incidence of Candidal infections is 2 to 21 per one lakh population depending on the country(1).Approximately 50% of Candidal infections are caused by *Candida albicans*(2).*Candida dubliniensis*was discovered in 1995 at Dublin, Ireland.*C. dubliniensis* have been overlooked and misidentified for a long time. This is because *C. dubliniensis* and its close relative *Candida albicans* share many features in common such as microscopic morphology and ability to form germ tubes in serum, production of blasto-conidia with pseudo-hyphae, true hyphae and chlamydospores used routinely by average clinical mycology laboratory to identify *C. albicans*. Of all *Candida albicans*identified, 4-10% is identified as*Candida dubliniensis* and 1in 10 to 20 cases could be misidentified.

Many phenotypic-based tests have been developed to differentiate*C.albicans* from *C.dubliniensis* like the absence of growth at 42°C and 45°C, characteristic rough-looking colonies and chlamydospores on STAIBagar, initial dark green colonies on CHROMagar Candida, non-fluorescent colonies on methyl-blue Sabouraud agar, turquoise smooth colonies on Candida ID agar, colony morphology on other differential media such as niger seed agar, simplified sunflower seed agar or tobacco agar, etc. In various publications, variable success has been reported and all have been insufficient alone for accurate differentiation of the two species.

To circumvent these difficulties, various molecular methods have been developed, polymerase chain reaction (PCR)(3) being the 'gold standard'. However, these tests are not readily applicable in most average mycology laboratories. So the concept of a reliable routine isolation medium that could be used instead of PCR in differentiation of the two species remains attractive.A novel medium, Hypertonic Xylose agar medium(HXAM) was developed by Abiroo Jan *et al* which was found equally sensitive and specific as PCR in differentiation of *C.dubliniensis* from *C.albicans*.(4)So In the Present study, we made an attempt to identify these Candida species using this medium in our area.

II. Material And Methods

This study was conducted in the Department of Microbiology of Government general hospital, Rangaraya medical college, Kakinada, Andhra Pradesh, India.

2.1. Processing of samples:

Around 124 Candida isolates from different samples were sub-cultured on Sabourauds dextrose agar and Germ tube testing was done.

2.2. Germ tube Testing:

Each isolate was inoculated in 0.5 ml serum and incubated at 37^0C and checked for Germ tube formation after one and half hour incubation.These Germ tube Positives were considered as Test strains.

2.3. Preparation of Hypertonic Xylose agar medium:

HXAM was prepared as follows.Composition of medium per litre includes Xylose – 10 g Sodium chloride – 65g Agar – 20g, Distilled water – 900ml. Medium is sterilized by Autoclaving for 15 min and cooled to 50^oC and yeast nitrogen base(6.8 g/lit) solution is prepared and added and mixed. Final pH adjusted to 6.3+/- 0.2. The medium is maintained at 25^oC.

2.4.Processing of Test strains:

Germ tube positive isolates were inoculated onto HXAM and growth observed after 48 hours of incubation at 28^oC. The *Candida* isolates that showed growth on this medium within 48 hours were considered as *C.albicans* and those which did not show any growth even after 7 days incubation were considered as *C. dubliniensis.*

Figure 1 : Hypertonic Xylose Agar Medium showing Growth of *Candida albicans* and not showing any growth of *Candida dubliniensis*

2.5.Antifungal susceptibility testing:

According to CLSI – M44 A2 guidelines for yeasts

- Media used: Mueller-Hinton agar + 2% Glucose and 0.5µg/ml Methylene blue dye medium. It is prepared and autoclaved and after cooling pH is maintained at 7.2 to 7.4
- Inoculum was prepared using 5 distinct colonies of yeast from 24 hour old culture and 5ml of 0.85% sterile physiological saline and its turbidity is adjusted to 0.5 McFarland standards (suspension of 1million to 5 million cells per ml)
- After streaking of inoculum onto the sterile dried test plates, Antifungal sensitivity pattern of *C. albicans*and *C. dubliniensis* was tested by disc diffusion technique using the drugs Fluconazole 25µg, Ketoconazole 10µg and Miconazole 30µg per disc concentration

III. Results

Among 124 Candida spp., 50 isolates showed Germ tube production and 74 strains did not. The Germ tube positive isolates were inoculated on Hypertonic Xylose Agar Medium for differentiation.

Table -1 Growth on Hypertonic Xylose Agar Medium(n = 50)

Growth	No.	%
Positive	42	84%
Negative	8	16%

Out of 50 study group, 42 (84%) candida isolates showed growth on HXAM at 48hrs of incubation at 28^oC.Remaining 8(16%) isolates didn't show any growth even after 7 days of incubation at 28^oC.

Table-2 Antifungal Susceptibility patterns of both Candida species.

Species	Fluconazole			Ketoconazole			Miconazole	
	S	I	R	S	I	R	S	R
C.albicans n=42	40	0	2	28	12	2	0	42
C.dubliniensis n=8nn=8	6	0	2	5	2	1	0	8

S- Sensitive, I - Intermediate and R - resistant

Both Candida species showed similar type of sensitivity pattern.

IV. Discussion

This study was taken mainly to differentiate *Candida albicans* and *Candida dubliniensis* using simple conventional method.

4.1 Xylose assimilation:

*C.albicans*assimilates xylose while *C. dubliniensis*does not.Gales *et al.*(5)Pincus*et al*(6)Loreto *et al.* have used methods like Vitek, API20C AUX, ID 32 C, Micronault‑Candida and showed variable results of differentiation. Using conventional Xylose disc assimilation method, Khan et al(7)observed that *C. dubliniensis*did not showed any assimilation and along with it Candida glabrata also showed similar results and declared that xylose assimilation should be done for Germ tube positive Candida only. Some false positives are also observed for *C. dubliniensis*organisms by Pincus*et al.*(6)So, using only xylose assimilation will not be sufficient for differentiation

4.2 Salt tolerance:

The regulation of specific stress genes have been differentiated between *C. albicans*and *C. dubliniensis.ENA21* gene, encodes sodium ion transporter, is strongly induced in *C. albicans*but not in *C. dubliniensis*(8). Alves et al(9)Chowdary et al(10)used Hypertonic Sabouraud Broth ,liquid medium to check for salt tolerance of the strains but any of the above didn't show clear cut results and they were not easily interpretable. Siveria-Gomes et al(11)observed false positives in this broth method also. Cassone et al. used agar based medium like YEPD agar in which salt is added which also didn't show any clear cut results. Later Akgül and Cerikçioğlu(12) used Modified Hypertonic Sabouraud dextrose agar which showed good results.

By viewing the results of these reactions separately , Abiroo Jan et al combined the properties of both reactions in single method and devised a novel medium 'HXAM' by using xylose into a carbon‑free hypertonic solid medium and found that all *C. albicans*showed visible growth on this medium at 48 h of incubation while none of the *C. dubliniensis*show.

Advantages:

- Both Xylose assimilation and salt tolerance tested with single medium
- Accuracy is more
- Reduced cost
- Reduced time and man power

Disadvantages:

- *C.glabrata* may be mistaken for *C.dubliniensis* for not showing any growth – over-come by considering Germ tube positives only.

Table 3- Comparison with Previous Study

Abiroo Jan et al.	**Present Study**
Sample size – 200	Sample size – 50
C. albicans – 186 (93%)	C. albicans - 42(84%)
C. dubliniensis – 14 (7%)	C. dubliniensis - 8 (16%)

The present study results were comparable to the results of Abiroo Jan et al.

V. Conclusion

Hypertonic Xylose agar medium is a simple, inexpensive in-house medium and is easy to prepare to differentiate *C.albicans* from*C.dubliniensis.*As overall isolation of *Candida dubliniensis*is very low in my study and other studies also, expensive and time consuming method like PCR-RFLP is not needed. This simple medium can be used in average mycology laboratories.

VI. References

[1]. Felix BongominSGROOaDWD. Global and Multi-National Prevalence of Fungal diseases- estimate precision. journal of fungi. 2017 september;(10.3390/jof3040057).
[2]. J. C. O. Sardi LSTBAMFA. Candida species: current epidemiology,pathogenicity, biofilm formation, natural antifungal. Journal of Medical Microbiology. 2013; 62(10.1099/jmm.0.045054-0).
[3]. Jan A BGFBFALMRS. MolecularIdentification of Candida dubliniensis among Candida albicans isolated from oral cavity of cancer patients using PCR RFLP in a tertiary care hospital in Kashmir, India. British microbiology research journal. 2016; 14(1-7).
[4]. Abiroo Jan GBBAFDAUKMMAKSRMS. Hypertonic Xylose Agar Medium: A Novel Medium for Differentiation of Candida dubliniensis from Candida albicans. Indian journal of medical microbiology. 2017 october - december; 35(518-21).
[5]. Gales AC PMHAJSSDCDa. Identification of Candida dubliniensis based on temperature and utilization of xylose and alpha methyl D glucoside as determined with the API 20C AUX and vitek YBC systems. Journal of clinical microbiology. 1999; 37(3804-8).
[6]. Pincus DH CDPWPASIGMa. Rapid identification of Candida dubliniensis with commercial yeast identification systems. Journal of clinical microbiology. 1999; 37(3533-9).
[7]. Khan Z ASCRJL. A simple xylose based agar medium for the differentiation of Candida dubliniensis and Candida albicans. Diagnostic microiological infectious diseases. 2012; 72(285-7).
[8]. Enjalbert B MGVCYTMDJea. Genome wide gene expression profiling and a forward genetic screen show that differential expression of the sodium ion transporter Ena21 contributes to the differential tolerance of Candida albicans and Candida dubliniensis to osmotic stress. Molecular microbiology. 2009; 72(216-28).
[9]. Alves SH MEdLSPOLSJAea. Hypertonic Sabouraud broth as a simple and powerful test for Candida dubliniensis screening. Diagnostic microbiological infectious diseases. 2002; 45(85-6).
[10]. Chowdhary A RHKTKSRPMea. Application of hypertonic Sabouraud glucose agar fordifferentiation of Candida dubliniensis from Candida albicans. Diagnostic Microbiological infectious diseases. 2011; 69(440-2).
[11]. Silveira Gomes F SDEESNOTMSea. Differentiation between Candida albicans and Candida dubliniensis using hypertonic Sabouraud broth and tobacco agar. journal of Brazilian society of tropical medicine. 2011; 44(457-60).
[12]. Akgül O CN. Hypertonic Sabouraud dextrose agar as a substrate for differentiation of Candida dubliniensis. Mycopathologia. 2009; 167(357 9.).

Dr RajyalakshmiGunti. "Hypertonic Xylose Agar Medium – A Novel Medium to Differentiate Candida DubliniensisFromcandidaAlbicans." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 5, 2019, pp57-60.