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Research Article

Identification and Frequency of Causative Organism in Oral Candidiasis in Early Childhood in Bangladesh

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Abstract

Introduction: Opportunistic infections may be caused by the part of the normal flora in the oral cavity called Candidiasis. This may happen in different circumstances and might be a matter of serious concern by comprising the host immunity in addition to augmented resistance to antifungal drugs. The study was aimed at using various clinical samples for appraising utility of HiCrome Candida Differential Agar to differentiate Candida species isolated. This is to be done on the basis of coloration and colony morphology. The study was conducted with an aim to establish a preliminary picture of the different Candida species causing oral candidiasis of the children by defining the existence and the influencing circumstances of the needs of community-based treatment for oral candidiasis.

Methodology: The study was conducted over a period of two year (January 2018 to December 2019) by collecting 148 number of Candida isolates from oral mucosa and tongue. The samples were being collected from the Dental Unit at Rajshahi Medical College Hospital outdoor which was further examined at the Molecular Pathology Laboratory of the Institute of Biological Sciences, University of Rajshahi. The use of chromogenic agar medium (HiCrome Candida differential agar) speciated the Candida isolates.

Result: Total 148 respondents, 23 of them were less than 1 year of age, 112 of them were between 1-3 years and 13 of them were between 4-6 years of old. The isolation of Candida albicans 140 (94.5%) predominated over non albicans Candida. Non albicans Candida species isolated were Candida tropicalis 108 (72.9%) followed by Candida krusei 68 (45.9%) and Candida parapsilosis 36 (24.3%).

Conclusion: As a convenient and rapid method of identification of Candida species HiCrome Candida differential agar is considered. Candida albicans was the major species isolated in the conducted study and children aged 1-3 year are most susceptible to recurrent oral candidiasis in the study. In choosing the proper antifungal agents, the sensitivity profile of Candida isolates is to be helpful as it may decrease the morbidity and mortality of the patients.

Keywords: Oral Candidiasis; Candida Albicans; HiCrome Candida Differential Agar; Antifungal Susceptibility Test; Minimum Inhibitory Concentration

Introduction

During the last decades, the occurrence of fungal infections has been increasing and in developed countries it is spreading more [1]. Candida species, which are part of the normal flora in the oral cavity, may cause opportunistic infections under various circumstances that compromise host immunity [2,3]. It gets often connected with inclining factors attributed to the organism and these cause chronic infections [4]. Candida albicans is the main reason behind causing candidiasis, the most common paediatric oral infections. Some other species of Candida like Candida parapsilosis, Candida krusei and Candida tropicalis can become hostile enough to increase patient morbidity and mortality attributable to oropharyngeal or general diffusion.

Pseudomembranous candidiasis is another form of presenting candida infection. It is categorized by white pseudomembranous cover in the oral mucosa and upper digestive tract. Other than this, critical atrophic candidiasis is distinguished by ache and swelling of the mouth or tongue, chronic hyperplastic candidiasis is categorized by consistent white scratches on the oral mucosa or tongue and angular cheilitis is characterized by lesions in the angles of the mouth which usually accompanies intra-oral candidiasis [5].

An occurrence or distinctive feature that has been related to the increased rate of afterward occurring disease is referred as a predisposing factor. The differences are important to be made as predisposing features which are associated with a disease but not the reason behind causing it. Ageing is also a matter which is associated with periodontal disease due to its increased incidence. Diabetes and immune suppressive disease due to different causes are adjustable factor as we may control them but they cannot be cured. Common local systemic predisposing factors are changes in epithelium, bad oral hygiene status, reduced salivary flow, loose- or tight-fitting dentures, corticosteroid inhalers, high sugar diet and common systemic predisposing factors are disorders of hormone, extreme age, endocrine disorders (e.g., diabetes), immunosuppression, dry mouth, extended antibiotic therapies, nutritional deficiencies [4].

Oral examination in regular interval and maintaining oral and dental hygiene will avert most cases of oral candidiasis. That is why the patients are required to be made aware of oral hygiene measures. Brushing the teeth, washing the buccal cavity, tongue, palate and dentures are the common maintenance of oral hygiene. Besides the use of anti-Candida solutions for instance Chlorhexidine or Hexetidine can enter those areas where the brush cannot. Additionally, the dentures are required to be removed at night time to clean it deliberately and immersed it in an antiseptic solution like Chlorhexidine [5].

Oral candidiasis is recently increasing due to extreme age, denture use due to cleft palate, diabetes, systemic steroid and antibiotic use, pernicious anemia, malignancy, radiation therapy on the head and neck, and cell-mediated immunodeficiency [6].

The aim of this study was to isolate and identify etiological agents and determine the frequency of oral candidiasis in most vulnerable age group. So, it is important to increase awareness among the people about oral disease and its predisposing factor to control oral candidiasis among children. So that my study plays an important role to control and prevent oral disease among the people of Rajshahi in Bangladesh. This study may also play a significant role to diagnose and cure oral candidiasis all over the world.

Methodology

Test isolates

The target population selected through a stratified random cluster sampling method, included all out patients age 1 month to 6 years from Paediatric Department of Dental Unit at Rajshahi Medical College Hospital Outdoor, who were especially having their tongue affected with white pseudomembranous plaque or else other having noticeable oral candidiasis. The samples were examined at the Molecular Pathology Laboratory, Institute of Biological Sciences, University of Rajshahi.

As a part of quality control, some reference strains of Candida albicans ATCC 90028, Candida tropicalis ATCC 705, Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used. Himedia Laboratories Pvt. Limited, Mumbai, India was the institution from where all the culture media control strains were attained.

Identification of isolates

Enzymatic substrates are contained in it relating to chromogenic composites. At the time of the substrate is cleaved by a specific enzyme, color is produced by the chromogenic substances. Yeast species produce the action of different enzymes which results in color disparity. This variation of color is useful for probable identification of diversified Candida species. HiCrome Candida differential agar is speciated depending on the variation of color offering to a rapid, suitable and consistent method with a view

to identifying clinically significant Candida species compared to cumbersome traditional methods. As a simple phenotypic test is replacement of molecular based assay in developing countries, HiCrome Candida differential agar can be accepted.

HiCrome Candida differential agar media was obtained commercially from Hi Media, Mumbai, India. Swabs were initially streaked on HiCrome Candida differential agar and incubated at 37°C for up to 48 hours for optimal development of color. HiCrome Candida differential agar allows differentiation of Candida species on the basis of color and colony morphology. Isolates forming light green colonies were identified as Candida albicans, blue to metallic blue Candida tropicalis, pink/purple, fuzzy colonies Candida krusei and cream-colored colonies as Candida parapsilosis [7].

Candia species	Inoculum (CFU)	Color of colony on HiCrome agar media	Colony morphology on HiCrome agar media
Candida albicans	50-100	Light green	Flat or even colonies
Candida tropicalis	50-100	Blue to metallic blue	Elevated colonies
Candida krusei	50-100	Purple / Pink	Fuzzy colonies
Candida parapsilosis	50-100	Cream to white	Smooth or wrinkled colonies

Table 1: Color and colony morphology of different Candida species on HiCrome Candida differential agar media [7].

Candida albicans and Candida dubliniensis species differentiation

Candida albicans and Candida dubliniensis are differentiated by their colony color and colony morphology on HiCrome Candida disparity agar media. There are some noticeable differences exist between Candida albicans and Candida dubliniensis. Amongst these differences, their ecological niches are not alike. Both Candida albicans and Candida dubliniensis are unusual in the gastro-intestinal tract of healthy individuals but for the oral cavity. Next, in terms of pathogenicity, Candida albicans there are major differences which are more pathogenic [8].

Results

One hundred and forty-eight (148) samples were collected between 2018 and 2019 from patients of Dental Unit at Rajshahi Medical College Hospital outdoor located in Rajshahi metropolitan area in Bangladesh. All respondents were age from 1 month to 6 years. Four Candida species Candida albicans, Candida tropicalis and Candida krusei and Candida parapsilosis were isolated on HiCrom Candida differential agar media.

Age of the respondents in years	Frequency	Percentage
< 1 year	23	15.5
1-3 years	112	75.7
4-6 years	13	08.8
Total	148	100.0

Table 2: Distribution of respondents by their age.

Total 148 respondents,23 of them were less than 1 year of age, 112 of them were between 1-3 years and 13 of them were between 4-6 years of old.

Figure 1 showing 52 (35.1%) of the respondents were male and 96 (64.9%) of the respondents were female.

In 148 sample; 140 sample has Candida albicans with 100 scanty, 26 light, 10 moderate and 4 heavy growth of colony, 108 sample has Candida tropicalis with 80 scanty, 22 light, 4 moderate and 2 heavy growth of colony, 68 sample has Candida krusei with 40 scanty, 26 light, 2 moderate and none of them have heavy growth of colony, 36 sample has Candida parapsilopsis with 20 scanty, 14 light, 2 moderate and none of them have heavy growth of colony shown in table 3.

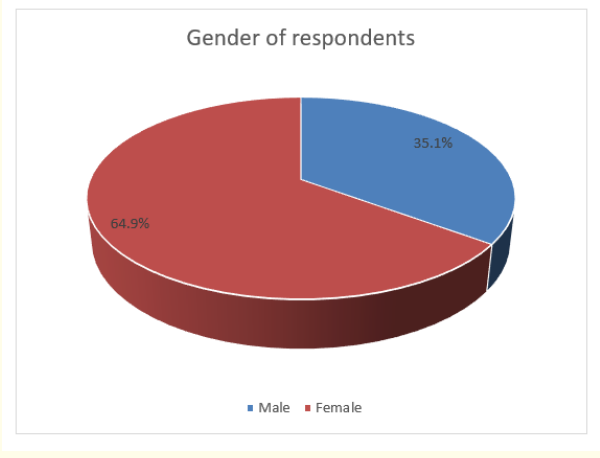

Figure 1: Distribution of respondents according to their gender.

Candida spp./Growth	Scanty (%)	Light (%)	Moderate (%)	Heavy (%)	Total = n, (%)
Candida albicans	100 (71.5%)	26 (18.5%)	10 (7.1%)	4 (2.9%)	140 (100%)
Candida tropicalis	80 (74.1%)	22 (20.3%)	4 (3.8%)	2 (1.8%)	108 (100%)
Candida krusei	40 (58.8%)	26 (38.3%)	2 (2.9%)	0 (0%)	68 (100%)
Candida parapsilopsis	20 (55.6%)	14 (38.9%)	2 (5.5%)	0 (0%)	36 (100%)

Table 3: Growth pattern results of all Candida species after the first inoculation.

Morphology of Candia species colony on selective/differential media

24-48 hours were taken at the temperature of 30˚C for incubation, isolates were injected onto HiCrome agar. Depending on the color and colony morphology, variation of Candida species specifically Candida albicans, Candida krusei, Candida tropicalis and Candida parapsilosis are allowed by HiCrome agar. Isolates forming light green colonies were identified as Candida albicans, blue to metallic blue Candida tropicalis, pink/purple, fuzzy colonies Candida krusei and cream-colored colonies as Candida parapsilosis (Figure 2).

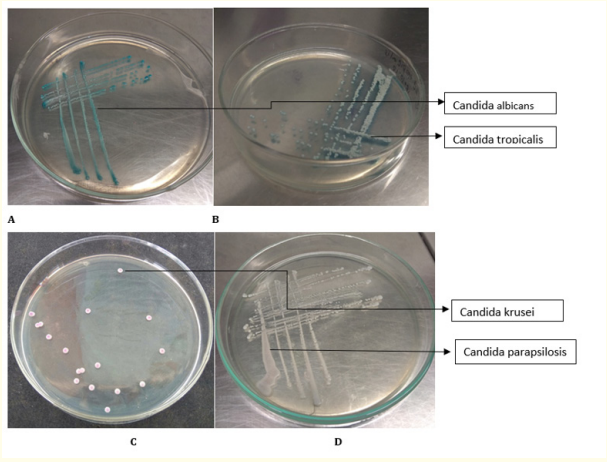

Figure 2: Colony morphology on HiCrome Candida differential agar media: A- Candida albicans, B – Candida tropicalis, C – Candida krusei, D – Candida parapsilosis.

Figure 3 shows in HiCrome Candida differential agar media Candida albicans show smooth light green to metallic green smooth colonies and Candida dubliniensis shows dark green to bluish green wrinkled colonies.

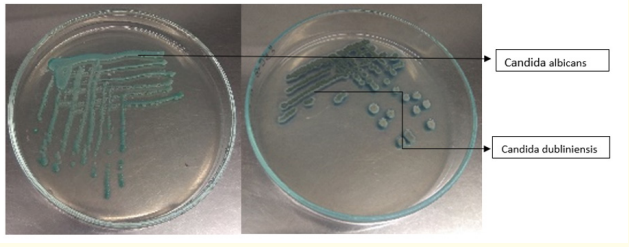

Figure 3: Color and morphology of colony of Candida albicans and Candida dubliniensis.

Distribution of Candida species recognized from clinical samples

One hundred and forty-eight (148) samples were collected from patients of Dental Unit at Rajshahi Medical College Hospital outdoor. Among them 140 (94.5%) isolates were identified as Candida albicans, 108 (72.9%) isolates as Candida tropicalis, 68 (45.9%) isolates as Candida krusei and 36 (24.3%) isolates as Candida parapsilosis (Figure 4).

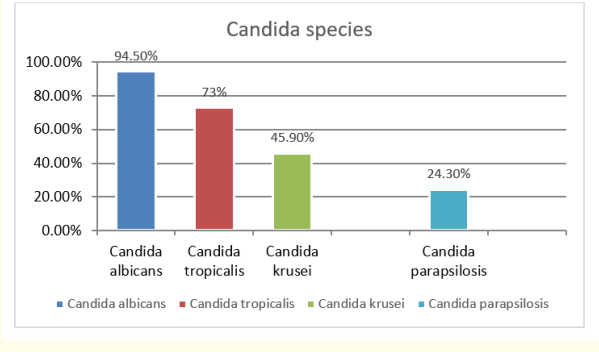

Figure 4: Distribution of Candida species recognized from clinical samples.

Distribution of Candida species by age of the respondents

As per table 4 shown below, Candida albicans was most widespread in the population, in the age group of 1 to 3 years. It is perceived that Candida tropicalis and Candida krusei were in this age group at comparatively greater quantity than in other age clusters.

Age	Candida albicans n, (%)	Candida tropicalis n, (%)	Candida krusei n, (%)	Candida parapsilopsis n, (%)
< 1 year	48 (34.3%)	36 (33.3%)	24 (35.3%)	12 (33.4%)
1-3 years	68 (48.6%)	56 (51.9%)	28 (41.2%)	20 (55.5%)
4-6 years	24 (17.1%)	16 (14.8%)	16 (23.5%)	4 (11.1%)
Total	140 (100%)	108 (100%)	68 (100%)	36 (100%)

Table 4: Candida species prevalence according to respondent's age.

In 140 isolates of Candida albicans 48 of them less than 1 year, 68 of them between 1-3 year and 24 of them were between 4-6 year, in 108 isolates of Candida tropicalis 36 of them less than 1 year, 56 of them between 1-3 year and 16 of them were between 4-6 year, in 68 isolates of Candida krusei24 of them less than 1 year, 28 of them between 1-3 year and 16 of them were between 4-6 year and in 36 isolates of Candida parapsilopsis12 of them less than 1 year, 20 of them between 1-3 year and 4 of them were between 4-6 year.

Distribution of Candida species by gender of the respondents

The objectives of this study were not genuinely formed depending on the distribution patterns of Candida species. Nevertheless, it is worth to be mentioned that these comparisons are deemed by the analysis of the data.

As per the summary of the table 5, the prevalence of Candida species is shown based on their gender. The women sheltering more species observe than males in Candida species colonization.

Spp. According to sex	Male n, (%)	Female n, (%)	Total n, (%)
Candida albicans	44 (31.4%)	96 (68.6%)	140 (100%)
Candida tropicalis	40 (37.1%)	68 (62.9%)	108 (100%)
Candida krusei	28 (41.1%)	40 (58.9%)	68 (100%)
Candida parapsilopsis	12 (33.3%)	24 (66.7%)	36 (100%)

Table 5: Candida species prevalence according to respondent's sex.

In 140 isolates of Candida albicans 44 of them were observed in male and 96 of them were from female, in 108 isolates of Candida tropicalis 40 of them were observed in male and 68 of them were from female, in 68 isolates of Candida krusei28 of them were observed in male and 40 of them were from female and in 36 isolates of Candida parapsilopsis12 of them were observed in male and 24 of them were from female.

Discussion

The conventional methods include the inoculation on corn-meal agar, biochemical assimilation and fermentation tests and thus the cost of mycology cultures is increased. Hence, these laboratories were being limited to the germ tube test for diagnosing the Candida albicans or non albicans Candida. It becomes impossible to select the proper agent for antifungal therapy. Prior to be mentioned that the monitoring of drug residence along with, a safe and effective drug treatment and the anticipation of resistance to antimicrobial therapy is not possible without standard diagnostic tools. Simple, cost effective and speedy method is required to be evaluated chromogenic medium and identifying Candida to species level. With a view to differentiating Candida species, Chromogenic agar is used as a up to date and more quick technique.

Oral candidiasis is an infection caused by oral Candida species; Candida albicans has been commonly detected, where- as Candida tropicalis, Candida parapsilosis and Candida krusei have been isolated less frequently [9]. Recently, however, as in our study, Candida albicans and Candida tropicalis are reported to cause oral infection, as a single species or as mixed causative agents [10-12].

According to our result, children are most vulnerable to oral candidiasis due to their lower immunity between age of 1-3 year because mother immunity persist in children for over a period of 6-12 months then children's own immune system begin to develop [13-16]. Malnutrition and drug resistance are two major causes of recurrent oral candidiasis in children between age of 1-3 year.

Light green colonies are produced by Candida albicans on HiCrome Candida differential agar in our study. Kumar., *et al*. Sukumaran., *et al*. reported all the samples of Candida albicans shows light green colonies on HiCrome agar as noted on Himedia, Mumbai, India [17,18]. It has been reported by Nadeem., *et al*. that green-colored colonies were showed by Candida albicans yet media used was CHROMagar Candida, (France) [19]. Blue to metallic blue colonies on HiCrome Candida differential agar is produced Candida tropicalis. The agreement with Sukumaran., *et al*. Manikandan., *et al*. and the agreement with Devi., *et al*. as well as the study represents all the specimens of Candida tropicalis showed blue to metallic blue colonies on HiCrome Candida differential agar [18,20,21]. Following that purple fuzzy colonies are produced by Candida krusei on HiCrome Candida differential agar. Based on the study, purple fuzzy colony on HiCrome Candida differential agar are produced by all the specimens of Candida krusei in accordance with Deaf., *et al*. and reported by Dharwad., *et al*. [22,23]. The study hereby represents the production of light pink to pink-colored colonies on HiCrome Candida differential agar by Candida parapsilosis which leads to complications in identification. Sivakumar., *et al*. reported that varying shades of pinkish, purple-colored colonies are produced by Candida parapsilosis nevertheless CHROMagar Candida (CHROMagar Company, Paris, France) was used as the chromogenic media [7].

Conclusion

In summary, we concluded that for successful treatment, species identification of Candida has a principal effect since it aids in ideal choice of the therapeutic drugs and ensure the utilization of HiCrome Candida differential agar media in a simple, rapid and inexpensive method. This identification will aid clinician in making quick choice on suitable antifungal agents. The utility of HiCrome agar in routine mycology laboratories may be increased by further modification in the agar and improvement in the observer skills. This may prevent the need for conventional identification methods. We also found that not all recurrent cases were associated with antifungal drug resistance in oral candidiasis. These recurrent cases might be managed by properly educating patient about personal hygiene and by keeping the affected area dry. An important role for the treatment of fungal diseases will be played by vitro susceptibility testing of the yeast to antifungal drugs in proper selection of antifungal drugs because there is vast exposure of drug-resistant species being the presence of the concern of incorrect empirical treatment. This will result in decreasing morbidity of patient and mortality.

Conflict of Interests

The authors declare no potential conflict of interests.

Ethical approval and consent to participate

Ethical approval was taken from the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) of Institute of Biological Sciences, University of Rajshahi. Memo No: 37 (21)/320/ IAMEBBC/IBSc.

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