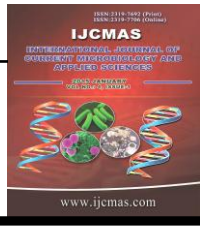
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 1067-1072

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 1067-1072**
http://www.ijcmas.com



Original Research Article

Prevalence and risk factors assessment of *Candida albican*s in tertiary health care institutions of Pakistan

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Keywords

Candida albicans, Oral Candidiasis, Antibiotic abuse

A B S T R A C T

Oral Candidiasis is an emerging threat worldwide in general and in Pakistan specially. It is alarming situatuion that bacterial isolation, characterization and susceptibility testing has not been provided in tertiary health care units in Pakistan including big and leading hospitals except a few well known like Agha Khan Hospital Karachi, which has a dedicated system and collection points present nationwide. Current study aimed to evaluate prevalence of candida albican (major fungus involved in oral fungal infection) in three major cities of the two densely populated provinces; Punjab (PU) and Khyber Pakhton khawah (KPK). Total of 503 clinical swab samples were collected from patients and further analysis was carried out. Prevalence rate of *C. albicans* was observed 41.94 % with three major risk factors; Antibiotic abuse, hygienic condition of oral cavity and alcohol consumption. This study indicates the high prevalence rate of *C. albican*, as well as antibiotic abuse. These two issues are very critical and needs to be addressed without any delay.

Introduction

Nature has made different barriers of defense in human beings including micro biota. More than 500 species of microbes has been isolated so far in normal gut flora. These microbes play a key role in colonization resistance against invading pathogens. *C. albicanss*, part of normal micro biota is an opportunistic pathogen. In conditions when normal flora is in its balance state, over growth of any of the specific microbe is repressed. Once this balance is overturned then superinfection of any of the species may take place. *C. albicans* overgrowth takes place in the absence of unbalance micro biota(Samantha Payne, 2003).Among microbial diseases, fungal diseases are emerging rapidly especially in last twenty five years. Candida

although a commensal is the most prevalent fungal pathogen causing both mucosal and systemic infections (Aher, 2014; Karima Sdoudi, 2014). Candida may cause urinary tract infections (UTIs) which accounts 10-15% of the total reported UTIs (Sanaa M. Ashour, 2015), male infertility (S. Sasikumar, 2013) vulvovaginal candidiasis (Saini, 2013; Solanki, 2014), Oropharyngeal candidiasis, Systemic Candidiasis and cutaneous candidiasis (Rajeev Shah, 2014). Catheterization for longer duration, diabetes mellitus, immunocompromized persons, deep burns, surgical procedures in the abdominal area, bone marrow transplantation, HIV , antibiotic treatment, gut perforation, malnutrition infants, administration of immunosuppressive agents may enhance the proliferation of *C. albicans* (Monika Staniszewska, 2012).

Due to antimicrobial abuse resistance of *C. albicans* against antifungal drugs is increasing gradually resulting in high morbidity and mortality rates (Korhalkar Anagha, 2014; Magda Mostafa Azab, 2014). Pakistan is least developed country. Very low budget amount is allocated for health care facilities. Tertiary health care hospitals are either completely deprived of advanced laboratory facilities or just have bacterial culturing labs. Very low attention has been laid down on fungal isolation, characterization, diagnosis of fungal infection and risk factors evaluation underlying fungal infection epidemics. Current study aimed to determine prevalence frequency of *C. albicans* in oropharyngeal infections and risk factors prevailing *C. albicans* infection in local population.

Materials and Methods

Present study consisted of total of 503 patients randomly selected from public sector tertiary health care hospitals in three big cities of densely populated provinces including on public sector institution of most neglected region of southern Punjab, Pakistan. Patients from all age groups and of both of the genders were included except those who are under the age 18 years and were healthy or were not suspected to harbor fungi in their oral cavity. After the collection of samples, a questionnaire was also dispensed to each patient to evaluate risk factors of *candida albican* presence.

Isolation and characterization of yeast

Clinical swab sample collection protocol previously adopted by Binita 2012 (Binita Srivastava, 2012) was followed. Sabouraud's dextrose agar media containing chloramphenicol 10% was used for culturing the swab samples. Culture plates were incubated for 24-48 hours. Plates having no yeast growth were incubated further for 72 hours. Colony forming units were determined. Morphology of the colony was determined by the protocol adopted by kausar Fatima 2014 (R.Rajendran, 2014). Smear was prepared and gram stained. Presence of psudohyphae and budding cells were observed to confirm the Candida presence.

Germ tube test

2-3 colonies were inoculated in human serum taken in test tube. Incubated on water bath for 2-3 hours at a constant temperature maintained at 37c. After incubation, glass slide was prepared by placing serum on glass slide and placed coverslip on it. Observations were made under low and high power objectives sequentially. Presence of germ tube in this incubation time was taken as a positive indication for *C. albicans* presence (R.Rajendran, 2014).

Carbohydrate fermentation test

Suspended a loopful of culture in presterilized distilled water. Prepared 2% media for glucose, lactose, sucrose, and maltose separately. 0.2ml of culture suspension was added in a test tube containing sugar media. Incubated for 48-72 hours at 30C. fermentation ability was indicated by gas entrapment and change in color in the test tube(R.Rajendran, 2014).

Statistical Analysis

IBM 20 was used for evaluation correlation between different risk factors and candida presence

Results and Discussions

Total of 503 clinical swab samples were analyzed. *C. albicans* positive samples were 211 and rests were *C. albicans* negative containing non albican species of Candida. In this study fifteen different categories of risk factors were evaluated by taking patient history and interview on a predefined and pre-formulated questionnaire (table 1). From these categories strong correlation was observed only in three categories. These are namely oral hygienic condition, alcoholic consumption and antibiotic usage category (Graph 1, 2 and 3 respectively).

Results depict that *C. albicans* growth was high in the patients who had a history of broad spectrum antibiotic treatment. In the presence of healthy and balanced normal micro biota, growth of *C. albicans* is controlled. "Colonization resistance" is a phenomenon in which normal floral inhibits and restricts the growth of any invading pathogen. Fatty acids especially of Short chain length are produced by this micro biota which produces immunity against invaders. Once this colonization resistance is imbalanced by antimicrobial therapy then overgrowth of opportunistic pathogen or of any foreign pathogen can takes place (Samantha Payne, 2003). Some bacteria produce such molecules which inhibit the growth of yeast. *Lactobacillus plantarum* produces bacteriocin like substance which suppress the growth of *C. albicans* (Katrin Stro¨m, 2002). More over when oral antibiotics are administered, some species of normal micro biota may die out. Competition for nutrients starts between the remaining species of microbes. This aggravates the situation resulting in the development of resistance of remaining microbes and results in super-infection of a specific microbe or microbes(Samantha Payne, 2003). Interesting phenomena has been demonstrated in a study which evaluated concentrated related modulation in growth and dissemination of Candida albican and non albican strains. It was observed that at low concentration morphogenetic transition is inhibited by alcohols and at high concentration gradient growth is retarded(Nitin M Chauhan, 2013). There were three alcoholics in our study. All the three patients were *C. albicans* positive. These results are consistent with the previous findings of Sanja 2013. Psychoactive substances including alcohol may augment the frequency of *C. albicans* in patients. Although prevalence frequency of non-albican species is also increased(Sanja Hadzic, 2013).

Oral hygienic category had also a great correlation with the *C. albicans* presence. Persons who maintain high hygienic conditions in their oral cavity have less chances of fungal presence as compared to those who had severely bad oral hygienic conditions. Predisposing factor which worsen the condition are also imbalance in the normal micro biota balance which maintains and controls the growth pattern of the microbes. Similar finding about the oral hygiene and increased *C. albicans* growth

has been reported by tahsin 2014 in children. Oral hygiene is directly proportional to the decrease in microbial count in oral cavity (Tahsin Celepkolu, 2014).Bad Oral hygienic conditions cause an increase in *C. albicans* growth (Assar, 2009; Catherine J. Binkley, 2009; Elerson Gaetti-Jardim Júnior, 2008; Pai, 2011; Saima Naseem, 2014).Some of the active ingredients present in formulation of pastes contain antimicrobial property. Microbial load is decreased rapidly on repeated use of such products. Triclosan also antagonizes microbial growth in general. Individuals who brush their teeth with triclosan containing products have low incidence rate of *C. albicans* also(J. Higgins, 2012).

Three categories of risk factors including oral hygienic condition, antibiotic use and alcoholism have been previously reported and are verified in our study. More over antibiotic abuse is indirectly evaluated giving a serious cautionary signal for health care associated personnel to lemmatize abuse of antibiotics. Prevalence of candida is increasing exponentially due to overwhelming antibiotic use. A comprehensive policy is required to be made for antibiotic usage. Also culture facilities in tertiary care hospital arerequired for fungal strains isolation and characterization.

Acknowledgment

Authors are thankful to Mr. Muhammad Naeem, Lecturer, University of Animal Sciences Lahore, Pakistan, Mr. Saif Ullah, Mr. Asad Ali Akbar Shirazi and Yaqoot Zahra for their valuable role in facilitation of this research.

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