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Detection of *Candida* species and Genotyping in Children with Diarrhea by Nested PCR

A Thesis
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1.Introduction.

Fungal diseases are a worldwide public health issue. The illnesses caused by fungi can range from superficial (athlete's foot), to deadly (blood stream infections). The three general classes of infections that the mycotic diseases branch of the centres for disease control is concerned about are opportunistic infections, community acquired infections, and hospital acquired infections (CDC(2012)Fungal Diseases.US Gov).

Opportunistic infections refer to infections that do not generally harm healthy individuals, but can cause massive damage to immunocompromised patients, including those with AIDS, individuals undergoing chemotherapy, or organ recipients.(CDC (2012) Fungal Diseases. US Gov)

Which is the most prevalent fungal pathogen for humans is *Candida albicans*. It is responsible for common clinical problems including oral thrush and vaginitis, but can also lead to life-threatening systemic infections in immunocompromised individuals, such as Auto immune diseases (AIDS) patients, resulting in 30-50% mortality rates. While several species of *Candida* are responsible for the deadly bloodstream infections, *C. albicans* is responsible for almost half (46%). For these reasons,chosen to focus our investigation on *C. albicans*.(Horn *et al.* , 2009).

Candida , which is the genus , comprises about 150 yeast species, the genus is composed of heterogeneous group of organisms, and more than 17 different *Candida* species are well-known to be aetiological agents of human infection; however, more than 90 % of invasive infections are caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* (Papon *et al.*, 2013). *Candida* is the part of normal flora of the human body colonizing various anatomical sites like oral cavity, digestive tract, vagina and skin(Seneviratne and Samaranayake,2008).

In cases where there is host debilitation, or where there is a change in the local environment promoting *Candida* overgrowth, this leads to *candida* infection (referred to as candidiasis) (Gow *et al.*,2011). There are numerous types of candidiasis including mucosal candidiasis, cutaneous candidiasis and systemic candidiasis .The *Candida* is a transition from a harmless commensal to disease causing pathogen depending on the host immune system and virulence factors of *candida* (Yang,2003).

Candidiasis is a mycotic infection caused by members of the genus *Candida*. Chiefly, *Candida albicans* is responsible for about (70–80%) of all *Candida* infections. The *Candida* is an opportunistic yeast pathogen which increases predominantly in patients with predisposing condition, including immunodeficiency such as HIV infections, prolonged use of broad-spectrum antibiotics, corticosteroids, diabetic patients and infections with other debilitating diseases.(Estrada-Mata E, *et al.* , 2016).

Candida yeasts which are generally present in healthy humans, are frequently part of the human body's normal oral and intestinal flora, and particularly on the skin; however, their growth is normally limited by the human immune system and by competition of other microorganisms, such as bacteria occupying the same locations in the human body(Mulley and Goroll , 2006).

Candida is requires moisture for growth, notably on the skin. For example, wearing wet swimwear for long periods of time is believed to be a risk factor (Goehring and Richard ,2008). In extreme cases, superficial infections of the skin or mucous membranes may enter into the bloodstream and cause systemic *Candida* infections(*MedlinePlus Encyclopedia* Vaginal yeast infection).

The role of *candida* is therefore potentially important as a common and potentially treatable disease . Prospective, hospital based studies were carried out to evaluate the prevalence and concentrations of *candida* and other yeast species in the stools of hospitalised children with and without diarrhea. An attempt was made to identify factors predisposing to intestinal carriage of yeasts(Ponnuvel *et al*., 1996).

Signs and symptoms of candidiasis vary depending on the affected area (Dolin, [edited by] Gerald *et al* .,2010).the main symptoms of gastrointestinal candidiasis in healthy individuals are anal itching, belching, indigestion, bloating, nausea, vomiting, gas, intestinal cramps, and gastric ulcers, diarrhea.(Martins *et al* ., 2014),(Wang *et al* ., 2014),(Erdogan and Rao 2015).

Diarrheal diseases occurring in early life (< 3 years) are an heterogeneous group of abnormalities including gastrointestinal infections, food hypersensitivity and allergy, immune dysregulation, and primary abnormalities of the enterocyte. Diarrhea is a leading cause of illness and death in children younger than 3 years worldwide, causing 1·3 million deaths every year . Despite defining etiology of diarrhea is critical to decide therapy and prevention strategy, the overall prevalence of early onset diarrhea was not clearly defined because of the large spectrum of etiologies and difficulties in patient selection. In younger children , acute diarrhea will lead to severe dehydration. Fluid loss and dehydration are the cause of death in nearly all children with acute diarrhea(Santosham *et al*., 2010).

The involvement of the various pathogens to diarrhea may differ substantially between regions depending on geographic , local meteorological, and socio-economic conditions. The principal reasons for the spread of diarrheal diseases are found in poor hygiene and sanitation, limited access to safe drinking water as well as inadequate education of health care providers and recipients (Curtis *et al*., 2000),(Thapar and Sanderson 2004).

Diarrheal infections are caused by different microbial agents such as bacteria, parasites, viruses , and fungi. However, in several developing countries, less attention is focused on viral and fungal agents. Diarrhea is the major cause of child morbidity and mortality in socio-economical developing countries. More than one million episodes of diarrhea occur every year among children under five years of age causing approximately 2.5million deaths(Kosek *et al* ., 2003),(O'Ryan *et al*., 2005).

Numerous microbial agents have been incriminated in diarrheal infections among which are enteric bacteria, parasites and viruses(Henry ,1991),(Babaniyi ,1991),(Bignan *et al* .,1991). *Candida*, *Trichosporon* and *Geotrichum* are fungi that have been reported to cause diarrhea (Talwar *et al* .,1990),(Danna *et al*., 1991).

Molecular method for direct identification of *Candida* species has been established to be an accurate and rapid method for the detection of candidal infections ((Roilides *et al*., 2004; Vendettuoli *et al*., 2008; Imran and Alshammry, 2016).

Different molecular methods for detection of candidia infection, have been used such as real time PCR(Liguori *et al*., 2007; Li *et al*., 2003),Multiplex PCR((Sampath *et al*., 2017),and nested PCR((Ahmad *et al*., 2002; Çerikçioglu *et al*., 2010).Polymerase chain reaction were based on amplifications of 18S and 28S rDNA regions have been used to identify diverse *Candida* species in clinical specimens. (Weerasekera *et al*., 2013; Makene, 2014).

Nested polymerase chain reaction (Nested PCR) is a modification of polymerase chain reaction intended to reduce non-specific binding in products due to the amplification of unexpected primer binding sites(. Elizabeth van Pelt- Verkuil *et al* ., 2008).

The aims of study :-

1. Genotyping detection of *Candida.albicans* isolated from Diarrheal children.
2. Subgenotype detection of *Candida.albicans*.

Objective of study:-

1. Isolation and phenotypic identification of *Candida* species by conventional methods.
2. Determination sensitivity and resistancy of *Candida*.albicans by making Antifungal susceptibility test
3. Molecular detection of *Candida.albicans* by using Nested PCR among children with diarrhea.
4. Determining the socio demographic factors (such as age ,weight, length ,duration of diarrhea ,residence , gender, mother education, water- source, type of milk feeding , the presence of child, clinical aspects, other diseases) associated with diarrheal disease in children.