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Mycological Causes of Diarrhea among Children in Diyala

A Thesis

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Partial Fulfillment of the Requirements for the Degree of Master of Science in
Medical Microbiology

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Dedication

To...

*My dear mother, who surrounds me with her
love and kindness, and never forgets me in
her sincere prayer.*

*My father, brother and sister, the source of
light in my life. They have shared the
moments of laughter and sorrow.*

*All who will benefit from this work even a
word.*

I lovingly dedicate this work

Ali

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Ali

Summary :

One hundred stool samples of children less than three years referred to AL-Batool Teaching Hospital, in Diyala province during the period (from 2nd October 2016 to 3rd December 2016). were collected sixty-four samples of them were diagnosed as *Candida* spp. infections by making a routine and confirmative diagnostic processes by direct microscopic examination of stool , cultured on Sabouraud dextrose agar (SDA) and microscopic examination of colonies.

The results revealed that, 24(37.5%) of isolates were *Candida albicans*;14(21.9%) isolates of *Candida glabrata*; 11(17.2%) isolates of *Candida parapsilosis*; 8(12.5%) isolates of *Candida krusei* and 7(10.9%) isolates of *Candida tropicalis*. The results of PCR study by detect the 25S rDNA showed that 20(83.3%) isolates belonged to the genotype A ; 2(8.3%) isolates belonged for each genotype B and genotype C of the *C. albicans*.

The results showed there are significantly ($P<0.05$) higher *Candida* infection rate among children with previous antibiotic use compared with those who had no previous antibiotic use , actually 38(73.1%) with more common *Candida* spp. recorded was *C. krusei* in a rate of (87.5%). While, the higher infection rate is among children consuming from tap water compared with those consuming another source, actually 60(68.9%) .The more common *Candida* spp. recorded was *C. glabrata* in a rate of (100.0%).The higher infection rate is among children consuming non-sterilized water compared to the children consuming sterilized water, actually 38(73.1%) .More common *Candida* spp. recorded was *C. glabrata* in a rate of (71.4%) and the higher infection rate between children use non-sterilized bottle compared to the children use sterilized bottle, actually 44(80.0%) with more common *Candida* spp. recorded was *C. parapsilosis* in a rate (81.8%).

Although, insignificant($P>0.05$),the results showed that *Candida* infection rate higher in male children compared to female children (66.7% vs. 60.5%).The *Candida* infection rate in (≥ 2) months patients high compared with other age groups , actually (77.8%).The rate of the infection was higher among patients who reside in rural areas compared to those residing in urban areas (65.6% vs. 61.1%).The infection rate was higher among patients without previous diarrhea compared to patients with previous diarrhea (64.5% vs. 62.5%). *Candida* infection rate was higher in children with chronic diarrhea compared to acute diarrhea (80.0% vs. 63.2%).The rate of the infection was higher among those on raw feeding(age more 2 years) (75.0%) compared to those on mixed or bottle or breastfeeding (68.4%, 63.1%, and 50.0%) respectively. *Candida* infection rate was higher in children who lived in the area open sewage without drainage, actually (67.3%) while children lived in the area open sewage with drainage (52.6%) and close sewage (35.4%). The rate of the infection was among patients whose mothers age is under 20 years (89.4%) compared with other age groups. The rate of the infection among patients whose mothers have primary education level was (76.5%) compared to those with other education level of mothers.

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List of abbreviations

Abbreviation	Meaning
AAD	Antibiotic associated diarrhea
AIDS	Acquired immune deficiency
BFI	Bacterial fungal interactions
BSI	blood stream infections
bp	base pair
<i>C. albicans</i>	<i>Candida albicans</i>
<i>C. non albicans</i>	<i>Candida non albicans</i>
CAC	chromogenic agar <i>Candida</i>
<i>Candida spp.</i>	<i>Candida species</i>
CDC	Center for Disease Control
CMA	Corn Meal Agar
Df	degree of freedom
DM	demethylase
DNA	Deoxyribo nucleic acid
EDTA	Ethylenediaminetetraacetic acid)
GIT	Gastrointestinal
GT	Germ tube
HIV	Human immunodeficiency virus
hrs	hours
ICU	Intensive Care Units
ITS	Internal transcribed spacer
LPCB	Lacto Phenol Cotton Blue
min	minutes
M	Molar (mol/L)
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
pH	power of Hydrogen
Rnase	Ribo nuclease
rpm	rounds per minute
rRNA	Ribosomal RNA
SAP	Secreted Aspartyl Proteinases
SDA	Sabouraud Dextrose Agar

sec	second
Sig	significant
SPSS	Statistical Package for Social Sciences
UV	Ultra violet
WHO	World Health Organization
YPD	Yeast extract Peptone Dextrose
μ	Micro

Chapter One

Introduction

1. Introduction

Candida species constitute a portion of the natural microbiota of the human mucosal, oral cavity, vagina, and gastrointestinal tract (Moran *et al.* 2012; Sardi *et al.*,2013).Several species, including *Candida albicans*, *C. dublinensis*, *C. glabrata*, *C. guilliermondii*, *C. Lusitaniae*, *C. parapsilosis* and *C. tropicalis*, can be found as part of the normal human commensal flora, especially in all sections of the gastrointestinal tract (Netea *et al.*,2008; Schulze and Sonnenborn,2009).

In normal healthy person, there is a balance between *Candida* species as a normal flora and the normal defense mechanism of the body (Ferrer, 2000),which will cause opportunistic infection in the presence of any of the predisposing factors like; diabetes mellitus, malnutrition (Conlon and Snyderman,2000), humidity, burn, HIV infection (Roitte *et al.*,1998), renal failure, endocrine disturbance (Guggenheimer *et al.*, 2000),cancer, indiscriminate usage of antibiotics (Daly *et al.*,1981),glucocorticoids and cytotoxic drugs (Roitte *et al.*,1998). However, in response to improving or disturbance in the sponsor security systems in the gut, like the intestinal microbiota, gut-associated immune system and the mucosal barrier, *Candida* spp. can convert from safe commensals into pathogens or disturbance in the host defense systems in the gastrointestinal, including the intestinal microbiota, gut-related immune system, and the mucosal preventive, *Candida* spp.can convert from harmless commensals into pathogens (Walker *et al.*,2009, Netea *et al.*,2008).

Candidiasis is primarily caused by *C. albicans*, while there has been a striking increase in the frequency of non-*albicans* *Candida* species in the last few years. The most important species which are considered pathogenic to human are *C.albicans*, *C. tropicalis*, *C. Kruse*, *C.glabrata*, *C.lusitaniae* and *C.viswanathii* (Chander,2002).Candidia Worldwide distributed in nature

(Morton and Harris,1975).Colonization of the gastrointestinal and genitourinary tract may occur during birth directly from the birth canal (Winner,1975), at some time during infancy or perhaps later in life, in which the source may be environmental like polluted fresh and marine water (Valdes-collazol *et al.*1987) soil, air (Meyer *et al.*,1984), plant (Ferrer,2000) , contamination of bedding, air of the hospital, wash basins and could be of human mucous membrane or gastrointestinal tract (Morton and Harris,1975).

Candida albicans is the more typical *Candida* species isolated from human stool. On the other hand, several reports have advised that *Candida albicans* may cause diarrhea,while other reports suggested reason that antibiotic-associated diarrhea (AAD) in young children (Danna *et al*, 1991). In recent years, the incidence of *Candida* spp. infections have increased. It has also been shown that *C.albicans* also causes diarrhea. Candidiasis in neonates does a serious and relatively common cause of late-onset sepsis associated with mortality. The recent study indicates that non-*albicans* infections are on the rise, which often accounts for more than 50 % of candidiasis found in the infected population.(Saravolatz *et al.*,2003).

In the developing world as a whole, about one-third of infant and child deaths are due to diarrhea. Dehydration causes approximately 70% of diarrheal deaths, the loss of much salts and water from the body, which needs water to maintain blood volume and other fluids to function properly (Gupta and Mahaj,2005). Underlying reasons for the spread of diarrheal are found in poor hygiene and sanitation; limited access to safe drinking water as well as unsuitable education of health care providers and recipients (Thapar and Sanderson,2004; Curtis *et al.*,2000). Mainly each child will suffer from diarrhea at a certain point, the potency for great dehydration is always concerned with electrolyte abnormalities and hypovolemia in a child with diarrhea. People with diarrhea often have a fever and stomach ache (abdominal cramps).The infectious

agents creating diarrhea can be enteric bacterias, parasites, viruses and fungi. Yeast like fungi are usually found in the gastrointestinal system in small numbers since their attachment and habitation to the mucosal surface is prevented by the anaerobic microflora. The prolonged use of antibiotics can cause an imbalance in defensive microbial flora in the gastrointestinal tract, leading to antibiotic-associated diarrhea (Krause *et al.*, 2003).

The yeasts have been reported in increased frequency and quantity in the stool of the patients, which can result from antibiotic treatment of diarrhea (Krause *et al.*, 2001). Although not commonly suspected clinically, such pathogenic yeast-like fungi can raise the severity of diarrhea-causing severe dehydration, malnutrition, and mortality in already debilitated patients especially, immune affected individuals, children, and older patients.

Discontinuing the antibiotic use, if required, administration of specific antifungal remedy can lessen morbidity and mortality in such patients (Krause *et al.*, 2003). For the treatment of fungal infection, many antifungal have been used such as compounds of polyenes and azoles. However, the random usage of these antifungal in the last few years helps with the appearance of resistant strains to many antifungal, in addition to side effects (AL-Hadithy, 1998; Cowan, 1999).

Aims of the study :

1. To isolation and identification of *Candida* spp. isolated from children suffering from diarrhea in Diyala province by routine laboratory procedures and molecular techniques based PCR.
2. To identification genotypes distributions of *Candida albicans*.
3. Study the distribution of the *Candida* spp. among children and to explore the effect of some relevant factors.