

## ***Candida* Species Induced Gastroenteritis in Aguata Urban, Anambra State**

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### **ABSTRACT**

Three hundred faecal samples of adult patients suffering from gastroenteritis in clinics and hospitals from Aguata urban were analysed for the presence of *Candida* species. The analyses were based on the cultural, morphological and biochemical characteristics, and extracellular enzyme production and activity. The percent isolates included *Candida albicans* 33%, *Candida tropicalis* 23%, *Candida lisitainiae* 15%, *Candida parasilosis* 11%, *Candida guilliermondii* 9%, *Candida kefyr* (pseudotropicalis) 7%, and *Candida Krusei* 2%. *Candida parasilosis* produced the enzyme amylase; *Candida Kefyr*, *Candida albicans* and *Candida tropicalis* produced lipase. Proteolytic activity was demonstrated by *Candida krusei*, *Candida kefyr* and *Candida albicans*. These isolates with the virulent factors are incriminated as the causative agents for gastroenteritis.

**Key words:** Gastroenteritis, lipase, opportunistic pathogen, pseudohypha

## INTRODUCTION

Medically important yeast are those fungi of primarily unicellular growth that are capable of producing or contributing to diseases of humans and animals. Although there are many genera and species of yeast, relatively few of these produce these diseases<sup>1</sup>. In most instances, the pathogenic yeasts are found among the deuteromycetes, or fungi imperfecti, that is fungi that do not exhibit the sexual or teleomorphic state in culture. Yeasts may also be classified as Ascomycetes or Heterobasidio mycetes depending on the method of their sexual reproduction. Some yeasts are considered as opportunistic pathogens because of their ability to initiate disease in immunosuppressed patients<sup>2,3</sup>

The most common yeast species acting as agents of human disease are *Candida albicans*, *Candida parasilosis* and *Cryptococcus neoformans*. Other emerging pathogens include *Malassezia furfur*, *Trichosporum beigeli*, *Rhodotorula* species, *Hansenula anomala*, *Candida lusitanae* and *Candida krusei*.

These opportunistic pathogens such as *Candida utilis* and *Candida lipolytica* that are environmental contaminants and industrially important have also been implicated. *Candida albicans* and other *Candida* species cause candidiasis. *Malassezia* species, for example *M.furfur* causes pityriasis versicolor while *Blastoschizomyces capitatus* causes invasive fungal disease in Leukemic patients<sup>4</sup>.

Gastroenteritis can be defined as the inflammation of gastrointestinal tract. This disease is mainly initiated by bacteria and protozoa while *Candida* species are present in gastro-intestinal tract as commensal<sup>5,6</sup>. With increase in the cases of gastroenteritis of unknown aetiology in Aguata urban, it became desirable to study the presence of opportunistic pathogenic *Candida* species in these cases.

## MATERIALS AND METHODS

A total of 300 faecal samples from adult patients of gastroenteritis as confirmed by the clinician, collected from 5 zones in Aguata urban were analysed for the presence of *Candida* species. The catchment hospitals in these zones are

- a. General Hospital Ekwulobia
- b. National Medical Centre Nkpologu
- c. Ogechukwu Hospital and Maternity Igboukwu
- d. Ijeoma Hospital and Maternity Umuoma
- e. Community Hospital Ezinifite

The faecal samples were placed in sterile bottles and

processed within 24 hours of collection.

## METHODS

Microscopic Observation of stool samples:

The stool samples were processed by emulsifying a small portion of the faeces in drop of sterile normal saline on a grease-free clean glass slide, covered with cover slip. Ethanol-ether concentration method (Davey&Crewe) of stool preparation was also, done<sup>7</sup>. Each prepared slide was examined under microscope using low power (x10) and high power objective lens (x40)

### Stool Culture

A small portion of the fresh stool sample was inoculated on a Petri dish and agar slopes containing Sabouraud Dextrose Agar (SDA) supplemented with Chloramphenicol (0.05mg/ml) and cyclohexamide (0.05mg/ml) Brain Heart Infusion Agar (BHIA) containing chloramphenicol (0.05mg/ml) and gentamycin (0.02mg/ml), using standard loop, cultures on SDA were incubated 28°C for 24 – 48 hours while cultures on BHIA were incubated at 37°C for 24 48 hours.

### Identification of Yeast Isolates

Identification procedures were based on the cultural, morphological and physiological characteristics of the organisms. The methods employed were adapted from these of Larone and Warren & Hazen<sup>2,6</sup> Identification studies were done with pure cultures. The studies include wet preparation using lactophenol cotton blue and Indian ink, germ tube and enzyme production tests.

### Wet preparation using Lactophenol Cotton blue and Indian ink

A drop of each of Lactophenol cotton blue (LP CB) and Indian Ink was placed on two well labeled clean glass slides respectively. With a sterile inoculation needle, two small portions of the culture were removed and placed on the drops. They were gently emulsified and covered with a cover slip and examined under the microscope with both the low power (x10) and high power (x40) objective lens for the morphology and the presence of capsule respectively.

### Germ tube test:

A very light suspension of the culture made in 1.0ml of sterile human serum, was incubated at 37°C for 2 hours. At the end of the incubation, a drop of yeast-serum mixture was placed on a clean grease-free slide and covered with a cover slip. The preparation was examined under (x10) and (x40) objective lens of

the microscope for germ tube production known as pseudo-phyphae

**Sugar fermentation and Assimilation test**

The methods of kerger-van Rij and Larone were employed for the sugar fermentation and sugar assimilation tests respectively.<sup>8,6</sup> The sugars used include dextrose, maltose, sucrose, lactose, galactose and trehalose.

**Screening of Isolates for Extracellular Enzyme Production Amylase**

To detect amylase production, nutrient agar medium containing 1.0% glucose (w/v) was used. The test organisms were inoculated on duplicate plates of the medium and incubated at room temperature for 3-10 days. The plates were flooded with Lugol's iodine solution and observed for clear zones around the colonies, which indicated amylase production.

**Lipase and Protease**

The methods of Tsubio et al. and Rinaldi were employed for the detection of Lipase and Protease production respectively.<sup>9,10</sup>

**Statistical Analysis:**

Analysis of variance (ANOVA) as was stipulated by Zar, was employed in statistical analysis of the data<sup>11</sup>

**RESULTS**

Out of 300 faecal samples analysed, 134 (%) samples of gastroenteritis patients contained *Candida* species. The isolates include *Candida albicans* (44) *Candida tropicalis* (31) *Candida lusitaniae* (21) *Candida parasilosis* (14) *Candida guilliermondii* (12) *Candida kefyr* (a) and *Candida krusei* (31).

**Table 1**, shows the distribution of *Candida* species based on age and sex. The numbers of cases positive for *Candida* species recorded, increased with increase in of the patients' ages. Between the ages of 60 – 90 years 16 cases of *Candida albicans* were isolated as against 5 cases between ages of 20 – 30 years. Similarly, the cases of *Candida* species isolates were more in males than in females. *Candida albicans* has more pathogenic attributes than other species except *Candida kefyr*.

**Table 2**, shows that *Candida albicans* and *Candida kefyr* produced lipase and protease while *Candida lusitaniae* produced amylase and protease activities and the rest of the *Candida* species produced only one enzyme each.

The Null hypothesis is rejected because there was significance difference between the mean values of the isolates amongst the age groups at P<0.05

**Table 1:** Distribution of *Candida* Species Based on Age and Sex of the patients

	<i>Candida albicans</i>				<i>Candida tropicalis</i>				<i>Candida lusitaniae</i>				<i>Candida parasilosis</i>				<i>Candida guilliermondii</i>				<i>Candida kefvr</i>				<i>Candida kruser</i>			
	Ages				Ages				Ages				Ages				Ages				Ages							
SEX	25-30	30-35	35-60	60-90	25-30	30-35	35-60	60-90	25-30	30-35	35-60	60-90	25-30	30-35	35-60	60-90	25-30	30-35	35-60	60-90	25-30	30-35	35-60	60-90	25-30	30-35	35-60	60-90
Male	4	7	8	13	1	5	6	8	2	4	4	5	1	1	5	2	1	2	3	4	0	1	3	4	0	0	2	0
Female	1	4	4	4	1	2	3	5	0	1	2	3	1	1	0	3	0	0	2	1	0	0	1	0	0	0	0	0
	5	11	12	16	2	7	9	13	2	5	6	8	2	2	5	5	1	2	4	5	0	1	4	4	0	0	2	1
Total	44				31				21				14				12				9				3			

**Table 2:** Extra cellular Enzymes production by *Candida* species

<u>Candida Species</u>	<u>Enzymes Amylase</u>	<u>Lipase</u>	<u>Protease</u>
<u>Candida albicans</u>	-	+	+
<u>Candida tropicalis</u>	-	+	-
<u>Candida lusitaniae</u>	+	-	+
<u>Candida parasilosis</u>	+	-	-
<u>Candida guilliermondii</u>	-	-	-
<u>Candida kefyr</u>	-	+	+
<u>Candida krusei</u>	-	-	+

Key: + = Positive - = Negative

It was observed that all the *Candida* spp grew on SDA but only *Candida albicans* gave positive reaction with germ tube test by producing pseudohyphae (germ tube) and chlamydospores

## DISCUSSION AND CONCLUSION

The result revealed the presence of *Candida* species in the patient population studied. These *Candida* species include *Candida albicans*, *Candida tropicalis*, *Candida Lusitanie*, *Candida parasilosis*, *Candida guilliermondii*, *Candida kefyr* and *Candida krusei*.

*Candida albicans* was more prevalent than others and it secreted lipase which was noted as pathogenic factor by the work done by Tsuboi et al<sup>9</sup>. The occurrence of these *Candida* spp correlated positively with the findings of other scientists Warren and Hazen, Oddos, Rinaldi and Cutler<sup>2,5,10,12</sup>. The higher incidence of *Candida* spp in older patients correlated positively with the finding of previous workers in crminating them as opportunistic pathogens. The isolated *Candida albicans* possessed both lipase and protease activities which are virulence attributes. *Candidal* infections are usually due to impaired epithelial barriers functions and occur in all age groups, but most common in new born and elderly<sup>13,14</sup>.

**In conclusion**, the result revealed that seven species of the genus *Candida* were isolated. It was also observed that the *Candida albicans* was more prevalent than others and there was significance difference between the mean values of the isolates amongst the age groups at  $P < 0.05$ . *Candida albicans* and *Candida kefyr* had both lipase and protease activities. *Candida albicans* being more prevalent and with the virulence attributes is then incriminated as the causative organism for the human gastroenteritis.

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