



Short Communication

Isolation and Identification of Fungi Microbiome in oral Cavity of Students in Ebonyi State University, Abakaliki, Nigeria

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Abstract

Microbiomes occupy different parts of the body including the oral cavity and they influence health and diseases. Studies on isolation and identification of culturable fungal microbiome prevalent in oral cavity of students in Ebonyi State University, Abakaliki was carried out in November, 2015. The aim of the study was to determine the most prevalent culturable fungi species inhabiting the oral cavity of healthy students. Swab sticks were employed for the collection of oral samples and inoculated onto potato dextrose agar (PDA) media and incubated for 72 hours at 25^oC, fungal species were isolated and identified. Fungi species identified were *Candida*, *Aspergillus*, *Fusarium*, *Blastomyces* and *Histoplasma*. The research revealed that *Candida* spp. and *Aspergillus* spp. were the most preponderant fungi organisms in oral cavity of healthy students.

Keywords: Fungi, Microbiome, Oral cavity, Diseases, Health.

Introduction

Microbiome refers to communities of microorganisms which inhabit different parts of the body. These organisms are diverse, fungi (mycobiome) constitute the dominant aspect of the microbiome, most of them are human pathogens¹. However their abundance and specific implication to diseases have received little attention over time. The human oral cavity contains different habitats such as; teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils, which are colonized by microbiomes. The microbiomes could be important in defensive mechanisms and metabolic processes². An undetermined fungal mycobiota inhabit the oral cavity of human. Some fungal mycobiota could be benign contributing to health, some contribute to destruction of tissues within the oral cavity and upper respiratory infections³. Majority of oral fungal infection have been reported to be due to *Candida albicans*, other fungal species associated with oral cavity diseases are becoming common. Most studies have characterized oral bacterial microbiota, the fungi aspects have received few attention³. Over 700 bacterial species have been identified in the oral cavity of human⁴. Research has shown that bacteria and fungal communities in oral cavities interfere and support themselves in several ways⁵.

Candida belong to healthy microbiome, but under certain circumstances, could cause life-threatening systemic infections, they also cause oral candidiasis and some significant human infections¹. Some species are less susceptible to commonly used antifungal drugs and this poses significant difficulties for effective treatment⁶. Reported prevalence of *Candida* species

was found to be high in elderly people⁷. This being influenced by the use of antibiotics, malnutrition, old age etc⁸.

Mouth is the major passage to internal system, food passes through the mouth to the digestive system, air also passes through the nostrils and oral cavity to the respiratory tract. This creates a continuous channel and could cause microbiomes in the mouth to spread to other neighboring sites. Presence of *Candida* and *Saccharomyces* species have been found to correlate with severity of hepatitis, pneumonia, cardiovascular diseases and other systemic diseases². The aim of the study was to isolate and identify fungi mycobiome in oral cavity of healthy students in Ebonyi State University, Nigeria.

Materials and Methods

Sample Collection: Fifteen students from Ebonyi State University, Abakaliki, Nigeria were randomly recruited for this study. Swab sticks were used to obtain samples after informed consent. Samples were preserved in the refrigerator prior to experiment. Media were prepared using Potato Dextrose Agar (PDA), poured aseptically into petri dishes and chloramphenicol added. The content of the swab sticks were dissolved with 2 ml of distilled water, homogenized and inoculated onto PDA media in petri dishes. They were incubated for 72 hours at 25^oC. Pure cultures of organisms were produced from the mixed culture and incubated for 72 hours at 25^oC. Thin smears of the organisms were prepared on microscope slides, two drops of lactose phenol cotton blue were dropped on the slide, covered with cover slip and viewed using light microscope at X40 magnification.

Results and Discussion

Five different culturable fungal species were identified from the oral cavity of 15 students. They were *Candida* (50%) *Aspergillus* (33.3%), *Fusarium* (23.3%), *Blastomyces* (10%) and *Histoplasma* (6.6%) (Table-1). Fungal species isolated were not diversified, compared to a study, which recorded 74 fungi in the oral cavity of healthy individual in Ohio, USA⁷. *Candida* were the most prevalent fungal mycobiome found among the subject. Some studies also found *Candida* as the most prevalent organism in mouth of healthy people and stated that *Candida* are dominant in the oral cavity of healthy people^{7,9}. Studies have shown that *Candida* expresses virulence factors and can cause different manifestations of oral candidiasis, hence the treatment of mucosal infections caused by *Candida* and the elucidation of the disease process have proven challenging¹⁰. *Candida* species present in the oral cavity may be unharmed but may become virulent, causing mucosal lesions, under certain conditions¹¹.

The colony forming unit varied in among the subject and predominated by brown and black colours respectively. Some students harboured more than one organism (Table-2). *Aspergillus* and *Fusarium* spp. were next to *Candida* in total abundance. The present research found *Candida*, *Aspergillus* and *Fusarium* as major opportunistic oral mucosal fungi species.

Aspergillus species are preponderant in both indoors and outdoors environment. Aspergillosis (a fungal infection caused by *Aspergillus* species) of the oral cavity is an uncommon condition which most frequently occurs in immunocompromised patients, such as those with haematological malignancies¹²⁻¹⁴. *Histoplasma* and *Blastomyces* were recorded very low; 6.6% and 10% respectively, they could be uncommon pathogen in the oral cavity. *Histoplasma capsulatum* cause Histoplasmosis, a deep mycosis¹⁵.

Table-1
Percentage frequency of fungal microbiome in the oral cavity of healthy students

| Fungi species | No of plates | Percentage frequency |
|--------------------|--------------|----------------------|
| <i>Candida</i> | 15 | 50 % |
| <i>Aspergillus</i> | 10 | 33.3 % |
| <i>Histoplasma</i> | 2 | 6.6 % |
| <i>Blastomyces</i> | 3 | 10 % |
| <i>Fusarium</i> | 7 | 23.3 % |
| Total | 30 | 99.9% |

Table-2
Number of colony forming unit in each sample

| Samples | Colour of colonies | No. of colony forming unit | Species |
|---------|--------------------|----------------------------|----------------------|
| A | Brown | 3 | <i>Candida</i> |
| B | Brown, milk | 3,2 | <i>Candida</i> |
| C | Dark | 4 | <i>Aspergillus</i> |
| D | Milk | 3 | <i>Candida</i> |
| E | Dark | 2 | <i>Aspergillus</i> . |
| F | Brown, Dark | 3,3 | <i>Aspergillus</i> |
| G | Dark, Milk | 2,3 | <i>Candida</i> |
| H | Milk, Dark | 2,2 | <i>Histoplasma</i> |
| I | Milk, Dark | 3,2 | <i>Blastomyces</i> |
| J | Dark, Brown | 2,2 | <i>Aspergillus</i> |
| K | Brown | 3 | <i>Candida</i> |
| L | Dark, Milk | 2,3 | <i>Histoplasma</i> |
| M | Brown, Dark | 3,3 | <i>Candida</i> |
| N | Dark, milk | 2,2 | <i>Blastomyces</i> |
| O | Milk | 3 | <i>Fusarium</i> |

A-O: represent student samples

Conclusion

The present research found dominant of *Candida* and *Aspergillus* spp. in oral cavity of healthy students. Their clinical relevance could be a step towards predisposing the host to opportunistic infections. Understanding the relationships between different fungal species as well as between fungi and other members of the oral microbiome will shed light on the pathogenicity of oral microbiomes and may lead to the discovery of some therapeutic approaches for the prevention and treatment of oral cavity infections.

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