Isolation and Identification of Fungi and Bacteria Microbiomes among Women Attending Federal Teaching Hospital Abakaliki, Ebonyi, Nigeria

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The research is self sponsord

Abstract

Bacterial and fungi are the most dominant microbiomes which form part of the normal flora of the mouth, skin, gastrointestinal tract and the vagina. These organisms cause infections when their normal balance are altered. Fifteen high vaginal swabs (HVS) samples were collected from women within the child bearing age; 15 -30 years in Ebonyi State Teaching Hospital, Abakaliki, Ebonyi State. The aim of the study was to isolate and identify bacterial and fungi pathogens associated with genital tract infections and examine their prevalence in the female cycle. Samples were obtained with swab sticks and cultured on potato dextrose agar (PDA). The organisms isolated were *Aspergillus* (41.1 %), *Beauveria* (11.7 %), *Staphylococcus* (5.8 %), *Penicillium* (5.8 %), Yeast (5.8 %) *Gibellula* (5.8 %), *Actinomycetes* (5.8 %), *Streptococcus* (2.9 %), *Cornularia* (2.9 %), Gram +ve bacilli (5.8 %), *Candida* (2.9 %) and Indeterminate (2.9 %). *Aspergillus* (41.1 %) and *Beauveria* (11.7 %) were the most prevalent fungi pathogens. Prevalence of *Aspergillus* was high during menstruation and ovulation periods in the female cycle with prevalence rate of 46.6 % and 38.8 % respectively. The research reveals that fungal organisms were more dominant among the subjects and could be the main cause of genital tract infection.

Keywords: Genital tract, vaginal swab, fungi, bacteria, patient, Ebonyi State

1. Introduction

The female genital tract provides an adequate conditions for the colonization of diverse microbiomes. They are subject to changes over a life time and influenced by developmetal and hormonal changes (Dodson and Friedrich, 1997) The monthly ovulation, menstruation, hygiene and sexual activities alter the ecosystem of vagina and this have significant implications for a woman's overall health. The primary colonizing bacteria of a healthy individual are of the genus *Lactobacillus*. They were first described as the gate keppers of the vaginal ecosystem and plays a crucial role in protecting the host from urogenital infections. Other species are *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus jensenii*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus vaginalis*, and *Lactobacillus salivarius* (Redondo-Lopez *et al*., 1990: Elshibly *et al.,* 1996).

Previous studies have revealed fungal pathogens associated with the female genital tract, they include C*andida albicans, C. tropicalis, C. krusei, C. famata, C. keyfri, C. glabrata, Aspergillus niger, Aspergillus flavus* and *Fusarium* spp. amongst others causing infections and reduced fertility in women, these fungi pathogens are widespread in the environment (Chan *et al.,* 1994). A number of bacterial and non-bacterial infections exist that affect the female reproductive tract and cause vaginal discharge. Most women usually complain of vaginal discharge, discomfort or offensive odor (Dodson and Friedrich, 1997). The bacterial pathogens associated with genital tract infection *include Neisseria Gonorrhea, E. Coli, Streptococcus pyogens, S. aureus, Klebsiella aerogenes,* etc (Chessbrough, 2000).

The use of antibiotics changes the normal flora of vagina, upset the normal balance of bacteria and allows other pathogenic organisms to gain a foothold, it also allows *Candida* to grow causing vagina *Candidiasis.* (Elshibly *et al.,* 1996). Almost all women have a type of yeast growing harmlessly in the vagina. In most cases, vaginal diseases could be inferred from vaginal discharge which is a common symptom in primary health care and is often the second most common gynaecological problem after menstrual disorders (Dodson and Friedrich, 1997). Vaginitis an inflammation of the vagina was described as one of the most common and serious female genital tract infections seen in hospitals in developing countries (Yarzever and Ibrahim, 2013). Vaginitis are common world wide but mostly in Africa and Asian countries (Azuzu and Osoba, 1990). Approximately 30 % of all vaginitis cases are caused by infection with *Candida* species *(*Adad *et al.,* 2001)

Candida are yeast like organisms that are part of the normal flora within the vaginal area, and in healthy individual, do not produce disease. The major transmission process includes sexual behaviours, non-sexual contact like handing of infected hospital consumables, using or exchanging clothes by adolescent and others (Lucas and Gilles, 2003). Other physical factors which can contribute to genital tract infections include douching, constantly wet vulva due to tight clothing, chemicals coming in contact with the vagina via scented tampons, poor personal hygiene and prolonged use of antibiotics which will kill the benign and beneficial bacteria allowing yeast overgrowth (Miurs, 1985; Spiegel, 1991).

Currently in developing countries, the most neglected area of healthcare is the management of genital tract disease particularly vaginitis among others which is an immense health problem in developed and developing countries (Dixon *et al*., 1996.

Poor social economic status, inadequate knowledge and shortage of effective treatment all contribute to the high incidence of genital tract infections (Tyada *et al.,* 1992, Burrow and Bueshing., 1999). Genital tract infection (both sexually and non-sexually) continue to constitute health burden in women in developing countries especially among women of child bearing age. It is therefore imperative to study the fungal and bacterial pathogens associated with the genital tract infections among females attending Federal Teaching Hospital, Abakaliki, Ebonyi Nigeria.

2. Materials and Methods

2.1 Study Population and Collection of Specimen

Fifteen patients from Federal Teaching Hospital Abakaliki , Ebonyi State were selected from record book of women that were consulted by doctors in out patient. Permission was obtained from the hospital and Patients consent was sort prior to sampling. Collection of vagina swab samples followed WHO/UNAIDS (2007) guidelines. After collection swab sticks were then placed back into the container, represented with alphabets, and their various ages also indicated on the container. The specimen were transported to the laboratory immediately, and stored in the refrigerator prior to medium preparation.

2.2 Medium Preparation

The PDA media were prepared from raw irish potato, two hundred gram of unpeeled potatoes were weighed and cut into smaller piece, washed and boiled with 250 ml of distilled water for 30 minutes on a stove. The boiled potatoes were sieved using muslin cheese cloth, the extracted water was poured into a beaker and residues disposed, 20 g of glucose and 15 g of agar power were weighed and added into the extracted water and then mixed gently. The mixture was autoclaved at 121^{o}C for 15 minutes and allowed to cool for some seconds and then poured aseptically into petri dishes.

2.3 Inoculation of Sample

An inoculum pool was made by adding 1 ml of distilled water to swab specimen to get a homogenized mixture. Then a sterile syringe was used to pipette the mixture and smear on the media. Media were incubated at room temperature for 72 hours, pure cultures of organisms were produced

2.4 Microscopy

Temporary slides were prepared by picking a thin mycelia onto a slide with a sterile needle, two drops of Lactose Phenol Cotton Blue (LPCB) were dropped on the specimen, covered with cover slip and examined using a light (Olympus Optical B045781) microscope at x 40. Bacteria were identified by Gram's method following the procedures of Yar'zever and Ibrahim (2013). The photomicrographs of some identified organisms were taken.

3. Result

The number of colony forming units identified varied in different patients and the most predominant colour of their colonies were black (Table 1 & Table 2). Most of the patients harboured more than one organisms, organisms identified were; *Aspergillus*, *Beauveri*, *Staphylococcus*, *Penicillium*, Yeast, *Gibellula*, *Actinomycetes*, *Streptococcus*, *Cornularia*, Gram +ve bacilli, *Candida* and indeterminate. Fourteen patients had *Aspergillus* (41.1 %), four patients had *Beauveria* sp (11.7 %), two had *staphylococcus* (5.8 %) etc (Table 3). Age group 21-25 years harboured more organisms 14(41.1 %) (Table 4). *Aspergillus* prevailed higher at menstruation and ovulation with 46.6 % and 38.8 % respectively (Table 5). The result revealed that patients in the menstruation group harboured more of fungal organisms than bacterial organisms, with *Aspergillus* (46.6 %) being the most prevalent and *Candida* (6.6 %) the least (Table 5).

Table 1: Fungi and bacteria isolated from genital tracts in plate 1

Sample	COC	NCSFU	ORGANISMS
A	Black, light brown	8	*Aspergillus* spp.
B	Brown	3	*Aspergillus* spp.
C	Black	Dominant	*Aspergillus* spp.
D	Milky, brown	2, 3	*Penicillium* spp., *Aspergillus* spp.
E	black	7	*Aspergillus* spp.
F	Light brown , black	13, 17	*Aspergillus* spp.
G	Black	1	*Gibellula* sp.
1	Pink, black	3, dominant	*Cornularia* spp.
2	Black	dominant	*Aspergillus* spp.
3	Brown, black	9,19	*Aspergillus* spp.,*Giberlulla* spp.
4	Black	8	*Beauveria* spp.
5	Black	3	*Gibellula* spp.
6	Black, pink	12, 4	*Penicillium* spp.
7	Milky	7	*Candida* spp.

A-G, patients in ovulation
1-7, patients in menstruation
COC, colour of colonies
NCSFU, Number of colony species forming unit

Table 2: Fungi and bacteria isolated from genital tracts in plate 2

Sample	COC	NCSFU	ORGANISMS
A	Black, milky	2	*Aspergillus* spp.
B	White, black	4,3	*Penicilliums* spp., *Beauveria* spp.
C	Black, yellow	Dominant ,12	*Aspergillus* spp., Actinomycetes sp.
D	Black, light brown	7, 2	*Aspergillus* spp.,Beauveria spp.
E	Black, light green	6, 2	*Aspergillus* spp., *Candida*
F	Dark, milky	6,7	*Aspergillus* spp., *Staphylococcus*
G	Brown	9	*Gibellula* sp.
1	Pink	4	*Cornularia* spp.
2	Black	9	*Penicillium* spp.
3	Black, brown	3,4	*Aspergillus* spp., *Candida* spp.
4	Light brown	2	*Beauveria* spp.
5	Brown	Dominant	*Aspergillus* spp.
6	Pink	8	*Gibellula* spp.
7	Black, brown	Dominant,3	*Actinomycetes* spp., *Aspergillus* spp.

A-G, patients in ovulation
1-7, patients in menstruation
COC, colour of colonies
NCSFU, Number of colony species forming unit

Table 3: Percentage of Fungi and Bacteria species isolated from female genital tracts

S/N	**Organisms**	**No. of Cases**	**% of Isolates**
1	*Aspergillus* spp.	14	41.1 %
2	*Staphylococus* spp.	2	5.8 %
3	*Beauveria* spp.	4	11.7 %
4	*Penicillium* spp.	2	5.8 %
5	Yeast	2	5.8 %
6	*Gibellula* spp.	2	5.8 %
7	*Actinomycetes* spp.	2	5.8 %
8	*Streptococcus* sp.	1	2.9 %
9	*Cornularia* sp.	1	2.9 %
10	*Gram +ve bacilli*	2	5.8 %
11	*Candida* sp.	1	2.9 %
12	Indeterminate	1	2.9 %
	TOTAL	**34**	**99.3 %**

Table 4: Prevalence of organisms according to patients age

S/N	**Organisms**	**15-20**	**21-25**	**26-30**
1	*Aspergillus* spp.	4	5	5
2	*Staphylococus* spp.	-	-	1
3	*Beauveria* spp.	1	2	1
4	*Penicillium* spp.	-	1	1
5	Yeast	1	-	1
6	*Gibellula* spp.	-	1	2
7	*Actinomycetes* spp.	1	-	1
8	*Streptococcus* sp.	-	-	1
9	*Cornularia* sp.	-	1	-
10	*Gram +ve bacilli*	1	2	-
11	*Candida* sp.	-	1	-
12	Indeterminate	-	-	1
	No infected	**7**	**14**	**13**
	% infected	**2.8 %**	**41.1 %**	**38.2 %**

Table 5: Percentage frequency of bacterial-fungal pathogens isolated from the female genital tract at different periods of the female cycle

S/N	ORGANISMS ISOLATED	NO. OF CASES(MENSTRUATION)	NO. OF CASES(OVULATION)
1	*Aspergillus* spp.	7(46.6 %)	7(38.8%)
2	*Beauveria* spp.	1(6.6%)	3(16.6%)
3	*Penicillium* spp	-	2(11.1%)
4	Yeast	2(13.3%)	-
5	*Gibellula* spp	-	2(11.1%)
6	*Staphylococcus* sp	-	1(5.5 %)
7	Actinomycetes	2(13.3 %)	-
8	*Streptococcus*	-	1(5.5 %)
9	*Cornularia* sp.	-	1(5.5 %)
10	Gram + Bacilli	2(13.3%)	-
11	*Candida* sp.	1(6.6%)	-
12	Indeterminate	-	1(5.5 %)
	Total	15(99.7%)	18(99.6 %)

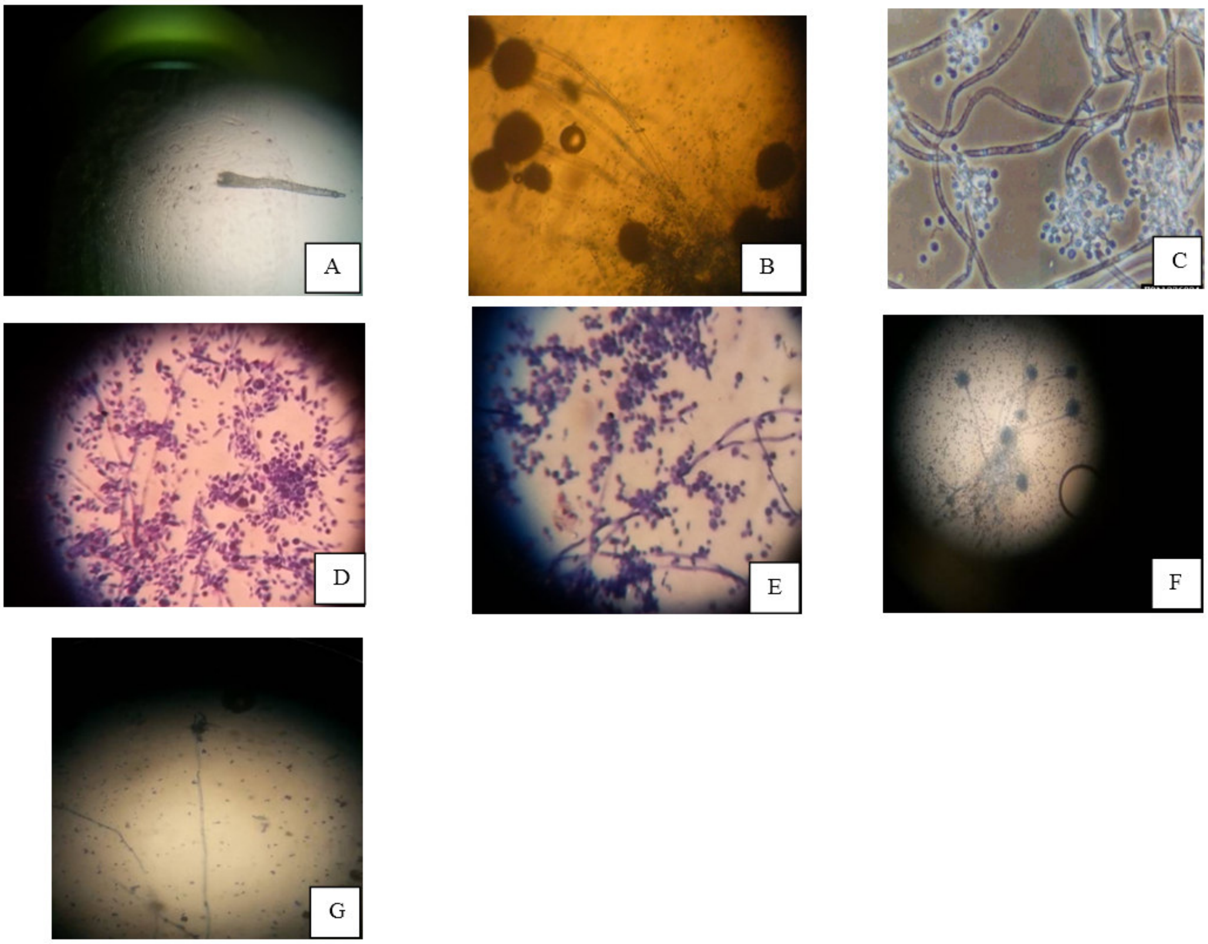

Figure 1: Photomicrograph of isolated organisms from the female genital tract.
A-*Cornularia* sp.
B-*Aspergillus* sp.
C-*Beauveria* sp.
D-*Staphylococci* sp.
E-Yeast
F-*Gibellula* sp.
G-*Penicillium sp.*

4. Discussion

The study found more prevalence of different fungal species among age group 21-25 years and 26-30 years. This finding is in consistent with that of Yar'zever and Ibrahim (2013), Orji (2015) who also found higher prevalence rate of organisms in genitall tract between the age group 21- 30 and 20-24 years. This could be as a result of more vulnerability of these groups to sexual activities or lack of awareness programs on prevention of genital infections, poor personal hygiene and overuse of antibiotics. Tyada *et al.* (1992), Burrow and Bueshing., (1999) stated that poor socio economic status, inadequate knowledge and improper medical treatment also contribute to the high prevalence of genital tract infections.. The age group 15-20 years, had less microbiome this may be as a result of their not yet being exposed to sexual activities compare to other ages, contrary to this finding, Edmund *et al* (1992) reported this age group (15-20) years to harbored more organisms.

Aspergillus spp. were the most prevalent organisms, this however disagrees with the reports made by other researchers (Yar'zever and Ibrahim, 2013: Isbor *et al,* 2015; Obidi *et al.,* 2009) who found *Candida* as the major etiological agent. *Aspergillus* is an opportunistic organism and people with impaired immune system are at higher risk of its oppurtunistic infection. *Aspergillus* species are associated with invasive infections in humans (Goodley *et al*., 1994). Gupta *et al.*, (2016) stated that immunocompromised hosts harboring leukemia or lymphoma and receiving chemotherapy and bone marrow transplant are vulnerable for infection by *Aspergillus* species. Also the high prevalence of *Aspergillus* may be associated with lack of knowledge and poor hygiene (Sallam,*et al* 2001; Lupiwa, *et al,* 1996; Hams, *et al*, 1996). *Aspergillus* is a genus composed of hundreds of mold species that can be found in different environments worldwide.Their spores are present in the air but usually do not cause disease unless it reaches an individual with a compromised immune system (Ventolini ,2014).

Candida sp. *are* yeast like organism that are part of normal flora within the vaginal area and in healthy individual do not produce disease, 30 % of all vaginitis cases are caused by infection with *Candida* species (Adad *et al*, 2001). In fourteen patients, only one had *Candida* sp. This present work therefore revealed fungal species as the lead etiological agent, only two bacteria species (*Staphylococcus* sp. and Gram +ve bacilli) were isolated.

Patients in the menstruation period harboured more organisms than those in ovulation period. *Aspergillus* spp. were the lead etiological agent in both periods, with slight difference among the two groups. At ovulation and menstruation periods of the female cycle, the body undergoes hormonal changes. High prevalence of *Aspergillus* spp. during menstruation may be due to improper hygienic process. The longer the pads, tissue or clothing materials used during menstrual period stay, the more stinky and conducive environment for proliferation of infectious organisms.

Two individuals had yeast organisms. Yeast are commensal organisms found in the skin, genital, gastrointestinal tracts and other mucosal sites in mammalians (Shokri *et al*.,2009). The bacteria pathogens were all Gram +ve organisms with *Staphylococcus* sp. and Gram +ve bacilli being the only bacterial pathogens identified. This could be an indicator that Gram +ve organism among other bacteria, could be the cause of most genital tract infection. Limited presence of *Candida* among the women suggest probably prolonged use of antibiotics. .

Conclusion

This research reveals that different patients harboured more than one organism with *Aspergillus* being predominant at ovulation and menstruation periods, it also revealed that fungal organism could be the main cause of genital tract infections as their prevalence were higher than bacterial organism. This study provides useful information for the diagnosis of female genital tract infection.

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