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# Distribution of Parasites of Digestive Tract of Humans in Onitsha Metropolis and Relationship with HIV Infection

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**Abstract:** Four hundred and seventy eight individual's who exhibited some manifestations of chronic and debilitating illness including persistent cough, skin cancer and dermatitis, multiple lymph adenitis, diarrhea and enteritis, genital sore, urethritis, vaginitis and weight loss were examined to establish relationships between human immuno-deficiency virus infection (HIV) and digestive tract pathogens in Onitsha metropolis. There was a significant relationship between HIV positive individuals and digestive tract diseases with HIV positive recording more digestive tract diseases with Candida albicans topping the table with 15.38% prevalence.

**Key words:** Candida Albicans · Parasites · Digestive Tracts and HIV

## INTRODUCTION

The digestive tract is one of the most common sites for clinical expression of human immune-deficiency virus infection [1, 2 and 3]. Many studies show that victims express oral Candidiasis, approximately one third of patients develop peri-rectal lesions due to Herpes Simplex virus [4], 30%-80% experience chronic or intermittent diarrhea. Several gastro intestinal diseases appear to be prevalent in these patients including visceral Kaposis sarcoma. Microsporidiosis, Crytosporidiasis, Cytomegalovirus of the digestive tract [5-8]. Many HIV Infected patients show symptoms of persistent diarrhea, fever and gastritis [9].

The cause of gastroenteritis may be due to proliferation of intestinal opportunistic parasites e.g protozoa, bacteria especially Salmonella species, fungi and viruses resulting from lowered immunity [10-12]. Persistent gastroenteritis with fever and other symptoms of AIDS including weight loss and dermatological manifestations should raise a warning signal to clinicians and other health workers. It is possible that persistent digestive tract disease may be indicative of HIV infection but the exact relationship between the two is not clear especially with regards to our Nigerian environment which may present different types of intestinal parasites from such types seen in caucatian HIV individuals [13, 14]. It was therefore necessary to evaluate intestinal parasites in HIV positive and negative individuals to determine the extent and distribution of the parasites.

## MATERIALS AND METHODS

**Sampled Population:** The study examined four hundred and seventy eight (478) individuals.

**Collection of Samples:** All information such as location, sex, age, occupation, marital and weights were obtained and data recorded. About 1-2grams of freshly passed stool sample were collected from each patient in a universal specimen container and labeled. Serum samples obtained from coagulated blood samples were used for HIV and Western blot analyses using Savyon Diagnostic Ashdod Israel and western blot for confirmatory test by Biorad Novo Blot from Paris, France respectively.

**Stool Routine Examination:** 0The stool samples were examined macroscopically for appearance prevalence of blood mucus and texture. A simple normal saline mount

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was made by picking a little quantity of the stool taken from suspicious sites and mounted in normal saline on a clean glass slide.

**Stool Concentration Technique:** All stool samples that yielded no cysts larva, ova or protozoa in the simple normal saline direct wet film examinations were subjected to concentration technique as follows:

About 1-2grams of stool were placed in a 20ml glass tubes. About 10ml of 10% normal saline was introduced in each 20ml glass tubes and sieved into small beaker using guaze strainers. This was transferred into 10ml glass tubes *i.e.* 6ml of suspension and 3ml of ether added, mixed well and centrifuged at 3000 rotation per minute for 1 minute.

By means of clean pasture pipettes, a few drops of the deposits were taken and mounted on slides, covered with cover slip and examined under low and high power objective lens of the microscope [10].

**Isolation of Digestive Tract Pathogens from Stool**

**Bacteria:** Portions of the stool were picked by means of sterile wire loop previously flamed over Bunsen burner and inoculated on Xylose lysine dextrose agar. The inocula were streaked out on the plates using the wire loop flamed in-between streaks to avoid crowded growths in the XLD agar. The plates were incubated at 37°C over night.

**Fungus:** Using a flamed wire loop, small portions of the stool samples were picked up and inoculated into Sabouraud agar plates, slants and slides in pairs and incubated for 14 days at 37°C also observing daily growths.

**Identification of Digestive Tract Bacterial Pathogens:** Yellow colonies growing on XLD agar with black centers were suspected *Salmonella* colonies. These suspected colonies were sub-cultured on Nutrient agar to stabilize the species and the pure growths subjected to biochemical identification as well as motility test by hanging drop method.

**Biochemical Identification of Digestive Tract Pathogens:** Sterile wire stabs were used to pick the suspected colonies and introduced into Durham fermentation tubes of glucose, lactose, sucrose and mannitol. Also, the wire stabs of suspected whoever was pierced through Triple Sugar Iron Agar (T.S.I.A) and sub-cultured onto Mackonkey agar to ensure purity. The fermentation tubes, TSIA tubes and plates were incubated at 37°C overnight. All colonies of suspected bacteria that were motile, fermented glucose and mannitol with acid and gas, lactose and sucrose not fermented, TSIA slant with alkaline (pink) and acid with gas but were identified as *Salmonella typhimurium*. The fermentation tubes with motile organisms that fermented glucose, did not ferment lactose and sucrose, acid mannitol and orange yellow acid (only) butt of TSIA were identified as *Salmonella typhi.*

**Serological Identification of Digestive Tract Bacterial Isolates:** A drop of saline was applied on clean glass slide and the bacteria colonies under test on Nutrient agar plates were collected with a sterile wire loop and emulsified on the slide to form moderate suspensions. Monovalent Salmonella type specific antisera (Oxford England) were added and steered slightly and observed for characteristic agglutination reaction.

**Identification of Digestive Tract Fungal Isolates:** The Sabourated agar slants and plates that grew suspected creamy colonies were tested as follows: The colonies were picked up with the tip of sterile capillary pipette and gently transferred and emulsified in 0.5ml sterile serum in a small test tube. Pooled human sera were used.

The above was repeated in a second tube using a known *Candida albicans* culture as positive control. The tubes were incubated at 37°C for $2\frac{1}{2}$ hours. The sera were mixed and transferred by means of pipette to a slide covered with coverslip. The slides were examined under high power and low powers of the microscope for the presence of short, lateral hyphal filaments (Green tube) formed by the yeast cells [10].

**Identification of Digestive Tract Parasites:** The X10 and X40 objectives of the microscopes were used to examine the slides that were mounted with emulsified stools and parasites identified pictorially continuously changing roundish structures with more than four nuclei identified as *Entamoeba histolytica.*

Roundish and occasionally oblong/oval flagellated highly motile cells were identified as *Trichomonas hominis*. By means of Pasteur pipette, drops of Lugol's iodine were applied to the slides to highlight and confirm the nuclear of *Entamoeba histolytica* [10].

Table 1: Distribution of Digestive Tract Pathogens in Onitsha Metropolis and Their Relations with HIV Infection

| | HIV positive | | | HIV Negative | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number Isolated | Prevalence Rate % | Total HIV positive | Number Isolated | Total HIV Negative | Total HIV Positive and Negative Isolates | Total No of HIV+ ve and –ve | Relative risk | Chi Sq. | Sig at P≤0.05 |
| Digestive Tract Isolates | | | | | | | | | | |
| *Trichomonas Hominis* | 25 | 14.79 | | 8 | | 33 | | 1.17 | 1.3 | Not significant |
| *Enta-Amoeba* | 21 | 12.43 | | 11 | | 32 | | 0.96 | 0.139 | Not significant |
| *Candida albicans* | 26 | 15.38 | | 16 | | 42 | | 0.87 | 0.97 | Not significant |
| *Salmonella typhi* | 6 | 3.55 | | 2 | | 8 | | 1.12 | 0.203 | Not significant |
| *Salmonella typhimurium* | 4 | 2.36 | | 2 | | 6 | | 0.98 | 0.043 | Not significant |
| Total | 82 | | 169 | | 309 | 121 | 478 | | | Not significant |

There was no significant relationship between individual digestive tract pathogen and HIV infection as shown in the Chi-Square values.

**Antibiotic Sensitivity Test:** Each of the isolates *S. typhimurium* and *S. typhi* were subjected to antibiotic sensitivity as follows: relevant antibiotic mentioned discs e.g *Gentamycin, Amoxycillin, Perflacin, Chloramphenicol, Ampicillin, Tetracyclin, Oflocin, Ciproxin, Sporidex Ciproval and Ceporex* were placed on each Mueller-Hinton sensitivity solid agar plates that has been previously seeded evenly with the test organisms diluted to $1x10^{4.5}$ organisms/ml, disc diffusion method and incubated over night at 37°C [11].

After incubation, the zones of inhibition surrounding the antibiotics sensitivity discs were taken as a measure of the inhibitory and bactericidal power of the drug against the particular test organisms and denoted as sensitive(s). The discs having the test organisms still growing around the discs were adjudged resistant.

Statistical comparison between digestive tract pathogens in HIV positive and negative individuals were carried out after the results were noted.

## RESULTS AND DISCUSSION

Digestive tract pathogens were isolated from some HIV positive and negative individuals who had no digestive tract manifestations. *Candida albicans* topped the list of these pathogens with a prevalence rate of 15.38%. Relative risk was 0.87 and Chi Square analysis 0.97. There was no significant relationship between HIV infection and *Candida albicans* prevalence. *Candida albicans* was also highest in prevalence in HIV negatives. Next in prevalence was *Trichomonas hominis*, in HIV positives and third in order of prevalence in HIV negatives. *Enta-amoeba histolytica* was third in prevalence in HIV positives and second in HIV negatives.

*Salmonella typhi* was fourth in HIV positives with 3.55% and equally fourth in HIV negatives (0.64%).

*Salmonella typhimurium* was the least in prevalence 2.36% in HIV positives and also the least in HIV negatives (0.64%). Total isolates was 48.52% out of the 169 HIV positives and 12.63% out of the 309 HIV negatives. Least prevalences were in 50 -59 years and 60 -69 years age group each recording zero isolate.

Highest prevalence of *Salmonella typhimurium* was recorded in 30-39 years 1.56% followed by 40 – 49 years, 0.93% next was 20-29 years 0.84%, least were 10-19 years, 50-59 years 60-69 years zero. Age group 20-29 years recorded highest prevalence of total pathogens 20.4%.

The twenty-three (23) cases of digestive tract disease yielded thirty-nine (39) pathogens distributed as seen in Table 1.

Some individuals expressed no digestive tract pathogens symptoms yet passed some pathogens in their stools. These are non-clinical individuals. When both clinical and non-clinical individuals were considered *Candida albicans* topped the list, 15.38% prevalence followed by *T. Hominis* 14.79% and *Ehistolitica* 12.43%, *S.Typhi* recorded 3.55% and the least being *S. typhimirium* 2.36% for HIV positive individuals.

Yeast cells (*Candida albicans*) proliferate in situation where the body loses immunity either by unrestricted use of cytotoxic drugs or in immune deficiency cases [11].

Contrary to our local environment especially Onitsha metropolis and perhaps Anambra State, the commonest pathogens identified in stools of HIV sufferers among Caucasians particularly in USA are *Pneumocystis carinii*. Cytomegalo virus of the digestive tract and *Crypsto-sporidium carinii*

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