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BIOCHEMICAL PROFILE AND RESISTANCE OF BACTERIAL AND FUNGAL AGENTS ISOLATED FROM DIARRHEA IN PEOPLE LIVING WITH THE HUMAN IMMUNODEFICIENCY VIRUS, N'DJAMENA

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ABSTRACT
Immunocompromised people such as HIV/AIDS patients are often subject to diarrheal diseases caused by bacteria and fungi. This study aimed at assessing the biochemical and antibiotic resistance profile of bacteria and fungi isolated from HIV patients. Stool samples were collected and analyzed according to standard clinical microbiology procedures for isolation of the etiological agents of diarrhea, using Hektoen, Mueller-Hinton and Sabouraud media. The filamentation assay, MGG staining, methylene blue and Amann's Lactophenol were used to identify different fungal agents. The biochemical characteristics of isolated fungi and bacteria were determined through agglutination tests, rack and API 20 E galleries. The susceptibility tests were performed by diffusion method on Casitone and Mueller-Hinton agar. The presence of bacteria was linked with CD4 count less than 50. The occurrence of fungi (85.49%) and bacteria (14.50%) isolates were significant (p = 0.001) and showed different range of sensitivity. Majority of bacterial strains were resistant to beta-lactams (50%) and sensitive (85%) to quinolones. The fungal strains were sensitive (83.63%) to the azole derivatives and resistant (96%) to the polyenes. Azole derivatives are more active than polyenes. The activity of fluconazole is limited. We recommend that prescribers recommend the use of 5-fluorocytosine, econazole and voriconazole which are more active and can be an alternative to fluconazole. The most active antibiotics are quinolones. Ciprofloxacin is the most active antibiotic and can be an alternative to nalidixic acid.

KEYWORDS: Diarrhea, HIV, microorganisms, biochemical, resistance, N'Djamena.

INTRODUCTION
Acute and chronic diarrhea in patients infected with the human immunodeficiency virus (HIV) constantly increased. During the last twenty years, there has been a significant increase in the number of nosocomial infections caused by pathogenic yeasts such as *Candida* of species. Though *Candida albicans* remains the main agent responsible for candidiasis with 50 to 70% of cases, there is a rise of non-albicans species that are often resistant to conventional treatments, such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. lusitaniae* (Mahideb, 2015). The resurgence of candidiasis is linked to the increase number of immunocompromised patients, the improvement of diagnostic techniques that can detect both deep or superficial fungal infections, and the rapid emergence of strains resistant to antifungal treatments (Chochillon and Forget, 2019). The poor management of patients in Africa is associated with the lack of

microbiological or even endoscopic diagnostic tools in the majorityof healthcare facilities. So, majority of cases are usually diagnosed at very advanced stages with severe immunosuppression and specific treatments are absent or unavailable (Pfaller et al., 2006). Therefore, mycoses in immunocompromised individuals such as HIV-positive patients represent a public health problem **(Vandeputte, 2008).** In fact, fungal infections are generally benign in immunocompetent individuals while inimmunocompromised individuals such as HIV/acquired immunodeficiency syndrome (AIDS) patient, fungal infections like dermatophytosis and candidiasis can persist for long periods (Hellard et al., 2000). *Candida* species caused 90% of digestive candidiasis. Candidiasis is usually caused bysaprophytic microorganisms that become pathogenic due to host defense mechanism failure as it is the case of HIV infection. The resulting diarrhea represents the second most important opportunistic infection in Africa (WHO, 2000). In Africa, 60 to 90% of HIV-infected patients have diarrhea (Hamidou et al., 2016). It is almost constant during the AIDS stage, where it causes disability with a big impact on the quality of life of the patient. Besides, diarrhea accelerates the decline of immunity in HIV-infected individuals (Vandeputte, 2008). Management of diarrhea, which is often the first reason for consultation, requires exact biological diagnosis and appropriate treatment (Slim. A, Bourdon, 1984). The microorganisms most often incriminated in diarrhea in HIV-infected people are parasites, fungal agents and bacteria. The fungal agents are involved in the degradation of the immune system and are responsible for nearly 40% of mortality (Laaid, 2009). In healthcare facilities, the increases in severe mycoses are directly related to the increase of iatrogenic factors. Advances in resuscitation, organ transplants and anticancer treatments increased the risk infections, with fungal infections being the most important. For a long time, only one molecule: amphotericin B (Fungizone) was available to treat severe mycoses due to his broad spectrum of activity on most yeasts and filamentous fungi (www.biomerieux.fr). In the 1980s, other systemic antifungals of the azole class appeared such as fluconazole (Triflucan®, Pfizer Laboratory) and itraconazole (Sporanox®, Janssen Laboratory). It was at this time that the AIDS epidemic was revealed, among other things, by numerous oropharyngeal and esophageal candidiasis, whose clinical failures with azoles contributed to the development of in vitro susceptibility tests (Robstaille and Fleutry, 2011). Digestive disorders can be manifested by chronic diarrhea or dyspepsia, requiring digestive biopsies for histopathology with etiological aims (Dieng et al., 2005, Botton et al., 1990).

Beta-Lactams are the most important and most widely used class of antibiotics in the world. This high utilization is due to their broad spectrum of action, low toxicity, effectiveness and relatively low cost (Bessimbaye et al., 2013). Therefore, the reduction of the effectiveness of these molecules in both human and veterinary pathologies is a serious concern. The most common incriminated bacterial strains include Meticillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae* with reduce sensitivity to Penicillin, Imipenem-resistant *Pseudomonas aeruginosa*, and Cephalosporin-resistant Enterobacteriaceae (Yusuf et al., 2013).

This study aims to evaluate the prevalence and biochemical profile of fungal and bacteria agents involved in diarrheal diseases in people living with HIV (PLHIV) and to determine the susceptibility of bacteria and fungi to respectively antibiotics and antifungal. The results of this work could serve as a good tool to advocate for appropriate prevention strategy and the implementation of effective antibiotic therapy against the etiological agents of diarrhea in PLHIV in Chad.

MATERIALS AND METHODS

Type, Site and duration of the study

This is a prospective etiological diagnosis of diarrhea in HIV-infected patients during a period of twelve (12) months from June 1, 2018 to May 31, 2019. Microbiological stool diagnostic tests were performed at the Bacteriology unit of Laboratory of Research, Diagnostics and Scientific Expertise (Labo-ReDES) of the Faculty of Human Health Sciences (FSSH) of the University of N'Djamena.

Ethical consideration

The research protocol used in this study received approval from the Ministry of Public Health with registration number: No124 / PR / PM / MSP / SG / DGAS / DSPELM / 09.

Similarly, people living with HIV (PLHIV) and suffering from diarrhea gave their informed consent to participate in the study after explaining the objectives of the study as well as the advantages and inconveniences of the survey through individual interviews.

Surveys

Consecutive non-probability sampling was performed, and the sample size was 531 patients. All HIV-positive people with signs of digestive disorders, naive to antifungal and antibiotics who agreed to participate in the study and followed up at the N'Djamena Center for Care (Center Al-Nadjma) and the Hospital of the day of the University Hospital Center of National Reference of N'Djamena (CHU-RN) were included in the study. Investigations were also conducted on: origin, sex, age, T4 cell count, hemoglobin level, antiretroviral (ARV) intake, and suspected origin of contamination (source of drinking water, food and the entire lifestyle of PLHIV.

Data processing

The data collected were computed and analyzed using Word 2013 and Excel 2013 software. The comparison of two qualitative variables was achieved using the chi-

square test (χ^2) analysis. The value of $p \leq 0.05$ was considered significant.

Stool examination

The stool samples were diluted in physiological saline and subjected to X10 and X40 microscopic examination with or without dye for detection of yeasts and molds. Microscopic observation was done either before or after culture. Examination of fixed and stained preparations was performed using May-Grünwald-Giemsa (MGG), methylene blue and Amann's Lactophenol (1.44). Lactophenol is particularly useful for washing the preparations made in methyl blue prepared with lactophenol. This allow to discolor the background and improve the contrast making it interesting for photomicrography. In addition, the viscosity of lactophenol limits the movement of objects during exposure. Conventionally, the detection of these associations is done by transplanting culture on Sabouraud medium containing triphenyl-2, 3,5-tetrazolium. Each species is more or less able to reduce this substance and released a colored compound. Colonies with different colors (from white to purplish red) should be considered as strains of different species, which should be subcultured and identified separately.

Isolation and characterization of bacteria and fungi

The isolation of germs was carried out after seeding stools on Hektoen agar, Sabouraud Chloramphenicol agar (Bio-Rad®) and incubate for 18 to 24 hours in a bacteriological oven at 37°C. After incubation, green and bluish colonies with or without a black center on Hektoen agar are suspected (*Salmonella*, *Shigella*). The whitish and blackish colonies on the Sabouraud chloramphenicol agar are suspected of mold (microscopic fungi of the genus *Aspergillus*, *Candida*...). Colonies on Sabouraud Chloramphenicol were seeded in 1 ml of one-day human serum and incubated in an oven at 37 ° C for 18 to 24 hours for the filamentation test. After 18 to 24 hours of incubation, 50µl of serum seeded were mounted between slide and cover slip for the microscopic search of filamentous yeasts at objective x 40. The yeasts showing filaments were identified with *Candida albicans*. *Aspergillus* and other fungi were also identified by their cultural characteristics and microscopic identification at the x 40 objective (Chabasse et al., 2002).

Colonies on Hektoen agar were subculture onto Mueller-Hinton (MH) agar for Gram stain, oxidase assay and antigenic studies. The agglutination test was performed according to the instructions of Kaufmann and White (Pilet et al., 1979) using sera: polyvalent anti-*Shigella* type 1, anti-flexneri, anti-boydii and anti-sonnei (Bio-Rad®) for *Shigella* research; anti-*Salmonella* (OMA, OMB, OMC and Vi) (Bio-Rad®) for *Salmonella* research.

The agglutination test with *E. coli* latex reagent was used following the manufacturer's instructions for the identification of *Escherichia coli* O157 H7.

The API 20 E gallery and the rack gallery allowed the identification of the biochemical characteristics of isolated bacterial and fungal.

The strains were stored at -80°C in the 15% glycerol brain-heart broth (Bio-Rad®) for future molecular characterization according to the guidelines of the National Committee on Clinical Laboratory Standards (NCCLS, 1998).

Drug susceptibility testing of Antibiotic and Antifungal

The manual method and the automated method using the Vitek Compact 15were used to determine the antibiogram and antifungigram:

Disk method

The disk method uses disks loaded with either the antibiotic or the antifungal drugs that are plated on an agar culture medium inoculated with bacteria or fungi by flooding or swabbing. The drug diffuses into the agar creating a zone of growth inhibition of the germ around the disc. Strains can be classified into sensitive (no growth), intermediate (low growth) or resistant (growth) depending on the diameter of the zone of inhibition.

A large number of antifungal are available in tablets:
- A semi-synthetic medium from the Institute Pasteur of Paris (I.P.P.) to test the action of 5-Fluorocytocin (5-FC) and Imidazole derivatives;
- A complex medium with casitone and yeast extract, also manufactured by I.P.P to test the polyenes (Ampphotericin B) and Imidazole derivatives (their zone of inhibition is clearer on this medium).

Mueller-Hinton (MH) agar was used to perform the antibiotic susceptibility test.

For each medium, a volume of 25 ml was used for a Petri dish 90 mm in diameter giving a thickness of 4 mm according to the work of Standardization Group of the Antibiogram of the French Society of Medical Microbiology (SFMM) and the European ASFT-EUCAST reference technique (Antifungal Susceptibility Testing - European Committee on Antimicrobial Susceptibility).

Antibiotics and antifungal used for susceptibility testing against isolated bacterial and fungal agents are summarized in the table 1.

Table 1: Antibiotic and Antifungal Drugs Used For Drug Susceptibility Testing.

Category	Class	Anti-infective	Dose/disc
Antibiotic (Bio-Rad)	Sulfamides	Cotrimoxazole (SXT)	25 µg
	Beta-Lactamines	Amoxicillin (AMX)	25 µg
		Amoxicillin + clavulanic acid (AMC)	20/10 µg
		Ceftriaxone (CRO)	30 µg
	Fluroquinolone	Ciprofloxacin (CIP)	5 µg
	Quinolone	Nalidixic acid (NA)	30 µg
	Cyclines	Tetracycline (TET)	30 µg
Antifungal (BioMérieux)	Azoles	5-Fluorocytosine (5-FC)	1 and 10 µg
		Econazole (ECZ)	50 µg
		Clotrimazole (CLZ)	50 µg
		Fluconazole (FCZ)	50 µg
		Voriconazole (VRZ)	50 µg
	Polyenes	Amphotericin B (AMR)	100 µg
		Nystatin (NYS)	100 IU

Automated method: Vitek Compact 15

The system includes the VITEK® 2 Compact instrument, a computer (workstation) and a printer. The software supplied with the VITEK® 2 Compact system includes data analysis and data management programs. A two-way computer interface automatically transfers the results to the user of the laboratory information system (LIS) and to various product and patient reports. A quality control system is available to validate a VITEK® 2 Compact system test kit. An Advanced Expert System (AES) system (clinical use) is available to allow systematic and on-line validation of results and interpretation of resistance phenotypes that have been demonstrated by antibiograms.

This technique determines the sensitivity of bacteria and fungi to antibiotics and antifungal in a semi-liquid medium. The VITEK®2 card contains 64 reaction wells containing 64 antibiotics at 64 concentrations and four antifungal (fluconazole, voriconazole, flucytosine and amphotericin B) at four different concentrations. The reading is performed at 660 nm. The growth rate is analyzed every 15 minutes in kinetics. Then, for each antibiotic, a specific algorithm converts the raw values (RTU) into a calculated MIC. To calculate the CMI, the controller checks the filling of the wells and then checks the raw values. It eliminates outlier values related to background noise and strains that are difficult to suspend in solution. Then, it determines the incubation time of the card. In the control well, it assesses the growth rate of the germ. The reading will stop if the growth in all wells is enough. This will be considered as the highest value that can be measured for this antibiotic. Finally, the MIC results are interpreted as S-I-R (sensitive-intermediate-resistant) according to the specific critical concentrations of the various committees.

The inoculum preparations for the isolation of bacterial and fungal agents were made according to the manufacturer's procedure. Using a dispensator, 3 ml of saline solution (Reference 1204, 500 ml, 0.45% NaCl) were distributed into the 5 ml tubes in a cassette. Then, a colony of the bacterium or yeast was suspended in 3 ml of saline solution using a Pasteur pipette andhomogenized. After what, the optical density was checked with the DensiChek McFarland and adjusted to 0.5-063 McF for bacteria Gram (-) and 1.80-2.20 McF for yeasts. Each sample were subjected to biochemical identification, antibiogram or antifungigram. A Gram (-) V1 221 pipette (0.5-250 µl) was used to dispense 145 µl of samples in 3 ml of antigenic saline solution (GN = Gram (-) and AST = corresponding Antibiotic) for each identification. Similarly, for yeasts, a Gram (+) V1 222 pipette (100-1000 µl) was also used to distribute 280 µl of sample in 3 ml of antifungal saline solution (YST = Yeasts identification and YST 10 = corresponding Antifungal). The biochemical, antibiogram or antifungigram identification cards were inserted into the suspensions arranged in the cassette and all was introduced into the VITEK2. Once the cassette is inserted into the VITEK2, the system was started to load and read the barcode of each card and seal them. Thereafter, the identification cassette was removed and VITEK2 proceeded with the analysis. The minimum inhibitory concentration of antimicrobials were provided by VITEK2 according to the European Antibiogram Committee (CAEU).

Determination of extended spectrum beta-lactamase (ESBL) phenotypes

Evidence for extended spectrum beta-lactamase (ESBL) phenotypes was also assessed on Muller-Hinton agar by the double disk synergy assay method according to the procedure of Jarlier et al. (1988). The tested strains were inoculated on the agar plate by the standard antibiogram method. The disks of cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), and aztreonam (30 µg) were placed 20 to 30 mm (center to center) from an amoxicillin disk. / clavulanic acid (20/10 µg) then incubated at 37°C. After 18 to 24 hours of incubation, the production of ESBL by the organism tested was based on the partial inhibition of ESBL by the acid clavulanic. The presence of any synergy between cefotaxime, ceftazidime, cefepime, aztreonam and clavulanic acid was characterized by a champagne-capped sharp.

RESULTS

Study population, biological and clinical parameters of patients

The study involved 531 subject, aged between 9 to 65 years, including 357 (67%) women representing the 2/3 of people PLHIV followed up at HIV healthcare center in N'Djamena, Chad, and 174 (34%) men. The average age of patients was 37 ± 28 years and 43% of them belong to the 20 to 30 age group. In this study, 276 (52%) of PLHIV were not infected with bacteria and fungi had a CD4 count greater than 500 / mm^3, while 218 (41%) PLHIV were infected with fungi had CD4 counts below 100 / mm^3 and 37 (7%) PLHIV infected with bacteria had a CD4 count less than 50 / mm^3 with hemoglobin levels ranging from 10 to 7 g / dl. There was no significant correlation between CD4 count and fungal infection (χ^2 = 0.23, ddl = 1, p = 0.50). Only 317 (60%) PLHIV were under antiretrovirals (ARV).

Prevalence of different opportunistic germs isolated from the stools of PLHIV

The table 2 shows the number of cases infected with opportunistic microorganisms. Out of the 531 cultures performed, 255 cultures were positive (48%) with 218 (85.49%) positive to fungi and 37 (14.50%) to bacteria (x^2 = 33.85, dd1 = 1, p = 0.001 significant difference).

Prevalence of infection of microorganisms by age group

The rates of bacterial and fungal infection were 12 (32.43%), 12 (25%), 3 (8.10%), 5 (16.21%), 5 (16.21%) and 51 (23.39%), 54 (25%), 50 (23%), 35 (16.05%), 28 (13%) respectively with regard to the age group; 9-19 years, 20-30 years, 31-41 years, 42-52 years and 52 years and older. The most affected age groups include those aged 20-30 and 52 and above with respectively 16.21% and 16.05% (Table 2).

Biochemical profile

Most of *Salmonella* Typhi isolates shared common biochemical characters: ONPG -, Urea -, TDA -, Simmons Citrate -, Indole -. H_2S +, ADH +/-, LDC +, ODC +, CIT +, GLU +, MAN +, SOR +, RHA +, ARA + and are all mobile. We also noticed that H_2S + was weakly positive after 24 hours of culture and more pronounced from 48 to 72 hours. The majority of *Shigella* isolates showed negative biochemical characters but were all glucose + and catalase +. The absence of gas in the presence of glucose is an indication of suspicion.

Shigella isolates were all motionless under microscopic observation.

Escherichia coli isolated showed ONPG +, ADH +, LDC +, IND + and were able to ferment most sugars.

Majority of fungi isolated were GLU +, SAC +, MAL + GAL + as shown in table 3.

Susceptibility of antifungal and antibiotics to fungi and bacteria isolates

Results from our investigation showed that, antifungal were sensitive in the average order of 53.57%, and 46.42% resistant to fungi isolates. The fungi isolates were 65% more sensitive to the azole derivatives, while 35% were resistant. On the other hand, they were much more resistant to the polyenes with 74.5% of occurrence and only 25.5% sensitive (Table 4).

Most isolated bacteria were resistant to cyclins (tetracycline), beta-lactamines (amoxicillin + clavulanic acid and ceftriaxone), and sulfonamides (cotrimoxazole) with respectively 46%, 50% and 68% (Table 4).

Table 2 shows the distribution by number of cases and prevalence of microorganisms isolated in the stool in patients with HIV infection.

Table 2: Distribution of microorganisms according to age group.

Microorganisms	**Age range**				
Bacterial agents	**9-19 years**	**20-30 years**	**31-41years**	**42-52years**	**52 years and +**
Shigella spp	-	-	-	-	1
Shigella flexneri	5	8	2	4	1
Salmonella Typhi	1	3	1	1	1
E. coli (O111+O55+O26)	6	-	-	-	-
Escherichia coli O157 H7	-	1	-	-	2
Total (%)	**12 (32.43)**	**12 (32.43)**	**3(8.10)**	**5 (16.21)**	**5 (16.21)**
agents fongiques					
Candida anbicans	31	18	19	23	16
Cryptococcus laurentii	3	7	2	1	3
Candida lipolytica	8	6	11	3	1
Candida crusei	-	6	7	-	1
Apergillus niger	4	10	2	1	3
Apergillus fumigratus	5	7	9	7	4
Total (%)	**51 (23.39)**	**54 (25)**	**50 (23)**	**35 (16.05)**	**28 (13)**

% = percentage, *E.coli* = *Escherichia coli*

Table 3 shows the biochemical profile of fungal and bacterial agents isolated in the stool in HIV-infected patients.

Table 3: Biochemical profile of fungal and bacterial agents with tested carbohydrates.

Microorganism	**Carbohydrate**																						
Fungal agent	**ONPG**	**ADH**	**LDC**	**ODC**	**CIT**	**H_2S**	**URE**	**TDA**	**IND**	**VP**	**GEL**	**GLU**	**MAN**	**INO**	**SOR**	**RHA**	**SAC**	**MEL**	**AMY**	**ARA**	**LAC**	**RAF**	**MAL**	**GAL**
Candida anbicans												+		-			+	-			-		+	+
Cryptococcus laurentii												+		+			+	-			-	+	+	+
Candida lipolytica												+		-			+	-			-	-	+	+
Candida krusei												+		+			+	-			-		+	+
Apergillus niger												+	+/-	+			+				-			
Apergillus fumigratus												+		-			+				-		+	+
Bacterial agent																								
Shigella spp	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+				-
Shigella flexneri	-	-	-	-	-	-	-	-	+/-	-	-	+	+	-	-	-	-	+/-	-	+/-				-
Salmonella Typhi	-	+/-	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-			-
E. coli (O111+O55+O26)	+	+	+	-	-	-	-	-	+	-	-	+	+	-	+	+	+	+	-	+	+			+
Escherichia coli O157 H7	+	+	-	-	-	-	-	-	+	-	-	+	+	-	-	+/-	-	-	-	+/-	+			+

+ = positive (use of carbohydrate by the microorganism); **- =** negative (non-use of carbohydrate by the microorganism) ; +/- = sometimes positive or negative.

NB: empty boxes: these are biochemical tests not carried out.

ONPG = Ortho-Nitro-Phényl-Galactopyranosidase; ADH b= Arginine Dihydrolase ; LDC = Lysine Décarboxylase, ODC = Ornithine Décarboxylase ; CIT = Simmons Citrate ; Urea H_2S=Dihydrogen sulfide ; TDA = Tryptophane Désaminase ; IND = Indole ; VP ; Vogues-Proskauer ; GEL = Gelatin ; GLU = Glucose ; MAN = Mannitol ; INO = Inositol ; SOR = Sorbitol ; RHA = Rhamnose ; SAC = Saccharose ; MEL = Melibiose ; AMY = Amygdalin ; ARA = Arabinose, Oxidase, LAC = Lactose, RAF = Raffinose ; MAL = Maltose ; GAL = Galactose.

Table 4 shows the sensitivity profile of fungal and bacterial agents isolated in the stool in HIV-infected patients.

Table 4: Sensitivity profile of fungal and bacterial agents to tested antibiotics and antifungal.

Microorganism	**Antibiotic**															**Antifungal**													
Fungal agent	**Nbre T**	**AMX**		**AMC**		**CRO**		**CIP**		**NAL**		**TET**		**SXT**		**AMR**		**ECZ**		**5-FC**		**FLZ**		**CLZ**		**NYS**		**VRZ**	
		S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Candida anbicans	107															39	68	57	50	87	20	41	66	49	58	11	96	65	42
Cryptococcus laurentii	16															5	11	12	4	14	2	13	3	9	7	1	15	12	4
Candida lipolytica	29															10	19	21	8	23	6	25	4	15	14	3	26	21	8
Candida krusei	14															6	8	11	3	14	0	13	1	2	12	6	8	13	1
Apergillus niger	20															9	11	14	6	17	3	12	8	11	9	3	17	16	4
Apergillus fumigratus	32															13	19	22	10	25	7	24	8	21	11	4	28	25	7
Total (%)	**218 (100)**															**82 (38)**	**136 (62)**	**137 (63)**	**81 (37)**	**180 (83)**	**38 (17)**	**128 (59)**	**90 (41)**	**107 (49)**	**111 (51)**	**28 (13)**	**190 (87)**	**152 (70)**	**66 (30)**
Bacterial agent																													
Shigella spp	1	0	1	1	0	1	0	1	0	1	0	1	0	0	1														
Shigella flexneri	20	11	9	13	7	14	6	20	0	16	4	13	7	9	11														
Salmonella Typhi	7	4	3	5	2	5	2	7	0	6	1	4	3	3	4														
E. coli (O111+O55+O26)	6	1	5	2	4	1	5	6	0	2	4	2	4	0	6														
Escherichia coli O157 H7	3	0	3	1	2	0	3	3	0	1	2	0	3	0	3														
Total (%)	**37 (100)**	**16 (43)**	**21 (57)**	**22 (59)**	**15 (41)**	**21 (57)**	**16 (43)**	**37 (100)**	**0 (0)**	**26 (70)**	**11 (30)**	**20 (54)**	**17 (46)**	**12 (32)**	**25 (68)**														

Nbre T=Nomber tested; Résistant = intermédiaire+résistant (I+R) ; sensible = S

Antibiotics : Cotrimoxazole/triméthoprim-sulfaméthoxazole (Cot/SXT: R < 10, 10 ≤ I≤ 18, S ≥ 19); Amoxicillin (AMX : R<11, 11≤ I ≤ 16, S≥ 17), Amoxicillin + clavulanic acid (AMC : R<11, 11≤ I ≤ 16, S≥ 17) Ceftriaxone (CRO : R< 15, 15 ≤ I≤ 20, S≥ 21) Ciprofloxacin (CIP : R< 21, S ≥ 25) ; Nalidixic acid (NA: R< 21, S ≥ 25) Tétracyclin (TET: R< 21, 21 ≤ I≤ 20, S ≥ 23) (CASMF, 2018).

Fungal : 5-Fluorocytosine (5-FC), Econazole (ECZ), Nystatin (NYS), Clotrimazole (CLZ), Fluconazole (FLZ), Voriconazole (VRZ) et Amphotéricin B (AMR).

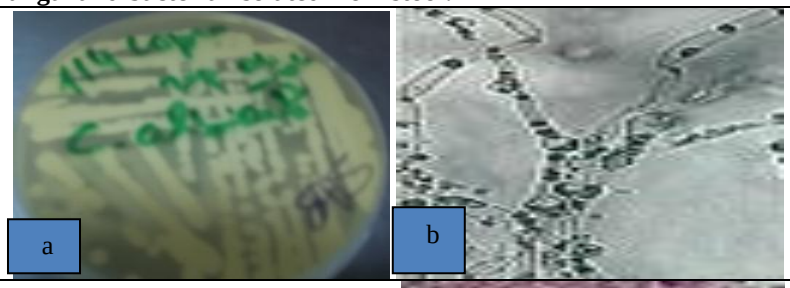
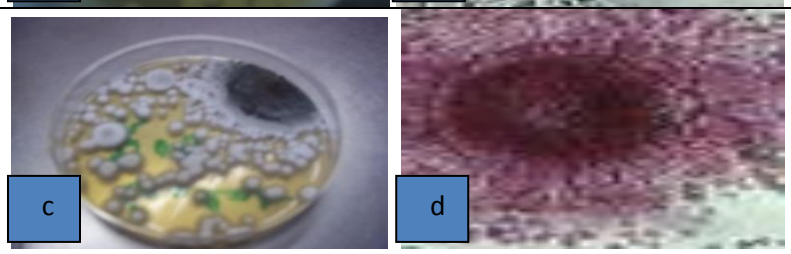
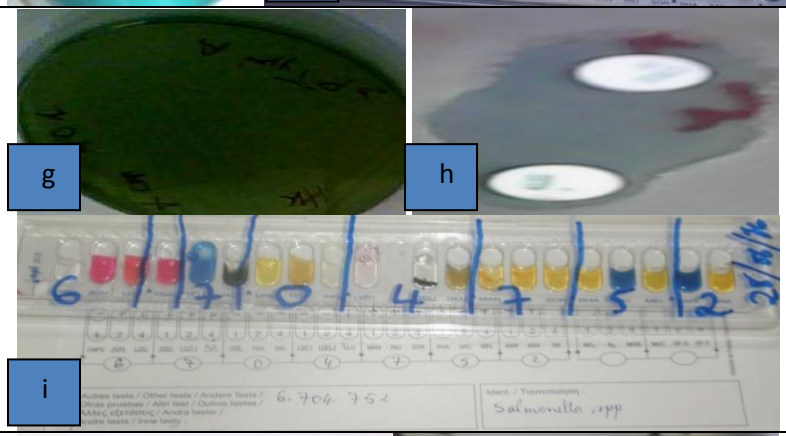
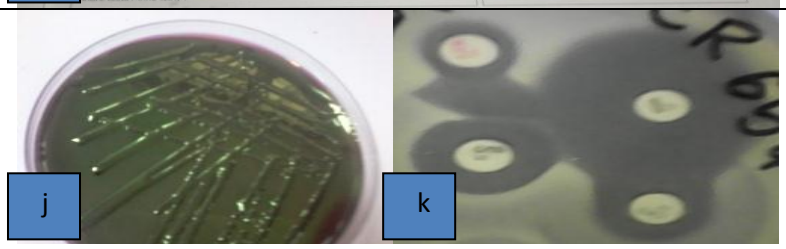
NB : cases vides : ce sont des tests de **sensibilité** non effectués.

Les CMI des antifongiques du VITEK2 sont issues des souches de contrôle de qualité : *Candida parapsilosis* ATCC® 22019 et *Candida krusei* ATCC® 6258(CLSI®)

Les CMI des antibiotiques du VITEK2 sont issues des souches de contrôle de qualité : *E. coli* ATCC® 25922, *E. coli* ATCC® 35218 et *Pseudomonas aerugginosa* ATCC® 27853 (CLSI®).

Table 5 shows the macroscopic and microscopic aspects of fungal and bacterial agents isolated in the stool in patients with HIV infection.

Table 5: Microscopic characteristics of fungal and bacterial isolated from stool.

1	a: Cottony colonies (whitish: *Candida albicans*) b : positive filament test showing *Candida albicans*	
2	C : Colonies black on the front with white halo and on the back the color is gray and fast growing. d : microscopic appearance of *Aspergillus niger* at magnification x 40 after MGG staining, Methylene Blue and Aman Lactophenol (1.44).	
3	e : Bluish colonies without black center f : Biochemical profiles (0.000.4000, 0.004.100) of *Shigella flexneri*	
4	g: Green colonies with a black center on the Hektoen environment h : picture illustrating the formation of a champagne cork indicating the production of ESBL by a strain of *Salmonella* Typhi i : biochemical profile (6,704,752; 7,044,552) on API 20 E with the identification of *Salmonella* Typhi	
5	J: Colonies of *Escherichia coli* (metallic reflection on the EMB agar) K: formation of double champagne cork illustrating the production of ESBLs by a strain of *Escherichia coli* (O111 + O55 + O26)	

Pictures (Dr Bessimbaye et al, 2019)

DISCUSSION

The number of women taking part to the survey consisted in the 2/3 of the overall sampled population of PLHIV with mean age of 34 ± 14 years old and 43% of them belonging to the 20 to 30 years aged group.

According to EDS-MICS 2014-2015, the prevalence of HIV among youth aged 15-24 years old is very low 1.1% with 1.4% for women and 0.7% for men, corresponding to a female to male ratio of 2 which means that in this age group, 200 women are infected per 100 men. This ratio is higher than that of the entire population aged 15-49 (ratio of 1.4). In women, the prevalence appears to

increase with age: from 1.2% between 15-19 years to 1.8% at 18-19 years, and 2.4% among 23-24 years old. In men, the prevalence varies irregularly, from 0.5% at age 15-17 to 0.3% at age 18-19, then increases slightly to 1.4% at 20-22 years and then decrease to 0.6% at age 23-24. This difference could be explained by the anatomical configuration of women that makes them more vulnerable, and their promptitude to consult in healthcare facilities (EDS-MICS, 2016).

During the first stage of HIV infection (primary infection), the viral load is high and the CD4 count decreases. But when the body produces antibodies against HIV (seroconversion), the viral load decreases and the CD4 count rises to a normal level. According to WHO, the progression of the disease passes through 3 different stages: a primary infection, an asymptomatic stage and the AIDS stage. About 95% of patients were at clinical stage 2 with 95%, symptomatic phase, but more than half (52% of them were not on ARVs) probably explaining the high frequency of gastrointestinal candidiasis. The third phase is the AIDS stage, characterized by lymphopenia with CD4 drop below 350 / mm3 or the presence of a clinical manifestation on the list of AIDS defining diseases (PNLS, 2006).

About 60% of patients surveyed were under antiretrovirals (ARVs) closed to the 48% found by Gonsu et al. (2014). However, ARV therapy does not affect co-infection and there was no significant association between CD4 count and fungal infections (x^2 = 0.23, dd1 = 1, p = 0.50).

It has been shown that people under ARVs are less prone to digestive candidiasis. In fact, the advent of highly effective antiretroviral combination therapies significantly reduced the incidence of oropharyngeal candidiasis in HIV-positive patients (Chabasse et al., 1999, Hamidou et al., 2016). On the other hand, the presence of bacterial species such as *Salmonella* Typhi and *Escherichia coli* was significantly associated with a CD4 level of less than 50 (x^2 = 24.499, ddl = 1, p = 0.001). A significant difference (x^2 = 33.85, ddl = 1, p = 0.001 significant difference) was observed between the prevalence of fungal infections (85.49%) and bacterial infections (14.50%). This might be explained by either the late arrival of patients at the treatment center or their precarious socio-economic conditions. During the investigation, 6 (1.1%) of cases of bacterial / fungal co-infection (*E. coli* / *C.albicans*), 4 (0.75%) cases of hematological diseases, including three cases of hemorrhages and a case of hypercoagulability with anemia (hemoglobin levels below 7g / dl). We could explain these hematological disorders by cytopenia including thrombocytopenia, deficits or even increases of some coagulation factors. Indeed, the physiopathology of HIV on the main actors of hemostasis (endothelium, platelets, coagulation) has been thought fully investigated (Stefano et al., 2011).

In these studies, we noted that the levels of factor VIII and fibrinogen are increased, and S protein levels are decreased HIV-infected patients in the AIDS stage compared with those infected non-AIDS stage patients. Some authors previously described of fibrinolytic system disorders such as elevated levels of tissue plasminogen activator, as well as its rapid inhibitor, PAI-1. Obviously, it was admitted that, the causes of these abnormalities are multiples including inflammatory syndrome, opportunistic infections, autoimmune diseases, neoplasia, and treatments (protease inhibitors) making it difficult analysis of the direct effects of HIV on these abnormalities in the HIV-infected patients. However, hemorrhagic complications seem to be rare compared to the tendency to thromboses (Jle.com/en/revues/hma/e-d). Complications consist mainly in infections due to opportunistic microorganisms (Table 2) and secondarily blood pathologies. The most common species found in the diarrhea of PLHIV were *C. albicans* (Table 2). In our study, malnutrition and chronic diarrhea among PLHIV are associated with a high risk of foodborne and waterborne diseases. Indeed, Nadlaou et al (2013) showed that malnutrition and chronic diarrhea among refugees is associated with a high risk of microorganisms in food and water. We recorded 7 cases of death (1.3%) out of 531 cases of diarrhea treated in PLHIV with a CD4 count <50 per mm^3. The ingestion of contaminated cider with animal faeces killed immunocompromised individuals in the United States (Juranek 1995, Helard et al.2000). Likewise, an epidemic in Milwaukee, through contamination of the public network, caused sickness in 400,000 subjects with several dozens of deaths among immunocompromised people (Kasmireczak 1994, Harrigan and Mccance 1976).

Our study identified fungal and bacterial infections in all studied age groups. The highest rates were observed in 20-30 years and 52 years and more age groups with respectively 16.21% and 16.05%. This could be explained by the vulnerability of PLHIV characterized by a gradual weakening of their immune system. In addition, majority of infected patients were women and it is well known that 3 out of 4 women will have a yeast infection at least once in their life (Slim and Bourdon, 2000).

The isolated fungi and bacteria fermented mostly GLU +, SAC +, MAL +, and GAL +. Glucose fermentation was a common biochemical characteristic observed in all fungi and bacteria isolated from stools of PLHIV. This could be explained by the fact that they are sharing a gene or plasmid allowing them to ferment glucose. Besides, similar biochemical characteristic of fungi and bacteria isolates have been reported (Slim and Bourdon 1984, Le Minor and Veron 1989).

In general, antifungal showed 53.57% sensitivity and 46.42% resistance to isolated fungi. The fungi isolated were 65% sensitive and 35% resistant to the azole derivatives tested. On the other hand, 74.5% fungi were

resistant to polyenes and 25.5%were sensitive. The overall high sensitivity of fungal strains to antifungal could be explained by the fact that the tested antifungal are not amongst the antifungal medicines available through the country. The most active antifungal are fluconazole (59%), voriconazole (70%), and flucytosine (83%), and all derived from azoles. More than half of the yeasts were resistant (62%) to Amphotericin B and 87% to Nystatin. This could be explained by the misuse of these two molecules in absence of laboratory evidence (Table 4). In addition, polyenes such as Nystatin and Amphotericin B were inactive (Mosoko and Affana, 2013).

A *Candida anbicans* isolate was resistant to these two tested antifungals (Table 4). The observed resistance phenotype could also be explained by the expression of mutated forms of cytosine permeases or cytosine deaminase (White et al., 1998). *Candida krusei*, is more and more involved in digestive mycosis by its resistance to fluconazole especially in neutropenic patients. *Candida krusei*that probably originate from gastrointestinal tract, causes 2 to 25% of fungemia. The most common clinical presentation of *C. krusei* septicemia is fluconazole-resistant fever. According to Shiraz in Iran in 2011, from 206 species isolated, *Candida glabrata* (16.6%) was the most resistant species to generally active ketoconazole reflecting a large number of small mutations (Kamiar et al., 2011). In fact, the haploid genome of *C. glabrata* possesses the highest probability of expressing a mutated gene, as well as the current medical context, advocating for prophylaxis in number of pathologies where the immunity of patients is affected, representing a favorable ground for the development of candidiasis and simultaneous selection of resistant isolates (Vandeputte, 2008).

Most of the isolated bacteria (Table 4) were resistant to cyclins (tetracycline), mid-order beta-lactamines and sulfonamides (cotrimoxazole) with rates of 46%, 47% and 68%, respectively. This could be due to self-medication, use of antibiotics in agriculture, breeding, or inappropriate prescriptions.

Cross-resistance has been observed in the class of beta-lactam antibiotics (amoxicillin, amoxicillin + clavulanic acid, ceftriaxone) on *Shigella*, *Salmonella*, *Escherichia* species that are naturally less sensitive. A level of resistance much higher than that of *Escherichia coli* O157 H7 (Table 4) which are resistant to amoxicillin 3 (100%). Such resistance could be related to the production of penicillinase, cephalosporinase and carbapenemase types-beta-lactamases (Duval 1999, Haukka and Siitonen).

Three out of seven strains of *E. coli* (O111 + O55 + O26) and a strain of *Salmonella* Typhi produced resistance phenotypes by ESBL production. Figures (i and 1) of Table 5 showed the champagne plug-shaped images between thethird-generation cephalosporin disks (C3G), and amoxicillin + clavulanic acid. The resistance phenotypes by ESBL production were also observed in *Escherichia coli* strains isolated from urine (Bessimbaye et al., 2015, Guelmbaye et al., 2015). The strong resistance observed with amoxicillin is probably due to a low-level penicillinase (amoxicillin + clavulanic acid and ceftriaxone). Antibiotics tested against bacteria were 68% resistant to cotrimoxazole. The phenomenon of resistance observed with cotrimoxazole on bacteria could be explained by the fact that it is one of the drugs commonly prescribed for the treatment of opportunistic infections in patients with HIV / AIDS.

In Chad, cotrimoxazole is used in primary prophylaxis to treat opportunistic infections for any HIV-infected patient whose CD4 count is less than or greater than 350 per mm3 (CNLS, 2006). In many resource-constrained countries, co-trimoxazole is widely used in the treatment of common infections, with the consequent increase in resistance to this drug. The resistance of non-typhoid *Salmonella* and *Pneumococcus* isolates to cotrimoxazole was reported in 44% and 52% of cases in Uganda (2004) and in Malawi in about 80% and 90% respectively (Van Oosterhout, 2005). The effectiveness of cotrimoxazole prophylaxis is unaffected by local prevalence and seems to be identical in strong (South Africa, Uganda and Zambia) or weak (Côte d'Ivoire) geographical areas. Meanwhile, the impacts of the use of cotrimoxazole on the evolution of drug resistance is poorly understood.

It is feared that extensive and prolonged use of cotrimoxazole prophylaxis may be associated with the development of drug resistance in common pathogens such as increased cases of penicillin-resistant pneumococci. As well, the use of cotrimoxazole is also thought to modify the fermentation flora of carbohydrates, which results in diarrhea associated with antibiotic therapy (Michel-Briand 1990, Mosoko and Affana 2004, Pereira et al. Santos, 2013).

CONCLUSION

People living with HIV are often victims of diarrheal diseases caused by microorganisms. Among the microorganisms identified, the fungal agents represent 85.49% of which the most frequently isolated species are *Candida albicans* thus showing a public health problem. Diarrheal diseases are linked to the general situation of infections related to the socio-economic conditions of PLHIV. Cases of co-infection have been noted, notably *E.coli* / *C.albicans* and increased as the CD4 count goes down. Patients under ARVs are less prone to diarrheal diseases such as gastrointestinal candidiasis. Fungi isolates are more susceptible to azole derivatives than polyenes. Bacteria isolate are more resistant to beta-lactamines than quinolones. Glucose fermentation is the main biochemical characteristic observed in both fungi and bacteria isolates. Glucose fermentation was a common biochemical characteristic observed in all fungi and bacteria isolates from the stools of PLHIV. In addition to the etiological agents of diarrhea, this study

has made it possible to highlight the hematological disorders caused by blood flow and blood clotting in PLHIV in a healthcare center in Chad.

COMPITING INTEREST

The authors declare no conflict of interest.

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