Original Article

Oral Flora and Routine Antibiotics Sensitivity of HIV Infected and Immune Competent Patients attending Yaoundé Central Hospital

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Abstract

Introduction: The normal human oral flora can be altered by certain systemic diseases such as Diabetes, HIV, Hypertension, Cancer, Sickle cell anemia. The aim of our study was to determine the oral micro flora and antibiotics sensitivity of HIV infected and non-HIV infected patients attending Yaoundé Central Hospital. **Methods:** This was a prospective cross sectional qualitative and comparative laboratory study carried out between the month of March 2016 to July 2016, conducted on thirty subjects divided into two groups of HIV infected patients and, a control group (Non-HIV infected patients). Ninety Specimens from 30 patients were collected from their saliva, gingival crevices and supra-gingival calculus. Microbial culture was done on specific culture media such as manitol, saburau, choccolat PV, choccolat VCN, EMB. This was followed by an enzymatic test (catalyze test, DNAse), while different species were purified and anti-biograms were done for the different isolates. **Results:** The age range of the patients recruited in the study was between 19-61 years; with a mean range of 34.30 years and a male: female ratio of 1:2. Tuberculosis was the only opportunistic infection in patients with HIV infection, *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus spp*, *Streptococcus β hemolytic*, *Streptoccocus spp.*, *Klebsiella pneumonia* were isolated in saliva, gingival crevices and supra gingival calculus of the patients. *Candida albicans* was found in high proportion in all salivary samples. Amongst the HIV infected patients, the most common commensal microorganism isolated was the *Streptococci spp.* with mean occurrence of 5 (33.33%); *K. pneumonia* was isolated in 14.28% of cases; we also have *Staphylococcus aureus* and *S. epidermidis* in 20% of cases. In the control groups, the *Streptococci spp.* were the most common with 33.33%, followed by *Streptococcus β hemolytic* 26.26%, *Klebsiella pneumonia* and *Staphylococcus epidermidis* 6.6% of case. HAART was generally found to reduce the bacteria load in the oral cavity. Candidiasis was the most common pathology found in the HIV group, followed by periodontal diseases as compared to the non-HIV group, where there was no candidiasis but periodontal diseases. Most bacteria isolated were found to develop resistance to Cotrimoxazole and Tetracycline, followed by Ofloxacine, Augmentin (amoxicillin and clavulanic acid) and kanamycin, the bacteria isolated were also sensitive to Minocycline, Levofloxacin, Ofloxacine, Gentamicin, Azetreoname, Cefoxine and Imipeneme. **Conclusion:** Though the antimicrobial load was higher in HIV patients, HAART tend to reduce the load of these microorganisms. Among routine antibiotics prescribed, most of the patients were found to develop resistance to Cotrimoxazole and Tetracycline. **Recommendation:** The same study should be carried out on routine HIV medications and prescription guidelines should be reviewed.

Keywords: Oral micro flora; HIV; Immunocompetent patients; Yaoundé

Introduction

The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans. More than 700 bacterial species or phylotypes, of which over 50% have not been cultivated, have been detected in the oral cavity. [1] Fundamentally the resident microbial flora is useful to human as it plays key roles in human physiology and regulates various interactions between the host and harmful organisms. They are also associated with several other important life processes such as industrial fermentation

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(e.g. the production of alcohol, vinegar and dairy products), antibiotic production, and can serve as vehicles for cloning in more complex organisms such as plants. [1] They are also useful in biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments microbial non microbial disease treatment. [2-4] In adverse conditions, however, these endogenous microbes may become opportunistic and cause disease as to their hosts especially when the immune system weakened.

The presence of nutrients, epithelial debris and secretions makes the mouth a conducive environment of the growth for ranges of microorganisms. The mouth contains different ecological combinations of microbial association which vary with age, general health status and ecological niches found on the tongue surface, saliva, and sub gingival calculus. In fact, the composition of the micro biota can be influenced by such host health status such as in HIV/AIDS, Diabetes, and Sickle cell anemia. Nearly three decades after its discovery, HIV infections remains one of the most common causes of death in Sub-Saharan Africa where it is incriminated in about 67% of the world's 33 million infected people. In Cameroon, the national HIV prevalence was estimated in 2014 to be at 5.5%. [5] Early recognition, diagnosis, and treatment of HIV-associated oral lesions may reduce morbidity rates. [6]

There is paucity of literature on oral microbial flora of HIV/AIDs patients in Cameroon. The present investigation was carried out to compare the composition of the oral microbial flora and antibiotics resistance of immune-compromised and immune-competent patients in Yaoundé Central Hospital.

Research Methodology

The study was a descriptive study conducted from March 2016 to July 2016. The study was carried out at Yaoundé Central Hospital, which is one of the major referral hospitals in Cameroon with a specialized HIV care center. Patients were selected by convenient sampling and all patients who had CD4 count less than 200 cells, aged above 18 years, certified to be HIV positive or negative, HIV patients who are freshly diagnosed and those on HAART. Patients who refused to give their consent and on antimicrobial mouth washes in the previous 12 hours were excluded.

The study was carried out in 3 stages; a data capture sheet was used for collecting the socio-demographic information, HIV status and the medications the patients is placed on. After which a full clinical examination was carried out and information filled in the data capture sheet and finally samples were collected from the patient for laboratory analyses.

Sample collection

Patient was asked to chew paraffin to stimulate saliva secretion which was directly collected in a test tube. Supra-gingival plaque was collected with sterile dental excavator by scraping the biofilm of the tooth surface. A sterile endodontic paper (paper point) was used to wicks up crevicular fluid from the gingival sulcus after careful isolation.

Laboratory analysis

Samples were plated/streaked on both selective and non-selective culture media and incubated under conducive environment for the growth of the target bacteria. After incubation was completed suspected bacterial colonies were selected and isolated for identifications. The clear soup-like liquid nutrient broth and nutrient agar were used as culture media. The media were sterilized by heating in an autoclave at 121°C under a pressure of 1 bar or for 15 minutes. All apparatus used from this point onwards must be sterilized by heat (glassware - 160°C for 2 hours) or exposed to radiation. Aseptic techniques were used to reduce the likelihood of bacterial contamination. After streaking, dilution of plating was used to identify the number of viable micro-organisms in a fixed amount of a liquid. Serial dilution involves repeatedly mixing known amounts of source culture with (sterilized) liquid. 1 ml added to 9 ml gives a 10-fold dilution; 1 ml added to 99 ml gives a 100-fold dilution. When fixed amounts of this dilution series are mixed with an appropriate agar and incubated, then different numbers of colonies were obtained. By working back from an easily counted plate and using the appropriate dilution factor, the number of micro-organisms in the original source culture was calculated. Biochemical identification was used to establish the enzymatic capabilities of bacteria isolate as well as isolate ability to grow or survive the presence of certain inhibitors (e.g. salts, surfactant, toxins, and antibiotics). Tests which were used were: catalase test, coagulase test, filamentous test. Sensitivity test was used to determine the profile of an organism's susceptibility/resistance to a panel of antibiotics.

Ethical considerations

Ethical clearance was given by the institutional review board of the Université des Montagnes and research authorization was taken from the Yaoundé Central Hospital.

Results

Of the 30 patients recruited in the study, each presented with 3 specimens totaling of 90 were processed. Females HIV positive were twice the number of male patients [Figure 1]. The ages of the patients ranged from 19 to 60 years with the mean age 37.06 ± 5 S.D. years for HIV-positive patients and 31.53 ± for HIV-negative group. A third (38.46%) belonged to the 31 to 40 years age group [Figure 2]. Out of the 14 HIV-positive patients, 5 (31.75%) had a CD4 cells count value between 151 and 200 CD4, 1 (7.14%) were found in the range between 1 and 50, 4 (28.57%) within 51 and 100 CD4 cells, and 4 (28.57%) in the range between 101 to 200 CD4 cells count [Figure 3].

Microorganisms identified. Eight groups of microorganisms were isolated from the saliva, gingival crevices and supra gingival calculus of patients selected [Table 1]. They were *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus spp*, *Streptococcus* β hemolytic, *Streptococcus spp.*, and *Klebsiella pneumonia*.

Saliva isolates

Candida albicans was isolated in the saliva of HIV patient with the highest rate (60%). In descending order, C. albicans

was followed by *Streptococcus spp.* (33.33%), *Staphylococcus aureus* (20%), *Klebsiella* and *Staphylococcus epidermidis* (13.3%). For the non-HIV patients we have *Candida albicans* was isolated in 20%, β hemolytic Streptococci and *Streptococcus spp.* (40%) each.

Gingival crevices isolates

In the gingival crevices of HIV-positive patients, *Candida albicans* was isolated from 40% of participants, *Klebsiella* from 6.67%, *Staphylococcus aureus* from 20%, *Staphylococcus epidermidis* from 13.33%) and *Streptococcus spp.* from 26.67%. For the HIV-negative, participants, *Candida albicans* was found in 13.33% and *Streptococcus spp.*, in 20%.

Supra gingival calculus

In the supra gingival calculus of HIV patients, *Candida albicans* was found in 33.33%, β hemolytic Streptococci in 13.33%, *Klebsiella* in 6.67%, *Staphylococcus aureus* in 26.67%, *Staphylococcus epidermidis* in 20% and *Streptococcus spp.* 13.33%. For non-HIV patients *Candida albicans* represent

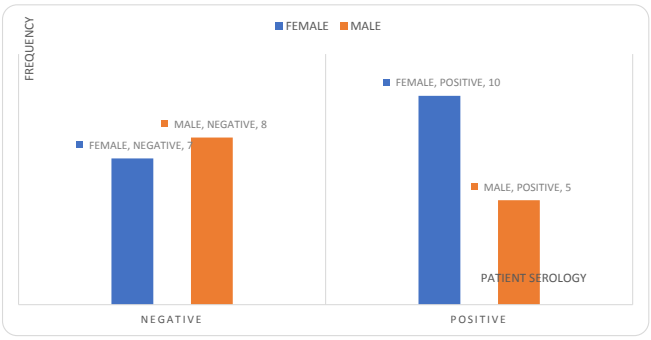

Figure 1: Sex distribution and serology of the studied population.

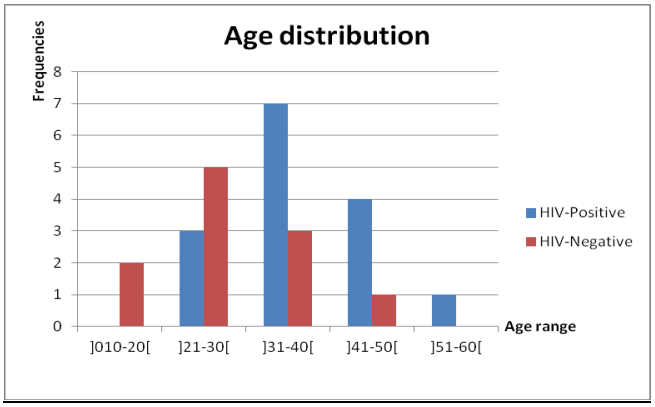

Figure 2: Age distribution of the studied population.

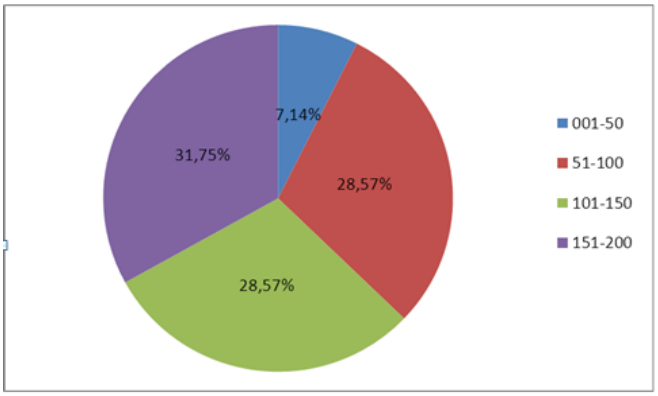

Figure 3: CD4 count range.

(6.67%), the same rates were observed for *Klebsiella* spp. and *Staphylococcus epidermidis*. This followed the *Streptococcus spp.*, (40%). [Figure 4].

Candida albicans, β hemolytic hemolytic Streptocicci, *Klebsiella*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus spp.* in HIV patients while *Candida albicans*,

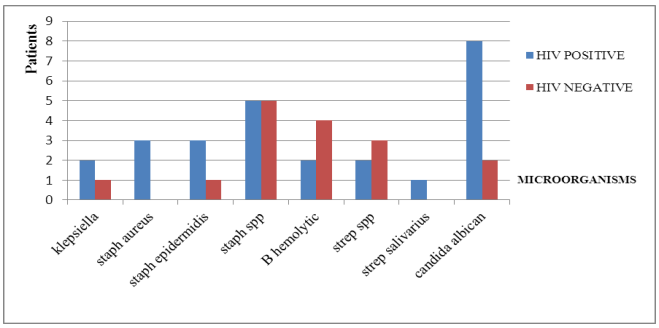

Figure 4: Comparative diagram of oral flora.

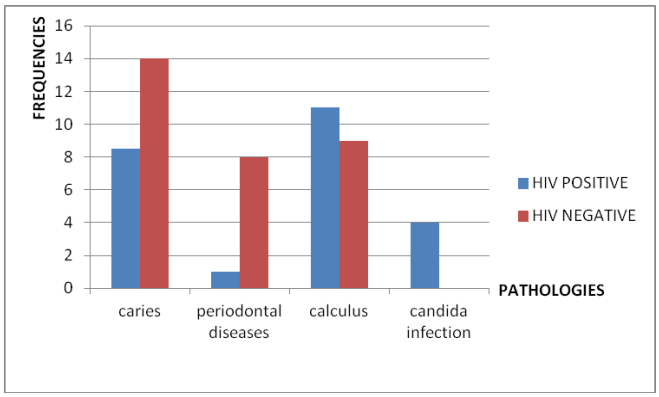

Figure 5: Pathologies.

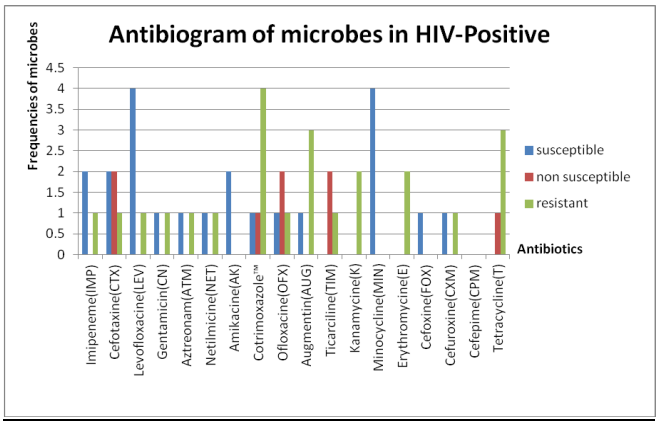

Figure 6: Susceptibility/resistance rates (HIV-positive).

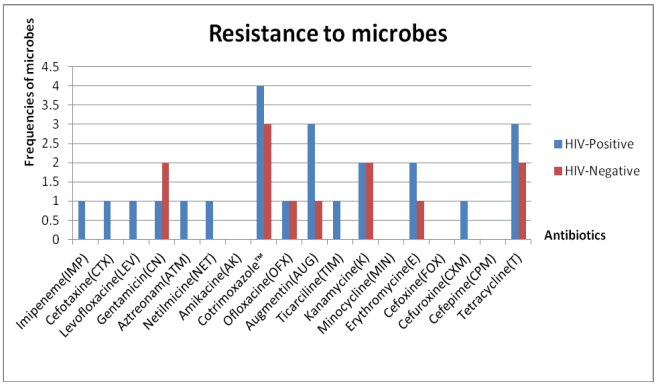

Figure 7: Susceptibility/resistance rates (Comparative diagram).

Table 1: Distribution of microbes in the studied population.

Oral Microorganisms	HIV-Positive (%)	HIV- Negative (%)	p-value
Candida albicans (SA)	60	20	0.01
Candida albicans (GC)	40	13.3	0.06
Candida albicans (SC)	33.3	6.7	0.04
β hemolytic (SA)	0.0	40	0.004
β hemolytic (GC)	0.0	26.7	0.024
β hemolytic (SC)	13.3	33.3	0.11
Klebsiella (SA)	13.3	0.0	0.12
Klebsiella (GC)	6.7	0.0	0.25
Klebsiella (SC)	6.7	6.7	0.50
Staphylococcus aureus (SA)	20	0.0	0.05
Staphylococcus aureus (GC)	20	0.0	0.05
Staphylococcus aureus (SC)	26.7	0.0	0.02
Staphylococcus epidermidis (SA)	13.3	0.0	0.12
Staphylococcus epidermidis (GC)	13.3	0.0	0.12
Staphylococcus epidermidis (SC)	20	6.7	0.17
Staphylococcus spp (SA)	0.0	0.0	0.0
Staphylococcus spp (GC)	0.0	0.0	0.0
Staphylococcus spp (SC)	0.0	0.0	0.0
Streptococcus salivarius (SA)	0.0	6.7	0.25
Streptococcus salivarius (GC)	0.0	0.0	0.0
Streptococcus salivarius (SC)	0.0	0.0	0.0
Streptococcus spp (SA)	3.3	40	0.36
Streptococcus spp (GC)	26.7	20	0.34
Streptococcus spp (SC)	13.3	40	0.06

Table 2: Microorganisms isolated base on the CD4 cells count.

CD4 Cells Count Range	1-50	51-100	101-150	151-200
Microorganisms	*-Candida albicans* *-Staphylococcus epidermidis*	*-Candida albicans* *-Klebsiella* *-Staphylococcus aureus* *-Staphylococcus epidermidis* *-Streptococcus spp.*	*-Candida albicans* *-B hemolytic* *-Staphylococcus aureus* *-Staphylococcus epidermidis* *-Streptococcus spp.*	*-Candida albicans* *-B hemolytic* *-Staphylococcus aureus* *-Streptococcus spp.*

β hemolytic spp, *Klebsiella*, *Staphylococcus epidermidis* and *Streptococcus spp.* in HIV-negative patients.

Candida albicans was most represented in HIV-positive patients (53.33%) while in HIV-negative patients it was found in 13.33%. For *Streptococcus spp.* was also found in similar amounts in HIV-positive and HIV-negative cases (33.33%). With regard to CD4 rates, from participants count between 1-50 cells, isolated were *Candida albican*, *Klebsiella* and *Staphylococcus epidermidis*. Between 51 and 100 cells microorganisms recovered includes mostly *Candida albicans*, *Staphylococcus epidermidis*, and *Klebsiella* and *Staphylococcus spp*. patients with CD4 cells range between 101-150 isolated organisms included *Streptococcus* spp., β hemolytic, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus spp*. [Table 2].

CD4 count higher than 104 were onserved in more than 50% of HIV-positve patients. CD4 151-200 we have *Candida albicans*, *Streptococcus* spp, β hemolytic, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus spp*. [Table 2].

Pathologies encountered. Of the 15 HIV patients recruited, 4 (26.66%) had Candida infection, 9 (60%) had caries, (6%) had periodontal disease and 11 (73%) had calculus. For HIV-negative patients, no Candida infection was found, 12 (80%) had caries, while periodontal disease and calculus was observed in 53.33% and 9 (60%, respectively [Figure 5].

Antibiotic susceptibility profile

Almost three quarters of the bacteria isolated 5 (71.42%) were resistant to cotrimoxazole and tetracycline, 2 (28.57%) were resistant to Ofloxacine, Amoxicillin/clavulanic acid and Kanamycine. All the microorganisms were sensitive (susceptible) to Minocycline, Levofloxacine, Ofloxacine, Gentamicine, Azthreonam, Cefoxine and Imipeneme [Figure 6].

Out of the 18 antibiotics used, HIV-Positive patients have developed resistance 14 antibiotics mean while the HIV-negative groups developed resistance to 07 antibiotics [Figure 7]. The majority of microbes identified were resistant to Cotrmoxazole, Augmentin, Erythromycine and Kanamycine and the rate of resistance was higher in the HIV-Positive group.

Discussion

In the current study, the HIV prevalence was two times higher in women than men; this is similar to a study carried out in Nigeria by Awofala and Ogundele, [7] who reported that HIV infection was higher in females than in males, although the male to female ratio was not up to 2:1 as seen in our study. Another study carried out in Cameroon by Mbanya et al. also showed a higher female to male prevalence of HIV, their reasons for this was because demographically women are more populated than men. [5] Also in this study the prevalence of HIV patient were higher amongst patients of the 31-40 years age groups. These

groups constituted the majority of adults who were not only the independent but also the sexually active.

Patients with CD4 count cells range of 151-200 were mostly represented in our study this is explained by the fact that most of the patient were taking antiretroviral therapy, which means that antiretroviral therapy increased the CD4 cells count of HIV patients. Grill et al. had earlier observed in their study that the number of CD4 count cells in HIV infected patients increased with HAART. [8]

The bacteria identified in HIV patients were similar to those identified in non- HIV patients, the only difference observed was that *Streptococcus* salivarus and *Staphylococcus epidermidis* were present in HIV patients and absent in non-HIV patients, elucidating that microorganisms identified were commensals constituting part of the normal oral flora. Machesh Chandra Hedge et al. in their study on the variation of microorganisms load between HIV and Non-HIV patients reported the same results. [4,6] The difference in the composition in micro flora in HIV and non-HIV patients is not really important because both groups have the same microorganisms. Though a similar result was reported byt Hedge et al., [9] they also identified *Actinobacter* spp. and Micrococcus spp. in the composition of oral flora in HIV patients which we did not identify in our study. These differences might be due to the exposure environment, oral, hygiene practices and dietary habits of the patients.

Candida albicans was the major microorganism isolated in the current study. Mushi and collegues (2014) carried out a review on thirteen original research articles on oral Candida infection/ colonization among HIV-infected African populations were reviewed. They reported a prevalence of OC that ranged from 7.6% to 75.3%. [10] The microbial load in HIV patients was higher than in non-HIV patients. This result can be explained by the fact that most HIV patients are immune compromised. As a result, microorganisms which at first commensal, consequently will become pathogenic Hedge et al. in their study this shift in oral microbial load between HIV and non-HIV patients. [8] The antibiotic sensitivity patterns in both the groups were compared in our study. Although increased bacterial resistance to first line antibiotics has been reported by Manfredi et al., in our study, we did not find any significant difference in the incidence of resistance. [11]

Co-trimoxazole is a prophylactic treatment that has a wide range of action against common bacteria, parasites, fungi and yeasts. As part of a minimum care package, UNAIDS/ WHO recommends co-trimoxazole prophylaxis for HIV-infected adults with symptomatic disease (WHO stage II, III or IV), or asymptomatic individuals with CD4 counts <500 cells/µl, and for all HIV-positive pregnant women after the first trimester. [12] From our study more than 2/3 (71.42%) bacteria isolated were resistant to Cotrimoxazole a compulsory medication in the basic HIV package in Cameroon. There were also resistant to Tetracycline (71.42%), Ofloxacine, Augmentin and Kanamycine (28.57%). Hedge et al. showed that Vancomycin-resistant *Enterococcus* increased in prevalence to 28.5%, whereas Methicillin Resistant *S. aureus* had increased to 59.5%. Resistance of *K. pneumonia* and *Enterobacter* spp. to third-generation cephalosporin increased to 20.6 and 31.1%, respectively. Resistance of *Pseudomonas aeruginosa* to Imipenem, Quinolones, and third-generation cephalosporin increased to 21.1, 29.5, and 31.9%, respectively. [9] The resistance of these micro-organisms calls for concern as it serves as a form of drug over use. Even if it is used as prophylaxis, the results calls for further research and review on the use of these antibiotics as opportunistic infections are the most common cause of death in patients with HIV and many of these are caused by commensal bacteria which are otherwise harmless in a normal individual.

Conclusion

Candida and *Staphylococcus aureus* infections were more prominent in HIV infected patients than non HIV infected patients with the highest concentrations in Saliva while β hemolytic spp were found more on HIC negative patients especially in their saliva and sub-gigival calculus. Calculus deposits and candida infections were higher in HIV infected individuals and periodontal diseases and dental caries were more in HIV negative patients. Seven bacteria species and one fungal specie in HIV group of patients, and five bacteria species plus one fungal specie in non-HIV group of patients. The main difference between the two groups of patient in quality of the *microbiota* was the absence of *Actinomyces* spp. and *Micrococcus* spp. in non-HIV group.

Dental caries, periodontal diseases, calculus and candidal infection for HIV infected the particularity was that we did not found candidal infection in non-HIV group. *Streptococcus* sp. was also prominent in HIV patients which are a normal commensal of the skin. There was no shift in the normal flora with a decrease in the immune competence assessed by CD4 cell count. More than 2/3 (71.42%) bacteria isolated were resistant to Cotrimoxazole, Tetracycline, Ofloxacine, Augmentin and Kanamycine. Ofloxacine, Amoxicillin/clavulanic acid and Kanamycine. All the microorganisms were sensitive (susceptible) to Minocycline, Levofloxacine, Ofloxacine, Gentamicine, Azthreonam, Cefoxine and Imipeneme.

Recommendations

- Dental prophylaxis should be done regularly to HIV infected individuals and strict infection prevention against opportunistic infections like oral candidiasis should be observed in dental clinics. Since candida and *Staphylococcus* infections were more prominent in HIV patient than non HIV patients, emphasis should be placed on their managements of newly diagnosed patients.
- Antibiotic sensitivity, antibiotic drug resistance and drug resistance monitoring on patients' needs a comprehensive research especially for prophylactic antibiotics.
- Evidence based therapy and medical laboratory analysis should be used for drug policies concerning these medications.

Consent

As per international standard or university standard written participant consent has been collected and preserved by the authors.

Conflict of Interest

The authors disclose that they have no conflicts of interest.

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