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Non-target oral bacterial resistance to Cotrimoxazole in HIV/AIDS patients living in South Western Uganda

Ihongbe[1] JC, [2]Moazzam ML, [3]Pazos V and [3]Agwu E.

[1]Department of Medical Laboratory Sciences, Babcock University, Illisan Remo, Ogun State, Nigeria. [2]Department of Public Health, School of Allied Health Sciences, Kampala International University (KIU) and [3]Disease Intervention and Management Failure (DIMAF) research group, Department of Medical Microbiology, Faculty of Basic Medical Sciences, KIU, Box 71, Bushenyi, Uganda

Correspondence: Ihongbe JC, PhD. ihongbe@yahoo.com ; Phone: +2348072319857.

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Abstract

Objective: This study was designed to assess the long-term effect of prolonged routine cotrimoxazole prophylaxis on non-target bacteria isolated from oral lesions of HIV/AIDS patients in South Western Uganda.

Method: Exactly 605 swabs (469 from females and 136 from males), were randomly collected from oral lesions of The AIDS Support Organization (TASO) HIV/AIDS patients in 4 Districts of Uganda. Sample processing was done aseptically using standard Microbiological techniques. Randomized Block Design ($\alpha = 0.05$) was used to compare both the prevalence and resistance of bacterial isolates.

Results: In Mbarara/Bushenyi districts, bacteria prevalence was 50.4%, followed by 20.8% in Rukungiri and 20.3% in Masaka districts. Most bacteria from Rukungiri showed 100% resistance. In Mbarara/Bushenyi, *S. aureus, B. catarrhalis* and Non-hemolytic *Streptococcus* showed 100% resistance while *B. cerius, S. aureus*, *E. coli* and *B. subtilis* showed 100% resistance in Masaka. Bacteria prevalence was significantly ($p<0.05$) dependent on location and district of isolation. Different bacteria isolates significantly ($p<0.05$) differed in their response to different antibiotics tested.

Conclusion: Despite its overall benefit, prolonged cotrimoxazole prophylaxis may have a long-term disadvantage such as the evolution of 100% resistance by non-target oral bacterial isolates recovered from HIV/AIDS patients living in Uganda.

Keywords: Cotrimoxazole resistance, Non-target oral bacteria, HIV/AIDS patients

Introduction

Opportunistic infections are a threat to lives and bacterial infections are well established major cause of morbidity and mortality among patients with HIV living in Africa (1-4). Co-trimoxazole, a fixed-dose combination of sulfamethoxazole and trimethoprim is a broad-spectrum antimicrobial agent used in the treatment and prevention of several opportunistic infections including a range of aerobic gram-positive and gram-negative organisms. The drug is widely available in both syrup and solid formulations at low cost in most places, including resource-limited settings.

Although WHO/UNAIDS issued a short-term statement on the use of co-trimoxazole prophylaxis for HIV/AIDS patients in sub-Saharan Africa in 2000 (5), most countries have not implemented this intervention. The reasons for the slow implementation of co-trimoxazole prophylaxis programs include the difference in causation and burden of HIV-related infections between well-resourced and resource-limited countries, the potential for development of drug resistance, and the lack of guidelines (6). It is widely used to lower disease incidences in HIV-infected patients. One example is the lowering of diarrhea, death, malaria, and hospitalizations by 30-70% during the time of co-trimoxazole prophylaxis than before in Uganda (7).

There has been fear over the limited evidence-based report for the efficacy of co-trimoxazole prophylaxis, particularly in areas with high levels of bacterial resistance to the drug (6). There is also concern that implementing wide and prolonged use of co-trimoxazole prophylaxis could lead to the development of multiple drug bacteria resistance to commonly available and affordable antibiotics (8-10). One study from Malawi (11) showed a significant increase in resistance of pharyngeal and fecal isolates from those receiving co-trimoxazole prophylaxes. Recent data from Uganda (7) found no significant changes in the bacterial resistance patterns of stool pathogens isolated from the family members of individuals on co-trimoxazole prophylaxis over two years. The impact of co-trimoxazole use in the evolution of drug resistance is uncertain especially in the microbial population for which the cotrimoxazole prophylaxis was not meant for (non-target). Resistant non-target resident microbial flora may pose danger to HIV/AIDS opportunistic infection management if they later take part in causing opportunistic infection in the course of HIV/AIDS disease progression. This study was therefore designed to investigate the effect of co-trimoxazole on non-target bacteria associated with oral lesions among HIV/AIDS patients in South Western Uganda.

Objective

The ultimate goal of this study is to design effective intervention (for sustainable co-trimoxazole prophylaxis) with data generated from monitoring the emergence of co-trimoxazole resistance by non-target bacteria associated with oral lesions among HIV/AIDS patients.

Materials and Methods

TASO centers of four South Western Uganda Districts located at Masaka, Rukungiri, Mbarara, and Bushenyi were used for this investigation. Patients who qualified for inclusion were: HIV seropositive; registered with TASO adults; were on daily co-trimoxazole prophylaxis and who have been clinically diagnosed with oral lesions (12, 3). This study was ethically approved by the Uganda National Council of Science and Technology; Kampala International University Research and Ethics Committee; TASO at both local and national level and TASO patients.

A total of 605 oral swabs were aseptically and randomly obtained from lesions in the oral cavities of 469 female and 136 male TASO HIV

infected and AIDS patients. The number sampled was guided by the upper limit required to give a 95% level of confidence at an expected prevalence of about 55% (3), using the precise prevalence formula: Sample size (n) = Z^2P (100-P)/D^2 (Epi-info version 3.2 database; 1995), where Z is a constant given as (1.96), P is expected prevalence (55%), and D is an acceptable error (5%). All samples were transported under the ice in a vaccine carrier to the Disease Intervention and Management Failure (DIMAF) research group, Department of Medical Microbiology, Kampala International University, Western Campus, Uganda, for analysis. All media were prepared under aseptic conditions and media performance control criteria reported by Cheesbrough, (13), were adopted. A standard protocol contained in our earlier report (14) was adopted in preparing blood and chocolate media for isolation and identification of oral *Streptococcus* sp. Thioglycolate agar was also inoculated and incubated anaerobically for the isolation of suspect anaerobic bacteria.

After 18-48 hours of incubation, suspect bacteria species of clinical significance were picked for further identification using standard Microbiological and biochemical methods (15, 16). Standard suspensions (0.5 McFarland) of identified bacterial colonies were inoculated onto plates of Mueller Hinton agar. Antibacterial susceptibility testing was done in line with the modified Clinical Laboratory Standards Institute guidelines for conducting Kirby-Bauer disk diffusion susceptibility testing of antibiotics (17). The modified criterion for antibiotic type selection and corresponding disc content for use in testing susceptibility of bacterial agents of mucosal infections which we reported earlier (18) and previously recommended by CLSI, (19) was adopted in this investigation.

After incubation at 35^0C for 18-24 hours, zone sizes were measured and interpreted using CLSI standards (20, 19). We determined the level of significance of data generated with Randomized Block Design (RBD), given as (Total Sum of Squares $_{(SST)}$ = Sum of Squares due to Different effects $_{(SSD)}$ + Error Sum of Squares $_{(SSE)}$), (21) for one observation per cell, (α=0.05).

Results

The identified oral lesions included: Acute Necrotizing Ulcerative Gingivitis (ANUG), Linear Gingival Erythemathous Banding (LGEB), Kaposi Sarcoma (KS), and Angular Cheilitis (AC). The results in (Table I), depict inter- (Between) and intra- (Within) district prevalence of bacterial isolates from 605 oral swabs collected from different HIV patients. Bacterial isolates were most prevalent in samples collected from Masaka, (83.2%) and Rukungiri, (82.9%), compared to the 68.2% prevalence recorded in Mbarara and Bushenyi Districts combined. The overall regional prevalence result (Table I) reveals *Streptococcus mutans* with the prevalence of 28.9% as the most prevalent bacterium, followed by 10.2 % *Staphylococcus aureus*, 6.1% *Branhamella catarrhalis,* and 5.6% *Escherichia coli* among others.

Comparing the bacteria prevalence from each district surveyed with those from other districts (Table I), *Streptococcus mutans* with a prevalence of 31.5% from Mbarara/Bushneyi Districts were the most predominant bacteria. This was followed by a 16.4% prevalence of *Proteus mirabilis* from Rukungiri District, 11.8% Non-hemolytic *Streptococcus* species from Mbarara/Bushenyi Districts, 11.3% *Staphylococcus aureus* from Rukungiri Districts, and 9.5% *Branhamella catarrhalis* from Masaka District. Out of 142 samples from Masaka District, 45(31.7%) *S. mutans* was the most prevalent bacteria followed by 16(11.3%) *Staphylococcus aureus,* 14(9.9%) *Branhamella catarrhalis,* 12(8.5%) *Klebsiella pneumonia,* and 10(7.0%) *Escherichia coli.* Non-hemolytic *Streptococcus* was not seen in samples from Masaka. While *S. mutans* was most prevalent 34 (22.4%), there were no diphtheroids and *S. pyogenes* from Rukungiri

District (Table I). Whereas *Streptococcus mutans* was most prevalent 96 (31.0%), there were no *Pseudomonas aeruginosa, Proteus mirabilis, Bacillus subtilis, and Bacillus cereus* seen in samples from the Mbarara district (Table I).

From (Table I), the resistance profile of co-trimoxazole by oral bacteria shows that *S. aureus,* Non-hemolytic *Streptococcus*, *B. subtilis,* and *B. cereus* were resistant (100.0%) to co-trimoxazole in all the four districts surveyed. In Masaka districts, in addition to *S. aureus,* Non-hemolytic *Streptococcus*, *B. subtilis,* and *B. cereus* which were 100.0% resistant, *E. coli* was the most resistant bacteria (100.0%), followed by 93.3% *S. mutans,* 75.5% *K. pneumoniae,* and 71.4% *S. pyogenes*. In Mbarara and Bushenyi Districts and in addition to *S. aureus* and other bacteria with absolute resistance, *Branhamella catarrhalis* showed 100% resistance in Mbarara and Bushenyi Districts. This was followed by 84.6% *Klebsiella pneumoniae* and 64.7 *S. pyogenes*. In Rukungiri District, apart from *Branhamella catarrhalis* with (77.8%) resistance, all the bacteria isolates were absolutely (100.0%) resistant to co-trimoxazole. Using RBD statistical tool, there were significant differences ($p<0.05$) when the prevalence of different bacteria strains isolated from the oral mucosa lesions was compared within districts (a) and between districts (b).

Discussion

"Non-target oral bacteria" is a term used in this manuscript to denote the fact that the cotrimoxazole prophylaxis given to all HIV/AIDS patients was usually not meant for the oral bacteria tested. HIV/AIDS is one of the fastest-growing threats to human development and the epidemic is more severe in Sub-Saharan Africa and Asia with about 40–50% of patients' having oral infections early in the course of the disease (22). Our observation of Acute Necrotizing Ulcerative Gingivitis (ANUG), Kaposi Sarcoma (KS), Linear Gingival Erythemathous Banding (LGEB), and Angular Cheilitis (AC) among the studied population is in line with earlier reports (22, 3). The pattern of the bacterial population (Table 1) we recovered from the identified oral lesions was significantly ($p<0.05$) dependent on factors such as the changes in the oral microbiota which may contribute to the oral complications associated with HIV disease; the host and/or environmental factors which may facilitate infection of oral epithelial cells with oral opportunistic pathogens and oral complications associated with the prolonged use of Highly Active Anti-Retroviral Therapy in HIV patients.

Members of the genus *Bacillus* can be isolated from many environments due to the longevity of the spores. The regulation of sporulation in *Bacillus* species is controlled in part by σ-factors, transcriptional activators, repressors, and the entry into sporulation is controlled by signals related to starvation, cell density, and cell cycle (23). *Bacillus* species are highly resistant to common antibiotics mainly due to spore formation. *Branhamella catarrhalis* is a β-lactamase producing Gram-negative diplo-*cocci*, uniformly resistant to vancomycin and clindamycin but susceptible to rifampin and erythromycin (24). *B catarrhalis* is a frequent cause of Lower Respiratory Tract (LRT) infections, sinusitis, otitis media, bacteremia, meningitis, keratitis, endocarditis, and suppurative arthritis. Diphtheria toxin is the fundamental had reported *C. diphtheria* as resistant to trimethoprim. Maple *et al.,* (26) had reported *C. diphtheria* as resistant to trimethoprim. Reduction of the global burden of oral disease may be dependent on effective prevention of oral manifestations of HIV/AIDS through: identification of HIV clinical diagnostic surrogates; research to improve treatment and prevention and mass education/dissemination of information on early detection of HIV up to the community level (22).

The overall impression from this study (Table 1) is that prolonged routine use of co-trimoxazole prophylaxis as broad-spectrum antibiotics against systemic opportunistic infection among the studied HIV/AIDS patients may have contributed to the development of 100% resistance by most non-target oral bacterial isolates (8-10, 6). The

observation of 100% co-trimoxazole resistance by *E. coli* in the Masaka district (Table 1) is higher than the 56.4% resistance we reported in Ekpoma Nigeria (18). This observed resistance of non-target oral *E. coli* may be similar to the pathovars of neurovirulent *E. coli*
with transferable resistance to one or multiple drugs richly present in human gastrointestinal tracts (27). Observed 100% resistance of most non-target oral bacteria (Table 1) may be due to trans-kingdom conjugation, cytoreduction, or recipient species picking up plasmid molecules released by the donor strains through cell lyses (28-30). The observed high non-target oral bacterial resistance to co-trimoxazole is contrary to the previous Uganda report of no significant changes in the stool bacterial resistance patterns of individuals on co-trimoxazole prophylaxis but is similar to a reported significant increase in resistance of pharyngeal and fecal isolates from those receiving co-trimoxazole prophylaxes in Malawi (11, 7).

The daily routine prophylactic administration of co-trimoxazole among the studied HIV/AIDS population may have impacted heavily in the development of the observed resistance and may also discourage the implementation of wide and prolonged use of co-trimoxazole prophylaxis which could likely lead to the development of resistance by our non-target oral bacterial isolates (8-10). This may be pointing to the emergence of uniform poly-microbial resistance to cotrimoxazole.

In the light of the observed resistance by non-target oral bacteria of HIV/AIDS patients and because data on constant CD4 cell count monitoring and randomized clinical trials needed to stop co-trimoxazole prophylaxis are not available in Uganda, stopping cotrimoxazole prophylaxis in the studied population may be discouraged until such data are available. The discontinuation of co-trimoxazole prophylaxis in HIV-infected individuals has also been considered in the context of drug toxicity, pregnancy, and immune restoration promoted by anti-retroviral therapy response (6). In these situations, discontinuation should be based on clinical judgment, considering the clinical, laboratory, adherence profile, and occurrence of severe adverse reactions (6).

Clinical evidence of immune reconstitution and clinical staging of HIV/AIDS patients alone may not be enough to recommend stopping co-trimoxazole prophylaxis in this circumstance of high non-target bacterial resistance. One possible alternative could lie in the usefulness of Ascorbic acid and Vitamin E, as a routine antioxidant regimen that protects the cells from oxygen free radicals and reactive oxygen intermediates, in people living with HIV (31). Again, this suggestion of ascorbic acid and vitamin E supplement tallies with current TASO practices of giving food supplements to its patients, to reduce malnutrition.

The present study may have shown that non-target microbial populations can develop resistance to antibiotics not intended for their elimination. Non-target oral bacteria developed resistance to cotrimoxazole in people living with HIV and undergoing co-trimoxazole prophylaxis. If this non-target resident flora becomes part of microbial agents of opportunistic infection in the studied population, treatment may become difficult. This study may be the first evidence-based report of high oral non-target bacterial resistance to co-trimoxazole in Uganda. More surveillance on co-trimoxazole resistance to other non-target microbial populations at other body sites is recommended to determine the relevance of continuing co-trimoxazole daily prophylaxis in people living with HIV/AIDS.

Conflict of Interest: None

Table 1: Prevalence/resistance profile of bacteria associated with oral lesions of HIV/AIDS patients in S/W Uganda

Organisms	**Number (%) Intra-district prevalence**				**Organisms**	**Cotrimoxazole (25µg) resistance**					
						Masaka		Rukungiri		Mbarara/Bushenyi	
	Mas. (n:148)	**Ruk.** (n:152)	**Mba:B** (n:305)	Total (%)		No: Tested	No.(%) Resist	No: Tested	No.(%) Resist	No Tested	No Resist
S. mutans	45 (30.4)	34 (22.4)	96 (31.5)	175 (28.9)	*S. mutans*	45	42 (93.3)	96	36 (35.5)	34	34 (100.0)
S. pyogen	7 (4.7)	0 (0.0)	17 (5.6)	24 (4.0)	*S. pyogen*	7	5 (71.4)	17	6 (64.7)	0	0 (0.0)
S. aureus	16 (10.8)	11(7.2)	35 (11.5)	62 (10.2)	*S. aureus*	16	16 (100.0)	35	35 (100.0)	11	11 (100.0)
N/h Strept	0 (0.0)	18 (11.8)	14 (4.6)	32 (5.3)	*N/H Strept*	0	0 (0.0)	14	14 (100.0)	18	18 (100.0)
P. aerugin	2 (1.4)	13 (8.6)	0 (0.0)	15 (2.5)	*P. aerugin*	2	1 (50.0)	0	0 (0.0)	13	13 (100.0)
K. pneumo	12 (8.1)	8 (5.3)	13 (4.3)	33 (5.4)	*K. pneumo*	12	9 (75.50)	13	11 (84.6)	83	83 (100.0)
P. mirabilis	4(2.7)	25 (16.4)	0 (0.0)	29(4.8)	*P mirabilis*	4	2 (50.0)	0	0 (0.0)	25	25 (100.0)
E. coli	10 (6.8)	5 (3.3)	19 (6.2)	34 (5.6)	*E. coli*	10	10 (100.0)	19	9 (47.6)	5	5 (100.0)
B. subtilis	4 (2.7)	2 (1.3)	0 0(0.0)	6 (1.0)	*B. subtilis*	4	4 (100.0)	0	0 (0.0)	2	2(100.0)
B. cerius	2 (1.4)	1 (0.7)	00 (0.0)	3 (0.5)	*B. cereus*	2	2(100.0)	0	0 (0.0)	18	18 (100.0)
B. catar	14 (9.5)	9 (5.9)	14 (4.6)	37 (6.1)	*B. catar*	14	9 (35.7)	14	14 (100.0)	9	2 (77.8)
Diphther	7 (4.7)	0 (0.0)	0 (0.0)	7 (1.2)	*Diphth*	7	2 (28.6)	0	0 (0.0)	0	0 (0.0)

***n :number**= Total number sampled.=, Mas=Masaka, Ruk = Rukungiri, Mba.= Mbarara, Bush. = Bushenyi. Using RBD, there were significant difference ($p<0.05$) when the prevalence of different bacteria strains in the oral mucosa were compared within districts (a) and between districts (b). S. mutans=Streptococcus mutans, S. pyogen= Streptococcus pyogenes, S. aureus= Staphylococcus aureus, N/h Strept=Non-hemolytic Streptococcus, P. aerugin=Pseudomonas aeruginosa, P. mirabilis: Proteus mirabilis, K. pneumo=Klebssiella, E. coli=Escherichia coli, B. subtilis-Bacillus subsidies, B. cerius: Bacillus cereus, B. catar: Branhamella catarrhalis and Diphther*

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