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Resistance, Minimum Inhibitory and Bactericidal Concentration Profiles of Oral Bacteria from HIV/AIDS Patients in South Western Uganda

Joseph Obiezu Chukwujekwu Ezeonwumelu[1,2,3*], Muhammad Ntale[4], Keneth Iceland Kasozi[5], Steve Okwudili Ogbonnia[6], Julius Kihdze Tanayen[3,7], Ezera Agwu[8], Chukwudi Onyeka Okonkwo[9], Ambrose Amamchukwu Akunne[2] and Frederick Byarugaba[10]

[1]Department of Pharmacy, Faculty of Medicine, Mbarara University of Science and Technology, P.O.Box 1410, Mbarara, Uganda.
[2]Department of Clinical and Biopharmacy, School of Pharmacy, Kampala International University, Western Campus, Box 71, Ishaka, Uganda.
[3]Kampala International University, Complementary and Alternative Medicine Research, (KIU-CAMRES) Group, Kampala, Uganda.
[4]Department of Chemistry, Makerere University, Kampala, Uganda.
[5]Department of Physiology, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Box 71, Ishaka, Uganda.
[6]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria.
[7]Department of Pharmacology and Therapeutics, Faculty of Medicine, Mbarara University of Science and Technology, P.O.Box 1410, Mbarara, Uganda.
[8]Department of Microbiology, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Box 71, Ishaka, Bushenyi, Uganda.
[9] Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Nnewi Campus, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria.
[10]Department of Microbiology, Faculty of Medicine, Mbarara University of Science and Technology, P.O.Box 1410, Mbarara, Uganda.

Authors' contributions

This work was carried out in collaboration among all the authors. Author JOCE initiated and designed the study. Authors JOCE, EA, AAA and FB collected the data. Authors MN, SOO, EA, COO and FB designed the study, wrote and corrected the protocol. Authors KIK, JKT and JOCE wrote the protocol and the first draft of manuscript, searched for literature, analyzed resistance, MIC and MBC data and read through the data and made corrections. Authors MN, SOO, EA and FB managed the experimental processes and read through and made corrections to the manuscript draft. Authors COO and AAA read through and made corrections to the manuscript draft.

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**Corresponding author: E-mail: nduezeh@gmail.com;*

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ABSTRACT

Background: The development of drug resistance is a major challenge in the management of microbial infections especially in immune-compromised (HIV/AIDS) patients.
Objective: This was to assess levels of antibacterial resistance; minimum inhibitory and bactericidal profiles of oral bacteria isolated from HIV/ AIDS patients in South Western Uganda and compare their levels with those of the reference organisms (control).
Methods: Bacterial isolates were grown on Mueller Hinton Agar, and biochemical tests were conducted using conventional and analytical profile index 20 sugar panel methods to identify strains. Antibiograms using modified Kirby-Bauer tube dilution and agar well diffusion methods were performed on purified isolates using antibiotic discs for resistance analysis and E-test strips for MIC and MBC analysis. Data were analysed using ANOVA with $p< 0.05$ considered statistically significant.
Results: All the tested bacteria except Salmonella pullorum and non haemolytic streptococcus showed 50 to 100% resistance to cotrimoxazole and erythromycin demonstrating resistance development in HIV/AIDS patients in rural communities of Uganda against commonly used antibacterials for management of opportunistic infections. *Staphylococcus aureus* and *Escherichia coli* were both >60% resistant to cotrimoxazole. *Pseudomonas aeruginosa* and *Bacillus cereus* were absolutely resistant (100%) to all the antibacterial agents used in this study. MIC and MBC levels for *S. aureus* when compared with *S. aureus* ATCC 25293 were highly related showing the level of ineffectiveness of the tested drugs ($p=0.235>0.05$ (MIC) and $p=0.409>0.05$ (MBC). High MIC and MBC levels of cotrimoxazole against *Pseudomonas aeruginosa* were followed by those of *Staphylococcus aureus*, perhaps associated with neutropenia and granulocyte dysfunction in human infections, necessitating appropriate dosage adjustments. Gentamycin and ceftriaxone had high MIC and MBC levels against *E. coli* respectively. Further analysis showed significance in ciprofloxacin against all the bacteria in its low MICs.
Conclusion: Bacterial resistance and poor drug efficacy in HIV/AIDS patients in rural communities are a major challenge in Uganda.

Keywords: Bactericidal concentration; bacterial inhibition; drug resistance; HIV/AIDS in Uganda.

ABBREVIATIONS

HIV = Human Immunodeficiency Virus; AIDS = Acquired Immunodeficiency Syndrome; MHA = Mueller Hinton Agar; API = Analytical Profile Index; MIC = Minimum Inhibitory Concentration; MICs = Minimum Inhibitory Concentrations; MBC = Minimum Bactericidal Concentration; MBCs = Minimum Bactericidal Concentrations; SPSS = Statistical Package for Social Sciences; S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; S. saprophyticus = Staphylococcus saprophyticus; S. mutans = Streptococcus mutans; S. pullorum = Salmonella pullorum; K. pneumoniae = Klebsiella pneumoniae; S. pneumoniae = Streptococcus pneumoniae; NH strep = Non haemolytic streptococcus; P. mirabilis = Proteus mirabilis; P. aeruginosa = Pseudomonas aeruginosa; B. cereus = Bacillus cereus; S = Sensitive; R= Resistant; I = Intermediate; p (p value) = Probability value; OIs = Opportunistic Infections; ART = Antiretroviral Therapy; KIU-WC = Kampala International University Western Campus; ATCC = American type culture Collections; CLSI = Clinical and Laboratory Standards Institute; ANOVA = Analysis of Variance; Genta = Gentamycin; Cipro = Ciprofloxacin; Erythro = Erythromycin;Cotrim = Cotrimoxazole;Ceftri = Ceftriaxone; MRSA = Methicillin Resistant Staphylococcus aureus; TASO = The AIDS Support Organisation and UNCST = Uganda National Council for Science and Technology.

1. INTRODUCTION

Uganda which is a developing country has been renowned for her efforts in combating the HIV/AIDS epidemic on the African continent [1]. The HIV/AIDS pandemic has become synonymous with opportunistic infections (OIs) whose control relies heavily on antimicrobial

agents [2]. The essence of treatment with antiretroviral agents (ARTs) against HIV/AIDS is to allow the already compromised immune system of the patient to recover. However, these ARTs are also under threat of resistance because different sub-types of HIV vary in their degree of response to the therapy [3–5]. Studies have shown that the development of the disease hinges on the inability of the cellular immunity to defend the body against the retrovirus [6]. In Uganda, studies have shown that community stigmatization, low level of public exposure and limited health facilities have been implicated in the continuous spread of HIV & AIDS in rural communities [7,8]. Moreover, the high demand for HIV health care services amidst limited funding has led to increased missed or late testing opportunities thus making the situation more complicated [9]. This indicates that the prevalence of the disease could be higher than what is currently reported at 7.4% [10] and the burden of the HIV and its associated opportunistic infections is bound to rise higher. In Uganda, the major opportunistic infections identified were shown by several studies to be of bacterial such as Streptococcus pneumoniae, Streptococcus mutans, non haemolytic streptococcus, *Staphylococcus aureus* and Haemophilus influenza; but non-typhoidal Salmonella, *Staphylococcus saprophyticus*, *Bacillus cereus*, *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* have also been implicated [11,12]; and fungal origin like Histoplasma, Cryptococcus, Toxoplasma, Zygomycoses, Geotrichoses, and Aspergillus [13,14,15,16]. Some forms of 2-3% oral lesion occur with common major recurrent ulcerations [17] and painful necrotizing stomatitis due to bacterial and yeast infections with the most commonly isolated being Candida albicans [18]. The clinical criteria for diagnosis of opportunistic infections considered were based on clinical evidence and guidelines such as clinical examination of candidiasis, TB screening algorithm for HIV-infected patients and patient history [19,20]. The presence of opportunistic pathogenic bacteria in HIV/AIDS positive persons requires immediate treatment with some antibacterial agents such as gentamycin, ciprofloxacin, erythromycin, cotrimoxazole and ceftriaxone. But absence of strong drug prescription policies in many African countries may make room for self-medication and use of sub-lethal drug doses by HIV/AIDS patients [21], thereby leading to antibiotic resistance and increased health burden [22–24]. The work of Kemajou et al. [25] in Nigeria among HIV positive patients with urinary tract infections has shown a significant magnitude of bacterial infection with multiple drug resistant *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* constituting a serious public health burden because of possibility of passing these resistant pathogens to a healthy population. The minimum bactericidal concentration (MBC) has been shown to be a more important and reliable guide in the assessment of antibiotic activity [26], because it shows drugs' ability to kill bacterial colonies at least dose unlike the minimum inhibitory concentration (MIC), which gives the least dose of an antibiotic required to inhibit bacterial growth [22]. The bactericidal activity of a drug is measured when MBC ≥ 4 MICs. It's important to evaluate the level of MBC/MIC in isolated bacterial strains in patients having HIV/AIDS from a rural community because rural communities of Africa are faced with severe health service shortages, and drug usage amongst patients and the general public is poorly regulated on the continent [23,24,27].

Noting that the major nosocomial bacterial infections amongst HIV/AIDS patients in the study location have been established [12], this study was conducted to assess the levels of resistance, MIC and MBC profiles of oral bacteria from HIV/AIDS patients in South Western Uganda and compare their levels with those of the reference organisms (control).

2. MATERIALS AND METHODS

2.1 Study Design

This was an experimental study in which previously isolated clinical oral bacterial isolates [12] were supplied by the Microbiology Laboratory freezing store KIU-WC room and analyzed.

2.2 Sample Size and Sampling Technique

"Out of 610 bacterial isolates identified in our previous study, [12], 100 bacterial isolates were systematically selected using a ratio of 1:6 (every 6th isolate selected) with the first isolate being randomly selected". Subsequently, 22 isolates of standard bacteria (a duplicate of each of the 11 isolates) were collected according to whether the bacterium was gram positive or gram negative, thereby making a total of 122 bacterial isolates.

2.3 Isolation and Identification of Bacteria

All 122 samples collected were grown on MacConkey agar, Chocolate agar and Blood agar to get fresh isolates and also confirm the identity of previously identified isolates [20,28–30], including the standard bacteria. The bacterial isolates were identified by using appropriate culture media, microscopy using gram stain, appropriate biochemical oxidase and catalase tests [31], chrom-agar orientation and carbohydrate assimilation tests using the analytical profile index testing kits (Biomerieux® SA France, INS005517) utilising apiwebTM identification software.

2.4 Susceptibility Testing

A bacterial suspension with a turbidity equivalent to 1.5 McFarland was made using overnight cultures and compared with turbidity standard and measured with a densitometer for certainty. The suspension was homogenized and used immediately. All purified isolates were subjected to susceptibility testing, followed by MIC and MBC measurements according to previously published methods [31]. Antibiograms of the purified bacterial isolates were carried out using Biomerieux® France antibiotics susceptibility discs for bacterial resistance while Minimum Inhibitory Concentration (MIC) was done with the aid of E-test strips [31]. The sensitivity tests for the bacterial strains were controlled with standard organisms of American Type Culture Collection (ATCC 25923 *Staphylococcus aureus* for pyogenic bacteria, ATCC 25922 *Escherichia coli* for enterobacteriaceae and ATCC 27853 *Pseudomonas aeruginosa*) for *Pseudomonas aeruginosa*. Antibiotics disc and its content used were as shown: ciprofloxacin (5 µg), erythromycin (15 µg), gentamycin (10 µg), ceftriaxone (30 µg) and cotrimoxazole (25 µg) discs. The suspension of 18-24 hr freshly sub-cultured bacterial strains were inoculated on freshly prepared Mueller Hinton agar, while respective antibiotic discs were carefully and specifically placed on the agar surface and incubated for 18-24 hours at 37℃. Then, zone of inhibition was measured with a transparent ruler and recorded. The results were interpreted as sensitive (S), intermediate (I) and resistant (R) using the CLSI (2007) guidelines. The susceptibility profiles (MIC and MBC) of the bacterial strains were carried out using Biomerieux® E-test strips (ciprofloxacin, erythromycin, gentamycin, and cotrimoxazole ceftriaxone). The MICs were determined by placing E-test strips on the freshly prepared Mueller Hinton agar inoculated with bacterial isolates, incubated for 18-24 hr and results recorded. MBC was determined using E-test method as described previously [27] on the bacterial isolates. The levels of comparison were considered between the 11 test bacterial isolates and their corresponding standard bacterial isolates and among the levels of resistance of the 11 bacterial isolates.

2.5 Data Analysis

Data were recorded in duplicates and univariate analysis was carried out. ANOVA test using SPSS version 20 was carried out to assess level of association between the test groups and standard reference organism. Statistical significance was measured at 95% confidence interval and a $p < 0.05$ was considered to be statistically significant.

3. RESULTS

The study showed that *Staphylococcus aureus* and *E. coli* had resistance against cotrimoxazole at 93.2% and 66.7% respectively. Amongst the Klebsiella pneumoniae, resistance was associated with cotrimoxazole at 66.7%. Streptococcus mutans had a resistance of 77.8% while Streptococcus pneumoniae had resistance of 85.8% against erythromycin. *Pseudomonas aeruginosa* and *Bacillus cereus* showed total resistance (100%) against all the antibacterials used except ceftriaxone. In addition, resistance by the bacterial isolates was found to be expressed against erythromycin and cotrimoxazole at 68.0% and 64.8% respectively as shown in Table 1. Analysis of variance (ANOVA) showed that there were statistically significant observations.

The antimicrobial drugs showed less effective levels of minimum inhibitory effects on *Staphylococcus aureus* compared to the more effective levels of inhibition on the reference organisms *Staphylococcus aureus* ATCC 25293, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The levels of MIC of all the drugs on *E. coli* (12.36 ± 15.55 µg/ml for gentamycin, 0.42 ± 0.48 µg/ml for ciprofloxacin, 0.01 ± 0.03 µg/ml for erythromycin, 0.00 ± 0.00 µg/ml for cotrimoxazole and 0.25 ± 0.16 µg/ml for ceftriaxone) were respectively comparable to their levels on *S. saprophyticus* (1.50 ± 0.00 µg/ml for gentamycin, 0.00 ± 0.00 µg/ml for ciprofloxacin, 0.00 ± 0.00 µg/ml for erythromycin, 0.00 ± 0.00 µg/ml for

cotrimoxazole and 0.25 ± 0.00 for ceftriaxone). Also, gentamycin and ciprofloxacin inhibited the growth of control *Pseudomonas aeruginosa* more effectively than their test counterparts (3.10 ± 4.11 µg/ml); (2.00 ± 2.83 µg/ml) and (0.13 ± 0.00 µg/ml; 0.25 ± 0.00 µg/ml) respectively. Importantly, gentamycin inhibited the growth of *Klebsiella pneumoniae* at the minimum concentration of 37.99 ± 96.16 µg/ml; followed by *Bacillus cereus* at 32.00 ± 0.00 µg/ml and *Streptococcus pneumoniae* at 27.50 ± 83.85 µg/ml but inhibited the growth of non haemolytic streptococcus at 0.13 ± 0.00 µg/ml as compared to its effects on reference bacteria at 0.06 ± 0.00 µg/ml. Ciprofloxacin and erythromycin exhibited respective inhibitory effects on *Bacillus cereus* at concentration of 16.00 ± 0.00 µg/ml and *Salmonella pullorum* at 12.00 ± 0.00 µg/ml. Cotrimoxazole and ceftriaxone had their corresponding MICs of 6.00 ± 8.49 and 2.34 ± 1.29 µg/ml on *Proteus mirabilis* and *Staphylococcus aureus* depicting low antibacterial effects and their bacterial inhibitory effects on *S. mutans* and *Salmonella pullorum* were individually 0.96 ± 2.24 µg/ml and 0.06 ± 0.00 µg/ml. All these imply that these drugs are more effective on the reference microorganisms than on the experimental microorganisms. This is shown in Table 2. Further analysis of variance showed statistical significance in ciprofloxacin activity against the microorganisms.

Ceftriaxone killed *Staphylococcus aureus* at an MBC of 0.01 ± 0.08 µg/ml, followed by ciprofloxacin at 9.46 ± 20.23 µg/ml, gentamycin at 28.89 ± 60.71 µg/ml, erythromycin at 67.09 ± 98.75 µg/ml and cotrimoxazole at 73.64 ± 106.53 µg/ml showing the relative bactericidal activities of the antibacterial agents. Whereas erythromycin killed *E. coli* at an MBC of 0.22 ± 0.67 µg/ml, this was followed by ceftriaxone at 4.22 ± 5.0 µg/ml, ciprofloxacin at 15.00 ± 27.79 µg/ml, gentamycin at 74.33 ± 103.89 µg/ml and then by cotrimoxazole at 227.56 ± 451.54 µg/ml. Furthermore, ciprofloxacin had the most effective MBC (4.00 ± 0.00 µg/ml) against *Pseudomonas aeruginosa* (32 ± 0.00 µg/ml) of all the test bacteria apart from the reference microorganisms while ceftriaxone also had a seemingly significant bactericidal activity against *Pseudomonas aeruginosa* (9.00 ± 0.0 µg/ml) at face value. However, comparisons showed that there were significant bactericidal observations in ciprofloxacin, erythromycin, cotrimoxazole and ceftriaxone while gentamycin did not have any statistically significant bactericidal observations against the isolates.

4. DISCUSSION

Maximum resistance to erythromycin and cotrimoxazole was demonstrated by the bacterial isolates in this study (Table 1) showing that antimicrobial resistance is a real threat in HIV/AIDS patients living in Uganda. Bearing in mind that cotrimoxazole prophylaxis is commonly used in Uganda to control opportunistic infections, the development of resistance against it amongst HIV patients raises major concerns on disease control within rural communities of Uganda [33]. This is in line with the previous concern raised in our previous study [12]. Results of the study conducted in Nigeria on HIV positive patients with urinary tract infections [25] revealed that HIV seropositive individuals exhibited significant levels of bacterial colonization with multiple drug resistant *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* which is similar with earlier findings [34]. This further confirms that immunocompromised status like HIV is a hot spot for multiple drug resistant pathogens to multiply relentlessly and become source of infection to other healthy population and this situation raises serious health concern. Most of the isolates were resistant to oxacillin, tetracycline, chloramphenicol and ampicillin. This high MDR antibiotic level also suggests a very high resistance gene pool due perhaps to gross misuse and inappropriate usage of antibacterial agents. The upsurge in antibiotic resistance noticed in the study [25] is in agreement with earlier work [35], where antibiotic abuse and high prevalence of self medication with antibiotics were identified as being responsible for the selection of antibiotic resistant bacterial strains [25]. Since a majority of drugs in the Ugandan market have not been under strict control and usage not under close monitoring by the regulatory bodies because of shortage of pharmaceutical personnel, it's clear that drug resistance is a major public health concern, though previously this had been ignored. *S. aureus* and *E. coli* were the most resistant bacterial isolates to cotrimoxazole. Moreover, resistance by *Pseudomonas aeruginosa* and *Bacillus cereus* was absolute since no antibacterial agent was effective against them. This is in agreement with a previous finding in which *Staphylococcus aureus*, *E. coli* and *P. aeruginosa* have been associated with high resistance [36]. *Staphylococcus aureus* is responsible for a majority of cases in health care systems [37], and the high level of resistance demonstrated in this study is a major

concern for Uganda health care providers and the country. In a previous study, susceptibility of *S. aureus*, *Klebsiella pneumonia*e and *E. coli* were found to be significant in children [38]. Our study has demonstrated that resistance is high in rural communities probably due to the population demographic differences and study area [39]. In Uganda, the control of HIV & AIDS has been associated with high rates of stigmatization, and low level of health care provision amidst the low staff levels in the region [40]. The increasing number of infections caused by methicillin resistant *S. aureus* (MRSA) strains is a major concern especially in HIV/AIDS patients who have been identified as high risk individuals [41].

In Uganda, no studies have been conducted to date comparing Minimum Inhibitory Concentrations (MICs) of series of antibacterials on microorganisms from HIV & AIDS patients. The findings are highly important for the Ugandan community, which heavily depends on antibiotics for management of OIs in HIV & AIDS patients [42], especially in rural communities. We demonstrated that the MIC of the drugs used was the least effective for *Staphylococcus aureus* amongst all micro-organisms tested as shown in Table 2 although all the drugs produced effective control against *Staphylococccus saprophyticus*. This is worrying since high MIC values of *S. aureus* have been associated with higher disease burden [43], which inevitably makes management least effective and efficient and more highly expensive coupled with extended hospitalization and re-investigation of cause of disease. This would imply that new drug options such as vancomycin should be used as replacements in a majority of health centres for management of oral infections due to *S. aureus* since it has been shown to be effective [22]. However, the development of treatment failure is a major challenge for HIV & AIDS patients especially in rural communities since pharmacokinetic indices of vancomycin have failed to correlate with clinical response as demonstrated in a previous study [44]. This would be due to differences in cellular (*in vitro*) and mammalian host (*in vivo*) responses to disease. In spite of the reported treatment failure, vancomycin is not only expensive and relatively unavailable in the rural settings, it is mainly administered parenterally and as such patients are liable to pains due to injection of vancomycin and would also require highly and rurally scarce skilled hands and expertise in its administration as an injectable.

Staphylococcus aureus was the least susceptible to gentamycin as gentamycin showed the least effective Minimum Bactericidal Concentration (MBC) against *Staphylococcus aureus* as shown in Table 3. The community isolates of *S. aureus* in this study demonstrated a persistent phenotype which would have been affected by the concentration of the antibacterials used [45]. This implies that the efficacy of the drugs used in the community against this community strain of *S. aureus* are inefficient, showing the need to review the antibiotic usage pattern in this community for the management of *S. aureus* related pathogenic infections in these patients. Moreover, the high prevalence of opportunistic infections in Uganda shows that the burden on the drug industry is enormous amongst HIV & AIDS patients [14–16].

This study also showed that cotrimoxazole was not effective against *Pseudomonas aeruginosa* isolates, following *Staphylococcus saprophyticus* in the ranks due to the levels of MIC realised in this study. *Pseudomonas aeruginosa* response to cotrimoxazole was followed by that of *Staphylococcus aureus*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections are associated with neutropenia and granulocyte dysfunction thus complicating the progression and management of HIV infection [46]. High levels of MIC on *Pseudomonas aeruginosa* have been associated with higher mortalities in children and the cut off points are expected to be lower for HIV & AIDS patients [47]. The levels of MIC reported in this study would be due to the development of genetic adaptations which are associated with HIV & AIDS [48]. This would be a reason to the contrast in observations from a previous study which showed that MICs of ciprofloxacin and gentamycin had significant effects on clearing *Pseudomonas aeruginosa* [49]. Different serogroups of *Pseudomonas aeruginosa* have different MIC activity; it's possible that a virulent serotype is prevalent in this community. This is because serogroup O:11 has been shown to be more resistant to ciprofloxacin and gentamycin and the need to switch antibiotics to include the appropriate carbapenems [50] especially for HIV& AIDS patients in the intensive care units of the regional health centres. *Pseudomonas aeruginosa* yielded to a high level of MBC of the drugs used necessitating their relative efficacy to be modulated.

Table 1. Susceptibility patterns of bacterial isolates

Bacteria	No. of isolates	S/R/I	Frequency (%) of antibacterial agent activity on test isolates				
			Genta (10 µg)	Cipro (5 µg)	Erythro (15 µg)	Cotrim (25 µg)	Ceftri (30 µg)
S. aureus	44	S	26 (59.1)	13 (29.5)	1 (2.3)	1 (2.3)	33 (75.0)
		R	18 (40.9)	23 (52.3)	41 (93.2)	41 (93.2)	11 (25.0)
		I	0 (0.0)	8 (18.2)	2 (4.5)	2 (4.5)	0 (0.0)
S. aureus ATCC 25293	12	S	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)
S. saprophyticus	1	S	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
		R	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
		I	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
E. coli	9	S	8 (88.9)	7 (77.8)	4 (44.4)	3 (33.3)	6 (66.7)
		R	1 (11.1)	1 (11.1)	5 (55.6)	6 (66.7)	3 (33.3)
		I	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)
E. coli ATCC 25922	8	S	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)	8 (100.0)
S. pullorum	1	S	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)
		R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
		I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
K. pneumoniae	9	S	5 (55.6)	5 (55.6)	1 (11.1)	3 (33.3)	8 (88.9)
		R	4 (44.4)	3 (33.3)	6 (66.7)	6 (66.7)	1 (11.1)
		I	0 (0.0)	1 (11.1)	2 (22.2)	0 (0.0)	0 (0.0)
S. mutans	9	S	9 (100.0)	6 (66.7)	0 (0.0)	4 (44.4)	7 (77.8)
		R	0 (0.0)	3 (33.3)	7 (77.8)	5 (55.6)	1 (11.1)
		I	0 (0.0)	0 (0.0)	2 (22.2)	0 (0.0)	1 (11.1)
S. pneumoniae	21	S	14 (66.7)	10 (47.6)	1 (4.7)	3 (14.3)	14 (66.7)
		R	6 (28.6)	11 (52.4)	18 (85.8)	16 (76.2)	7 (33.3)
		I	1 (4.7)	0 (0.0)	2 (9.5)	2 (9.5)	0 (0.0)
NH streptococcus	1	S	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)
		R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
		I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
P. mirabilis	2	S	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	2 (100.0)
		R	0 (0.0)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)
		I	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
P. aeruginosa	2	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
		R	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)
		I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Bacteria	No. of isolates	S/R/I	Frequency (%) of antibacterial agent activity on test isolates				
			Genta (10 µg)	Cipro (5 µg)	Erythro (15 µg)	Cotrim (25 µg)	Ceftri (30 µg)
P. aeruginosa ATCC 27853	2	S	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)
B. cereus	1	S	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
		R	1 (100.0)	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)
		I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	122	S	88 (72.1)	66 (54.1)	31 (25.4)	39 (32.0)	95 (77.9)
		R	32 (26.2)	44 (36.1)	83 (68.0)	79 (64.8)	26 (21.3)
		I	2 (1.6.5)	12 (9.8)	8 (6.6)	4 (3.2)	1 (0.8)

KEY: S. aureus = Staphylococcus aureus, ATCC = American Type Culture Collection, S. saprophyticus = Staphylococcus saprophyticus, E. coli =Escherichia coli, S. pullorum = Salmonella pullorum, K. pneumoniae = Klebsiella pneumoniae, S. mutans = Streptococcus mutans, S. pneumoniae = Streptococcus pneumoniae, NH streptococcus = Non haemolytic streptococcus, P. mirabilis = Proteus mirabilis, P. aeruginosa = Pseudomonas aeruginosa, B. cereus = Bacillus cereus.

Table 2. Minimum inhibitory concentration (MIC) of isolated organisms

Organism	MIC against common drugs (Mean ±SD (µg/ml))					
	Gentamycin	Ciprofloxacin	Erythromycin	Cotrimoxazole	Ceftriaxone	Distilled water
S. aureus	15.84 ± 33.74	0.35 ± 0.51	1.65 ± 6.68	5.16 ± 17.43	2.34 ± 1.29	0.00 ± 0.00
S. aureus ATCC 25293	0.06 ± 0.00	0.13 ± 0.00	0.13 ± 0.00	0.50 ± 0.00	0.0 ± 0.00	0.00 ± 0.00
S. saprophyticus	1.50 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.00	0.00 ± 0.00
E. coli	12.36 ± 15.55	0.42 ± 0.48	0.01 ± 0.03	0.00 ± 0.00	0.25 ± 0.16	0.00 ± 0.00
E. coli ATCC 25922	0.13 ± 0.00	1.00 ± 0.00	0.50 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00
S. pullorum	1.00 ± 0.00	0.25 ± 0.00	12.00 ± 0.00	0.00 ± 0.00	0.06 ± 0.00	0.00 ± 0.00
K. pneumoniae	37.99 ± 96.16	0.52 ± 0.49	1.14 ± 3.02	1.14 ± 3.02	0.22 ± 0.14	0.00 ± 0.00
S. mutans	3.39 ± 5.95	0.97 ± 1.02	6.40 ± 9.10	0.96 ± 2.24	0.11 ± 0.11	0.00 ± 0.00
S. pneumoniae	27.50 ± 83.85	0.45 ± 0.49	0.07 ± 0.16	2.96 ± 7.88	0.20 ± 0.15	0.00 ± 0.00
NH streptococcus	0.13 ± 0.00	0.25 ± 0.00	0.13 ±0.00	1.00 ± 0.00	0.50 ± 0.00	0.00 ± 0.00
P. mirabilis	8.06 ± 11.23	0.22 ± 0.04	0.00 ± 0.00	6.00 ± 8.49	0.16 ± 0.13	0.00 ± 0.00
P. aeruginosa	3.10 ± 4.11	2.00 ± 2.83	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00
P. aeruginosa ATCC 27853	0.13 ± 0.00	0.25 ± 0.00	0.05 ± 0.00	0.20 ± 0.00	0.06 ± 0.00	0.00 ± 0.00
B. cereus	32.00 ± 0.00	16.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00

KEY: S. aureus = Staphylococcus aureus, ATCC = American Type Culture Collection, S. saprophyticus = Staphylococcus saprophyticus, E. coli = Escherichia coli, S. pullorum = Salmonella pullorum, K. pneumoniae = Klebsiella pneumoniae, S. mutans = Streptococcus mutans, S. pneumoniae = Streptococcus pneumoniae, NH streptococcus = Non haemolytic streptococcus, P. mirabilis = Proteus mirabilis, P. aeruginosa = Pseudomonas aeruginosa, B. cereus = Bacillus cereus and Water = Distilled water

Table 3. Minimum bactericidal concentration (MBC) of isolated organisms

Organism	Minimum bactericidal concentration against common drugs (Mean ±SD (µg/ml))					
	Gentamycin	**Ciprofloxacin**	**Erythromycin**	**Cotrimoxazole**	**Ceftriaxone**	**Water**
S. aureus	28.89 ± 60.71	9.46 ± 20.23	67.09 ± 98.75	73.64 ± 106.53	0.01 ± 0.08	0.00 ± 0.00
S. aureus ATCC 25293	1.0E-13 ± 0.00	7.1E-15± 0.00	256.00 ± 0.00	4.5E-13 ± 0.00	3.18 ± 9.12	0.00 ± 0.00
S. saprophyticus	4.00 ± 0.00	32.00 ± 0.00	128.00 ± 0.00	1024.00 ± 0. 00	1.3E-14±0.0	0.00 ± 0.00
E. coli	74.33 ± 103.89	15.00 ± 27.79	0.22 ± 0.67	227.56 ± 451.54	4.22 ± 5.02	0.00 ± 0.00
E. coli ATCC 25922	8.00 ± 0.00	32.00 ± 0.00	512.00 ± 0.00	424.00±170.83	22.00 ± 5.66	0.00 ± 0.00
S. pullorum	16.00 ± 0.00	32.00 ± 0.00	00.00 ± 0.00	0.00 ± 0.00	24.00 ± 0.00	0.00 ± 0.00
K. pneumoniae	40.67 ± 83.56	0.00 ± 0.00	56.89 ± 170.67	131.78 ± 341.33	17.33±20.47	0.00 ± 0.00
S. mutans	131.11± 35.85	31.22 ± 44.89	114.22±185.68	6.67 ± 11 .31	10.22±11.29	0.00 ± 0.00
S. pneumoniae	27.81 ± 40.43	6.67 ± 15.54	13.14 ± 55.76	79.24 ± 137.12	20.57±18.24	0.00 ± 0.00
NH streptococcus	64.00 ± 0.00	256.00 ± 0.00	512.00±0.00	256.00 ± 0.00	8.8E-16±0.0	0.00 ± 0.00
P. mirabilis	16.00 ± 22.63	32.00 ± 45.25	256.00±362.04	64.00 ± 90.51	14.00±14.14	0.00 ± 0.00
P. aeruginosa	32.00 ± 0.00	4.00 ± 0.00	512.00 ± 0.00	192.00 ± 90. 51	9.00 ± 9.90	0.00 ± 0.00
P. aeruginosa ATCC 27853	64.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9. 00 ± 9.90	0.00 ± 0.00
B. cereus	32.00 ± 0.00	128.00 ± 0.00	256.00±0.00	128.00 ± 0.0 0	16.00 ± 0.00	0.00 ± 0.00

Key: S. aureus = Staphylococcus aureus, ATCC = American Type Culture Collection, S. saprophyticus = Staphylococcus saprophyticus, E. coli = Escherichia coli, S. pullorum = Salmonella pullorum, K. pneumoniae = Klebsiella pneumoniae, S. mutans = Streptococcus mutans, S. pneumoniae = Streptococcus pneumoniae, NH streptococcus = Non haemolytic streptococcus, P. mirabilis = Proteus mirabilis, P. aeruginosa = Pseudomonas aeruginosa, B. cereus = Bacillus cereus and Water = Distilled water

The levels of MIC of the drugs used in this study against *Escherichia coli* were comparable to their levels of MIC on *Staphylococcus saprophyticus* in that they both showed high titres. These pathogens are often associated with urinary tract infections [51], and the fact that they were isolated in nasal-oral samples shows the evolutionary pattern of the pathogens in these communities. In the management of these infections, appropriate dosages have been recommended since sub-MIC levels have been shown [52] to have an effect of increasing *S. saprophyticus* colonization in cell cultures, thus showing the need for clinicians to be at par with the appropriate dosage for effective management of these microbes especially in HIV & AIDS patients. This study has been the first to demonstrate MIC titres from these patients against oral microbes in HIV & AIDS patients from a rural community. Gentamycin was not as effective as ceftriaxone due to high MBC values against *E. coli*. This correlated positively with the MIC activity thus showing the reduction in efficacy of a majority of the drugs used in the study.

Further analysis with ANOVA showed statistical significance in ciprofloxacin ($p < 0.05$) against all the microbes depicting effective inhibitory effect. Ciprofloxacin is a third generation fluorinated quinolone structurally related to nalidixic acid. Bacterial resistance to ciprofloxacin develops infrequently, both *in vitro* and clinically, except in the setting of pseudomonal respiratory tract infections [53]. Ciprofloxacin resistance is a major threat which is bound to be realised under irrational drug usage due to its frequent use. Also, the resistance to other antibacterials was high. Increasing antibacterial resistance trends indicate that it is imperative to rationalize the use of antimicrobials in the community and also use these conservatively. Moreover, the MBCs of ciprofloxacin, erythromycin, cotrimoxazole and ceftriaxone were significant ($p<0.05$) against the microbial isolates.

The poor regulation [23] of antimicrobial agents seems to be the leading cause of the high resistance and low drug potency demonstrated in this study. Amidst the existing challenges, opportunistic infections are bound to flourish especially among HIV & AIDS patients in rural communities [21], thus leading to increased public spending by the central government on health care which ultimately leads to a heavy tax burden onto the citizens [54] of Uganda.

5. CONCLUSION

The study was able to demonstrate the development of resistance by oral isolated microbes in HIV & AIDS patients in a rural community of Uganda. *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* were the major pathogens isolated which affected the drug potency. *Staphylococcus aureus* showed the highest MIC and MBC activity thus leading to low drug potency. *E. coli* followed and activity against *Pseudomonas aeruginosa* was relative. Since these were orally isolated pathogens, the study has been able to illustrate the role of oral hygiene in HIV & AIDS persons in the management of opportunistic infections.

Therefore, we recommend a further study on the serogroups, serotypes of the isolated microbes to determine the resistant strains would help to shed more light on the epidemiological pattern of the strains responsible for these observations. Since rural communities are today laden with varied supplies of antibacterials in the market, it is really important to carry out a study on these bacteria with practically existing antibacterials in the rural drug shops in the Uganda market. Moreover, rural communities of Uganda rely heavily on ethnomedical plants for management of opportunistic infections; a study to identify the potency and level of resistance to selected plant species of community relevance should be conducted.

CONSENT

Written consent was sought and obtained from the Kampala International University Microbiology Research Laboratory, Ishaka, Bushenyi where the isolates were being kept with the consent of the former researcher on these organisms.

ETHICAL CONSIDERATIONS

Ethical approval was sought and obtained from The AIDS Support Organisation (TASO) Kampala, Uganda National Council for Science and Technology (UNCST) and Mbarara University of Science and Technology Institution's Research and Ethics Committees. These clearances enabled us to use the clinical isolates supplied by the Microbiology Department, KIU_WC.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Slutkin G, Okware S, Naamara W, Sutherland D, Flanagan D, Carael M, Blas E, Delay P, Tarantola D. How Uganda reversed its HIV epidemic. AIDS Behav. 2006;10(4):351–360.
2. Dockrell DH, Edwards S, Fisher M, Williams I, Nelson M. Evolving controversies and challenges in the management of opportunistic infections in HIV-seropositive individuals. J. Infect. 2011;63(3):177–186.
3. Ataher Q, Portsmouth S, Napolitano LA, Eng S, Greenacre A, Kambugu A, Wood R, Badal-Faesen S, Tressler R. The epidemiology and clinical correlates of HIV-1 co-receptor tropism in non-subtype B infections from India, Uganda and South Africa. J. Int. AIDS Soc. 2012;15(1):2.
4. Spacek L, Shihab HM, Kamya MR, Mwesigire D, Ronald A, Mayanja H, Moore RD, Bates M, Quinn TC. Response to antiretroviral therapy in HIV-infected patients attending a public, urban clinic in Kampala, Uganda. Clin. Infect. Dis. 2006;42:252–259.
5. Devi SB, Ningshen R, Arvind G. Burden of opportunistic infections in HIV / AIDS patients in the highly active antiretroviral therapy Era. A Regional Institute of Medical in Human Immunodefic. Virus. 2008;5–8.
6. Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, Pertel T, Ranasinghe S, Lindqvist M, Davis I, Lane K, Rychert J, Rosenberg ES, Piechocka-Trocha A, Brass AL, Brenchley JM, Walker BD, Streeck H. HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. Sci. Transl. Med. 2012;4(123):123-25.
7. Tsai AC, Bangsberg DR, Kegeles SM, Katz IT, Haberer JE, Muzoora C, Kumbakumba E, Hunt PW, Martin JN, Weiser SD. Internalized stigma, social distance, and disclosure of HIV seropositivity in rural Uganda. Ann. Behav. Med. 2014;46(3):1–17.
8. Mburu G, Ram M, Siu G, Bitira D, Skovdal M, Holland P. Intersectionality of HIV stigma and masculinity in eastern Uganda: Implications for involving men in HIV programmes. BMC Public Health. 2014;14: 1061.
9. Wanyenze RK, Kamya MR, Fatch R, Mayanja-Kizza H, Baveewo S, Sawires S, Bangsberg DR, Coates T, Hahn JA. Missed opportunities for HIV testing and late-stage diagnosis among HIV-infected patients in Uganda. PLoS One. 2011;6(7): e21794.
10. UNAIDS. The HIV And AIDS Uganda Country Progress Report; 2014.
11. Hidron AI, Kempker R, Moanna A, Rimland D. Methicillin-resistant *Staphylococcus aureus* in HIV-infected patients. Infect. Drug Resist. 2010;3:73-86,.
12. Agwu E, Ihongbe JC, Ezeonwumelu JO, Lodhi MM. Baseline burden and antimicrobial susceptibility of pathogenic bacteria recovered from oral lesions of patients with HIV/AIDS in South- Western Uganda. Oral Sci. Int. 2015;12(2):59–66.
13. Ramos-Gomez FJ, Flaitz C, Catapano P, Murray P, Milnes AR, Dorenbaum A. Classification, diagnostic criteria, and treatment recommendations for orofacial manifestations in HIV-infected pediatric patients. Collaborative Workgroup on Oral Manifestations of Pediatric HIV Infection. J. Clin. Ped. Dent. 1999;23:85-96.
14. Rubaihayo J, Tumwesigye NM, Konde-Lule J. Trends in prevalence of selected opportunistic infections associated with HIV/AIDS in Uganda. BMC Infect. Dis. 2015;15(1):187.
15. Agwu E, Ihongbe JC, Tirwomwe JF, Pazos V, Tirwomwe M, Casadesus L. Appraisal of oral lesions status of HIV / AIDS patients in South Western Uganda. Braz J Oral Sci. 2008;7(26):1591–1595.
16. Tirwomwe JF, Rwenyonyi CM, Muwazi LM, Besige B, Mboli F. Oral manifestations of HIV/AIDS in clients attending TASO clinics in Uganda. Clin Oral Invest. 2007;11:289–292.
17. Muzyka BC, Glick M. Major aphthous ulcers in patients with HIV disease. Oral Surg. Oral Med. Oral Pathol. 1994;77:116–20.
18. Korting HC, Ollert M, Georgii A. *In vitro* susceptibilities and biotypes of *Candida*

albicans isolates from the oral cavities of patients with human immunodeficiency virus. J. Clin. Microbiol. 1989;26:2626–2631.

19. Iroezindu MO, Ofondu EO, Hausler H, Wyk BV. Prevalence and risk factors for opportunistic infections in HIV patients receiving antiretroviral therapy in a resource-limited setting in Nigeria. J. AIDS Clin. Res. 2013;S3:002.
DOI: 10.4172/2155-6113.S3-002
20. Hewitt DJ, Mcdonald M, Portenoy RK, Rosenfeld B, Passik S, Breitbart W. Pain syndromes and etiologies in ambulatory AIDS patients. Pain, 1997;70:117–123.
21. Bajunirwe F, Arts EJ, Tisch DJ, King CH, Debanne SM, Sethi AK. Adherence and treatment response among HIV-1-infected adults receiving antiretroviral therapy in a rural government hospital in Southwestern Uganda. J. Int. Assoc. Physicians AIDS Care. 2009;8(2):139–147.
22. van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: A systematic review and meta-analysis. Clin. Infect. Dis. 2012;54(6):755–71.
23. Celletti F, Wright A, Palen J, Frehywot S, Markus A, Greenberg A, de Aguiar RAT, Campos F, Buch E, Samb B. Can the deployment of community health workers for the delivery of HIV services represent an effective and sustainable response to health workforce shortages? Results of a multicountry study. AIDS. 2010; 24(Suppl 1):S45–S57.
24. Dieleman M, Bwete V, Maniple E, Bakker M, Namaganda G, Odaga J, van der Wilt GJ. I believe that the staff have reduced their closeness to patients: An exploratory study on the impact of HIV/AIDS on staff in four rural hospitals in Uganda. BMC Health Serv. Res. 2007;7:205.
25. Kemajou TS, Ajugwo AO, Oshoma CE, Enabulele OI. Antibiotic resistance of bacterial isolates from HIV positive patients with urinary tract infection (UTI) in Port Harcourt, Nigeria. J. AIDS Clin. Res. 2016;7:594.
DOI: 10.4172/2155-6113.1000594
26. Petrus EM, Tinakumari S, Chai LC, Ubong A, Tunung R, Elexson N, Chai LF, Son R. A study on the minimum inhibitory concentration and minimum bactericidal concentration of nano colloidal silver on food-borne pathogens. Int. Food Res. J. 2011;18(1):55–66.
27. Greer G, Akinpelumi A, Madueke L. Improving management of childhood malaria in Nigeria and Uganda by improving practices of patent medicine vendors. Arlington, Va. 2004;1:1–66.
28. Andrews JM. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 2001;48(Suppl 1):5–16.
29. Yilmaz MT. Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains. Turkish J. Med. Sci. 2012; 42(Suppl. 2):1423–1429.
30. Hadacek F, Greger H. Testing of antifungal natural products: Methodologies, comparability of results and assay choice. Phytochem. Anal. 2000;11(3):137–147.
31. Cheesbrough M. District laboratory practice in tropical countries. 2006;Part 1.
32. Bolmström A. Determinations of minimum bactericidal concentrations, kill curves, and postantibiotic effects with the Etest technology. Diagn. Microbiol. Infect. Dis. 1994;19(3):187–195.
33. Mermin J, Lule J, Ekwaru JP, Downing R. Hughes P, Bunnell R, Malamba S, Ransom R, Kaharuza F, Coutinho A, Kigozi A, Quick R. Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members. AIDS. 2005;19(10):1035–1042.
34. Adeyemi FM, Akonai KA, Oyemiyi TT, Kassim OO, Blessing IE, et al. Molecular characterization and antibiotic resistance profiles of bacterial isolates cultured from HIV seropositive patients. Arch. Clin. Microbiol. 2015;6:1-12.
35. Adegoke AA, Tom Mvuyo, Okoh A, Steve J. Studies on multiple antibiotic resistant bacteria isolated from surgical site infections. Sci. Res. Essays. 2010;5:3876-3881.
36. Piggot TJ, Holdbrook DA, Khalid S. Electroporation of the *E. coli* and *S. aureus* membranes: Molecular dynamics simulations of complex bacterial membranes. J. Phys. Chem. B. 2011;115(45):13381–13388.
37. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB,

Talan DA. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N. Engl. J. Med. 2006;355(7):666–74.

38. Musiime V, Cook A, Bakeera-Kitaka S, Vhembo T, Lutakome J, Keishanyu R, Prendergast AJ, Lubwama S, Robertson V, Hughes P, Nathoo K, Munderi P, Klein N, Musoke P, Gibb DM. Bacteremia, causative agents and antimicrobial susceptibility among HIV-1-infected children on antiretroviral therapy in Uganda and Zimbabwe. Pediatr. Infect. Dis. J. 2013;32(8):856–62.
39. Parkhurst JO, Lush L. The political environment of HIV: Lessons from a comparison of Uganda and South Africa. Soc. Sci. Med. 2004;59(9):1913–1924.
40. Chan BT, Weiser SD, Boum Y, Siedner MJ, Mocello AR, Haberer JE, Hunt PW, Martin JN, Mayer KH, Bangsberg DR, Tsai AC. Persistent HIV-related stigma in rural Uganda during a period of increasing HIV incidence despite treatment expansion. AIDS. 2015;29(1):83–90.
41. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: Epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. 1997;10(3):505–20.
42. Mermin J, Lule J, Ekwaru JP, Downing R, Hughes P, Bunnell R, Malamba S, Ransom R, Kaharuza F, Coutinho A, Kigozi A, Quick R. Cotrimoxazole prophylaxis by HIV-infected persons in Uganda reduces morbidity and mortality among HIV-uninfected family members. AIDS. 2005;19(10):1035–1042.
43. Van Hal SJ, Lodise TP, Paterson DL. The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: A systematic review and meta-analysis. Clin. Infect. Dis. 2012;54(6):755–771.
44. Clemens EC, Chan JD, Lynch JB, Dellit TH. Relationships between vancomycin minimum inhibitory concentration, dosing strategies, and outcomes in methicillin-resistant *Staphylococcus aureus* bacteremia. Diagn. Microbiol. Infect. Dis. 2011;71(4):408–414.
45. Lechner S, Lewis K, Bertram R. *Staphylococcus aureus* persisters tolerant to bactericidal antibiotics. J. Mol. Microbiol. Biotechnol. 2012;22(4):235–244.
46. Pitrak DL, Tsai HC, Mullane KM, Sutton SH, Stevens P. Accelerated neutrophil apoptosis in the acquired immuno-deficiency syndrome. J. Clin. Invest. 1996;98(12):2714–2719.
47. Tamma PD, Turnbull AE, Milstone AM, Hsu AJ, Carroll KC, Cosgrove SE. Does the piperacillin minimum inhibitory concentration for *pseudomonas aeruginosa* influence clinical outcomes of children with pseudomonal bacteremia? Clin. Infect. Dis. 2012;55(6):799–806.
48. Smith EE, Buckley DG, Wu Z, Saenphimmachak C, Hoffman LR, D'Argenio DA, Miller SI, Ramsey BW, Speert DP, Moskowitz SM, Burns JL, Kaul R, Olson MV. Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. Proc. Natl. Acad. Sci. USA. 2006;103(22):8487–92.
49. Zhanel GG, Kim SO, Davidson RJ, Hoban DJ, Nicolle LE. Effect of subinhibitory concentrations of Ciprofloxacin and gentamicin on the adherence of *Pseudomonas aeruginosa* to Vero cells and voided uroepithelial cells 250. Chemother. 1993;39(0009–3157):105-111.
50. Vitkauskiene A, Skrodeniene E, Dambrauskiene A, Bakšyte G, Macas A, Sakalauskas R. Characteristics of carbapenem-resistant *Pseudomonas aeruginosa* strains in patients with ventilator- associated pneumonia in intensive care units. Medicina (B. Aires). 2011;47(12):652–656.
51. Eriksson A, Giske CG, Ternhag A. The relative importance of *Staphylococcus saprophyticus* as a urinary tract pathogen: Distribution of bacteria among urinary samples analysed during 1 year at a major Swedish laboratory. APMIS. 2013;121(1): 72–78.
52. Erdeljan P, MacDonald KW, Goneau LW, Bevan T, Carriveau R, Razvi H, Denstedt JD, Cadieux PA. Effects of subinhibitory concentrations of ciprofloxacin on Staphylococcus saprophyticus adherence and virulence in urinary tract infections. J. Endourol. 2012;26(1):32–7.
53. Campoli-Richards DM, Monk JP, Price A, Benfield P, Todd PA, Ward A. Ciprofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs. 1988;35(4):373–447.

54. Geng EH, Bwana MB, Muyindike W, Glidden DV, Bangsberg DR, Neilands TB, Bernheimer I, Musinguzi N, Yiannoutsos CT, Martin JN. Failure to initiate antiretroviral therapy, loss to follow-up and mortality among HIV-infected patients during the pre-ART period in Uganda. J. Acquir. Immune Defic. Syndr. 2013;63(2): e64–71.

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