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Efficacy of Commercially Used Antibacterial Agents against Oral Bacteria Associated with HIV/AIDS Patients in South Western Uganda

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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 KKI, 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. All authors read and approved the manuscript for publication.

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ABSTRACT

Aims: This was to determine efficacy and resistance profiles against commonly used commercial antibacterial agents in Uganda in the management of oral pathogens in HIV/AIDS patients.
Study Design: This was an experimental study.
Place and Duration of Study: Microbiology Laboratory, Mbarara University of Science and Technology, Mbarara, Uganda between September 2015 and February 2016.
Methodology: Bacterial isolates were tested against commercial antibacterial agents in Uganda. Drug shops, pharmacies and hospitals were purposively and conveniently sampled. Drugs commonly used for the management of opportunistic infections amongst HIV/AIDS patients were purchased and used in the laboratory for susceptibility, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using standard protocols.
Results: All the bacterial isolates showed mean total resistance above 60% against erythromycin [85 isolates (69.7%)] and cotrimoxazole [79 isolates, (64.8%)]; with injectable gentamicin [97 isolates (79.5%)] and ceftriaxone [105 isolates (86.0%)] displaying high susceptibility; and ciprofloxacin [65 isolates (53.3%)] showing moderate susceptibility. This shows that national policy on effective regulation of these antibacterial agents needs to be revised to ensure that the situation is reversed. Gentamicin showed increased significant mean activity ($P^{***} < .005$, ANOVA, multiple comparisons) in MIC and MBC when compared with the other antimicrobial agents.
Conclusion: Gentamicin was highly efficacious in this study and resistance of these oral bacteria to common commercial antibacterial agents is a major public health burden especially among Uganda HIV/AIDS patients. Improving drug regulation activities will reduce antibacterial resistance and treatment failures. We recommend a survey on the reasons for efficacy of gentamicin against all the commercially available antimicrobials used in this study.

Keywords: Antibacterial resistance; drug efficacy; oral bacteria; HIV and AIDS; drug policy; Uganda.

ABBREVIATIONS

HIV : Human Immunodeficiency Virus
AIDS : Acquired Immunodeficiency Syndrome
MHA : Mueller Hinton Agar
API : Analytical Profile Index
MIC : Minimum Inhibitory Concentration
MBC : Minimum Bactericidal Concentration
MBCs : Minimum Bactericidal Concentrations
p (p value) : Probability value
NDA : National Drug Authority
KIU-WC : Kampala International University Western Campus
NCCLS : National Council for Clinical and Laboratory Standards
CLSI : Clinical and Laboratory Standards Institute
ANOVA : Analysis of Variance
TASO : The AIDS Support Organisation and
UNCST : Uganda National Council for Science and Technology.

1. INTRODUCTION

In a majority of developing countries including Uganda, the food and drug industries have been overly liberalized leading to passive regulation of pharmaceutical agents in a majority of communities [1]. This implies that individuals in communities can access medications without necessarily having a prescription from the hospital/health clinic. This is highly alarming in immune compromised individuals due to the risk of development of drug resistance [2]. In the management of opportunistic infections among HIV/AIDS patients, resistance to commonly used commercially available drugs would be a major blow on the promotion of health within this population [3,4]. Oral lesions in HIV/AIDS patients of South Western Uganda have been studied previously [5] and those due to bacterial pathogens have been shown to be the most prevalent in the region [6]. Moreover, recent findings from the region in the same population have shown that resistance to common

antibacterial agents is a real threat facing the medical profession and this patient population in the region [7], however, no information is available to date on the status of the efficacy of the available drugs in the market. This is important since great variations may exist between studies using antibacterial discs and the commercially available antibacterial agents needing effective regulation and monitoring by drug regulatory bodies. This implies that differences do exist between *in vivo* and *in vitro* studies, thus the need to get clear picture on the resistance burden in the region.

In Uganda, regulation of the drug industry is under the National Drug Authority (NDA), which is responsible for controlling drug supply and consumption within the country, although this has been marred by a lot of challenges [8, 9]. After 12 years of its incorporation, NDA still finds it challenging to enforce a majority of its major objectives, thus leading to increased exploitation of the vacuum by the general public in the use of drugs that are substandard [1]. In addition, regulating drug supply and distribution with a few personnel and large population of unregistered and improperly registered drug shops and pharmacies still continues to be the NDA's major challenges in the execution of its duties. This shows that consumers may be exploited by private drug outlets due to this on-going uneasy battle by the NDA in combating uncontrolled supply, distribution, dispensing and administration of antibacterial agents in Uganda. Also, the limited number of drugs within the Ugandan market has been faced with a major limitation on pharmacovigilance as a result of limited national funding, limited trained human resource in the ministry, and poor coordination of national activities [10]. These weaknesses have subsequently created an open window for exploitation by the drug shops and pharmaceutical industries, which has been a major contributory factor to the development of drug resistance in Uganda [2,7].

Drug resistance to common antibacterial agents amongst HIV/AIDS patients of Uganda has been reported [6,7], however, information on the level of efficacy and resistance against commercially available antibacterials in the Ugandan market is still limited to date. This is because majority of the studies have been done in the laboratory with antibacterial discs and not antibacterials practically present in the market. Some of these commercial antibacterial agents when compared with the highly standardised antibacterial discs may be resisted by these oral pathogens of HIV/AIDS patients possibly due to availability of fake or substandard drugs in the market. This is conceivably subject to poor current good manufacturing practices, poor distribution and storage facilities. This seeming disinterest in studies on commercially available antibacterial agents leads to limited studies and information on the commonly available drugs currently in circulation in the market. This study would provide basic information which would help enforce policy in Uganda for effective drug regulation and promotion of health especially among HIV/AIDS patients. The objective of the study was to determine the efficacy of common commercial antibacterial agents and resistance patterns of oral bacterial isolates from HIV/AIDS patients in South Western Uganda against selected commercial antibacterial agents.

2. MATERIALS AND METHODS

2.1 Study Design

The study design was experimental. Clinical oral bacterial isolates which had earlier been isolated [6] were provided by the Microbiology Laboratory cold store room of KIU-WC and analyzed.

2.2 Sample Size and Sampling Technique

A systematic-random sampling technique was used in selection of a sample of 100 bacterial isolates from 610 bacterial isolates identified in one of the previous studies [6]. Each bacterial isolate was selected in every 6^{th} isolate (1:6). The first bacterial isolate was selected at random. Successively, a duplicate isolate of the standard bacteria was selected using gram negative or positive criteria for each set of the bacteria under study: Two isolates of ATCC 25923 *Staphylococcus aureus* were selected to compare the activity of each of the pyogenic bacteria (that is, two isolates of reference ATCC 25923 *Staphylococcus aureus* for comparison with *Staphylococcus aureus*, two isolates of reference ATCC 25923 *Staphylococcus aureus* for comparison with *Staphylococcus saprophyticus*, two isolates of reference ATCC 25923 *Staphylococcus aureus* for comparison with *Streptococcus mutans*, two isolates of reference ATCC 25923 *Staphylococcus aureus* for comparison with *Streptococcus pneumoniae*, two isolates of reference ATCC 25923 *Staphylococcus aureus* for comparison with non haemolytic streptococcus, and two isolates of

reference ATCC 25923 *Staphylococcus aureus* for comparison with *Bacillus cereus* making a total of 12 isolates of reference ATCC 25923 *Staphylococcus aureus*; then two isolates of ATCC 25922 *Escherichia coli* were selected for comparison with each of the enterobacteriaceae which included *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella pullorum* and *Proteus mirabilis* making a total of eight isolates of reference ATCC 25922 E*scherichia coli*; and finally two isolates of ATCC 27853 *Pseudomonas aeruginosa* were selected for comparison with *Pseudomonas aeruginosa*, thus making a grand total of 122 bacterial isolates used in the study (100 isolates of test bacteria and 22 isolates of reference bacteria).

2.3 Selection of Commercial Antimicrobial Agents

Commonly used commercial antibacterial agents were selected by purposive and convenience sampling in Bushenyi District specifically in Bushenyi – Ishaka Municipality and Mbarara District specifically in Mbarara Municipality of South Western Uganda. Sampling was conducted in pharmacies, drug shops and hospitals within these Districts. Antibacterial agents selected were known to be used for the management of opportunistic infections amongst HIV/AIDS patients. The antibacterials used with their strengths were gentamicin injectable (40 mg/ml) and erythromycin suspension (25 mg/ml) purchased from a Drug Shop at Ishaka; cotrimoxazole suspension (8/40 mg/ml) purchased from a Pharmacy at Ishaka; ceftriaxone injectable (200 mg/ml) purchased from a Pharmacy at Mbarara; and ciprofloxacin injectable intravenous fluid (2mg/ml) purchased from a Hospital at Ishaka Town.

2.4 Isolation and Identification of Bacteria

MacConkey agar, Chocolate agar and Blood agar were used as the primary media to grow the 122 samples to get fresh isolates. The identities of the previously identified isolates and standard bacteria were re-identified and ratified by growing the isolates on the suitable culture media (MacConkey agar, Chocolate agar and Blood agar) and applying microscopic gram stain, suitable oxidase and catalase biochemical tests [11–15], chrom-agar orientation and carbohydrate assimilation tests utilising the analytical profile index (API) testing kits (Biomerieux® SA France, INS005517) employing apiweb TM identification software.

2.5 Susceptibility Testing

A 1.5 McFarland standard was used to prepare a bacterial suspension from an overnight culture with an equivalent turbidity standard measured with a densitometer for clarity. The suspension was made uniform with a homogenizer and instantly used. The sensitivity, MIC and MBC of all purified isolates were measured in accordance with methods described by Cheesbrough [11]. Antibacterial activities of the antibacterial agents against the purified bacterial isolates were determined with NCCLS (now called CLSI) [16] modified Kirby-Buaer tube dilution and agar well diffusion methods for bacterial sensitivity and resistance patterns, MIC and MBC [11]. The 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* were used as the controls for the tests.

2.6 Preparation of Broth for MIC

The suspension of 18-24 hour freshly sub-cultured bacterial strains was inoculated on freshly prepared Mueller Hinton agar (MHA) on which holes were made with a sterile cork borer. The sensitivity and resistance patterns, MIC and MBC were determined following the standard conventional method [11]. The specific concentrations of the antibacterial agents prepared by their respective manufacturers in 1 mL of water for injection were each used as stock solutions in sterile plain tubes. They included gentamicin (40 mg/ml), ciprofloxacin injectable intravenous fluid (2 mg/ml), erythromycin suspension (25 mg/ml), cotrimoxazole suspension (8/40 mg/ml) and ceftriaxone injectable (200 mg/ml)). A 200 µL of the stock solution of the antibacterial agent was pipetted into each of the holes made on the media, incubated for 18-24 hours and then zones of inhibition were measured with a transparent ruler and recorded for analysis of sensitivity and resistance patterns. Afterwards, serial dilutions ranging from 0.5 to 0.004 strength of each of the stock solutions were made using 1mL of distilled water in each case [17,18]. Then, 200 µL of each dilution was pipetted with a micropipette into respective holes made on the agar plate for agar well diffusion starting with the lowest dilution. The plates and the tubes were incubated at 37℃ for 18-24 hours,

checked for growth and zone of inhibition in the petri dishes.

2.7 MIC and MBC Determination

MIC was determined by recording the smallest concentration of the drug that inhibited growth of microorganisms. But MBC could not directly be determined from the tubes because of high turbidity in the tubes. Hence, samples were taken with sterile swab sticks from cleared zones of inhibition taking cognizance of each specific dilution on the petri dishes and then smeared onto a fresh Mueller Hinton agar medium and incubated for 18-24 hours and then results were checked for MBC as the smallest concentration of the drug that killed the organisms i.e. the plate in which the smallest concentration of the drug disallowed the re-growth of the organisms [11].

2.8 Data Analysis

The duplicates of the data were entered in MS Excel and analysis was conducted using Graph Pad Prism Version 6. Information was expressed as mean ±SEM. One-way ANOVA was conducted and significance was considered when $P < .05$. Multiple comparisons were conducted and a Tukey's test was used to determine sources of variation between groups at 95% significance.

3. RESULTS

3.1 Susceptibility and Resistance Patterns of Oral Bacteria

Major resistance was recorded with erythromycin and cotrimoxazole at [39 (88.6%)] and [37 (84.1%)] respectively for *Staphylococcus aureus* isolates; at [1 (100%)] for *Staphylococcus saprophyticus* and [9 (100%)] for *Klebsiella pneumoniae*; at [8 (88.9%)] and [7 (77.8%)] respectively for *E. coli;* at [7 (77.8%)] and [6 (66.7%)] respectively for *Streptococcus mutans*; at [15 (71.4%)] and [14 (66.7%)] respectively for *Streptococcus pneumoniae*; and at [2 (100%)] and [1 (50%)] respectively for *Proteus mirabilis*. *Salmonella pullorum* was [1 (100%)] resistant to erythromycin, cotrimoxazole and ceftriaxone. *Pseudomonas aeruginosa* demonstrated resistance to all the antibacterial agents except ceftriaxone whereas *Bacillus cereus* was resistant to all the antibacterial agents except gentamicin and ceftriaxone. However, antibacterial activity was observed with gentamicin and ceftriaxone at [33 (75%)] for *Staphylococcus aureus* isolates; at [6 (66.7%)] and [8 (88.9%)] respectively for *Klebsiella pneumoniae* isolates; at [8 (88.9%)] for *Streptococcus mutans* isolates; at [14 (66.7%)] and [21 (100%)] respectively for *Streptococcus pneumoniae* isolates and at [1 (100%)] for *Staphylococcus saprophyticus*, at [2 (100%)] for *Proteus mirabilis* and at [1 (100%)] for *Bacillus cereus* isolates. Gentamicin and ciprofloxacin had [1 (100%)] antibacterial activity against *Salmonella pullorum* isolates. Ciprofloxacin also had [1 (100%)] activity against only one isolate of *Staphylococcus saprophyticus*. Ceftriaxone recorded [21 (100%)] activity against isolates of *Streptococcus pneumoniae* and [2 (100%)] against isolates of *Pseudomonas aeruginosa*. Gentamicin, ciprofloxacin and ceftriaxone scored antibacterial activity against *E. coli* isolates at [8 (88.9%)], [6 (66.7%)] and [6 (66.7%)] respectively. Non haemolytic streptococcus [1] and all the reference bacteria such as *Staphylococcus aureus* ATCC 25923 [12], *Escherichia coli* ATCC 25922 [8] and *Pseudomonas aeruginosa* ATCC 27853 [2] isolates yielded 100% to all the antibacterial agents used. Gentamicin, ciprofloxacin and ceftriaxone displayed respective mean total sensitivities of [97 (78.5%)], [65 (53.3%)] and [105 (86%)]. Erythromycin and cotrimoxazole showed mean total resistance of [85 (69.7%)] and [79 (64.8%)] respectively as shown in Table 1.

3.2 Minimum Inhibitory Concentration (MIC)

Major isolates were gram positive bacteria and few were gram negative bacteria. Among these, their efficacy and resistance against the common antibacterial agents in the market as compared to their controls and other groups were noted. Statistical significant differences in MIC activity were noticed in only gentamicin activity against gram positive *Staphylococcus aureus*, *Streptococcus mutans* and *Streptococcus pneumoniae* and gram negative *Escherichia coli* and *Klebsiella pneumoniae* isolates (One-way ANOVA, $P < .05$) as shown in Table 2. From table 1, ceftriaxone demonstrated efficacy against most of the bacteria except *Salmonella pullorum.* However, from table 2, the MIC data showed non significant differences in activities of ceftriaxone, ciprofloxacin, erythromycin and cotrimoxazole. Tukey's multiple comparison tests further revealed significant differences in MIC activities on *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae* when gentamicin bactericidal

activity was compared with ciprofloxacin, erythromycin, cotrimoxazole and ceftriaxone (Table 4).

3.3 Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration (MBC) was reached and was statistically significant in gentamicin (ANOVA, *P< .05*) and this was in response to *Staphylococcus aureus* [*P**** = *.0001*], *Streptococcus pneumoniae* [*P**** = *.0001*], *Escherichia coli* [*P*** = *.0006*] *and Klebsiella pneumoniae* [*P**** = *.0001*] (Table 3). Equally, Tukey's multiple comparisons showed gentamicin to be the only commercial antibacterial agent with statistical significant differences in bactericidal activities of *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae* when gentamicin bactericidal activity was compared with ciprofloxacin, erythromycin, cotrimoxazole and ceftriaxone (Table 5). Erythromycin and cotrimoxazole respectively did not have any bactericidal activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella pullorum*, *Staphylococcus saprophyticus*, *Bacillus cereus* and *Proteus mirabilis*. Ciprofloxacin and ceftriaxone exhibited no bactericidal activity against *Bacillus cereus* and *Staphylococcus saprophyticus* respectively. Besides the above stated bactericidal activities of gentamicin, the other commercially available antibacterial agents did not record any statistically significant bactericidal activity with analysis of variance and Tukey's multiple comparisons of the means as shown in tables 3 and 5.

4. DISCUSSION

Resistance (> 60%) against commonly used and commercially available antibacterial agents was demonstrated in this study with gentamicin proving to be the most commonly used effective antibacterial agent in the Ugandan market. There is a worldwide pointer to frequent unbefitting use of antibacterials [19]; Perhaps contributing to antibacterial resistance, which is reaching worrying heights in Southern and Eastern Europe [20]. Ignoring the issue of antibacterial resistance leads to unfavourable medical consequences, including considerable ecological and economic implications [21-24]. This study has shown that liberalization of drugs use, drugs manufacturing, distribution and warehousing in developing countries results to non observance of existing rules and regulations in daily routine transactions and activities. This may culminate in poor current good manufacturing practices leading to production of fake or substandard drugs which are not adequately and regularly checked by regulatory authorities; Thus creating impunity and disregard for standards in the drug industry, drug supply and distribution chains. Hence, this is a major threat to the sustainability of the drug industry and above all, a public health concern as a result of passive regulations and their poor implementation in the drug industry and distribution sector in Uganda [1]. This situation can be further compounded by several factors such as frequent availability and uncontrolled access to antibacterial agents by the populace without prescription in private pharmacies and drug shops, lack of updated national guidelines for antimicrobial use for primary care physicians, self storage of antibacterials in home pharmacies without permission, improper guides and instructions, improper storage conditions in pharmacies and drug shops, unstreamlined health system, insatiable self health seeking behaviours, undue methods of supply and dispensing of antibacterials, different attitudes and levels of knowledge about antibacterial agents among various patients and their cultures and beliefs [25]. Sometimes, the reason for inefficacy may be improper storage as shown in a study in Serbia where antibiotics were found to be most commonly stored in the refrigerator without such indication, in the kitchen or bathroom where they were either exposed to unusual temperatures, heat and moisture thereby causing their accelerated spoilage. Sometimes, these drugs might have expired and been relabelled by unscrupulous dealers. Sometimes, they might have been damaged in transit or by exposure to excessive light in transit or storage without prior knowledge preceding use in treatment. This, hence, may result to failure in treatment and this failure can be misconstrued and misinterpreted as resistance [25]. These issues plausibly lead to increased spending on drugs that are not working in immune-compromised patients. The ministry would also have to spend more on worthless drugs in the HIV/AIDS community, since more costly drugs would have to be procured [2,3].

Table 1. Susceptibility and resistance patterns against commercial antimicrobial agents in study area

Bacteria	No. of isolates (N)	S/R/I	Antibacterial agent				
			Genta (40 mg/ml)	Ciprof (2 mg/ml)	Eryth (25 mg/ml)	Cotri (8/40 mg/ml)	Ceftri (200 mg/ml)
Staphylococcus aureus	44	S	33 [a] (75.0) [b]	18 [a] (40.9) [b]	1 [a] (2.3) [b]	5 [a] (11.4) [b]	33 [a] (75.0) [b]
		R	11 [a] (25.0) [b]	20 [a] (45.5) [b]	39 [a] (88.6) [b]	37 [a] (84.1) [b]	11 [a] (25.0) [b]
		I	0 [a] (0.0) [b]	6 [a] (13.6) [b]	4 [a] (9.1) [b]	2 [a] (4.5) [b]	0 [a] (0.0) [b]
SA ATCC 25293	12	S	12 [a] (100.0) [b]	12 [a] (100.0) [b]	12 [a] (100.0) [b]	12 [a] (100.0) [b]	12 [a] (100.0) [b]
Staphylococcus saprophyticus	1	S	1 [a] (100.0) [b]	1 [a] (100.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	1 [a] (100.0) [b]
		R	0 [a] (0.0) [b]	0 [a] (0.0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]	0 [a] (0.0) [b]
		I	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
Escherichia coli	9	S	8 [a] (88.9) [b]	6 [a] (66.7) [b]	1 [a] (11.1) [b]	0 [a] (0.0) [b]	6 [a] (66.7) [b]
		R	0 [a] (0.0) [b]	2 [a] (22.2) [b]	8 [a] (88.9) [b]	7 [a] (77.8) [b]	0 [a] (0.0) [b]
		I	1 [a] (11.1) [b]	1 [a] (11.1) [b]	0 [a] (0.0) [b]	2 [a] (22.2) [b]	3 [a] (33.3) [b]
Escherichia coli ATCC 25922	8	S	8 [a] (100.0) [b]	8 [a] (100.0) [b]	8 [a] (100.0) [b]	8 [a] (100.0) [b]	8 [a] (100.0) [b]
Salmonella pullorum	1	S	[a]1 (100.0) [b]	1 [a] (100.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
		R	0 [a] (0) [b]	0 [a] (0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]
		I	0 [a] (0) [b]	0 [a] (0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
Klebsiella pneumoniae	9	S	6 [a] (66.7) [b]	3 [a] (33.3) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	8 [a] (88.9) [b]
		R	3 [a] (33.3) [b]	5 [a] (55.6) [b]	9 [a] (100.0) [b]	9 [a] (100.0) [b]	0 [a] (0.0) [b]
		I	0 [a] (0.0) [b]	1 [a] (11.1) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	1 [a] (11.1) [b]
Streptococcus mutans	9	S	8 [a] (88.9) [b]	4 [a] (44.4) [b]	2 (22.2) [b]	3 (33.3) [b]	8 (88.9) [b]
		R	1 [a] (11.1) [b]	3 [a] (33.3) [b]	7 [a] (77.8) [b]	6 [a] (66.7) [b]	1 [a] (11.1) [b]
		I	0 [a] (0.0) [b]	2 [a] (22.2) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
Streptococcus pneumoniae	21	S	14 [a] (66.7) [b]	9 [a] (42.9) [b]	3 [a] (14.3) [b]	4 [a] (19.0) [b]	21 [a] (100.0) [b]
		R	3 [a] (14.3) [b]	5 [a] (23.8) [b]	15 [a] (71.4) [b]	14 [a] (66.7) [b]	0 [a] (0.0) [b]
		I	4 [a] (19.0) [b]	7 [a] (33.3) [b]	3 [a] (14.3) [b]	3 [a] (14.3) [b]	0 [a] (0.0) [b]
Non haemolytic streptococcus	1	S	1 [a] (100.0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]
		R	0 [a] (0) [b]	0 [a] (0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
		I	0 [a] (0) [b]	0 [a] (0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
Proteus mirabilis	2	S	2 [a] (100.0) [b]	1 [a] (50.0) [b]	0 [a] (0.0) [b]	1 [a] (50.0) [b]	2 [a] (100.0) [b]
		R	0 [a] (0.0) [b]	0 [a] (0.0) [b]	2 [a] (100.0) [b]	1 [a] (50.0) [b]	0 [a] (0) [b]
		I	0 [a] (0.0) [b]	1 [a] (50.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0) [b]
Pseudomonas aeruginosa	2	S	0 [a] (0.0) [b]	0 [a] (0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	2 [a] (100.0) [b]
		R	2 [a] (100.0) [b]	2 [a] (100.0) [b]	2 [a] (100.0) [b]	2 [a] (100.0) [b]	0 [a] (0.0) [b]
		I	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
Pseudomonas ATCC 27853	2	S	2 [a] (100.0) [b]	2 [a] (100.0) [b]	2 [a] (100.0) [b]	2 [a] (100.0) [b]	2 [a] (100.0) [b]
Bacillus cereus	1	S	1 [a] (100.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	1 [a] (100.0) [b]

Bacteria	No. of isolates (N)	S/R/I	Antibacterial agent				
			Genta (40 mg/ml)	Ciprof (2 mg/ml)	Eryth (25 mg/ml)	Cotri (8/40 mg/ml)	Ceftri (200 mg/ml)
		R	0 [a] (0.0) [b]	1 [a] (1.00.0) [b]	1 [a] (100.0) [b]	1 [a] (100.0) [b]	0 [a] (0.0) [b]
		I	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]	0 [a] (0.0) [b]
Total	122	S	97 [a] (79.5) [b]	65 [a] (53.3) [b]	30 [a] (24.6) [b]	36 [a] (29.5) [b]	105 [a] (86.0) [b]
		R	20 [a] (16.4) [b]	38 [a] (31.1) [b]	85 [a] (69.7) [b]	79 [a] (64.8) [b]	13 [a] (10.7) [b]
		I	5 [a] (4.1) [b]	19 [a] (15.6) [b]	7 [a] (5.7) [b]	7 [a] (5.7) [b]	4 [a] (3.3) [b]

KEY: Genta = Gentamicin; Ciprof = Ciprofloxacin; Eryth = Erythromycin; Ceftri = Ceftriaxone; S = Sensitive; R = Resistant; I = intermediate sensitive ; E. coli = Escherichia coli, SA = Staphylococcus aureus; and ATCC = American Type Culture Collection; [a] = Number of bacteria; [b] = Percentage of bacteria.

Table 2. Mean MIC of commercial antibacterial agents against oral bacteria in the study area

Bacteria isolated	N	Genta	Ciprof	Eryth	Cotri	Ceftri	Water
				Mean MIC ± SEM (µg/ml)			
Staphylococcus aureus	44	14.79±2.7****	2.73±1.02	0.41±0.14	3.11±1.50	0.16 ±0.02	0.00±0.00
Escherichia coli	9	2.69±0.55****	0.13±0.03	0.48±0.30	0.11±0.06	0.22± 0.05	0.00±0.00
Streptococcus mutans	9	3.16±0.56***	1.14±0.48	0.58±0.36	0.89±0.59	0.12±0 .03	0.00±0.00
Klebsiella pneumoniae	9	2.27±0.68***	0.51±0.28	0.00±0.00	0.00±0.00	0.10±0 .03	0.00±0.00
Streptococcus pneumoniae	21	3.64±0.25****	0.74±0.20	0.71±0.25	1.12±0.40	0.20 ±0.03	0.00±0.00
Pseudomonas aeruginosa	2	0.40±0.00	0.10±0.00	0.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00
NH streptococcus.	1	4.00±0.00	0.20±0.00	2.50±0.00	0 .40±0.00	0.06±0.00	0.00±0.00
Proteus mirabilis	2	22.0±18.0	0.11±0.09	0.00±0.00	20.0±20.0	0.16±0.09	0.00±0.00
Staphylococcus saprophyticus	1	0.40 ± 0.00	0.20 ±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Salmonella pullorum	1	0.40 ± 0.00	0.20 ±0.00	0.00±0.00	0.00±0.00	0.13±0.00	0.00±0.00
Bacillus cereus	1	4.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00
Pseudomonas aeruginosa ATCC 27853	2	0.40±0.00	0.00±0.00	0.03±0.00	0.40±0. 00	0.06±0.00	0.00±0.00
Escherichia coli ATCC 25922	8	0.04±0.00	0.00±0.00	0.25±0.00	0.40±0. 00	0.06±0.00	0.00±0.00
Staphylococcus aureus ATCC 25923	12	0.40±0.00	0.02±0.00	0.25±0.00	0.40±0 .00	0.00±0.00	0.00±0.00

*KEY: Genta = Gentamicin; Ciprof = Ciprofloxacin; Eryth = Erythromycin; Cotri = Cotrimoxazole; Ceftri = Ceftriaxone; N = number of isolates; ATCC = American Type Culture Collection and Water = Distilled water; (ANOVA, P < .001); Multiple comparisons (P*** < .05) when compared with all groups.*

Table 3. Mean MBC of commercial antimicrobial agents against oral bacteria in study area

Bacteria isolated	N	Genta	Cipro	Eryth	Cotri	Ceftri	Water
		MBC	Mean ± SEM				
Staphylococcus aureus	44	18.42±2.79***	2.12±0.79	1.26±0.73	1.08±0.84	0.31±0.04	0.00±0.00
Escherichia coli	9	24.0±6.33***	3.58±2.07	0.28±0.28	4.44±4.44	4.89±2.86	0.00±0.00
Streptococcus pneumoniae	21	22.67±4.07***	7.44±1.99	8.33±2.64	4.00±2.62	0.60±0.14	0.00±0.00
Klebsiella. Pneumoniae	9	18.67±6.77***	0.47±0.29	0.00±0.00	0. 00±0.00	0.36±0.00	0.00±0.00
Pseudomonas aeruginosa	2	4.00±0.00	1.00±1.00	0.00±0.00	0.00±0.00	2.00±0.00	0.00±0.00
Salmonella pullorum	1	4.00±0.00	2.00±0.00	0.00±0.00	0.00±0.00	0.40±0.00	0.00±0.00
Staphylococcus saprophyticus	1	4.00±0.00	2.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Non Haemolytic streptococcus	1	40.00±0.00	2.00±0.00	25.00±0.00	40.00±0.00	0.50±0.00	0.00±0.00
Proteus mirabilis	2	40.00±0.00	1.10 ± 1.27	0.0 ± 0.00	0.00±0.00	0.31±0.27	0.00 ±0.00
Streptococcus mutans	9	19.58 ± 19.44	5.16 ± 8.52	3.28 ± 8.21	8.89±17.64	0.32±0.22	0.00±0.00
Staphylococcus aureus ATCC 25923	12	4.0±0.00	0.4±0.00	2.5±0.00	4.0±0.00	0.04±0.00	0.00±0.00
Escherichia coli ATCC 25922	8	0.4±0.00	0.02±0.00	4.0±0.00	4.0±0.00	0.13±0.00	0.00±0.00
Pseudomonas aeruginosa ATCC 27853	2	4.0±0.00	2.0±0.00	0.0±0.00	0.0±0.00	2. 0±0.00	0.00±0.00
Bacillus cereus	1	40.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.00±0.0 0	0.00±0.00

*KEY: Genta = Gentamicin; Ciprof = Ciprofloxacin; Eryth; Erythromycin; Cotri = Cotrimoxazole; Ceftri = Ceftriaxone; N = number of isolates; N = number of isolates, ATCC = American Type Culture Collection and Water = Distilled water; ANOVA (P< .05); Multiple comparisons (P***< .05 when compared with all groups.*

Table 4. MIC confident intervals and P values from Tukey's multiple comparisons test (α<0.05)

Bacterium under consideration	Antibacterials under comparison							
	Gentamicin vs. Ciprofloxacin		Gentamicin vs. Erythromycin		Gentamicin vs. Cotrimoxazole		Gentamicin vs. Ceftriaxone	
	P value	95% confidence interval	P value	95% confidence interval	P value	95% confidence interval	P value	95% confidence interval
Staphylococcus aureus	P**** = 0.0001	1.433 -3.690	P**** = 0.0001	1.085 - 3.342	P**** = 0.0001	1.453 - 3.710	P**** = 0.0001	1.347 - 3.604
Streptococcus mutans	P* = .0237	0.1934 –3.842	P** = .0021	0.7534 - 4.402	P** = .0084	0.4423 - 4.091	P*** = .0002	1.213 - 4.862
Streptococcus pneumoniae	P**** = .0001	1.895 - 3.911	P**** = .0001	1.916 - 3.932	P**** = .0001	1.468 - 3.484	P**** = .0001	2.434 - 4.450
Escherichia coli	P**** = .0001	1.535 - 4.150	P**** = .0001	1.865 - 4.480	P**** = .0001	1.448 - 4.063	P**** = .0001	1.643 - 4.257
Klebsiella pneumoniae	P** = .0051	0.1934 - 3.842	P*** = .0002	0.9251 - 3.608	P*** = .0002	0.9251 - 3.608	P*** = .0002	1.213 - 4.862

Table 5. MBC confident intervals and P values from Tukey's multiple comparisons test (α<0.05)

Bacterium under consideration	Antibacterials under comparison							
	Gentamicin vs. Ciprofloxacin		Gentamicin vs. Erythromycin		Gentamicin vs. Cotrimoxazole		Gentamicin vs. Ceftriaxone	
	P value	95% confidence interval	P value	95% Confidence interval	P value	95% Confidence interval	P value	95% Confidence interval
Staphylococcus aureus	P**** = .0001	10.90 - 21.69	P**** = .0001	11.77 - 22.56	P**** = .0001	11.94 - 22.73	P**** = .0001	12.71 - 23.50
Streptococcus mutans	P* = .0231	1.901 - 36.57	-	-	-	-	-	-
Streptococcus pneumoniae	P*** = .0008	4.930 - 25.53	*P*** = .0018	4.034 - 24.63	P**** = .0001	8.368 - 28.97	P**** = .0001	11.77 - 32.37
Escherichia coli	P** = .0042	5.065 - 35.78	P*** = .0007	8.365 - 39.08	P** = .0066	4.198 - 34.91	P** = .0083	3.754 - 34.47
Klebsiella Pneumoniae	P** = .0011	5.966 - 30.43	P*** = .0008	6.433 - 30.90	P*** = .0008	6.433 - 30.90	P** = .0010	6.072 - 30.54

This study has been able to provide more insight into the challenges faced by the national drug regulatory agencies such as NDA in this regard. This study also gives insight into the complications and impediments associated with such regulatory ineptitude. The use of commercially available antimicrobial agents in the market would probably influence policy change for the enactment of up- to-date drugs' laws in Uganda. This study has also provided further value and understanding of antibacterial resistance in the context of associated contributory factors in HIV/AIDS patients in Uganda shedding more light on the reason for the previous study [7]. This has further validated the findings in the previous study [7], that resistance of opportunistic infective agents against publicly availed drugs in Uganda is a major public health threat [6,26]. NDA has faced a couple of challenges in enforcing its major objectives nationwide as a result of conflicting governmental policies in the drug industry, and inimically unexplained and uncomfortable prolonged delay in the passage of necessary drug control bills into laws and enabling legislations by the Parliament of Uganda [1]. The high rate of unlicensed drug shops has continuously made this difficult, amidst a misplaced priority and limited budget from the central government to fund its monitoring activities [10]. This implies that majority of drugs are bound to be abused by the general public since access to drugs does not necessarily require a prescription from the doctor [27,28].

These operational conditions in Uganda, have led to the exploitation of the general public who would easily misunderstand dosage instructions or simply not adhere to treatment regimens [29,30]. Information on the ability of HIV/AIDS patients to effectively avoid misunderstanding instructions in the region is limited till date, thus showing the NDA still has a lot of work to conduct within Uganda. Once more information is obtained, strategies on how best to help communities would be exploited and these would help to arrest or reduce the escalating problems being expounded in these studies [6,7]. This is important since a majority of oral isolates identified in these studies are gram positive and should be easy to control through an improved national policy plan by the responsible authorities.

Gentamicin showed significant MIC and MBC activities probably due to its low usage by patients and low sales by the drug shops and pharmacies in Uganda. This can be attributable to the injectable nature of gentamicin associated with pain and phlebitis at the site of injection and discomforting adverse effect of ototoxicity and fear of renal toxicity. Gentamicin is a major broad spectrum antibacterial agent in the therapeutic class of aminoglycosides and it is rarely recommended for wider public usage for management and control of infections especially amongst HIV/AIDS patients because it is orally non-absorbable and can only be injected which patients dislike. This shows that the limited use was not because of strict control by regulatory authorities hence demanding the role of NDA in Uganda to be strengthened to ensure that challenges met are removed for increased consumer protection, through improved drug regulation [1]. Improved pharmacovigilance would help narrow the gap being created in the efficacy of available commercial antibacterial agents in Uganda against the threat being posed by the high trend of resistance especially in immuno-compromised patients.

5. CONCLUSION AND RECOMMENDA-TIONS

Bacterial resistance against commercially available antibacterials in the Ugandan market has been demonstrated in this study. This would be due to challenges faced by the regulatory authorities within the nation. Gentamicin was the most efficacious agent identified in this study with a statistical difference and this might be due to its low usage among the general public, possibly owing to its mode of administration as an injectable. Improvement in drug regulatory policies would probably lead to reduced development of resistant microbial strains and treatment failures in Uganda. We strongly recommend a survey on the reason for high efficacy of gentamicin on the oral bacteria of HIV/AIDS patients in South Western Uganda to be conducted.

CONSENT

Written consent was sought and secured from the Kampala International University Microbiology Research Laboratory, Ishaka, Bushenyi where the isolates were being kept with the permission of the scientist who previously researched on these organisms.

ETHICAL APPROVAL

Ethical approval was pursued and acquired from the 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 allowed us to use the clinical isolates supplied by the Microbiology Department, KIU_WC.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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