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Original article

In-vitro inhibitory effect of lactic acid bacteria isolated from Kunun zaki against multi-drug resistant diarrhogenic bacteria in HIV patients in Jos, Nigeria

Solomon Musa [1], John Otumala Egbere [1], Collins Emeka Odu [2], Margaret Musa Danladi [3], Isaac Amechi Onyimba [4], Michael Dashen [1], Edo Godwin Ochiheche [5], Hemen Winefred Nguhemen [5], Amos Obaje Ogaji [1], Anayochukwu Chibuike Ngene [*6]

1- *Department of Microbiology, Faculty of Natural Sciences, University of Jos, Nigeria.*
2- *Department of Biological Sciences, Faculty of Natural Sciences, University of Maiduguri, Nigeria.*
3- *Department of Microbiology, Plateau State University, Bokkos, Nigeria.*
4- *Department of Science Laboratory Technology, Faculty of Natural Sciences, University of Jos, Nigeria.*
5- *Department of Science Laboratory Technology, Fidei Polytechnic, Gboko, Nigeria.*
6- *Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.*

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ABSTRACT

Background: Claims from locals in Nigeria hold that Kunun zaki has some medicinal properties. The study was therefore carried out to investigate the inhibitory effect of lactic acid bacteria (LAB) on multidrug resistant diarrheagenic bacteria in human immunodeficiency virus (HIV) patients. **Method:** Twenty-five stool samples of seropositive HIV patients from Plateau State Specialist Hospital confirmed to have chronic diarrhea were collected aseptically and bacteria were isolated and identified using microscopic and biochemical techniques. The antibiotics susceptibility tests of the isolates were also carried out using the disc diffusion method to determine drug resistance of the bacteria. The lactic acid bacteria (LAB) used were isolated and identified using standard bacteriological techniques and analytical profile index (API) kits. Diarrheagenic bacteria which showed multiple resistance to antibiotics were tested against lactic acid bacteria using agar well diffusion method. **Results:** The results showed that *Shigella* spp (36.0%), *Salmonella* spp (16.0%) and *Escherichia coli (E. coli)* (48.0%) were the diarrheagenic bacteria isolated from the HIV patients. The pathogens were most resistant to ampicillin (60%) and least resistant to tarivid (8%). *Lactobacillus brevis*, *Lactobacillus lactis*, *Lactobacillus casei* and *Lactobacillus plantarum* isolated from Kunun zaki demonstrated antibacterial activity against the pathogens with the effect of the two lactic acid bacteria (*L. lactis* Gb3ii and *L. plantarum* Ar1) being significantly higher than the individual LAB used respectively. **Conclusion:** Lactic acid bacteria from Kunun zaki had demonstrated antibacterial effects against multidrug resistant pathogens, hence could be potential probiotics for inclusion in the fermentation of Kunun zaki that HIV patients could consume.

Introduction

Since lactic acid bacteria (LAB) can thrive well above 38ºC, they are formally referred to as thermophiles. In fermented foods and beverages, probiotic bacteria such as lactic acid bacteria create compounds that prevent the growth of pathogenic,

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* *Corresponding author:* Anayochukwu Chibuike Ngene
E-mail address: ngene.anayochukwu@mouau.edu.ng

non-pathogenic, and spoilage organisms [1]. The capacity of lactic acid bacteria to produce harmful chemicals including organic acid, diacetyl, hydrogen peroxide, and bacteriocin may explain their antibacterial action. Lactic, acetic, and propionic acid are among the organic acids. Hydrogen peroxide is oxidative and bactericidal. A brief peptide or protein called bacteriocin that has bactericidal properties is produced by lactic acid bacteria. Bacillus or Clostridium spores cannot grow on Nisin (bacteriocin), which is known to be bactericidal to Gram-positive bacteria [2]. Studies have been carried out to understand the effects of probiotics on the regulation of the immune response and potential applications for disease prevention over the past 20 years, with an increase in interest in the health effects of probiotic consumption in both food and pharmaceutical companies [2]. The health advantages of probiotics are not new; they have long been found in traditional foods like cheese, yogurt, milk, and salty seafood and have been employed for nutritional purposes. People then became aware of the positive advantages of eating fermented foods on their health [3].

Probiotics can saturate our digestive system with beneficial microbes that can balance out the undesirable ones and protect us from a variety of illnesses. These bacteria include lactobacilli, streptococci, clostridia, coliform, and bacteroides. Improving health may be a useful strategy for preventing us from contracting various diseases [3]. Thus, probiotics may help the host's health by modulating the immune system [4], restricting pathogen colonization [5], and managing metabolic and inflammatory problems of the gut [6]. Probiotics are beneficial for restoring normal gut permeability, mechanical integrity, and homeostasis as well as for lowering antibiotic-associated diarrhea after antibiotic therapy [7].

Clinical studies have validated some of the effects attributed to probiotics. Probiotics have also been shown to be useful in treating conditions like allergies, diarrhea, inflammatory bowel disease (IBD), and the prevention of upper respiratory tract infections [7,8]. Additionally, intestinal flora imbalances brought on by environmental pollutants, poor nutrition, host genetics, stress, and way of life [9,10].

Numerous studies have shown that the human immune system is negatively impacted by the human immunodeficiency virus (HIV), particularly the cluster differentiation (CD4)+ T-cells, and that HIV infection is characterized by dysbiosis of the gut microbiota, changes to the intestinal barrier, and systemic inflammation [11]. Gut-resident bacteria have the ability to influence the mucosal immune system, and altering the mucosal innate immune system can lead to the establishment of a dysbiotic pro-inflammatory group responsible for chronic inflammation in the mucosa and periphery [12]. Reduced CD4 cell counts have been linked to the development of oral lesions, and human immunodeficiency virus infection considerably affects total microbial colonization as well as the microbiota composition in the oral cavity [13].

In order to improve immune functions in HIV-infected individuals, including those receiving short-term antiretroviral therapy, interventions in HIV-positive patients are required to restore the integrity of the immune system of gut-associated lymphoid tissue (GALT). Using probiotics may help to restore gut barrier functions, remodel the microbiome, and help to reduce bacterial translocation and pro-inflammatory cytokine production (ART) [14].

The host's microbiota may be modulated by probiotics, mucosal barrier functions may be improved, and the immune system may be modulated, among other strain-related mechanisms [15]. Since not all of the implicated processes are fully understood, probiotic clinical use must be coupled to probiotic strain and dosage in order to determine their efficiency under certain conditions [16]. Understanding probiotic-specific mechanisms and choosing probiotic strains in connection to the target patient's unique pathogenic and clinical abnormalities have both been the subject of studies, and more are currently being undertaken [17].

According to reports, foods that have been fermented with lactic acid have a pH level below 4, which is also adequate to stop the formation of the majority of food-borne diseases. The majority of diseases have critical pH ranges where they cannot develop. However, other factors such as the ambient temperature, the presence of undissociated acids, and the food's ability to act as a buffer also influence how much bacteria are hindered by low pH values. The bacterial cell may absorb the un-dissociated acid, lowering the intracellular pH and inhibiting metabolic activity. However, extremely high concentrations (up to 10^9 cfu/1ml) of lactic acid

bacteria are required in order to generate an acidic medium that is strong enough to prevent the growth of bacterial pathogens [18].

Materials and methods

Study design

A cross-sectional study was carried out between the periods of February, 2020 and May, 2020 at the Plateau State Specialist Hospital located at Jos North Local Government Area of Plateau State, Nigeria.

Study population

A total of twenty-five patients examined at Plateau State Specialist Hospital, Jos confirmed to be HIV seropositive, to have chronic diarrhea volunteered for participation in this study. Not included in this study were people who were HIV/AIDS negative.

Ethical clearance

Ethical clearance was obtained from the ethical committee of the Plateau State Specialist Hospital, Jos, Plateau State (NHIEC/09/23/2010b). Verbal and written consents were sorted for from all the study population. After verbal consent was obtained, socio-demographic and clinical information were obtained from all participants using pre-structured questionnaires.

Stool sample collection

The stools were collected in sterile containers with the observance of required precautions to avoid contamination. The samples were transported in cool box containing ice packs to the G-Impact 360º Diagnostic and Research Centre of Public Health Interventions and Industrial Development, Jos for processing.

Isolation and identification of diarrhogenic bacteria

Stool culture

All stool samples were taken to the lactic acid bacteria oratory and were cultured within 30 minutes of collection on Salmonella-Shigella agar and MacConkey agar. The plates were incubated at 37ºC for 24 hours and the isolated organisms were identified based on colonial morphology, Gram staining, and biochemical characteristics.

Gram staining

Each colony was smeared on a clean grease-free glass slide and were allowed to air dry after which the smear was heat fixed by passing it a few times over Bunsen flame. The air-dried, heat-fixed smear was placed on a staining rack and was flooded with crystal violet (primary stain) and was allowed to stand for 60 seconds after which the slide was washed gently with clean water. The slide was flooded with Gram's iodine (mordant) and allowed for 60 seconds and was washed gently with clean water. The smear was decolorized with acetone for 15 seconds after which the slide was flooded with neutral red (counter-stain) and was allowed for 60 seconds. The slide was gently wash with clean water and was then blotted dry gently using cotton wool. The slide was examined microscopically using the x100 objective of the microscope after the application of a drop of immersion oil.

Biochemical tests

Pure isolates were subjected to biochemical test as described by [19].

Indole test

A sterile test tube containing 4 ml of peptone was aseptically inoculated with a growth from a pure 18 – 24 hours culture and the tube was incubated at 37ºC for 24 – 28 hours. Aliquots of 0.5 ml of Kovac's reagent was added to the broth culture and was observed for the presence or absence of red ring.

Methyl red test

Organisms from an 18 – 24 hours pure culture were lightly inoculated into peptone water contained in a sterile test tube and the medium was incubated aerobically at 37ºC for 24 hours. Following 24 hours of incubation, 1 ml of the broth was gently transferred into a sterile test tube and 2 to 3 drops of methyl red indicator was added and was observed for red colour immediately.

Voges proskauer test

Organisms from an 18 – 24 hours pure culture were lightly inoculated into peptone water contained in a sterile test tube and the medium was incubated aerobically at 37ºC for 24 hours. Following 24 hours of incubation, 2 ml of the broth was gently transferred into a sterile test tube. 6 drops of 5% alpha-napthol was added to the tube and was mixed well to aerate after which 2 drops of 40% potassium hydroxide was added and mixed well. The tube was observed for a pink-red colour at the surface within 30 minutes with a vigorous shaking within the 30 minutes period.

Citrate test

Citrate medium was prepared, sterilized and cooled in slanted position. The slant was streaked back and forth with a well-isolated colony using an inoculating needle. The tube was aerobically incubated at 37°C for 24 hours after which the

medium was observed for colour change from green to blue along the slant.

Triple sugar iron test (TSI)

A well-isolated colony's top was picked using a straight inoculation needle, and the colony was then injected on triple sugar iron agar slant by first stabbing through the medium's center to the tube's bottom, and then streaking the agar slant's surface. The tube's cap was left off, and it was incubated at 37°C in free air for 24 hours before the medium's reaction was observed.

Antibiotic susceptibility test

Antibiotic susceptibility tests were carried out on the isolates using the disc diffusion method and the antibiotics used included tarivid (OFX, 10µg), peflacine (PEF, 10µg), ciproflox (CPX, 10µg), augmentin (AU, 30µg), gentamycin (CN, 10µg), streptomycin (S, 30µg), ceporex (CEP, 10µg), nalidixic Acid (NA, 30µg), septrin (SXT, 30µg) and ampicillin (PN 30µg). The test isolate was emulsified in peptone and the turbidity was compared to that of 0.5% McFarland's standard. Aliquots of 0.5 ml of the suspension was placed on the surface of the nutrient agar plate and a sterile glass rod was used to evenly spread the suspension over the entire surface of the agar plate. The antibiotic discs were aseptically placed on the surface of the emulsified agar plates and were incubated at 37ºC for 24 hours. After incubation, the zones of inhibition around the antibiotic discs were measured and interpreted based on the breakpoint criteria of the Clinical and Lactic Acid Bacteria oratory Standards Institute (CLSI). Isolates showing resistance to three or more categories of antibiotics were considered as multi drug-resistant bacteria [20].

Isolation of lactic acid bacteria (LAB) from Kunun zaki

Sample collection

A total of ten Kunun zaki was bought from terminus market in Jos Plateau State and transported immediately to microbiology laboratory, University of Jos for the isolation of lactic acid bacteria.

Media preparation for lactic acid bacteria isolation

Modified De Man, Rogosa and Sharpe (MRS) agar media (Hi-Media) was used to isolate lactobacilli from samples. The MRS agar medium was prepared according to the manufacturer's instruction. 62.2 g of MRS agar was dissolved in distilled water (1 L) and heated to dissolve. The solution was sterilized by autoclaving at 121°C for 15 min and used for isolation of the lactic acid bacteria.

Serial dilution

One millilitre of Kunun zaki was dispensed aseptically into a sterile test tube to which 9 ml of sterile distilled water had been previously added. The mixture was shaken to homogenize the Kunun zaki solution and a dilution factor of 10^{-1} was obtained. Then 1 ml of this dilution (10^{-1}) was pipetted and dispensed aseptically into another sterile test tube containing 9 ml of sterile distilled water to make a mixture of one in hundred dilutions (10^{-2}). The process was repeated until a dilution of seven-fold was obtained (10^{-5}).

Inoculation

Exactly 0.1 ml each of the two dilutions (10^{-4} and 10^{-5}) was dispensed into clean and sterile petri dish in duplicates. This was followed by the addition of 10 ml of the molten and sterile lactic agar medium. The plate was rotated carefully to allow even distribution of the inoculum within the medium. This was then allowed to set and solidify. The plates were incubated at 30ºC anaerobically for 24-48 h and observed for growth. Distinct colonies were picked and subcultured severally until pure cultures were obtained. This was then used for morphological and cultural characterization [21].

Identification of suspected lactic acid bacteria isolates

Identification of suspected lactic acid bacteria isolates as described by [22].

Gram staining

Gram staining was carried out on the isolates. A smear of the culture was made on a clean grease free slide lactic acid bacteria labelled with each isolate code and heat fixed to dry. The smear was then stained with crystal violet for 60 seconds after which it was rinsed in water. Few drops of Lugol's iodine solution (Gram's iodine) was added and allowed for 60 seconds. The smear was decolourized with 95% ethanol for 30 Sec. and immediately rinsed with tap water. The slide was counter stained with carbon fuchsin for 1 min and rinsed with water and then dried with Whatman filter paper. Gram-positive cells are purple while Gram-negative cells are red [19].

Biochemical characteristic of the isolates

Catalase test

A microscopic slide was placed inside a petri dish. Using a sterile inoculating loop, a small amount of

microorganism from 24-hour pure culture was placed onto the microscopic slide. 3% H_2O_2 solution was added to each of the slides and a portion of the bacterial colony was mixed with it. Production of bubble indicated the presence of catalase enzyme in the bacteria [23,24].

Sugar fermentation test

Sugar fermentation was used to test the ability of the bacteria to ferment sugar such as lactose (disaccharide), sucrose (disaccharide), sorbose (Monosaccharide) and mannitol (an alcoholic sugar). The based medium of peptone substrate (0.5-1%) was prepared and 1% Andrade's indicators was added. The medium was dispensed into 5 sterile Durham tubes and autoclave. The sterile medium was inoculated with broth culture and incubated at 35^{o}C for 24-48 h. The culture tubes were observed for gas and acid productions [25,26].

Identification of suspected lactic acid bacteria isolates using analytical profile index (API) Kit

A. Preparation of the strips

Each full test consisted of 5 strips, each with 10 numbered tubes. Lactic acid bacteria were added to an incubation box (with tray and cover) and a package of strips. On the tray's lengthy flap, isolates group and strain numbers were written. The inoculation pan was filled with water (tap water). Only the small dents in the plastic of the tray, which were filled with water, were held upside down to drain extra water. The two lengthy strips (0-19 and 20-39) were unwrapped, divided into four shorter strips (0-9, 10-19, 20-29, and 30-39), and then arranged all four in a logical sequence in the incubation tray. The last smaller strip (numbers 40–49) was removed from the packing and placed on the tray as well.

B. Preparation of the inoculum

1. A heavy suspension of bacteria was prepared and to determine how much bacteria to use for inoculation.

One millilitre of sterile water was added into a sterile tube, and all the bacteria were picked up from a plate using a swab and were transferred into the tube to make a heavy suspension of the bacteria. Resuspend by vortexing.

The number of drops was determined to get a 2McFarland turbidity, as follows:

5ml of water was added into a 13ml glass tube and drops of the bacterial suspension was transferred into the glass tube to get a suspension with a turbidity equivalent to 2 McFarland. The number of drops were recorded (n=1).

2. Bacterial suspension was prepared for the inoculation of API 50 strips.

An ampule of the API 50 CHB/E medium was opened as follows:

The white plastic cap was pushed down as far as it will go while the ampule was held vertically in one hand. The ampule's cap was removed by pressing the thumb tip forward while it was positioned on the striated portion of the cap. The cap was then slowly taken off.

The bacterial suspension created in step 1 was added to the ampule in two times the quantity of drops required to achieve a 2 McFarland turbidity (i.e., 2n drops).

C. Inoculation of the strips

The incubation box slightly tilted forward. The tubes were filled (not the upper part with the hole) with the inoculated medium. The tip of the pipette was placed against the side of the cupule to prevent formation of bubbles. The strips were incubated for 24 and 48 hours at 30°C. When a carbon source was metabolized, the medium was acidified and the red indicator in the medium changed to yellow (Tube number 25 is different: it will change from red to black). The colour was recorded after 24 and 48 hours of incubation. When a positive result becomes negative at the second reading, only the first reading was taken into account.

Determination of the inhibitory effect of lactic acid bacteria on multi-drug resistant diarrhogenic bacteria

Lactic acid bacteria were tested against bacterial isolates showing resistance to antibiotics using agar well diffusion method. The lactic acid bacteria used are *Lactobacillus brevis* and *Lactobacillus lactis*. The diarrhogenic bacterial isolates were emulsified in peptone and the turbidity was compared to that of 0.5% McFarland's standard. 0.5 ml of the suspension was placed on the surface of the nutrient agar plate and a sterile glass rod was used to evenly spread the suspension over the entire surface of the agar plate. The plate was first drilled using a cork borer, and then the holes were filled with lactic acid bacteria suspensions. The plates were incubated for 24 hours at 37 °C. The zones of inhibition around the holes were measured and analyzed following incubation [22].

Statistical analysis

Statistical analysis was carried out using microsoft Excel 2010. The data obtained were presented in tables and graphics form.

Results

Isolation and identification of diarrhogenic bacteria from HIV patients

Macroscopic and microscopic features of bacterial isolates from HIV positive patients in Jos

Bacterial colonies with different morphology including differences in size, shape, colour and elevation were observed. Morphological characterization revealed the isolates to be coccibacilli rod. The colonies had convex elevations however, majority had short slender rods shape while few others were long slender rod shaped. The diameter (0.1-0.2 mm) was observed in all. All the isolates were translucent with color ranging from colourless with black pigment to pinkish. In order to characterize the isolates, Gram staining and biochemical tests were performed. Result showed that all the isolates were Gram-negative. In microscopic analysis, the isolated bacteria were all mucoid rods and with convex elevations (Appendix 1).

Biochemical characteristics of bacterial isolates from HIV positive patients in Jos

The biochemical characterization showed that the colony characteristics of the isolates were different from each other. Four isolates 1,4,5 and 6 had acid but no gas production; while the other isolates had both acid and gas production. All of the isolates utilized glucose for energy source. Isolates 7,8,10,13,14,15,17 and 18 had acid and gas production for sucrose utilization whereas isolates 7-11, 13-18 and 21 showed acid and gas production for lactose utilization (Appendix 2-3). Methyl red (MR) was positive for all isolates while VP was negative for all isolates. Citrate test was also found to be negative for all isolates. There was utilization of fructose, maltose, sucrose, lactose, mannose and arabinose. From these biochemical tests, the possible organisms were identified to be *Shigella* spp, *Salmonella* spp and *E. coli*.

Prevalence of *E. coli, Salmonella* spp and *Shigella* spp isolated from HIV positive patients in Jos

Figure 1 showed the prevalence of *E. coli*, *Salmonella* spp and *Shigella* spp isolated from HIV positive patients in Jos. *Escherichia coli* had the highest prevalence 12(48%) followed by *Shigella* spp 9 (36%) while the least was *Salmonella* spp 4(16%).

Antibiotic sensitivity and resistance of bacterial isolates from HIV positive patients in Jos

Table 1 showed the antibiotic sensitivity and resistance of bacterial isolates from HIV positive patients in Jos. Isolated bacteria were found to be most susceptible to tarivid (OFX) with susceptibility percentage of 92% followed by ciproflox (CPX) with a susceptibility percentage of 88% while amplicin (PN) had the least with a susceptibility percentage of 40% **(Table 6).** Amplicin (PN) had the highest resistance with resistance percentage of 60% followed by nalidixic acid (NA) with a resistivity percentage of 48% while the least was tarivid (OFX) with resistance percentage of 8% **(Figure 2).**

Susceptibility and resistance of *E. coli, Salmonellai* spp and *Shigella* spp isolated from HIV positive patients in Jos to antibiotics

Table 2 represents the susceptibility and resistance of *E. coli, Salmonellai* spp and *Shigella* spp isolated from HIV positive patients in Jos to antibiotics. *Salmonella* spp showed the least susceptibility of 12% followed *Shigella* spp with 32% while the highest susceptibility was *E. coli* with 36%. *Salmonellai* spp and *Shigella* spp has the least resistance value of 4% followed by *E. coli* with a resistance value of 12% **(Figure 3).**

Macroscopic and microscopic features of lactic acid bacterial isolates from Kunun zaki sold in Jos

The morphology of different lactic acid bacteria such as size, shape, colour and elevation were observed. Results revealed the isolates to be coccobacilli rod. The colonies had raised elevations with a range of diameter (0.1-0.2 mm) observed in all.

Identification of lactic acid bacteria using API-kit

Table 3 shows the API of the isolated lactic acid bacteria, where the following lactic acid bacteria were identified as *Lactobacillus brevis* (86.6% accuracy), *Lactobacillus lactis* (93.8% accuracy), *Lactobacillus plantarum* (90% accuracy), and *Lactobacillus paracasei* (84.4% accuracy).

In-vitro inhibitory effects of lactic acid bacteria on multi- drug resistant bacterial isolates from HIV positive patients in Jos

In **table (4)** the invitro inhibitory effects of lactic acid bacteria on multi-drug resistant bacterial isolates from HIV positive patients in Jos is

represented. The mixed lactic acid culture tends to have stronger anti-bacterial activity against the isolated bacteria with isolate 12 showing the highest zone of inhibition (35mm) followed by isolate 9 with 33mm zone of inhibition. Isolates 14 and 21 were resistant to lactic acid bacteria culture since their zones of inhibition were 0 mm.

Table 1. Antibiotic sensitivity and resistance of bacterial isolates from HIV positive patients in Jos.

Isolate/Zone of Inhibition in mm	**S**	**PN**	**CEP**	**OFX**	**NA**	**PEF**	**CN**	**AU**	**CPX**	**SXT**	**MARI**	**R %**	**S%**
Shigella spp	23.50	21.00	20.50	18.50	18.50	17.00	16.50	20.50	19.00	15.50	0.00	0.00	100.00
***Salmonella* spp**	13.50	1.50	0.00	8.00	2.00	3.00	2.50	2.00	7.00	0.00	0.90	90.00	10.00
Salmonella spp	20.00	3.50	20.00	20.00	10.50	20.00	20.00	10.50	20.00	9.50	0.40	40.00	60.00
Shigella spp	20.00	8.50	9.50	20.00	9.00	20.00	20.00	10.00	20.00	20.00	0.40	40.00	60.00
Shigella spp	11.00	9.50	11.00	20.00	20.00	10.00	20.00	20.00	20.00	20.00	0.40	40.00	60.00
Shigella spp	11.00	9.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	0.20	20.00	80.00
Escherichia coli	20.00	9.50	20.00	20.00	9.50	20.00	20.00	20.00	20.00	20.00	0.20	20.00	80.00
Escherichia coli	10.00	8.50	8.50	20.00	9.50	20.00	20.00	10.00	20.00	9.50	0.60	60.00	40.00
Escherichia coli	11.00	1.00	9.00	20.00	8.50	20.00	8.50	11.00	20.00	20.00	0.60	60.00	40.00
Escherichia coli	20.00	10.00	9.00	20.00	8.50	20.00	8.50	20.00	20.00	20.00	0.40	40.00	60.00
Escherichia coli	20.00	9.00	10.00	20.00	8.50	20.00	20.00	20.00	20.00	9.00	0.40	40.00	60.00
***Shigella* spp**	9.50	0.00	0.00	1.50	0.00	0.00	0.00	0.00	1.00	0.00	1.00	100.00	0.00
Escherichia coli	10.00	11.00	10.00	20.00	9.00	20.00	20.00	10.50	20.00	20.00	0.50	50.00	50.00
Escherichia coli	1.00	0.00	1.00	20.00	8.50	8.50	20.00	8.50	9.00	1.00	0.80	80.00	20.00
Escherichia coli	22.00	20.00	20.00	19.50	18.00	19.00	21.50	17.50	20.50	18.00	0.00	0.00	100.00
Escherichia coli	20.50	19.00	19.50	20.50	17.50	22.50	21.00	20.00	21.00	18.00	0.00	0.00	100.00
Escherichia coli	20.50	0.00	0.00	21.00	16.50	18.00	20.00	16.50	19.50	17.00	0.20	20.00	80.00
Escherichia coli	19.00	16.50	15.50	19.00	17.50	18.50	20.00	18.50	17.50	18.00	0.00	0.00	100.00
Salmonella spp	20.50	20.50	17.00	21.50	16.50	18.00	20.00	21.50	17.00	20.50	0.00	0.00	100.00
Salmonella spp	20.50	18.50	19.00	20.00	18.50	20.00	20.50	19.50	19.00	18.50	0.00	0.00	100.00
Escherichia coli	18.50	0.00	17.00	16.50	0.00	0.00	0.00	0.00	21.00	0.00	0.60	60.00	40.00
Shigella spp	21.50	16.50	18.00	19.50	18.00	20.50	17.00	20.50	21.00	21.00	0.00	0.00	100.00
Shigella spp	21.50	20.00	18.50	20.00	21.00	20.50	21.00	19.50	20.50	20.00	0.00	0.00	100.00
Shigella spp	20.50	19.50	20.50	21.00	20.50	21.00	19.50	21.50	19.50	20.50	0.00	0.00	100.00
Shigella spp	24.00	20.50	22.00	21.50	20.00	21.00	20.00	21.00	20.00	21.00	0.00	0.00	100.00
Key:	OFX = Tarivid			PEF = Reflacine			CPX = Ciproflox						
	AU = Augmentin			CN = Gentamycin			S = Streptomycin						
	CEP = Ceporex			NA = Nalidixic Acid			SXT = Septrine						
	PN = Amplicin												
	MARI = Multiple Antibiotics Resistance Index								S = Susceptibility R = Resistibility				

The organisms in bold and underlined were the multi-drug resistant used to test with the lactic acid bacteria. Kirby-Bauer disc diffusion susceptibility test protocol R $\leq$13mm, I =14-17mm, S $\geq$ 18mm

Table 2. Cumulative number of bacteria isolates that were multi-drug resistant based on each species.

S/NO	Isolates	Total number of isolates in the species	Number of resistant isolates	Percentage of resistant isolates
1	*Shigella* spp	9	3	33.33
2	*Salmonella* spp	4	2	50.00
3	*E. coli*	12	7	58.33
4	Total	25	12	Not applicable

Table 3. Identification of Lactobacillus species based on carbohydrate fermentation profiles using API 50 CHL database.

Lactic Acid Bacteria Isolates API Identification	Identity accuracy (%)
Gb8 *Lactobacillus brevis*	86.6
Gb3ii *Lactobacillus lactis*	93.8
Gb2 *Lactobacillus lactis*	79.8
Gb3i *Lactobacillus plantarum*	87.6
Gb4 *Lactobacillus paracasei*	84.4
Ar1 *Lactobacillus plantarum*	90.0
Tm9 *Lactobacillus lactis*	90.0
Tm8 *Lactobacillus lactis*	71.9
Tm3 *Lactobacillus plantarum*	79.9
Gb1 *Lactobacillus plantarum*	80.0

I.D: Identity (%), the percentages following the scientific names of species represent the similarities from the computer-aided database of API-web™ API 50 CHL V5.1 software.

Table 4. *In-vitro* inhibitory effects of lactic acid bacteria on multi- drug resistant bacterial isolates from HIV positive patients in Jos.

Isolate	Zone of inhibition (mm) due to *Lactobacillus lactis* (Gb3ii)	Zone of inhibition (mm) Due to *Lactobacillus plantarum* (Ar1)	Zone of inhibition (mm) due to mixed culture (Gb311 and Ar1)
Salmonella spp	12.5±0.5	15±0.5	21±0.5
Escherichia coli	16.5±0.5	18±0.5	26±0.5
Escherichia coli	14.2±0.5	19±0.5	24±0.5
Escherichia coli	6±0.5	7±0.5	7±0.5
Shigella spp	5±0.5	6±0.5	5±0.5

Figure 1. Prevalence of *E. coli*, *Salmonella* spp and *Shigella* spp isolated from HIV positive patients in Jos.

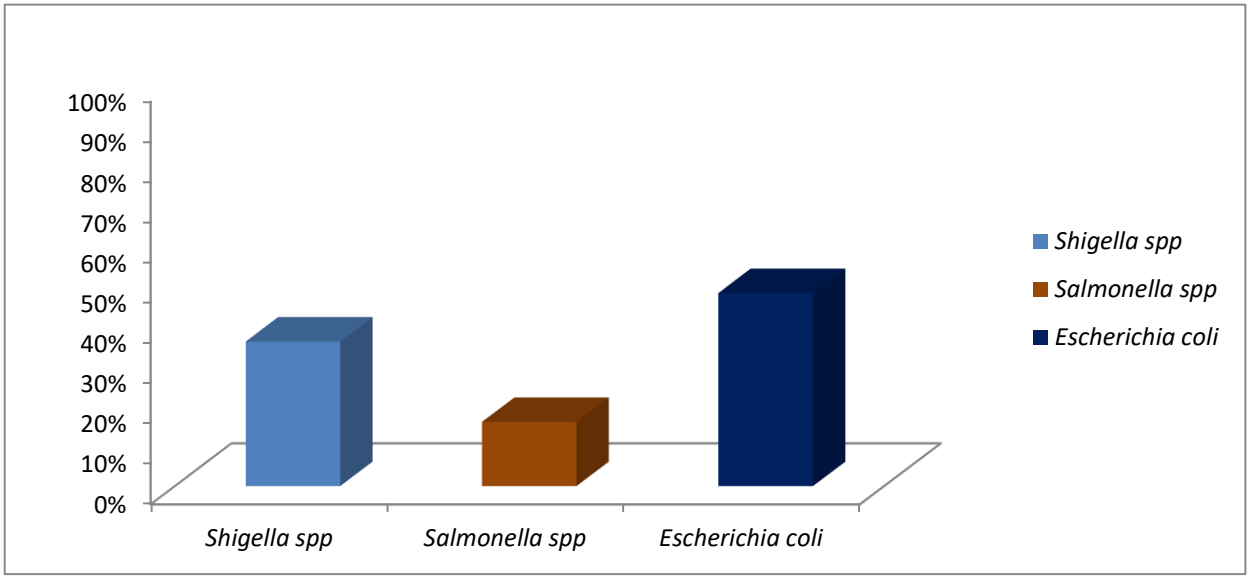
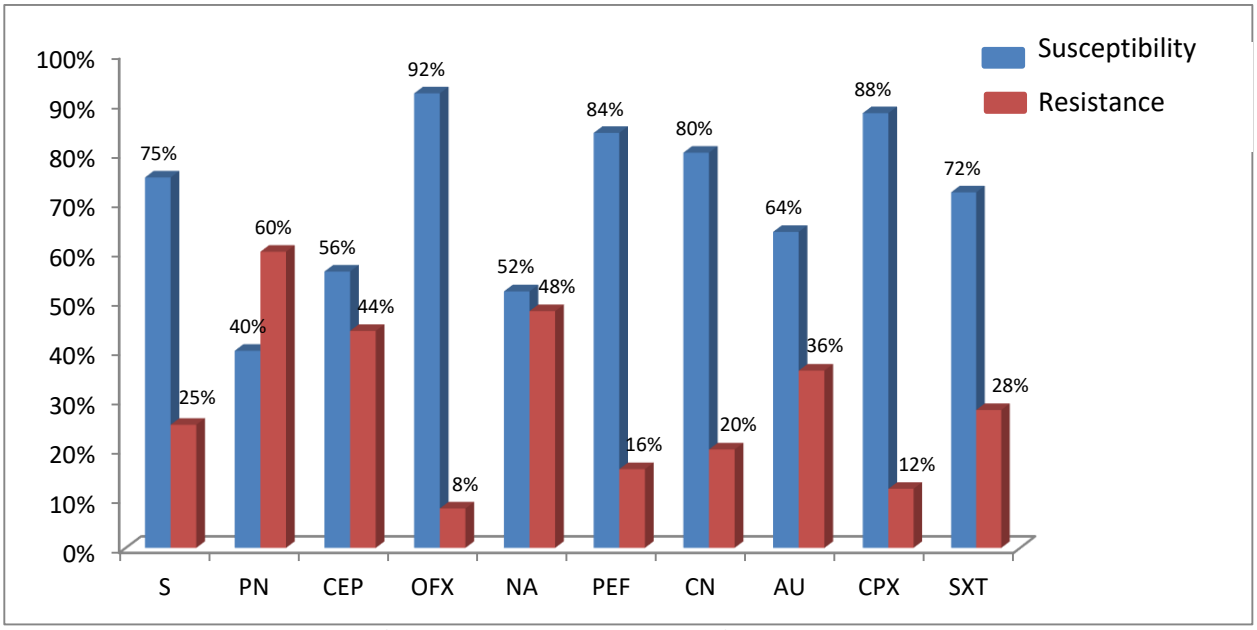


Figure 2. Percentage susceptibility and resistance of bacteria isolated from HIV positive patients in Jos to various antibiotics.



OFX = Tarivid , SXT = Septrine, PEF = Reflacine, PN = Amplicin, CPX = Ciproflox, S = Streptomycin, AU = Augmentin, CN =Gentamycin, CEP = Ceporex, NA = Nalidixic acid.

Discussion

Prealence of diarrhogenic Bacteria isolated from HIV patients in Jos

In this research, *E. coli* had the highest prevalence, followed by *Shigella* spp while the least was *Salmonella* spp. This supports the research findings of [27], on the identification of enteric pathogens in HIV-positive patients with diarrhoea in Northern India where enteropathogenic *E. coli*, and *Campylobacter jejuni*, were identified in the stools of diarrhogenic stools of HIV patients. *Escherichia coli* is a normal commensal in the gastro intestinal tracts of humans and hence it is expected to be prevalent among the enterobacteria identified. However, *E. coli* is becoming increasingly an opportunistic pathogen in immune-compromised individuals such as the people living with HIV. *Escherichia coli* O157:H7 has been known to be highly antibiotic resistant and causes haemolytic uremic syndrome in 5% of population [28].

Multidrug resistant diarrhogenic bacteria

An organism is said to be multi-drug resistant when it is not susceptible to at least one antimicrobial agent in three or more categories of antibiotics. In this study, *Salmonella* spp, *Shigella* spp, *E. coli* were isolated from stool samples of HIV patients in Jos showed resistance to tarivid, augmentin, ceporex, ampicilin, septrin, peflacin, gentamycin, nalidixic acid, streptomycin and septrin, as similar trends was reported by [29] on molecular epidemiology and antimicrobial susceptibility of clinical *Staphylococcus aureus* from healthcare institutions in Ghana. Multi-drug resistance in microorganisms is a significant issue on a national and international scale. Alternatives to antibiotics that have negligible to no significant adverse effects have been proposed by numerous studies. One of the most amiable probiotic strains known to mankind since the dawn of time is lactic acid bacteria. The current work exhibits the inhibitory activity of lactic acid bacteria consortium against MDR clinical isolates in an effort to combat the spread of antibiotic resistance among microorganisms [30]. Due to a lack of access to drinkable water and poor hygiene standards in most rural communities, the issue of diarrhea, a typical complication of HIV infection, is made even worse. Rivers, ponds, wells, and streams are the sources of drinking water for the villagers in the study areas' rural settlements. Such water sources are untreated and faecally contaminated, making them key conduits for the spread of diseases like diarrhea that are waterborne [27]. The indiscriminate use of modern antibiotics in the treatment of diarrhogenic bacterial infections among HIV patients is beginning to fail and, in most cases, leads to increase in antibiotic resistance. This study revealed the possibility of sourcing potent antimicrobial agents from lactic acid bacteria as the isolates elicited appreciable antibacterial activity against the clinical pathogens [31,32].

Lactic acid bacteria (LAB) from Kunun zaki

Out of 120 Kunun-zaki samples, 25 Lactic Acid Bacteria were isolated and the identified Lactic Acid Bacteria in this study were *Lactobacillus brevis, Lactobacillus lactis, Lactobacillus paracasei* and *Lactobacillus plantarum*, which are organisms that cause fermentation in Kunun-zaki as reported by [3]. Although all of their isolates based on probable identification were *Lactobacillus* spp. from traditional drink Kunun zaki enriched with paddy rice and sweet potatoes, the identification of these organisms from Kunu zaki was close to being in agreement with those isolated by [33]. This outcome is also consistent with research by [34], who identified comparable organisms from millet and sorghum sold in Nkwo-Achara Market, Abia state, after they underwent fermentation.

Probiotic properties of lactic acid bacteria

In this study, all the isolated lactic acid bacteria showed some level of probiotic potential while the highest probiotic potential was observed in *Lactobacillus lactis*. This is consistent with research findings from [35], which found that probiotic strains of *L. fermentum* RM28 and *E. faecium* RM11 inhibited colon cancer cell proliferation at rates of 21-29% and 22-29%, respectively. These results raise the possibility that both strains could be used as probiotics in functional foods. The results of this study corroborate those of [36], in which 26 isolates of lactic acid bacteria were purified and tested for their antimicrobial activity against seven human pathogenic MTCC strains, including three test fungi (*Aspergillus fumigatus, Aspergillus* sp., and *Candida albicans*) and four test bacteria strains (two Gram-negative namely *Escherichia coli*, *Salmonella enterica* ser. Typhi and two Gram-positive *Staphylococcus epidermidis* and *Bacillus amyloliquifaciens*). Out of the 26 isolates, eight were chosen for further probiotic potential study because their antibacterial activity was proven to be effective against the greatest number of tested pathogens.

Inhibitory effect of lactic acid bacteria on the multi-drug resistant diarrhogenic bacteria

This study shows that lactic acid bacteria were found to have a potential to inhibit the growth of all tested diarrhogenic bacteria pathogens. The diarrhogenic bacteria isolates showed different susceptibility, the mixed culture of lactic acid bacteria tends to have stronger anti-bacterial activity against the tested diarrheagenic bacterial. All the diarrhogenic bacteria isolates were inhibited both individually and in mixed form. This is similar to [37] who reported the inhibition zone of supernatant of lactic acid bacteria against human pathogens ranged from 9.25 ± 0.35mm to 11.8 ± 0.35 mm. Furthermore, [38] discovered that *Salmonella* spp. and *E. coli* O157:H7 growth is inhibited by lactic acid, even at low concentrations (2 mg/mL). Similarly, [39] showed that lactic acid at a concentration of 9 mg/mL inhibits the growth of *Salmonella Typhimurium* (inhibition zone of 22.6 mm) and *P. aeruginosa* (inhibition zone of 22.5 mm), while against *E. coli* and *S. aureus*, lactic acid was ineffective [40]. Moreover, [38] found that lactic acid even at low concentration (2 mg/mL) inhibits the growth of *Salmonella spp*. and *E. coli* O157:H7. Similarly, [39] showed that lactic acid at a concentration of 9 mg/mL inhibits the growth of *Salmonella Typhimurium* (inhibition zone of 22.6 mm) and *P. aeruginosa* (inhibition zone of 22.5 mm), while against *E. coli* and *S. aureus* lactic acid was ineffective. Several authors suggested that lactic acid is more efficient antibacterial agent than acetic, citric and propionic acid [41,42]. Other studies indicate higher efficiency of sorbic and benzoic acid than lactic acid [32,43].

Conclusion

According to the results obtained in this study, it can be concluded that *Lactobacillus plantarum* and *Lactobacillus lactis* isolated from Kunun zaki drink, a locally fermented beverage has strong antimicrobial activity against a wide range of diarhogenic bacteria which are clinical pathogens. Cultivation of these isolates under optimum condition could serve as potential source of antibacterial agents (like lactic acid, hydrogen peroxide, bacteriocins and others) having preservation as well as probiotic activity. These can reduce and control different human health problems caused by pathogens among HIV patients. Therefore, isolation and screening of lactic acid bacteria from potential locally prepared Kunun zaki is one of the basic sources for the discovery of new potential lactic acid bacteria for controlling and treatment of infectious diseases to improve the health quality of human beings most especially HIV patients. Lactic acid bacteria from Kunun zaki had demonstrated antibacterial effects against multidrug resistant pathogens, hence could be potential probiotics for inclusion in the fermentation of Kunun zaki that HIV patients could consume.

Recommendations

Based on the finding of the present study, a further in vivo test is recommended to evaluate the therapeutic capability of lactic acid bacteria in detail. In vitro tests are therefore only the first step of predicting the effect of probiotics against diarrhogenic bacteria among HIV patients. Further studies in the form of clinical trials are needed to determine the real efficacy of probiotics and further studies are needed to provide more details on the combined effects of different natural antimicrobial agents with lactic acid.

Conflict of interest

The authors report no conflicts of interest.

Financial disclosures

None.

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