

Original Research Article

ANTIBIOTIC SUSCEPTIBILITY AND INTESTINAL EPITHELIAL CELL ADHESION PATTERN OF HOMOFERMENTING LACTIC ACID BACTERIA (LAB) ISOLATED FROM KUNU-ZAKI, A SPONTANEOUSLY FERMENTING NIGERIAN CEREAL BEVERAGE

ABSTRACT

This study evaluated the antimicrobial susceptibility patterns of eight homofermenting Lactic acid bacteria (LAB) isolated from ready to drink kunu-zaki produced using spontaneously fermenting cereal grains of *Digitaria exilis* (Acha), *Sorghum bicolor* (Sorghum) and *Pennisetum americanum* (Millet) in composite and non-composite proportions. Adhesion of identified LAB strains was evaluated *in vitro* using guinea pig intestinal epithelial cell scraping. LAB strains were also tested for sensitivity to five different antibiotics following the modified standard Kirby-Bauer procedure on MRS agar (pH 7.4) using the disc diffusion technique with Amoxicillin (AMX 25µg), Erythromycin (ERY 10µg), Ciprofloxacin (CPX 10µg), Cotrimoxazole (COT 25µg), and Gentamycin (GEN 10µg). For quality control of the antibiotics, sensitive reference strains *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 obtained from the Nigeria Institute of Medical Research were used. Antimicrobial substances produced by the eight LAB isolates inhibited the growth of four selected human pathogens *in vitro*. All eight LAB isolates were resistant to Amoxicillin, Gentamycin and Ciprofloxacin. *L. plantarum*²⁶, *L. paracasei ssp paracasei*³⁹ and *Pediococcus damnosus*³² were resistant to Erythromycin whilst all others were susceptible. *L. plantarum*²⁶ and *L. paracasei ssp paracasei*³⁹ displayed multiple drug resistance to all antibiotics tested. All LAB isolates demonstrated high *in-vitro* intestinal epithelial cell adhesion potential. Lactic acid bacteria are present in ready to drink kunu-zaki as viable strains and information regarding their antibiotic reaction pattern is scanty. This study suggests that LAB antimicrobial activity may not be affected if kunu zaki is consumed simultaneously with antibiotic therapy. However, if these LAB strains are intended for use as kunu-zaki starter cultures, it is important that they should be further carefully examined for inability to transfer antibiotic resistance genes to food pathogens.

Keywords: antimicrobial, lactic acid bacteria, antibiotics, susceptibility, resistance

Introduction

34 The lactic acid bacteria (LAB) are a group of Gram positive bacteria, non-respiring, non-spore
 35 forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of
 36 carbohydrates. They are the most important bacteria in desirable food fermentations, being
 37 responsible for the fermentation of sour dough bread, sorghum beer, all fermented milks, cassava
 38 (to produce *gari* and *fufu*) and most "pickled" (fermented) vegetables. Historically, bacteria from
 39 the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* are the main species
 40 involved. Several more have been identified, but play a minor role in lactic fermentations
 41 [7]. Lactic acid bacteria are usually found in decomposing plants and lactic products. Lactic acid
 42 is the major metabolic end product of the carbohydrate fermentation. LAB are a large group of
 43 fermentative, anaerobe facultative, aerotolerant microorganisms which are usually present in the
 44 gut of humans and other animals, raw vegetables, meat and meat products, and cereals [7]. In
 45 animals, their numbers may vary with the species, the age of the host, or the location within the
 46 gut [11]. In the food industry, lactic acid bacterial strains are widely employed either as starter
 47 cultures or as non-starter lactic acid bacteria. Furthermore, owing to their probiotic properties,
 48 several LAB strains have found use as adjunctive cultures in foods and feed [19]. Increasing
 49 commercial interest for functional food containing probiotics, has increased scientific interests in
 50 these products. Lately, the researches in the food biotechnologies have centered on careful
 51 isolation and selection of new "safety" strains of *Lactobacillus* that might both ensure the
 52 microbiological security of the food as well as bring benefits to the consumer's health [5]. One
 53 of the anxieties which has evolved over the use of LAB as starter cultures, however, is resistance
 54 to antibiotics. Antibiotics are one of the great discoveries against bacterial infections.
 55 Unfortunately bacteria can fight back by being resistant towards antibiotics. . From a human
 56 perspective, resistance to antibiotics is an un-desirable ability of microorganism. Antibiotic
 57 resistance refers to the ability of microorganisms to withstand the bacteriostatic and bactericidal
 58 effects of antibiotics and provides a survival benefit to the invading microorganisms. Under such
 59 circumstances, it is difficult to eliminate the infection caused by these microorganisms if and
 60 when it happens. The mechanisms by which microorganisms exhibit resistance to antibiotics
 61 include drug inactivation or modification, alteration of target site, alteration in the metabolic
 62 pathway, and reduced drug accumulation [10]. The development of antibiotic resistance in
 63 bacteria is of public concern in view of the fact that a patient could develop antibiotic resistance
 64 by contacting a resistant microorganism or the emergence of a microorganism in the patient's

body when treatment with antibiotic begins [8]. Even though a health beneficial microorganism such as LAB with antibiotic resistance may have the advantage of not being affected by the antibiotic when a consumer takes it together with the antibiotic, however, such an organism with antibiotic resistance could eventually become an opportunistic pathogen. This could be highly detrimental [26] to the infected host and the transfer of antibiotic resistant genes from the health beneficial food LAB to food pathogens may occur. Some species of LAB commonly used in the food industry or naturally occurring in raw food materials have been found to be resistant to glycopeptides antibiotics [30]. Genes conferring resistance to several antimicrobials (including Chloramphenicol, Erythromycin, Streptomycin, Tetracycline, and Vancomycin) located on transferable genetic elements (plasmids and transposons) have already been characterized in lactococci [26].and lactobacilli [15] from foods.Frequency of spontaneous mutation to kanamycin and streptomycin was reported to be very high for an appreciable number of lactobacilli [10]. Currently there are lots of data on the prevalence of antibiotic resistance and the mechanisms implicated in clinical bacteria [10] [15]. It is therefore important that strains intended for use in the food systems should also be carefully examined for antimicrobial susceptibility/resistance.Kunu-zaki is a traditional beverage made from spontaneously fermenting cereals. The beverage originated from Northern Nigeria but has found wide acceptability in all parts of the country. Cereals used for kunu-zakiinclude sorghum. millet or maize in non-composite proportions. Regardless of the cereal used, kunu-zakiwild fermentation is largely dominated by Lactic acid bacteria as reported by several researchers in the past [2] [4] [13] [14]. The spontaneous fermentation production process however usually results in kunu zaki of inconsistent quality from batch to batch of each production. The use of starter cultures, which would produce a uniform fermented kunu-zaki product quality remains yet unexplored. Yet this drink has become very popular in a country referred to as the most populous African nation.The hypothesis behind this present work therefore is that it would be possible to select strains with antimicrobial properties which can be used as starter cultures for kunu-zaki. The aim of this current study was therefore to evaluate theantibiotic reaction pattern of homo-fermentingLAB strains isolated from the fermenting slurries of kunu zaki.. The information obtained from this study could contribute to the potential use of these LAB isolates in the food and pharmaceutical industries

MATERIAL AND METHODS

Laboratory Production of Kunu-zaki

Sorghum; (*Sorghum bicolor*), Millet, (*Pennisetum americanum*) and Hungry rice (locally known as Fonio or Acha) *Digitaria exilis* grains were obtained from the Nigeria Cereal Research Institute in Ibadan, Oyo State, Nigeria. The grains were cleaned, weighed and washed before steeping in distilled water. 200 grams of cereal grains were used for the kunu-zaki production. A control experiment was set up with distilled water without the grains. For the kunu-zaki made from composite grains, an equal weight of grains was used for each part. The laboratory production method followed the traditional process of kunu-zaki fermentation.

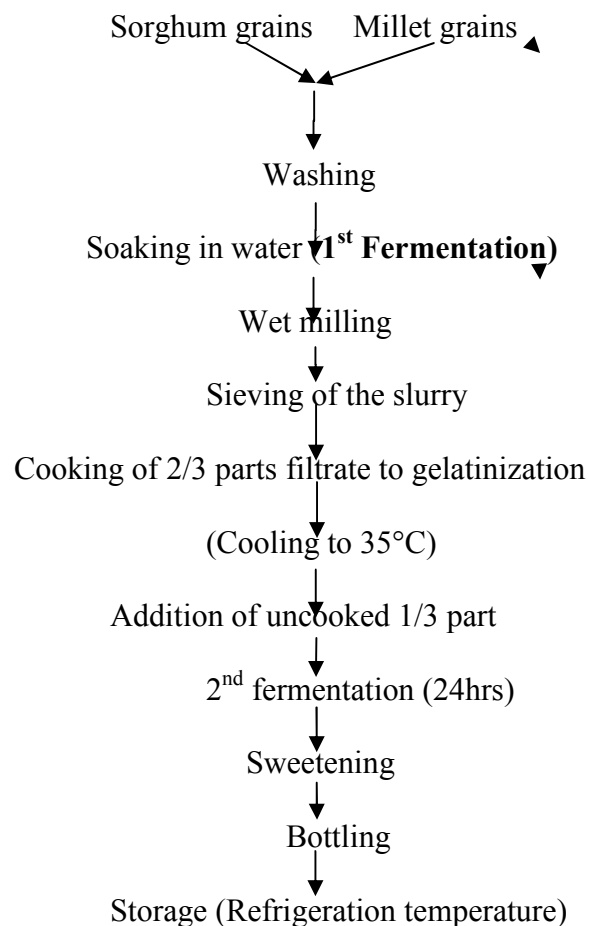


Figure 1: Flow diagram for the traditional processing of kunu-zaki from composite grains

Isolation of the Lactic acid bacteria

The isolation of the Lactic acid bacteria was done following the pour plate technique. Samples were obtained directly from the 72 hours fermenting mash of the kunu-zaki made from each composite cereal type. The samples were collected in sterile carriers and stored on ice until delivery to the laboratory. Once delivered to the laboratory, they were kept under refrigeration temperature until ready for analysis.

The Pour plate technique was used to isolate the organisms. The kunu-zaki samples were used directly, diluted to 10^{-1} , 10^{-2} and 10^{-3} using sterile peptone water. 1 ml aliquot of the samples and dilutions were plated into MRS (deMann, Rogosa and Sharpe) agar (pH 6.2 and pH 5.5). The plates were incubated at 37 °C for 3 days under anaerobic conditions (in an anaerobic jar using Oxoid anaerogen compact). The use of the medium was to isolate and enumerate the lactobacilli present in the sample. After incubation, individual colonies were selected and transferred into sterile broth mediums. The streak plate technique was used to obtain pure colonies. The pure colonies isolates were examined according to their colony morphology, catalase reaction and gram reaction. The Gram positive and catalase negative cocci and bacilli colonies were stored in glycerol as lactic acid bacteria.

Morphological, physiological and biochemical examination of LAB

The morphological, physiological and biochemical examination of the isolates were determined by the standard procedure of gram staining, catalase test and gas production test according to methods offered by Bulut, 2003.

Identification of Isolates

The means of identification used was the API 50 CHL medium intended for the identification of the genus *Lactobacillus* and related genera.

Determination of antibiotic susceptibility

Standardised culture suspensions (0.5 MacFarland standard, equivalent to cell density of 10^8 cfu/ml) was spread evenly on the surface of the MRS agar plate (Oxoid) using a sterile cotton swab.. The inoculated plate was allowed to dry before placing the diffusion discs containing antibiotics onto the surface of the inoculated agar plates. Susceptibility of the eight isolates to 5 types of antibiotics was performed by the disc diffusion method as used by [27]. Commercially available antibiotics discs (Oxoid) containing Amoxicillin (25µg), Erythromycin (10µg), Ciprofloxacin (10µg), Cotrimoxazole (25µg) and Gentamycin (10µg) were used. Precaution was taken to ensure uniform contact between the antibiotic disc and agar plate. After 36 hours incubation at 30-32°C under anaerobic conditions, inhibition zone diameters were measured. Inhibition zone diameters were measured inclusive of the diameter of the discs. Results were expressed as sensitive S, intermediate, I, and resistant, R, respectively according to the cut-off points given by the manufacturer [21].

Adhesion Assay

Intestinal epithelial cells were collected by scrapping the intestinal wall of healthy guinea pigs with a cytobrush and suspending at pH 7.0 in MEM (Eagles Minimal Essential Media). The suspension was kept under refrigeration until the adhesion assay. For the adhesion assay, bacterial suspension was standardised by harvesting the LAB isolate from a 12 hour culture and washing twice with saline (0.8% NaCl) solution, once with MEM (pH 7.0) and re-suspension in MEM to obtain a final concentration of 10^7 cfu/ml ($OD_{540}=0.15$). The bacterial suspension (500µl) was then added to 500µl of guinea pig intestinal cell suspension and incubated at 37°C for 1 hour under low agitated conditions (35 rpm). The control was prepared by substituting the bacterial suspension with MEM. In order to remove non adherent bacteria, tubes were centrifuged for 10 minutes at 800 rpm and the supernatant discarded. The pellets were re-suspended in 1ml MEM and washed three times under the same conditions. Bacterial binding to the epithelial cells was examined by light and phase contrast microscopy using differential staining. The total number of cells, cells with associated bacteria and bacteria adhered to the cell was quantified and expressed as

- a) Percentage of adhesion: - the number of epithelial cells with bacteria adhered divided by the total number of epithelial cells observed x 100.

b) Adhesion index: - The total number of bacteria attached to cells divided by the number of cells with bacteria adhered.

Antimicrobial activity

After 18 hours incubation, active cultures were spotted on the surface of MRS agar plates. The plates were incubated to grow the cultures for 24 hours at 37°C under anaerobic conditions. Overnight indicator reference pathogen inoculated to soft agar containing 0.7% agar was overlaid on the MRS plates. These plates were incubated according to the appropriate condition for the indicator organism. At the end of incubation, the inhibition zone around the spotted isolates was measured. Isolates which gave an inhibition zone bigger than 1mm was determined to have antimicrobial activity. Data on the zone of inhibition obtained against the different bacterial pathogens was statistically analysed and significance evaluated at 5% level.

212 RESULTS

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216 **TABLE 1: ADHESION ASSAY OF LAB ISOLATE TO INTESTINAL EPITHELIAL**
 217 **CELLS OF GUINEA PIGS**

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NAME OF LAB ^{CODE}	ADHESION (%)	ADHESION INDEX	FERMENTINGPARENT CEREAL
<i>P.damnusus</i> 2 ³²	80	15	Sorghum-Millet
<i>L.plantarum</i> 1 ³³	65	18.4	Sorghum-millet
<i>L.paracasei ssp paracasei</i> 1 ⁶	100	22	Sorghum-Millet
<i>L.paracasei ssp paracasei</i> 2 ²⁹	55	14.5	Acha-sorghum
<i>L.plantarum</i> 1 ²⁶	80	15	Sorghum
<i>L.paracasei ssp paracasei</i> 3 ³⁹	75	6.8	Sorghum-Millet
<i>P.pentosaceus</i> 2 ²⁷	60	12	Millet
<i>L.plantarum</i> 1 ¹²	70	24	Sorghum-Millet

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222 **Table 2: ANTIMICROBIAL ZONE OF INHIBITION (mm) OF API 50 CH TEST KIT IDENTIFIED LAB ISOLATES**
223 **AGAINST SELECTED HUMAN PATHOGENS**

Antimicrobial zone of inhibition (mm)				
Reference pathogens				
LAB significant taxa	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>
<i>L. plantarum</i> ³³	21.00±1.00 ^{def DEF}	24.17±0.76 ^{gDEFGH}	23.17±1.26 ^{efghiDEFGH}	17.83 ^C ±0.76 ^{defg}
<i>P. pentosaceus</i> ²⁷	28.00±1.00 ^{jk GHJ}	22.17±0.28 ^{9^f EFG}	22.33±1.15 ^{cdefgCDEFG}	17.83 ^E ±0.29 ^{defg}
<i>P. damnosus</i> ³² <i>p</i>	19.67±1.53 ^{de BC}	20.67±0.58 ^{de BCDEF}	18.67±0.71 ^{abcAB}	0.00 ^A ±0.00 ^a
<i>L. paracasei ssp para2</i> ²⁹	11.00±1.00 ^{b B}	22.50±0.5 ^{f EFGH}	23.83±0.76 ^{cdefEFGH}	12.83 ^C ±0.29 ^b
<i>L. plantarum</i> ²⁶	22.83±1.76 ^{gh DEFGHI}	26.27±1.00 ^{hiGHI}	21.07±1.10 ^{ghiBCDEF}	24.17 ^{HJ} ±0.29 ^b
<i>L. paracasei ssp para1</i> ⁶	28.00±1.00 ^{jk IJ}	24.00±0.76 ^{gFGH}	25.00 ±1.32 ^{ijkGH}	14.17 ^C ±0.29 ⁱ
<i>L. paracasei ssp para3</i> ³⁹	23.33±2.8 ^{hiH}	20.00±1.00 ^{dBCDEF}	29.00±.00 ^{bcdeHI}	14.00 ^C ±0.50 ^{bcd}
<i>L. plantarum</i> ¹²	15.00±1.0 ^{cBC}	20.50±0.89 ^{deBCDEF}	20.17±0.28 ^{bcdefBCD}	18.00 ^{CD} ±0.52 ^{efg}

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226 Values are means of duplicate readings.

227 Values with the same superscripts across columns in small letters and down rows in capital letters are not significantly different

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TABLE 4: ANTIBIOTIC SUSCEPTIBILITY/RESISTANCE OF LAB ISOLATES

	25µg	10µg	10µg	25µg	10µg
NAME OF LAB / ^{CODE}	Amoxicillin	Erythromycin	Ciprofloxacin	Cotrimoxazole	Gentamycin
<i>L.plantarum</i> 1 ³³	R	13±0.7	R	12±1.1	R
<i>L. paracasei ssp para</i> 1 ⁶	R	15±1.4	R	R	R
<i>L.paracasei ssp para</i> 2 ²⁹	R	7±1.1	R	R	R
<i>L. plantarum</i> 1 ²⁶	R	R	R	R	R
<i>L.paracasei ssp para</i> 3 ³⁹	R	R	R	R	R
<i>P. pentosaceus</i> 2 ²⁷	R	13±1.1	R	9±2	R
<i>L. plantarum</i> 1 ¹²	R	10±1.1	R	R	R
<i>P. damnosus</i> ³²	R	R	R	14±1	R
<i>S. aureus</i>	R	R	20±2	17±1	R
<i>E. coli</i>	8±1.1	R ^{NIT}	22±1	R	12±0.3

Values (mm) represent means of duplicate readings with standard deviations.

R= Resistant; NIT= Nitrofurantoin;

The interpretive criteria used according to the manufacturer's instructions were

Zone diameter (mm)

Susceptible : $\geq 10\text{mm}$

Intermediate: 6-9mm

Resistant: $\leq 5\text{mm}$

Resistant breakpoint = 5mm

Susceptible breakpoint= 10mm

Table 3: PHYSICAL AND BIOCHEMICAL PROPERTIES OF IDENTIFIED LAB CANDIDATES

Name of LAB isolate	Homo / Hetero fermenter	Growth @ 10°C	Growth @ 15°C	Growth @ 45°C	Growth in 4% NaCl	Growth in 6.5% NaCl
<i>Lactobacillus plantarum</i> ¹³³	HM	+	+	-	+	-
<i>Pediococcus pentosaceus</i> ²²⁷	HM	+	+	+	+	+
<i>Pediococcus damnosus</i> ²	HM	+	+	-	-	-
<i>Lactobacillus paracasei</i> ssp <i>para</i> ¹⁶	HM	+	+	-	+	+
<i>Lactobacillus plantarum</i> ¹²⁶	HM	+	+	+	+	-
<i>Lactobacillus paracasei</i> ssp <i>para</i> ²⁹	HM	+	+	-	+	+
<i>Lactobacillus paracasei</i> ssp <i>paracasei</i> ³⁹	HM	+	+	-	+	+
<i>Lactobacillus plantarum</i> ¹¹²	HM	+	+	-	+	-

+ = Positive; - = Negative; HM = Homofermenter

DISCUSSION

Table 1 shows the adhesion percentage of the various LAB isolates to intestinal cells *in vitro*. The highest adhesion in this study was demonstrated by *L.paracasei ssp paracasei*⁶. Adhesion to the GI tract has been widely used as a criterion for the selection of health benefitting lactobacilli since many authors have presented adhesion as an important criterion that should be used when choosing potential health benefitting bacteria [9] [16]. The adhesion is one of the strategies used by many bacteria to maintain stable numbers at different ecological niches. Some studies have shown that the high capability of adhesion of a certain microorganism indicates that they can compete for nutrients with a higher efficiency than those nonadherent bacteria. The adhesion to epithelial surfaces is a critical step in the colonization of *Lactobacillus* and one of the suggested mechanisms by which they could protect the intestines from the colonization of pathogens is by their competition for the receptor sites for adhesion. All the LAB investigated in this study gave high adhesion abilities. This agreed with the work of [25] and was quite encouraging especially when Table 2 is considered. Table 2 shows that all the homo fermenting LAB isolates were able to inhibit indicator pathogens with high inhibition zones. These results agree also with the findings reported by [3] & [22] where LAB isolates from various fermented foods displayed antimicrobial activity towards *S.aureus* and *E.coli* respectively. The findings of this study suggest that these LAB isolates from kunu-zaki would be beneficial to the gastrointestinal tract of humans when kunu-zaki is consumed. This conclusion also tallies with the observations from the work of [11]. Table 3 displays the physical and biochemical properties of these LAB isolates. The findings indicate that *Pediococcus pentosaceus* 2²⁷ was able to grow at all conditions of temperature and NaCl concentrations tested. Majority of African spontaneously fermented foods take place at room temperatures and kunu-zaki is no exception. Halting the fermentation process requires the use of high temperatures after which cooling takes place before “back-slopping”. It is therefore important to study the ability of the LAB to grow at different cooling temperatures [23]. From this study, *Pediococcus pentosaceus* 2²⁷ and *L.plantarum* 1²⁶ were both able to grow at 45⁰C. This would make both LAB isolates likely candidates as starter cultures for the large scale production of kunu-zaki under controlled fermentation conditions. All LAB isolates with the exception of *Pediococcus damnosus* were able to grow at 4.5% NaCl concentrations. The introduction of NaCl is also sometimes used in African fermentations to halt fermentation processes [23]. In the event of choice of starter cultures for use in controlled

301 fermentations, ability to withstand NaCl concentrations becomes important [19]. The introduction
 302 of probiotic (health benefitting) products into the market, and the use of specific probiotic
 303 organisms as starter cultures, has however constantly raised the question of antibiotic resistance
 304 and what possible interactions may occur if a consumer is taking a probiotic food simultaneously
 305 with antibiotic therapy [20]. Plasmids are genetic elements independent of the cell chromosome.
 306 Over time, some plasmids have developed genes that make them resistant to selected antibiotics.
 307 Plasmids are easily transferred between cells, and this allows the efficient spread between
 308 bacteria of resistance to an antibiotic [26]. Antibiotics originally were substances produced by
 309 fungal microorganisms to eliminate competition from bacteria for survival reasons. However, the
 310 number of antibiotics now available and their widespread use in prescription drugs and for
 311 general disinfection purposes has raised concerns about the potential for the development of
 312 antibiotic resistance. Table 4 shows the antibiotic reaction pattern of the LAB isolates to five
 313 selected antibiotics commonly used to treat infections in Nigerian clinics. In this study, all the
 314 LAB isolates were resistant to Amoxicillin, Gentamycin and Ciprofloxacin. However, all the
 315 isolates were susceptible to Erythromycin except *L. plantarum* 1²⁶ (isolated from fermenting
 316 Sorghum), *L. paracasei* sspparacasei3 and *P. damnosus*. *P. damnosus* 2³², *P. pentosaceus* 2 and
 317 *L. plantarum* 1³³ were however susceptible to Cotrimoxazole. The resistance of these LAB to
 318 antibiotics, however beneficial, nonetheless raises concerns of the possibility of the ingested
 319 LAB becoming antibiotic-resistant from related intestinal bacteria that already have acquired
 320 resistance, or more seriously, the reverse—a product containing antibiotic-resistant LAB bacteria
 321 that pass antimicrobial resistance genes or genes that encode for virulence factors onto resident
 322 bacteria [18]. A probiotic should preferably not possess any antibiotic resistance and if it does, it
 323 should be unable to transfer the antibiotic resistance genes [18]. In this research work, 62.5% of
 324 the isolates were sensitive to Erythromycin and 25% were sensitive to Cotrimoxazole. All eight
 325 LAB isolates were resistant to Amoxicillin and Gentamycin. For quality control of the antibiotics
 326 used during the study, sensitive reference strains including *Staphylococcus aureus* ATCC 25923
 327 and *E. coli* ATCC 25922 were used. These strains were obtained from the Nigerian Institute of
 328 Medical Research NIMER. The antibiotics tested are antibiotics commonly used in various
 329 clinical treatments in the country. These findings contradict the findings reported by [17]. In
 330 Ethiopia, [17] reported the susceptibility patterns of selected LAB isolated from *wakalim*, a
 331 traditional Ethiopian fermented beef sausage. Most LAB tested were susceptible to antibiotics

and they conclude that those strains isolated from traditional fermented foods could have application as a starter cultures for large scale production of the traditional product and/or as probiotic to enhance health of the consumers. Strains intended for use in the food systems as starter should be carefully examined for antimicrobial susceptibility [29]. Some scientists argue that the intrinsic resistance of lactic acid bacteria to many antibiotics may be considered as advantageous for those isolates with probiotic potential. The logic is that such resistance could be helpful for sustainable utilization of the strains in human intestine to maintain the natural balance of intestinal microflora during antibiotic therapy [17]. However, there is the danger of transferring multiple drug resistance to pathogens in the intestinal environment. The susceptibility of the probiotic LAB isolates to the clinically important antimicrobials, on the other hand is overall beneficial as it minimises the chances of disseminating resistance genes to pathogens both in the food matrix and/or in the gastrointestinal tract [1]. Actually, the European Food Safety Authority summary report of 2005 explains that antibiotic resistance is not a safety issue *per se*, it only becomes a safety issue when horizontal transfer is concerned. Intrinsic resistance may arise because the candidate microorganism may lack the target for the antibiotic. This will raise less concern in a safety evaluation. Instead, a microorganism that has acquired mobile DNA encoding for an enzyme that modifies or destroys an antibiotic should attract more attention. Furthermore, it is also important to recognise that antibiotics differ in their clinical and veterinary importance [12]. Lactobacilli are known to possess naturally a wide range of antibiotic resistance but in most cases the antibiotic resistance is not of the transmissible type [18]. Of the antibiotics tested by [24], indigenous lactobacilli were resistant to cloxacillin, penicillin and streptomycin. This is comparative to the pattern revealed in this our study. Also, [1] investigated the antimicrobial pattern of LAB isolated from fermented milk in Ethiopia. Their work noticed resistance to Methicillin by all strains of LAB tested but they concluded that the LAB could not consist of reservoirs for transmissible genes of Erythromycin or Streptomycin resistance based on their susceptibility. Homo-fermenters are usually preferred to hetero fermenters as choice starter cultures for fermented foods since the end product of their metabolism is lactic acid only. The result of this study documents findings on the antibiotic resistance pattern of these eight homo-fermenting LAB present in ready to drink kunu-zaki. If these strains are therefore intended for use in kunu-zaki especially as starter cultures, it is important that they should be further carefully examined for inability to transfer antibiotic resistance genes to food pathogens.

363

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