In-vivo anti-diarrhea assessment of Bacteria Isolates from selected Fermented Foods in Mitigating *Escherichia-Coli* Induced-Diarrhea in Wistar Rats

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ABSTRACT

Antibiotic resistance is a major challenge in the control of diarrhea worldwide. And probiotics that could serve to ameliorate these comes with high cost, and its effectiveness is marred by non-compliance due to drug form presentation. In this study, probiotics were investigated in Nigerian indigenous fermented foods. Bacteria were isolated from Ogi, Kunu and Nunu using pour-plate method on MRS agar. Agar well diffusion method was used to screen the isolates for antibacterial activities against the test organism, Escherichia coli (E. coli). Fecal consistency of Wistar rats was the indices used to assess the therapeutic and preventive potentials of the LAB isolates. The isolates were identified using 16SRNA sequencing. Among 128 bacterial isolates, 26 inhibited the test organism with diameter of inhibition zones ranging from 2.00 to 12.00 mm, and 13 of these showed lactic acid bacteria (LAB) properties. All the 13 isolates resolved the diarrhea induced in Wistar rats with the most active Vagococcus fluvialis within 24hrs of administration. Twelve of the isolates were identified as LAB belonging to the genera Enterococcus, Pediococcus, Weisella and Vagococcus. The non-LAB isolate was Bacillus sp. The study concluded that indigenous fermented foods are rich in probiotic LAB that can serve as alternative to antibiotics in the treatment and prevention of diarrhea due to gastrointestinal infections. The study recommends the use of these organisms for the production of fermented foods that could be used as therapeutic and preventive diets for gastrointestinal diseases.

KEYWORDS

Antimicrobial, Escherichia coli, infection, lactic acid bacteria, probiotics and therapeutic

I. INTRODUCTION

Diarrhea remains a major cause of mortality among under-age children (mostly under 5years) around the world, especially in developing world (Khedid *et al*., 2019). Diarrhea is a condition characterized by the passage of loose or watery stools or an increased frequency of stools. It is a form of gastrointestinal disease caused by a variety of bacteria, parasites and viruses consumed through contaminated food or water, or spread from person to person as a result of poor hygienic practices (Vieira*, et al*., 2018).

Management of diarrhea in patients have focused mainly on dietary recommendations, oral rehydration therapy and medications (David *et al*., 2019) each of which has various shortcomings. Dietary recommendations have brought much confusion about optimal foods for patients with diarrhea (Khedid *et al*., 2019). The apathy towards oral rehydration therapy (ORT) which was developed as a safer, less expensive, and easier alternative to intravenous fluids has been linked to side effects such as nausea and vomiting (David *et al*., 2019). Also, great concerns over the development of antibiotic resistance and antibiotic-associated diarrhea discourages their use in the treatment of gastrointestinal diseases (Khedid *et al*., 2019).

Probiotics has been suggested as an alternative means of treating and preventing diarrhea especially in children (Busayo *et al*., 2018) due to the absence of side effect in its usage (Lin and Pan, 2017). Many strains of lactobacilli, bifidobacteria, enterococci and yeast such as *Saccharomyces boulardii* are packaged as commercial probiotics, and have gained prominence among the elites in most advanced countries. One of the most well documented effects of probiotics, especially LAB is the reduction of the diarrhea period and prevention of diarrhea in patients including children (Van Belkum *et al*., 2019; Yaneisy *et al*., 2019).

Many years ago, research on the link between certain food and health benefits has been ongoing which has led to the realization that the human diet includes largely of fermented products, acquired from plant or animal sources. These fermented products have for years now been appreciated and consumed globally in most part of the world as a fundamental part of the human diet (David *et al*., 2019). More so, the practice of food fermentation has been driven by indigenous microorganisms in the raw ingredient all through Africa which control the dietary accessibility, organoleptic value and security of the end products (Owusu-Kwarteng *et al*., 2018). Lactic acid bacteria have for a while now, because of their great function in most fermented foods, been the center of attention for researchers (Khedid *et al*., 2019) and have been isolated from numerous fermented foods for their use as probiotics and functional food resources (Solieri *et al*., 2018). Food and mainly the fermented products are considered an important vehicle for the entrance of living microorganisms into the human body according to David *et al*., 2019.

The health benefits of probiotics have not been fully exploited in the third world countries such as Nigeria, due to the high cost of the commercially packaged forms. In addition, majority of patients that comes up with diarrhea have apathy for the drug form presentation of the probiotics resulting in non-compliance, and with consequences on the effectiveness of the probiotics. This study investigated the efficacy of probiotics in indigenous fermented foods in the treatment and prevention of diarrhea as a means to address the issues of cost and non-compliance in the administration of probiotics.

A. Impact Statement

The important of this research lies in investigating, diagnosing and treating antibiotic-resistance strains of bacteria causing diarrhea using isolated strains from fermented foods, such as lactic acid bacteria.

II. MATERIALS AND METHODS

Fermented food samples: Nunu, Kunu and Ogi samples were the indigenous fermented foods used in this research. They were purchased from different sellers at different locations in Ilorin and its metropolis systematically.

A. pH analysis of fermented food samples

The pH is a unit of measurement for the acidity or alkalinity of a medium using a logarithmic scale with seven as neutral, where lower values are more acidic and higher values are more alkaline. The pH of the samples was measured by using calibrated pH meter (digital tester pen pH meter). The beaker was cleaned with distilled water before use. The mixture of the sample was made in the beaker by adding 10 ml of the sample to 100 ml of distilled water and the calibrated pH meter was inserted into the solution. The reading was observed at 25 ℃ and recorded when the reading on the pH meter was stabilized.

B. Test organisms

Clinical isolate identified as *Escherichia coli* was obtained from the culture collection section of the Pathology Department of the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria.

C. Probiotics

Potential probiotics were isolated from "nunu", "kunu" and "ogi" samples all of which are Nigeria indigenous fermented foods. Ten samples of each of the three fermented foods were purchased from retailers at different locations in Ilorin, Nigeria, into sterile sample bottles. Bacteria were isolated from each sample after serial dilution, by plating on MRS agar using the pour plate method. Isolates were characterized based on their morphology, biochemical and sugar fermentation tests.

Assay for antibacterial activities of isolates:

D. Preparation of cell-free supernatants

A loop full of each of the isolates were inoculated into separate McCartney bottles containing 20 ml of MRS broth and incubated at 37 ºC for 48 hours. After incubation, each broth culture was centrifuged at 5,000 x g using cold centrifuge. The supernatant was filtered through membrane filter and the resultant filtrate was kept at 4 ºC until required.

E. Antibacterial assay

The agar-well diffusion method was used to screen the isolates for antibacterial activity following the method used by Kos, 2018. Indicator lawns were prepared by inoculating 20 ml of Mueller Hilton molten agar media (45 ºC) with 100 µl, of 0.5 McFarland of an overnight-incubated culture of test organism before pouring into Petri-dishes and allowing to solidify. Three wells were bored into the seeded agar using sterile 5 mm diameter cork-borer and the wells were sealed with two drops of sterile molten agar. Aliquots, 50 µl of the cell-free supernatant of the isolate was placed into each of the wells. Plates were incubated in upright position, at 37 ºC for 24 h. The plates were observed for zones of inhibition. Diameter of inhibition zones around wells were measured in the plate using meter rule and the mean was taken as the antimicrobial activity of the isolates.

F. Determination of antimicrobial principle of isolates

Test was run to determine whether lactic acid, hydrogen peroxide or bacteriocin were produced as the antimicrobial responsible for the inhibition by the isolates. The isolates were inoculated in MRS broth. After incubation at 37℃ for 24hrs, cultures were centrifuged and cell-free supernatants (CFS) were subjected to three different treatments: (i) non-neutralized, (ii) neutralizing by addition of 5M NaOH to exclude organic acid inhibitory effects (pH 6.5), (iii) treatment of neutralized supernatants in (ii) with catalase to a concentration of 1mg/ml for 1hr at 25° C to exclude H_2O_2 inhibitory effects and (iv) excluding the inhibitory effects of bacteriocin, which is a form of protein by treating the neutralized supernatants in (iii) with proteinase K to a concentration of 1mg/ml. The different supernatants were filtered through a 0.22µm Millipore sterile filter. An aliquot of 60 µl of the final supernatant was filled in nutrient agar (0.9% agar) which was used to fill holes bored on Mueller Hinton medium that had been seeded with active test organisms (10^6CFU/ml). The plates were pre-incubated at 4 ℃ for 2hrs and then were incubated for 18hrs at 37 ℃ and zone of inhibition was checked by measuring the diameter (mm). Triplicate trial assays were carried out.

G. Determination of antidiarrheal activities of isolates in Wistar rats

Isolates that were Gram positive, catalase and oxidase negative, and showed high and broad antimicrobial activity against the test organisms were selected for the in vivo therapeutic and preventive antibacterial activities in Wistar rats.

H. Experimental animals

One hundred and thirty-eight (138) male albino rats (*Rattus norvegicus*) aged 5–6 weeks used in this study were purchased from IBK animal house in Offa, Kwara State, Nigeria. The rats were randomly distributed into three groups of 45 rats each. The remaining three rats were used as control. The 45 rats in each group were subdivided into 15 subgroups each containing 3 rats that were housed together in one cage. Cages were labelled according to the test organisms and the treatment as shown in table 1. The rats were kept at room temperature to acclimatize for 14 days prior to the commencement of the experiment. All the rats received standard pellet feed (Vital feed) and water *ad libitum* throughout the period of the study.

I. Induction and treatment of diarrhea in Wistar rats

The rats were fasted 24 hours prior to the commencement of the experiments. The test bacteria used were *Clostridium difficile, Salmonella typhi* and *Escherichia coli*. Suspensions of the bacteria were prepared using physiological normal saline, and adjusted to 0.5 McFarland. The rats were orogastrically dosed with 1 ml (10^6CFU) of the suspension to induce diarrhea in them (Adebolu, 2018). The fecal products of the rats were observed daily for changes in appearance from the pellet form to the soft and watery forms which was used as indicator of diarrhea induction. Treatments were commenced immediately the soft and watery fecal materials were noticed, by administering 1ml (10^6CFU) of 0.5 McFarland of the isolated probiotic bacteria orogastrically, daily, for the rest of the 21-day period used for the experiment. Time of change in fecal consistencies were observed in grouped rats after the administration of test organisms, and after the therapeutic treatments.

The number of days before the appearance of watery stools after diarrhea induction was taken as the level of prevention by the probiotics. The longer the delay of diarrhea by an isolate the higher its preventive activity.

Table 1: Grouping of Wistar rats for therapeutic and preventive treatments of diarrhea

Rat Group	Therapeutic Study (E. coli induced diarrhea)	Preventive Study (E. coli induced diarrhea)
Control Group 1 (CG)	Three rats fed with the standard pellet feed + water only.	Three rats fed with the standard pellet feed + water only.
Control Group 2	ECG: E. coli dosed rats fed with pellet feed + water only.	Rats fed with pellet feed + water and dosed with E. coli on day 8.
Control Group 3	ECGLa: E. coli dosed rats fed with pellet feed + water + L. acidophilus.	Rats fed with pellet feed + water with addition of L. acidophilus for the first 7 days, and dosed with E. coli on day 8.
Treatment Groups 1 to 13	E. coli dosed rats fed with pellet feed + water + isolated probiotics individually.	Rats fed with pellet feed + water with addition of isolated probiotics individually for the first 7 days, and dosed with E. coli on day 8.

III. IDENTIFICATION OF LAB ISOLATES

Genomic DNA was extracted from the cultures, amplified, sequenced, purified and results were obtained by a BLAST (Basic Local Alignment Search Tools) search (NCBI) using BLASTN 2.2.31+. The bacterial isolates were characterized by sequencing the 16S rDNA. The universal primers 27F and 1492R are used to amplify the 16S target region as shown in Table 2.

Table 2: 16S Primers sequences

Primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACT

A. Evolutionary analysis

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-10272.68) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1607 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

B. Statistical analysis

The data gathered were collected in triplicates and processed using one-way analysis of variance (ANOVA), SPSS 20.0.

C. Ethical Approval Number

UERC/ASN/2018/2025/1414 dated 10/09/2025.

IV. RESULTS

Twenty-six isolates showed inhibitory activities against *E. coli* with diameter zones of inhibition ranging from 5.0 – 12.0mm as shown in table 4 from the 128 isolates isolated from the fermented food samples as shown in Figure 1.

Table 3: pH of the fermented food samples taken before isolation of bacteria

Sample No.	Nunu (pH)	Kunu (pH)	Ogi (pH)
1	4.11 ± 0.01a	3.61 ± 0.01ab	3.42 ± 0.01d
2	4.13 ± 0.01ab	3.39 ± 0.21b	3.38 ± 0.01a
3	4.21 ± 0.01bc	3.57 ± 0.01a	3.41 ± 0.01c
4	4.13 ± 0.01ab	3.54 ± 0.01a	3.44 ± 0.01e
5	4.45 ± 0.01d	3.51 ± 0.01a	3.47 ± 0.01f
6	4.52 ± 0.01d	3.54 ± 0.01a	3.41 ± 0.01c
7	4.23 ± 0.01bc	3.57 ± 0.01a	3.39 ± 0.01b
8	4.29 ± 0.12c	3.59 ± 0.01a	3.40 ± 0.01b
9	4.12 ± 0.01a	3.55 ± 0.01a	3.43 ± 0.01d
10	4.25 ± 0.01c	3.56 ± 0.01a	3.42 ± 0.01d

Note: Means sharing the same superscript letter (a, b, c, d) are not significantly different (Tukey HSD, α =0.05)

Table 4: Cellular, morphology and biochemical reaction of E. coli

Biochemical Tests	Reactions (E. coli)
Gram reaction	-
Catalase	+
Citrate utilization (Simmon's citrate agar)	-
Sugar fermentation	+
Gelatin liquefaction (Nutrient gelatin)	-
Indole Production	+
Nitrate Reduction	+
Urease (Urea broth)	-
Voges-Proskauer	-
Methyl Red	+
Motility (SIM medium)	+
Oxidase	-

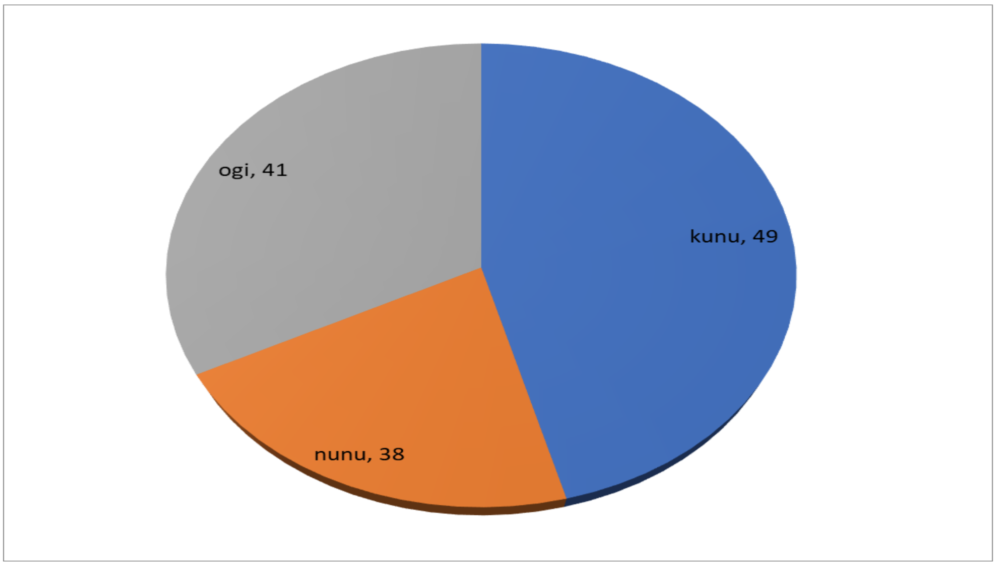

Figure 1: Number of isolates obtained from the different fermented food sample

Table 5: Isolates Zone of inhibition

Isolates	Zone of inhibition (mm)
NN3	8.0
NN5	10.0
NN7	8.0
NN12	12.0
NN17	10.0
NN21	9.0
NN23	10.0

KN1	8.0
KN2	8.0
KN3	9.0
KN6	5.0
KN8	8.0
KN26	10.0
KN31	9.0
KN33	10.0
KN38	8.0
KN43	9.0
KN45	8.0
OG2	8.0
OG3	10.0
OG6	11.0
OG14	5.0
OG19	7.0
OG26	2.0
OG32	10.0
OG36	5.0

Table 6: Diameter zones of inhibition of the cell-free supernatants of the bacterial isolates against *E. coli*

Isolate	**Gram**	**Shape**	**Motility**	**Catalase**	**MR**	**Indole**	**Oxidase**	**KOH**	**Glu**	**Fru**	**Lac**	**Suc**
NN3	+	C	-	-	+	-	-	-	+	+	+	+
NN5	+	C	-	-	+	-	-	-	+	+	+	+
NN7	-	C	-	+	-	-	-	+	+	+	-	+
NN12	+	C	-	-	+	-	-	-	+	+	+	+
NN17	+	R	-	-	+	-	-	-	+	+	+	+
NN21	-	C	-	+	-	-	+	+	-	-	-	-
NN23	+	R	-	+	-	+	-	-	-	+	+	+
KN1	+	C	-	-	+	-	-	-	+	+	+	+
KN2	+	C	-	-	+	-	-	-	+	+	+	+
KN3	+	C	-	-	+	-	-	-	+	+	+	+
KN6	+	C	-	+	-	-	-	-	-	+	-	+
KN8	+	R	-	+	-	-	-	-	+	+	+	+
KN26	-	C	-	+	-	-	+	+	+	+	-	+
KN31	+	R	-	+	-	+	-	-	-	-	+	+
KN33	+	C	-	-	+	-	-	-	+	+	+	+
KN38	+	C	-	-	+	-	-	-	+	+	+	+
KN43	+	C	-	-	+	-	-	-	+	+	+	+

KN45	+	R	-	+	-	-	+	-	+	+	-	+
OG2	+	C	-	-	-	-	-	-	+	+	+	+
OG3	-	C	-	-	-	+	-	+	-	+	-	-
OG6	+	C	-	-	+	-	-	-	+	+	+	+
OG14	-	C	-	+	+	-	-	+	-	-	-	-
OG19	+	R	-	+	-	-	+	-	+	+	-	+
OG26	-	C	-	-	+	-	-	+	+	+	+	+
OG32	+	C	-	-	+	-	-	-	+	+	+	-
OG36	-	C	-	+	-	-	+	+	-	-	+	+

NB: All the bacteria are suspected to be lactic acid bacteria except NN17.

Table 7: Inhibition of *Escherichia coli* by crude, NaOH neutralized, catalase treated and proteinase K treated cell-free-extracts of the isolates

Isolates	Crude Extract	Neutralized Extract	Catalase Treated	Proteinase K Treated
NN3	12.0±1.000	2.0±0.000	4.0±1.000	0.0±0.000
NN5	10.0±1.155	4.0±0.000	6.0±1.155	0.0±0.000
NN12	13.0±1.000	3.0±0.000	4.0±1.325	0.0±0.000
NN17	9.0±1.000	3.0±1.000	5.0±1.000	0.0±0.000
KN1	13.0±1.214	3.0±1.000	7.0±1.115	0.0±0.000
KN2	7.0±0.226	4.0±0.000	6.0±1.000	0.0±0.000
KN3	4.0±1.000	3.0±0.000	2.0±0.000	0.0±0.000
KN33	3.0±1.000	2.0±0.155	3.0±0.000	0.0±0.000
KN38	5.0±1.000	2.0±1.000	3.0±1.000	0.0±0.000
KN43	13.0±0.577	5.0±1.000	5.0±0.557	0.0±0.000
OG2	9.0±1.000	4.0±0.000	6.0±1.000	0.0±0.000
OG6	7.0±1.115	3.0±1.000	4.0±1.000	0.0±0.000
OG32	8.0±1.000	2.0±0.577	3.0±1.000	0.0±0.000

Values are means of 3 replicates experiment ± SD.

In-vivo analysis of probiotic potential of the LAB isolates from fermented food samples shows antidiarrheal effect in albino rats as shown in table 7, 8 and 9. Preventive effect of the LAB isolates was shown in Table 10. Identification of the LAB isolates KN1, KN2, KN3, KN33, KN38, KN43, OG2, OG6, OG32, NN3, NN5, NN12 and NN17 at molecular level was shown on table 11.

Table 8: Changes in the feacal consistency of Wistar rats treated with isolates after *Escherichia coli* -induced diarrhea

Day	CG	EG	ENN 3	ENN 5	ENN 12	ENN 17	EOG 2	EOG 6	EOG 32	EKN 1	EKN 2	EKN 3	EKN 33	EKN 38	EKN 43	Ela
1	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
2	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
3	p	w	w	p	w	w	p	w	w	w	p	p	w	p	w	w
4	p	w	w	w	w	w	w	w	w	w	w	p	w	p	w	w
5	p	w	w	w	s	w	w	w	w	w	w	w	w	w	w	s
6	p	w	w	w	s	w	w	s	w	w	w	w	w	w	w	s
7	p	w	w	w	s	w	w	s	w	w	w	w	s	w	s	s
8	p	w	s	w	s	s	w	s	s	s	w	w	s	s	s	p
9	p	w	s	s	p	s	s	p	s	s	s	w	s	s	s	p
10	p	w	s	s	p	s	s	p	s	s	s	s	s	s	s	p
11	p	w	s	s	p	s	s	p	s	s	s	s	p	s	s	p
12	p	w	s	s	p	s	s	p	s	s	s	s	p	s	s	p
13	p	w	s	s	p	s	s	p	s	s	s	s	p	s	s	p
14	p	w	s	s	p	s	s	p	s	s	s	s	p	p	s	p
15	p	d	s	s	p	s	p	p	s	p	s	s	p	p	s	p
16	p	d	s	s	p	s	p	p	s	p	s	s	p	p	p	p
17	p	d	s	p	p	s	p	p	p	p	p	s	p	p	p	p
18	p	-	p	p	p	p	p	p	p	p	p	p	p	p	p	p
19	p	-	p	p	p	p	p	p	p	p	p	p	p	p	p	p
20	p	-	p	p	p	p	p	p	p	p	p	p	p	p	p	p
21	p	-	p	p	p	p	p	p	p	p	p	p	p	p	p	p

Key:

p = pellet-like stool; w = watery stool; s = semi-solid stool; d = death of rat.
CG= group of rats fed with grower mash only without being dosed with *E. coli.*

E-G= group of rats dosed with 1ml of 10^5cfu/ml of *E. coli* and fed with grower mash.

ENN (3, 5, 12&17) = group of rats dosed with 1 ml of 10^5 cfu/ml of *E. coli* and fed with isolates NN (3, 5, 12&17) respectively.

EOG (2, 6&32) = group of rats dosed with 1ml of 10^5cfu/ml of *E. coli* and fed with isolates OG (2, 6&32) respectively.

EKN(1,2,3,33,38&43)= group of rats dosed with 1ml of 10^5cfu/ml of *E. coli* and fed with isolates KN(1,2,3,33,38&43) respectively.

Ela= group of rats dosed with 1ml of 10^5cfu/ml of *E. coli* and fed with *Lactobacillus acidophilus*.

Table 9: Change in the feacal consistency of Wistar rats fed with isolates prior to diarrhea induced by *Escherichia coli*

Observation of feacal product texture for period of 21 days after inoculation

Day	CG	G-E	NN3E	NN5E	NN12E	NN17E	OG2E	OG6E	OG32E	KN1E	KN2E	KN3E	KN33E	KN38E	KN43E	La-E
1	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
2	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
3	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
4	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
5	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
6	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
7	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
8	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
9	p	w	p	p	p	p	p	p	p	p	p	p	p	p	p	p
10	p	w	p	p	p	p	p	p	p	p	p	p	p	p	p	p
11	p	w	p	p	p	w	p	p	p	p	w	p	p	w	w	p
12	p	w	w	w	p	w	p	p	p	w	w	w	w	w	w	p
13	p	w	w	w	p	w	p	p	p	w	w	w	w	w	w	p
14	p	w	w	w	p	w	p	p	p	w	w	w	w	w	w	p
15	p	w	w	w	p	w	w	p	p	w	w	w	w	w	w	p
16	p	w	w	w	p	w	w	p	w	w	w	w	w	w	w	p
17	p	w	s	w	p	w	w	w	w	w	w	w	w	w	w	p
18	p	w	s	s	s	s	w	w	w	w	s	w	w	s	w	p
19	p	d	s	s	p	s	s	w	w	s	s	w	s	s	s	p
20	p	d	s	s	p	s	s	s	s	s	s	s	s	s	s	s
21	p	d	s	s	p	s	s	s	s	s	s	s	s	s	s	p

Key:

p = pellet-like stool; w = watery stool; s = semi-solid stool; d = death of rat.
GC= group of rats fed with grower mash only without being dosed with *E. coli.*
G-E= group of rats fed with grower mash and dosed with 1ml of 10^5cfu/ml of *E. coli.*
NN (3, 5, 12&17) E= group of rats fed with isolates NN (3, 5, 12&17) respectively and dosed with 1 ml of 10^5 cfu/ml of *E. coli*.
OG (2, 6&32) E= group of rats fed with isolates OG (2, 6&32) respectively and dosed with 1ml of 10^5cfu/ml of *E. coli*.
KN(1,2,3,33,38&43)E= group of rats fed with isolates KN(1,2,3,33,38&43) respectively and dosed with 1ml of 10^5cfu/ml of *E. coli*.
La-E= group of rats fed with *L. acidophilus* and dosed with 1ml of 10^5cfu/ml of *E. coli*.

Table 10: BLAST prediction

S/No.	Sample ID	Organism Identity	% Identity	Accession No. (BLAST Hit)
1	K1=KN1	Weissella cibaria Uga49-1	99.74	DQ294961.1
2	K2=KN2	Enterococcus faecium HN-N32	98.95	FJ378687.1
3	K3=KN3	Enterococcus durans KCTC13289	97.57	CP042598.1
4	K4=KN33	Pediococcus pentosaceus K15E	98.05	OM936151.1
5	K7=KN38	Pediococcus pentosaceus NRIC123	100	AB362605.1
6	K8=KN43	Pediococcus pentosaceus NRIC123	100	AB362605.1
7	OG1=OG2	Enterococcus durans DpS11	99.54	AB362601.1
8	OG3=OG6	Enterococcus gallinarum FDAARGOS728	99.67	CP046307.1
9	OG4=OG32	Enterococcus faecium HB-1	99.34	CP040878.1
10	N1=NN3	Enterococcus faecium HN-N32	98.62	FJ378687.1
11	N3=NN5	Enterococcus lactis HBUR51084	100	OR502326.1
12	N5=NN12	Vagococcus fluvialis AN7	99.84	MN560003.1
13	N6=NN17	Bacillus safensis BRM1	99.54	CP043404.1

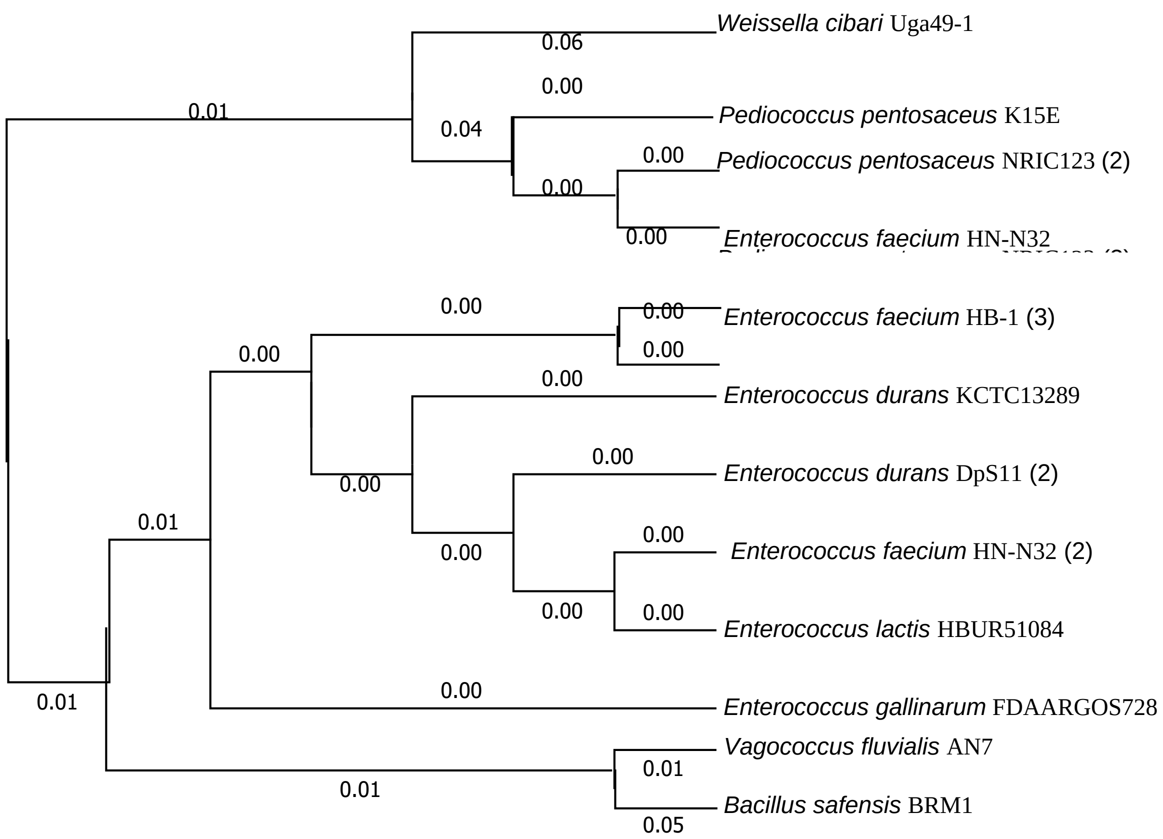

Figure 2: Neighbor joining tree analysis of the phylogenetic relationships of bacteria isolates

V. DISCUSSION

Ten samples of each fermented foods, Nunu, Kunu and Ogi as shown in Table 3, were collected systematically from various sellers at different locations in Ilorin and were transported to the Microbiology laboratory of Microbiology department in the University of Ilorin, Kwara state, Nigeria. The pH of the samples was analyzed and the results were as shown on Table 3. Ogi samples had the pH range of 3.38 - 3.47 which made it more acidic than other samples which were Kunu and Nunu with pH ranges of 3.51 - 3.60 and 4.10 - 4.52 respectively. The pH of Nunu was less acidic compared to other samples due to high protein content which breaks down to nitrogenous compound which neutralized the acidity in the medium during fermentation. LAB found in milk and other fermented food products catabolize glucose in two ways: homofermentative and heterofermentative according to Kandler in 2017. Firstly, homofermentative LAB: Glucose components via EMP pathways (Embden Meyerhof Parnas) producing 90% lactic acid and 10% CO2. Secondly, heterofermentative LAB: Glucose components via HMP (Hexose monophosphate) producing lactic acid, ethanol, acetaldehyde, diacetyl, exopolysaccharide and CO2. Lactic acid bacteria help fermented products (cheese, yoghurt, butter, gari, lafun, ogi, nunu, kunu, beer etc.) to gain their own aroma, smell and structure (Caplice and Fitzgerald, 2018).

The total of 128 bacterial cultures that were isolated using MRS agar as shown on figure 1 were named according to the name of samples (NN- Nunu, KN- Kunu and OG- ogi) and serial number of orderly collections of the sample Many bacteria are used as starter cultures for the industrial processing of fermented products and also, in this research work non-starter lactic acid bacteria can originate from the raw material and the environment as in accordance to Bintsis, 2018). One of the most important characteristics of probiotics is as protection against pathogens in the intestinal tract of the host. In addition to all of these, probiotics can also be widely used in many fields like pharmaceuticals in pharmacology.

The isolation, identification, and characterization of novel LAB strains have two benefits. The first is to reveal the characteristic taxonomy of the LAB and the second is to obtain promising beneficial and functional probiotic LAB according to Gusils *et al*. (2019). These LAB were evaluated for their functional traits, probiotic properties and ability to inhibit the growth of pathogenic or diarrheal-inducing bacteria. The antimicrobial activity which is the most probiotic properties of LAB isolates, of all the isolates was examined against *Escherichia coli* to screen them for further research work. *E. coli* was clinical sample isolated from diarrhea patients, obtained from the pathology department of University of Ilorin teaching hospital (UITH). The confirmation of identity of the pathogenic organisms was carried out using conventional method of identification and the results revealed the organisms to be *E. coli* according to Bergy’s manual as shown on Table 4.

The zone of inhibition ranged from 2-12 mm in diameter as shown in Table 5. The isolates were able to inhibit the selected indicators at variable degrees, particularly NN12 with the highest zone of inhibition of 12mm against *E. coli*. Similar findings were reported by Akabanda *et al.* (2018) on antimicrobial activity of selected lactic acid bacteria isolated from Nunu against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* where variable degree of inhibition zones on the microorganisms were observed. According to Yang *et al*., (2018), microorganisms which produce antimicrobial compounds, are capable of inhibiting the growth of Gram-positive and Gram-negative bacteria. Such lactic acid bacteria antimicrobial effect was also reported by Adesokan *et al*., (2018) where lactic acid bacteria cell free supernatant was used to inhibit the growth of *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Proteus vulgaris*. Obadina *et al.* (2018) also demonstrated the antagonistic effects of *Lactobacillus plantarum* against *Staphylococcus aureus, S. typhi, E. coli and Bacillus subtilis.* There was no disagreement in the antagonistic activity shown against pathogens by the LAB strains. In agreement with the finding in this research work, Reuben *et al*., 2019, Shin *et al*., 2018, Taheri *et a*l., 2019 and Olufemi *et al*., 2018 reported antagonistic activity against wide spectrum of pathogens by LAB isolated. Conversely, Yaneisy *et al*., 2019, Busayo *et al*., 2017, Kizerwetter-Swida and Binek, 2018 reported antagonistic activity by strains of LAB against Gram-positive pathogens (including *Clostridium perfringens* and *Staphylococcus aureus*) and Gram-negative pathogens (including *E. coli* and Salmonella). Spanggaard *et al*., 2018 stated that pathogen antagonism by probiotics was the major influential factor hindering pathogenic bacteria to establish in the GIT, and this indicates that a significant contribution to the control of pathogens is expected when lactic acid bacteria are used as probiotics. Those isolates that were active against *E. coli* were 26 in number and were selected for further lactic acid bacteria properties and probiotic potential assessments as shown in Table 5.

The cellular characteristics involved Gram's reaction and motility test and the result reveal that all the isolates were not motile but not all are positive to Gram's staining. The biochemical tests the isolates were subjected to reveal that not all the 26 isolates possessed lactic acid bacteria properties. The biochemical tests adopted in this research work were catalase production test, KOH test (string test), sugar hydrolysis, starch hydrolysis, etc. and the results were recorded as shown on table 6. Biochemical analysis showed that 13 of the isolates were catalase-negative, were able to ferment all the tested sugars, KOH negative, gram positive and were unable to digest starch. This was in agreement with the work done by Carr *et al*., 2018; Mathur and Singh, 2017 that stated that lactic acid bacteria used in conventional fermented foods are gram-positive, facultative anaerobes, catalase negative, immobilized lacking cytochromes.

Table 7 shows the antimicrobial principle of lactic acid bacteria isolates. The crude extracts of all the LAB isolates showed high zone of inhibition ranging from 6.0 – 12.0mm against *E. coli*. Absence and reduction in the zone of inhibition was observed in the crude extracts that were neutralized, treated with catalase and proteinase K to remove the acidic, hydrogen peroxide and bacteriocin respectively from the crude extracts, against the pathogenic organisms. The extracts that were treated with proteinase K had no inhibitory activity against all the pathogenic organisms. This revealed the importance of bacteriocin as the major factor of inhibitory activity of the LAB isolates against the pathogenic organisms. Probiotic bacteria produce various compounds, such as organic acids (lactic and acetic acids), bacteriocins, and reuterin, which are inhibitory to pathogen's growth in the gastrointestinal tract of the Wistar rats. Antagonistic activity by LAB were sustained by the secretion of different antimicrobial substances including organic acids (lactic, acetic etc.), bacteriocins, alcohols, hydrogen peroxide, antimicrobial peptide etc. as reported by Venkatasatyanarayana *et al*., 2019.

The substances responsible for the antagonistic activity by most promising LAB probiotic strains selected as revealed in this study were organic acid and bacteriocin. When the pH of the CFS was neutralized, all the LAB lost their antagonistic activity against the pathogens examined. Similarly, Gusils *et al*., 2019 reported the complete loss of antagonistic activity by 100 LAB strains isolated from GIT of pigs against pathogens when the pH of CFS was neutralized. LAB strains from poultry also lost their inhibitory action after pH neutralization as reported by Karim *et al*., 2018. Lin and Pan, 2017 reported constant antimicrobial activity within the pH range from 1.0 to 4.0 but complete loss of activity at 5.0 to 11.0 pH. In the same vein, Blajman *et al*., 2018 reported no zones of inhibition against pathogen tested when the CFS of LAB strains isolated from poultry were adjusted to pH 6.5. Furthermore, this study revealed that hydrogen peroxide was not responsible for antagonistic activity by the selected LAB as there was no effect when the supernatants of the LAB were treated with catalase. This research work findings showed that the antimicrobial activity by the LAB strains was as a result of the secretion of organic acids, bacteriocins or other natural antimicrobial substances as shown on Table 7.

In-vivo analysis of probiotic potential of the LAB isolates from fermented food samples shows antidiarrheal effect in albino rats as shown in table 6. These studies on antidiarrhea effect of these isolates were based on observing a significant reduction in stool frequency and stool consistency after LAB isolates from fermented food samples therapy was conducted as shown in Table 8. The groups of albino rats that were induced with the selected pathogenic organisms (*Escherichia coli*) to have diarrhea which appeared on different days after inoculation ranging from day 2-3 stop stooling watery feacal product while feeding them with 10^5cfu/ml of isolates daily immediately there is appearance of watery stool. The variation in the day of appearance and disappearance of watery stool in the groups of rats when induced and treated respectively, can be attributed to different physiological status of their bodies. The group of albino rats, EG that were induced with the pathogenic test microorganism, *E. coli* but were not fed with the isolates, continue stooling and died of diarrhea infection while the CG group which served as control group had no symptom of diarrhea.

NN12 showed the fastest level of treatment compared to others LAB isolates as there were early disappearance of watery feacal product in the groups of rats fed with NN12 as shown in table 7. NN12 showed almost the same effect in the treatment of diarrhea as *Lactobacillus acidophillus*, a defined probiotic LAB use in a commercial probiotic product with trade name called Linex as shown in table 8. Preventive effect of the LAB isolates was shown in Table 9. The occurrence of death in them was as a result of lack of nutrients due to loss of appetite and dehydration resulted from water loss in their body through watery fecal products observed in them.

In the groups that were fed with the test LAB isolates after the appearance of watery feacal products as a result of inducement of diarrhea in them using the pathogenic microorganisms, the later reduction in moisture content of the feacal products that was observed was due to amelioration effects in the groups of rats. The ameliorative effects in these groups of rats, showed the anti-diarrhea potentials of microorganisms present in fermented Kunu, Ogi and Nunu which is the major confirmatory probiotic potential of the isolates.

It was found that the LAB isolates of KN1, KN2, KN3, KN33, KN38, KN43, OG2, OG6, OG32, NN3, NN5, NN12 and NN17 are kin to Weissella cibaria Uga49-1, Enterococcus faecium HN-N32, Enterococcus durans KCTC13289, Pediococcus pentosaceus K15E, Pediococcus pentosaceus NRIC123, Pediococcus pentosaceus NRIC123, Enterococcus durans DpS11, Enterococcus gallinarum FDAARGOS728, Enterococcus faecium HB-1, Enterococcus faecium HN-N32, Enterococcus lactis HBUR51084, Vagococcus fluvialis AN7 and Bacillus safensis BMR1 with percentage identity of 99.74, 98.95, 97.57, 98.05, 100.00, 100.00, 99.54, 99.67, 99.34, 98.62, 100.00, 99.84 and 99.54 respectively (Table 10) as it was stated by Crowley et al., 2013 that Nowadays, lactic acid bacteria are reported to cover 17 genera: Aerococcus, Alloiococcus, Dolosigranulum, Enterococcus, Globicatella, Carnobacterium, Lactobacillus, Lactococcus, Leuconostoc, Melissococcus, Lactosphaera, Oenococcus, Pediococcus, Tetragenococcus, Vagococcus, Streptococcus and Weisella.

Although all the isolates showed antimicrobial activity against the test organism (*E. coli*) in the Wistar rats and resulted to treatment and prevention of diarrhea in them, *Vagococcus fluvialis* AN7 had the highest effectiveness in the treatment and prevention of diarrhea in Wistar rats. The effectiveness found in *Vagococcus fluvialis* AN7was closely related to the effectiveness found in defined probiotic culture of *Lactobacillus acidophilus.* The molecular identification revealed that all the 13 isolates were LAB except isolate NN17 (*Bacillus safensis* BRM1), although had high antimicrobial activity against *E. coli,* which was a Bacillus.

The phylogenetic grouping indicated that strains having similar sequences were clustered in the same group and presumably were considered as close relatives in reference to Alp and Kuleasan, 2020.

VI. CONCLUSION

In conclusion, this study reveals the significant role of the isolates from the fermented foods in treating and preventing *E. coli* induced diarrhea. These potentials exhibited by the bacteria isolates gave them the potential to ameliorate and prevent diarrhea in the Wistar rats used in this study. *Kunu, Nunu* and *Ogi* are rich in the presence of LAB which were incriminated to involve in the fermentation of those foods. Also, since fermented foods are dominated by lactic acid bacteria which have probiotic potentials, this eventually confers their therapeutic effect against diarrhea and makes them become functional foods.

RECOMMENDATIONS

Probiotics present in the fermented foods is recommended for the management of diarrhea in diarrhea patients. So various diarrhea patients should be encouraged to consume indigenous probiotics than long-lasting chemotherapeutics. Also, the isolates obtained from this study showed good probiotic potential therefore, they could be used as starter culture while preparing probiotic-based food products.

a) Highlight of Findings

- *a)* 128 bacteria were isolated from the 3 selected indigenous fermented food samples.
- *b)* Only 26 isolates had antimicrobial activity against selected intestinal pathogenic organisms.
- *c)* 13 of the selected isolates were characterized to be LAB.
- *d)* The LAB isolates treated and prevent
- *e)* *coli*-induced-diarrhea in Wistar rats
- *f)* All the isolates were identified to be LAB molecularly except *Bacillus safensis* BMR1.
- *g)* *Vagococcus fluvialis* AN7 showed antidiarrhea potential similar to *Lactobacillus acidophilus*, a defined probiotic bacterium.
- *h)* LAB present in the fermented food possess probiotic potential and have ability to treat and prevent diarrhea in
- *i)* diarrhea patients. Therefore, fermented foods can be used as prophylaxis for diarrhea.

b) Data availability statement: All the data used in this research paper are primary data and no new data were generated or analysed in support of this research.

c) Authors contribution: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, validation, visualization, writing original draft and writing review and editing: Adigun Musibau Adeleke. Supervising: Saliu Bolanle Kudirat.

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