International Journal of Food Microbiology 305 (2019) 108251

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Occurrence of bacteria and endotoxins in fermented foods and beverages from Nigeria and South Africa

Ifeoluwa Adekoya[a,*], Adewale Obadina[b], Momodu Olorunfemi[c], Olamide Akande[d], Sofie Landschoot[e], Sarah De Saeger[f], Patrick Njobeh[a]

[a] Department of Biotechnology and Food Technology, University of Johannesburg, Johannesburg, South Africa
[b] Department of Food Science and Technology, Federal University of Agriculture, Abeokuta, Nigeria.
[c] Department of Botany, University of Ibadan, Ibadan, Nigeria
[d] Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria
[e] Department of Applied Bioscience Engineering, Ghent University, B-9000, Belgium
[f] Centre of Excellence in Mycotoxicology and Public Health, Ghent University, B-9000, Belgium

ARTICLE INFO

Keywords:
Fermented foods
Gram-negative bacteria
Endotoxins
Safety
Nigeria
South Africa

ABSTRACT

In Africa, fermented foods and beverages play significant roles in contributing to food security. Endotoxins are ubiquitous heat stable lipopolysaccharide (LPS) complexes situated in the outer cell membranes of Gram-negative bacteria. This study evaluated the microbiological quality of fermented foods (*ogiri*, *ugba*, *iru*, *ogi* and *ogi baba*) and beverages (*mahewu* and *umqombothi*) from selected Nigerian and South African markets. The bacterial diversity of the fermented foods was also investigated and the identity of the isolates confirmed by biochemical and molecular methods. Isolate grouping was established through hierarchal clustering and the samples were further investigated for endotoxin production with the chromogenic Limulus Amoebocyte Lysate assay. The total aerobic count of the samples ranged from 5.7 to 10.8 Log CFU/g. Fourteen bacteria genera were detected with most of the isolates being members of the Enterobacteriaceae family. *Sphingomonas paucimobilis* and *Escherichia coli* were the dominant Gram-negative bacterial species detected. There were considerable variations in the concentrations of endotoxins produced and the lowest endotoxin concentration was found in *ogi* (4.3×10 EU/g) and the highest in *iru* (5.5×10^4 EU/g) while, 44% of *umqombothi* samples had endotoxins. *Ogi baba* samples had better microbial quality than other samples due to its reduced bacterial load and endotoxin levels. There was a strong positive ($r = 0.714$, $r = 0.996$) and significant ($p < 0.01$) correlation between the endotoxin levels and bacterial loads of the samples. Some previously unreported species of bacteria found in the fermented foods included *Aeromonas haemolyticus* and *Rhizobium radiobacter*. This is the first comprehensive report on endotoxins in fermented foods and beverages in Africa. Furthermore, the occurrence of pathogenic bacteria and toxins in the foods and beverages is of serious concern that calls for immediate action.

1. Introduction

The incidence of food-borne infections has increased globally within the past few years with a significant number of human population at risk (Cho et al., 2011) and Gram-negative and Gram-positive bacteria pathogens are at the epicentre of most reported cases (Sudershan et al., 2014). Globally, fermented foods and beverages have proven to be critical in the sustenance of the nutritional status of people due to their availability, richness of nutrients, affordability, and ease of processing. They are consumed almost daily across Africa by both infants and adults. They are produced from different substrates; maize (*Zea mays*), sorghum (*Sorghum bicolor*), melon (*Colocynthis citrullus*), African oil bean (*Pentaclethra macrophylla* Benth), and locust beans (*Parkia biglobosa*). The examples of fermented foods and beverages indigenous to Africa include *ogi*, *ogi baba*, *ugba*, *iru*, *ogiri*, *mahewu* and *umqombothi*. *Ogi*, *ogi baba*, *mahewu*, and *umqombothi* are products of lactic acid fermentation of maize and sorghum, with *ogi* and *ogi baba* being indigenous to Nigeria and consumed as breakfasts and weaning foods while *mahewu* and *umqombothi* are beverages both popularly consumed by black South Africans.

The microorganisms involved in the fermentation of these products (*Ogi*, *ogi baba*, *mahewu*, and *umqombothi*) are mainly lactic acid bacteria (*Lactococcus lactis*) and yeasts (*Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*) (Odunfa and Oyewole, 1998). *Ogiri* (substrate:

* Corresponding author.
E-mail address: olotu.ifeoluwa@gmail.com (I. Adekoya).

https://doi.org/10.1016/j.ijfoodmicro.2019.108251
Received 5 November 2018; Received in revised form 10 June 2019; Accepted 11 June 2019
Available online 15 June 2019
0168-1605/ © 2019 Elsevier B.V. All rights reserved.

melon), *ugba* (substrate: African oil bean), *iru* (substrate: locust beans) are condiments derived from alkaline fermentation of proteinaceous oily seeds usually consumed in Nigeria and also exported to other countries in the world including South Africa. *Ugba* is also used as a snack and as a basal ingredient for salad preparation. Despite the benefits of fermented foods, they have been demonstrated to be regularly contaminated with pathogenic microorganisms including Gram-negative bacteria (Aworh, 2008; Ogunshe et al., 2012). A study by Ogunshe and Olasugba (2008) revealed that 66% of *iru* ($n = 1125$), 82.4% of *ogiri* ($n = 148$) and 93.9% of *ugba* samples ($n = 115$) obtained from the Middle Belt region of Nigeria were contaminated with coliforms. Similarly, Nwachukwu et al. (2014) recovered *Klebsiella*, and *Pseudomonas* spp. from *ogiri*, thus consumption of these foods may pose a health risk to consumers.

Apart from the fact that Gram-negative bacteria in fermented foods may cause infections, they can be toxigenic, producing endotoxins in foods. Endotoxins are ubiquitous heat stable lipopolysaccharide (LPS) complexes that can be found at the outer cell membranes of Gram-negative bacteria (Adam et al., 2014). The potency of endotoxin varies amongst different bacterial species but *E. coli* produces LPS with extremely high endotoxin activity, hence it is often used as the model organism (Raetz and Whitfield, 2002). Human exposure to endotoxins can lead to many health complications such as septic shock, microvascular abnormalities development, multiple organ failure, disseminated intravascular coagulation and necropsy (Kalita et al., 2017). The incidences of endotoxin in foods have been poorly investigated and most studies conducted on endotoxins cover sparse food categories such as milk. Endotoxin levels from 40 to 5.5×10^4 endotoxin unit (EU)/g were found in infant milk manufactured in nine countries (South Africa, Holland, Spain, Switzerland, USA, Belgium, Ireland, Slovenia, and United Kingdom) by Townsend et al. (2007).

There is a need for surveillance of fermented foods to ensure consumer safety and the interaction of severe factors associated with their production particularly in Africa. Such factors include poor handling practices, unstandardized processing methods, the participation of various microbiota and unhygienic display practices, which may facilitate their contamination with pathogenic microorganisms and their toxins. Since endotoxins are contaminants that pose a threat to human health, their presence, as well as that of their causative agents (Gram-negative bacteria) in fermented foods need to be investigated. Therefore, the prime objective of this study was to screen for pathogenic bacteria using molecular-based methods and quantify the levels of endotoxins in fermented foods and beverages from Nigeria and South African markets.

2. Materials and methods

2.1. Materials

2.1.1. Reagents

Pierce Limulus Amebocyte Lysate (LAL) chromogenic endotoxin quantification kit (88282) was purchased from Separations Pty Ltd., South Africa. Nutrient agar (CM003), Thiosulfate-citrate-bile salts-sucrose agar (TCBS) (CM0333), Plate count agar (CM0325), *Salmonella* agar (CM 0099), peptone water (0009), cephaloridine, fucidin, and cetrimide (CFC) selective agar supplement (SR0103), *Pseudomonas* agar (CM0929) and MacConkey agar (CM0007) were purchased from Oxoid Ltd., Basingstoke Hants, England. Columbia Sheep blood agar plates (M1013) were purchased from BioMerieux, South Africa. Other reagents used were of analytical grade. Glassware used in the LAL assay was rendered pyrogen free by heating at 140 °C for 4 h.

2.1.2. Fermented food and beverage samples

Cluster sampling method was followed to collect different fermented foods and beverages from sellers in Southwest, Nigeria and Gauteng, South Africa between February 2015 and July 2016. Within Southwest Nigeria, maize gruel (*ogi*, $n = 18$), sorghum gruel (*ogi baba*, n = 18), locust bean (*iru*, $n = 36$), African oil bean seed (*ugba*, n = 18), and melon (*ogiri*, n = 18) were collected. Within Gauteng, South Africa, *ogi*, (n = 18), *iru* (n = 36), *ugba* (n = 18), *ogiri* (n = 36), maize meal (*mahewu*, n = 18), and traditional cereal-based opaque beer (*umqombothi*, n = 18) were collected. The samples were collected in sealed sterile containers maintained on ice bags, transported to the Food, Environment, and Health Research Group (FEHRG) Laboratory, University of Johannesburg, South Africa, stored for 3 days at 4 °C prior to microbiological and endotoxin analysis.

2.2. Methodology

2.2.1. Microbiological analysis

The fermented food samples were blended with a sterile laboratory blender and 10 g of each sample was homogenized in 90 mL sterile 0.1% peptone water for 30 s to a homogenous suspension and the samples serially diluted. Both selective and general-purpose medium were used for bacteria isolation. For the isolation of total aerobic bacteria and *Vibrio* spp., 0.1 mL aliquot from each sample was plated on Nutrient and TCBS agar, respectively, and the plates were incubated at a temperature of 37 °C for 24 h. For the enumeration of total coliform and *Salmonella* spp., 0.1 mL of aliquot was plated on MacConkey and *Salmonella* agar, respectively. The incubation temperature was 37 °C and plates were examined after 24 h. For *Pseudomonas* spp. isolation, 0.1 mL of aliquot was plated on solidified *Pseudomonas* agar with CFC selective supplement. The incubation temperature was set at 25 °C and plates examined after 48 h. Control plates which had no inoculum were also prepared and all analyses were carried out in triplicates. The colonies were counted using a colony counter, and mean bacterial load was calculated and results expressed as the logarithm (base 10) of the colony forming unit per gram of sample (Log CFU/g), only counts < 300 colonies were recorded.

Furthermore, pure isolates were sub-cultured on Nutrient agar plates, incubated at 37 °C for 24 h. and their micromorphological characteristics examined on an optical microscope (Olympus CX40, Micro-instruments, New Zealand). Purified cultures were maintained on nutrient agar slants, which were kept as stock cultures at 4 °C. For each pure isolate, Gram staining was carried out to further categorize the purified bacteria culture as either Gram-positive or Gram-negative as described by Brenner et al. (2005). The Gram-negative bacteria cultures were subjected to biochemical tests through VITEK 2 Compact system. Furthermore, the identities of both categories of bacteria (Gram-positive and Gram-negative) were established using molecular biology method. Broadly, the identities of the isolated bacteria were established through a combination of their morphological characteristics, biochemistry, and 16S sequencing.

2.2.2. Microbial identification using the VITEK 2 compact instrument

The VITEK 2 instrument (BioMerieux, North Carolina, USA) utilizes advanced colorimetric measurements, which are dependent on verified biochemical tests procedures such as carbon utilization, growth resistance and enzymatic actions to establish the identity of a microorganism. The use of VITEK 2 Compact for microbial identification has been reported and endorsed by several authors (Quesada et al., 2010; Pincus, 2006; Ligozzi et al., 2002). For the VITEK 2 Compact identification procedure, Gram-negative bacteria isolates were sub-cultured on Columbia Sheep blood agar to ensure their viability and purity. Culture suspensions were prepared in sterile saline after calibrating the Densi-Chek instrument (BioMerieux) at turbidity between 0.53 and 0.63 McFarland standard. Gram-negative test cards (21341) were automatically filled with the prepared inoculum suspensions, sealed and incubated at 35.5 °C for 18 h in the VITEK 2 instrument. The optical densities of the cards were read every 15 mins and the results obtained were evaluated against those available in the VITEK database to establish the identity of the unknown organisms. Final identification of

excellent (96–99%), very good (93–95%), good (89–92%) and acceptable (85–88%) were considered to be correct, whereas some isolates were with non-reactive bio pattern or unidentified. The identities of all the representative bacterial isolates except for those with excellent/very good identification were further confirmed by DNA sequencing.

2.2.3. DNA extraction, Polymerase Chain Reaction (PCR) and sequencing

2.2.3.1. DNA extraction. Genomic DNA analysis was performed using a Fungal/Bacterial DNA extraction kit (Zymo Research (ZR) Corporation, Southern California, USA) as recommended by the manufacturer. Pure bacterial culture (0.5 mL) was centrifuged at 10,000 ×*g* for 3 mins to pellet the cells. The supernatant was discarded and the bacterial cells (50–100 mg) were suspended in a 1.5 mL ZR Bashing Bead™ lysis tube containing 200 µL of phosphate buffer saline (PBS). The tube was inserted into a beater for 5 mins, followed by 1 min centrifugation at 10,000 ×*g*. The supernatant (400 µL) was transferred to a Zymo-Spin™ IV spin filter in a collection tube, with the repetition of the centrifugation step. To the filtrate, 1.2 mL of binding buffer was added and 800 µL of the mixture was transferred to a Zymo-Spin™ IIC column in a new collection tube and centrifuged at 10,000 ×*g* for 1 min. This step was repeated twice, followed by the addition of 200 µL pre-wash buffer and 500 µL DNA wash buffer. The column was transferred to a sterile 1.5 mL microcentrifuge tube and 100 µL DNA elution buffer added and centrifuged at 10,000 ×*g* for 30 s to elute the DNA.

2.2.3.2. Amplification of 16S rRNA gene by Polymerase Chain Reaction. The 16S rRNA gene-based universal primers: 27F (5′-AGAG TTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTA CGACTT-3′) were used for the amplification of the DNA (Lane, 1991; Turner et al., 1999). A thermocycler (Mastercycler pro384, Eppendorf, USA) was utilized for the amplification, the PCR products were held at 4 °C and electrophoresed on a 2% agarose gel along with a DNA ladder (Fermentas Life Science, Lithuania). The bands representing the amplified products were viewed with a gel documentation system (Vacutec, Florida, South Africa).

2.2.3.3. Sequencing of the PCR products. The PCR products were gel extracted using Zymoclean gel DNA recovery kit. The extracted products were sequenced in the forward and reverse directions (Applied Biosystems, Thermofisher Scientific, Big Dye terminator kit v3.1, Carlsbad, California, USA) and cleaned with ZR-96 DNA clean-up kit. The cleaned products were run on an ABI 3500 xL Genetic Analyser (Applied Biosystems) and the data generated (.abi files) were analysed on the CLC Bio Main Workbench 7. This was followed by a BLAST search on the National Center for Biotechnology Information (NCBI) database to establish the equivalence of the fragments with existing sequences (Stephen et al., 1997). Sequences generated in this study were also submitted to the GenBank (data references: Supplementary file).

2.2.4. Phylogenetic analysis

The phylogenetic association of bacterial sequences from *iru* including reference strains was obtained from a Neighbour-Joining analysis of Saitou and Nei (1987). The bootstrap consensus tree was inferred from 1000 replicates (Felsenstein, 1985). The evolutionary distances were computed with the Maximum Composite Likelihood method (Tamura et al., 2004). Fifteen nucleotide sequences were analysed and the final data set had 246 positions after the elimination of gaps and missing data. The phylogenetic and molecular evolution analyses were conducted in MEGA7 (Kumar et al., 2016).

2.2.5. Hierarchal clustering

The fermented food samples were clustered hierarchically based on their bacterial profile through simple matching distance based on the method established by Choi et al. (2009). Data generated was represented with heat maps where color codes reflect the presence (dark grey) or absence (grey) of a certain bacterium. The R software package version 2.15.3 was used (R Core Team, 2013, R Foundation for Statistical Computing, Vienna, Austria).

2.2.6. Endotoxin analysis

All samples were homogenized using a sterile mechanical blender. Endotoxins was analysed with the chromogenic LAL test kit, which consisted of endotoxin standard, LAL, chromogenic substrate and endotoxin-free water following the manufacturers' instruction (Separations, South Africa). The minimum dilution of each sample was 1:10 with pyrogen-free water, while the maximum dilution was 1:1,000,000. The pH of the samples was adjusted to 7 ± 1 with 0.1 M NaOH or 0.1 M HCl. 96-Well plates were placed on a heating block for 10 mins and temperature maintained at 37 °C. Samples and standards (50 µL each) were dispensed into the wells, the plates were covered with the lid and placed in an incubator for 5 mins at a temperature of 37 °C. Afterward, 50 µL of LAL was added to each well, the plates were shaken gently for 10 s and incubated at 37 °C for 10 mins after which time, 100 µL of chromogenic substrate solution was added. To the mixture, 50 µL of 25% acetic acid was added and the absorbance of the mixture was measured using a microplate reader (ELX800, Cole Palmer, Illinois, USA) set at a wavelength of 405 nm. Assay range was from 0.1 to 1.0 EU/ml and the concentration of endotoxin in each sample was established using a standard curve which was prepared by spiking known amount of endotoxin standards (0.1–1.0 EU/mL) into endotoxin-free water. Endotoxin-free water (50 µL) was used as a blank. The positive control was also prepared at the midpoint concentration of the standard curve (0.5 EU/mL) as well as the enhancement/inhibition control. The samples were tested over at least five dilutions and values were expressed as endotoxin units (EU) per g of sample. All analyses were carried out in triplicate.

2.2.7. Data analysis

A descriptive statistic of bacteria counts and endotoxin levels (mean, frequencies, and percentages) was performed using the Microsoft Office Excel 2010 (Redmond, WA, USA). Data generated were subjected to a one-way analysis of variance (ANOVA) with the SPSS version 23.0 (IBM Corporation, New York, USA) and the Tukey's test was used to separate differences amongst the means at 5% confidence level. In addition, Pearson's correlation coefficient between the bacterial load and endotoxin levels of the fermented foods obtained from Nigeria and South Africa was calculated.

3. Results

The overall bacterial composition of fermented foods from Nigeria and South Africa markets are given in Table 1. As seen, total aerobic counts (TAC) of all the samples were > 5 Log CFU/g, with *iru* samples from South Africa having the highest mean TAC (10.8 Log CFU/g). *Vibrio* spp. and *Pseudomonas* spp. were recovered from all the analysed *umqombothi* samples with mean counts of 2.3 and 2.9 Log CFU/g, respectively. A total of 499 bacterial isolates were identified in the samples (*ogi* = 45 isolates, *ogi baba* = 11 isolates, *ogiri* = 100 isolates, *mahewu* = 22 isolates, *iru* = 191, *ugba* = 95 isolates, *umqombothi* = 35 isolates).

Furthermore, 13 bacteria genera were detected in those fermented food samples from Nigeria (Table 2), meanwhile 14 genera were found within the South African samples (Table 3), most of which belonged to the *Enterobacteriaceae* family. Amongst the Gram-negative bacteria identified, *E. coli* was detected only in *iru* (15%) and *ogiri* (33%) from both countries, while *P. luteola* was found in 17% of *mahewu* samples. Additionally, 67% of all *ugba* samples were positive for *B. subtilis*, but only 3 bacteria genera (*Sphingomonas*, *Pantoea* and *Bacillus*) were found in *ogi baba*. However, other species within these genera were found in other samples including *ogi*, *mahewu*, and *iru*.

Table 1
Mean bacterial loads of fermented foods obtained from Nigerian and South African markets in Log CFU/g.

Source	Samples		Total aerobic count	Total coliform count	*Salmonella* count	*Pseudomonas* spp. count	*Vibrio* spp. count
Nigeria	*Ogiri*	Mean	9.0	3.2	2.2	2.12	2.6
		Range	7.3–10.6	2.5–4.5	1.0–3.0	1.0–3.5	1.0–2.8
	Iru	Mean	10.8	3.0	2.0	2.2	2.3
		Range	6.6–11.6	2.0–3.7	1.0–3.5	1.0–4.3	2.0–2.6
	Ugba	Mean	6.7	2.0	1.1	2.5	2.2
		Range	6.0–9.6	1.3–3.7	0–1.6	2.3–2.7	1.5–2.6
	Ogi	Mean	7.8	1.1	1.28	2.5	2.5
		Range	5.9–9.6	0–2.5	0–2.3	1.5–2.8	1.0–2.9
	Ogi baba	Mean	5.7	1.1	2.2	2.2	2.3
		Range	3.0–7.0	0–2.6	0–3.3	1.0–1.7	0–2.8
South Africa	*Ogiri*	Mean	8.5	2.3	2.2	2.6	2.3
		Range	6.0–11.12	1.5–3.5	1.6–2.9	1.7–3.6	1.0–3.5
	Iru	Mean	10.8	3.1	2.3	2.7	2.8
		Range	7.3–11.1	2.8–4.5	0–2.7	1.9–3.5	1.6–3.3
	Ugba	Mean	8.7	2.5	1.2	2.5	2.6
		Range	6.6–10.3	1.0–3.6	1.0–1.7	1.5–3.8	1.0–3.5
	Ogi	Mean	7.3	1.2	2.3	2.2	2.3
		Range	5.7–9.3	0–1.8	0–2.9	1.0–2.8	1.6–3.0
	Mahewu	Mean	8.4	2.2	2.0	1.1	2.1
		Range	5.3–10.7	0–2.8	0–3.3	1.0–3.7	1.0–2.8
	Umqombothi	Mean	8.3	2.2	2.2	2.9	2.3
		Range	6.8–9.3	0–3.5	1.5–2.8	2.0–3.7	1.5–2.9

The bacteria isolates that gave pink color during preliminary identification (Gram staining) was categorized as Gram-negative and were screened following 47 biochemical tests on the VITEK instrument, the results of some of the bacteria with very good and excellent identification are presented in Table 4. Accordingly, *A. salmonicida*, *S. paucimobilis*, and *P. luteola* were amongst the bacteria identified with

Table 2
Incidence rates of bacteria isolated from fermented foods sold in Nigerian markets.

Bacteria	*Ogi* N = 18	*Ogi baba* N = 18	*Ogiri* N = 18	*Ugba* *N* = 18	*Iru* N = 36	Accession numbers
	% + ve samples	% + ve samples	% + ve samples	% + ve samples	% + ve samples	
Gram-negative bacteria						
Acinetobacter haemolyticus	–	–	–	17	–	KT027777.1
Acinetobacter iwoffii	–	–	–	11	–	AB859068.1
Escherichia coli	–	–	22	–	19	JF892819.1
Escherichia coli	–	–	6	–	–	AJ875445.1
Pantoea spp.	–	17	–	–	6	EF469213.1
Pasteurella canis	–	–	–	–	14	AY634676.1
Serratia marcescens	33	–	–	28	–	KU894791.1
Vibrio vulnificus	11	–	57	–	11	KT823473.1
Vibrio parahaemolyticus	–	–	11	–	–	DQ345442.1
**Aeromonas salmonicida*	–	–	–	11	3	97%*
**Pseudomonas luteola*	–	–	11	17	8	95%*
**Pantoea spp.*	22	–	16	–	–	94%*
**Rhizobium radiobacter*	–	–	16	–	–	94%*
**Serratia plymuthica*	–	–	–	–	8	93%*
**Sphingomonas paucimobilis*	17	22	22	17	14	93%*
Gram-positive bacteria						
Bacillus cereus	17	–	–	–	–	CP012691.1
Bacillus altitudinis	–	–	11	–	–	MG855711
Bacillus pumilus	17	–	–	–	8	MG855694
Bacillus spp.	–	–	–	17	–	MG855696
Bacillus oleronius	–	–	–	–	17	MG855705
Bacillus subtilis	–	–	22	78	67	KP224305.1
Bacillus safensis	17	–	–	22	22	MG855704
Bacillus anthracis	–	–	–	11	–	MG855713
Bacillus kochii	–	–	–	–	8	MG855709
Bacillus spp.	–	22	–	–	11	MG855693
Bacillus spp.	–	–	17	–	–	MG855714
Enterococcus faecium	–	–	22	–	–	KU898955.1
Enterococcus faecalis	–	–	–	–	8	MG855691
Enterococcus faecalis	–	–	11	22	8	MG855708
Enterococcus gallinarum	–	–	17	6	–	KU196084.1
Enterococcus casseliflavus	–	–	11	–	–	KT630829.1
Paenibacillus spp.	–	–	–	–	6	MG855710
Paenibacillus spp.	17	–	–	–	–	MG855699

%: Percentage, +ve: positive, *represents bacteria with excellent/very good identification on the VITEK instrument and their corresponding percentages.

Table 3
Incidence rates of bacteria isolated from fermented foods sold in South African markets.

Bacteria	*Ogi* *N* = 18	*Mahewu* N = 18	*Ogiri* N = 18	*Ugba* N = 18	*Iru* *N* = 36	*Umqombothi* N = 18	Accession numbers
	% + ve samples	% + ve samples	% + ve samples	% + ve samples	% + ve samples	% + ve samples	
Gram-negative bacteria							
Acinetobacter haemolyticus	–	–	–	11	–	–	KT027777.1
Acinetobacter iwoffi	–	–	–	17	–	–	AB859068.1
Cronobacter Sakazakii	–	–	–	–	–	44	AB274299.1
Escherichia coli	–	–	28	–	8	–	JF892819.1
Escherichia coli	–	–	17	–	3	–	KU880540.1
Pantoea spp.	–	17	39	33	–	–	EF469213.1
Pasteurella canis	–	–	–	–	6	–	AY634676.1
Serratia marcescens	–	11	–	6	–	–	KU894791.1
Serratia nematodiphila	–	–	–	–	–	22	FJ662869.1
Shigella flexneri	6	–	–	–	6	–	KX146471.1
Vibrio vulnificus	11	–	17	–	11	11	KT823473.1
Vibrio parahaemolyticus	–	–	11	–	–	–	DQ345442.1
Cronobacter dublinensis	11	–	–	–	–	–	KR347471.1
**Rhizobium radiobacter*	–	–	22	11	9	–	94%*
**Sphingomonas paucimobilis*	17	44	22	17	25	39	95%*
**Serratia plymuthica*	–	–	–	–	11	–	93%*
**Pseudomonas luteola*	–	17	–	28	19	11	95%*
Gram-positive bacteria							
Bacillus cereus	11	–	–	–	36	17	MG855712
Bacillus spp.	6	–	–		–	–	MG855707
Bacillus cereus	–	17	–	–	–	–	KR709243.1
Bacillus altitudinis	11	–	17	–	17	–	MG855697
Bacillus xiamenensis	–	–	–	11	–	17	MG855692
Bacillus toyonensis	–	–	–	3	–	–	KX129781.1
Bacillus pumilus	11	17	22	–		22	KU844041.1
Bacillus oleronius	–	–	–	–	11	–	MG855706
Bacillus halotolerans	–	–	–	–	22		MG855697
Bacillus subtilis	–	–	17	56	78	–	MG855715
Bacillus safensis	17	–	–	–	–	–	MG855700
Enterococcus durans	–	–	17	–	–	–	MG855702
Enterococcus faecium	–	–	11	–	11	–	MG855695
Enterococcus faecalis	–	–	28	44	8	–	MG855701
Paenibacillus spp.	–	–	17	17	–	–	MG855703
Paenibacillus polymyxa	–	–	–	–	–	11	MG855716

%: Percentage, +ve: positive, *represents bacteria with excellent/very good identification on the VITEK instrument and their corresponding percentages.

excellent identification of 97%.

For the phylogenetic construction, as an example amongst other fermented food analysed, the 16S rRNA gene sequences of the bacteria from *iru* were grouped into three monophyletic clades, which indicated the closeness of the sequences to one another as well as reference sequences (Fig. 1). The bootstrap frequencies of the sequences ranged from 49% to 100%. *Shigella sonnei* was shown to be closest relative of *Enterococcus cloacae* in clade 1 and majority of the species in clade 2 and 3 had excellent bootstrap support. All the previously unreported *Bacillus* spp. strains (https://goo.gl/JQzC1o, https://goo.gl/3R4HXu, https://goo.gl/kgm7Y1,https://goo.gl/7sjnTk) belonged to the same monophyletic clade. Members of a taxon are usually inseparable based on 16S rRNA gene and the evolutionary relationship depicted amongst the isolate makes molecular investigation of microbial diversity in foods of uttermost importance. The occurrence of the isolates in all the food materials irrespective of location was very diverse and at different frequencies as shown in the heat maps represented in Figs. 2 and S1.

Endotoxins levels were equally quantified in similar samples and the determined mean levels are presented in Table 5 and as observed, there were considerable variations as established for bacterial contamination. For example, amongst the positive samples, the lowest contamination level was 42.90 EU/g recovered from *ogi* but the highest level of 5.5×10^4 EU/g was detected in *iru* from Nigeria. There were significant differences ($p < 0.05$) in the mean endotoxin levels in *iru*, *ugba*, and *ogiri* from both Nigeria and South Africa. Most of the samples positive for endotoxin had levels below 1.0×10^3 EU/g whereas the incidence of endotoxin contamination in *umqombothi*, *mahewu*, and *ogi baba* was 44%, 28% and 22%, respectively, as shown in Table 5. Generally, *iru*, and *ogi* from Nigeria had higher incidence rate of aerobic bacteria, Gram-negative bacteria and endotoxins contamination than their South African counterparts. There was a strong positive and significant correlation between the bacterial load and endotoxin levels of the fermented foods obtained from Nigeria ($r = 0.714$, $p < 0.01$) and South Africa ($r = 0.996$, $p < 0.01$).

4. Discussions

4.1. Bacterial flora of fermented foods from Nigeria and South Africa markets

Fermented foods and beverages play a significant role in the diet of African populations based on their availability, affordability, and contribution to addressing protein-calorie malnutrition as well as micronutrient deficiencies, which are common. These products are commonly manufactured following various traditional processing methods but must still be safe for consumption. In our study, the quality of fermented foods and beverages from both Nigerian and South African market outlets was established. It was found that mean TAC of all the samples exceeded the microbiological criteria (> 4 Log CFU/g) for foodstuffs according to EC (No 2073/2005) (EC, 2005). The presence of microorganisms beyond tolerable levels is detrimental and could cause food-borne diseases in the form of food poisoning or intoxication. Increasingly in Africa, it seems there is a high cost paid on the negligence of circumspection to food safety and quality as several outbreaks of

Table 4
VITEK biochemical tests results of selected Gram-negative bacteria isolated from fermented foods.

Biochemical tests	Abb.	*Aeromonas salmonicida*	*Sphingomonas paucimobilis*	*Serratia plymuthica*	*Pseudomonas luteola*	*Rhizobium radiobacter*	*Pantoea* spp.
Ala-phe-pro-arylamidase	APPA	+	−	−	−	−	−
Adonitol	ADO	−	−	−	−	−	−
L-Pyrrolydonyl-arylamidase	PyrA	−	−	+	−	−	+
L-Arabitol	IARL	−	−	−	−	−	−
D-Cellobiose	dCEL	−	+	+	−	+	+
ß-Galactosidase	BGAL	−	+	−	−	−	−
H2S production	H2S	−	−	+	+	+	−
ß-*N*-acetyl-glucosaminidase	BNAG	−	−	+	−	+	+
Glutalyl arylamidase pNA	AGLTp	−	−	−	−	−	−
D-Glucose	dGLU	−	+	+	+	+	+
γ-Glutamyl-transferase	GGT	−	−	−	−	−	−
Fermentation/glucose	OFF	−	−	−	−	−	−
ß-Glucosidase	BGLU	−	+	+	+	+	+
D-Maltose	dMAL	−	+	+	+	+	+
D-Mannitol	dMAN	−	−	+	−	+	+
D-Mannose	dMNE	−	+	+	+	+	+
ß-Xylosidase	BXYL	−	−	−	−	−	−
ß-Alanine arylamidase pNA	BAlap	−	−	−	−	−	−
L-Proline arylamidase	ProA	−	−	−	−	−	−
Lipase	LIP	−	−	−	−	−	−
Palatinose	PLE	−	+	+	−	−	−
Tyrosine arylamidase	TyrA	−	−	+	−	−	−
Urease	URE	−	−	−	−	−	−
D-Sorbitol	dSOR	−	−	+	−	+	−
Saccharose/sucrose	SAC	−	+	+	−	+	+
D-Tagatose	dTAG	−	−	−	−	+	−
D-Trehalose	dTRE	−	+	+	+	+	+
Citrate (sodium)	CIT	−	−	−	−	−	−
Malonate	MNT	−	−	−	−	−	−
5-Keto-d-gluconate	5KG	−	−	−	−	−	−
L-Lactate alkalinisation	ILATk	−	−	−	−	−	−
α-Glucosidase	AGLU	−	−	+	−	−	−
Succinate alkalinisation	SUCT	−	−	−	−	−	−
ß-*N*-acetyl galactosiminidase	NAGA	−	−	−	−	−	−
α-Galactosidase	AGAL	−	+	+	−	+	+
Phosphatase	PHOS	−	−	−	−	−	−
Glycine arylamidase	GlyA	−	−	−	−	−	−
Ornithine decarboxylase	ODC	−	−	−	−	−	−
Lysine decarboxylase	LDC	−	−	−	−	−	−
L-Histidine assimilation	IHISa	−	−	−	−	−	−
Coumarate	CMT	−	+	+	+	+	+
ß-Glucoronidase	BGUR	−	−	−	−	−	−
O/129 resistance	0129R	−	−	+	+	−	+
Glu-gly-arg-arylamidase	GGAA	−	−	−	−	−	−
L-Malate assimilation	IMLTa	−	−	−	−	−	−
Ellman	ELLM	−	−	−	−	−	−
L-Lactate assimilation	ILATa	−	−	−	−	−	−

Abb.: Abbreviation, −: Absent, +: Present.

food poisoning linked to microbial contamination are often reported (Blumberg et al., 2011; Neil et al., 2012; Malangu, 2014). They manifest as acute gastroenteritis, abdominal discomfort, pain, diarrhea and even death (Kimmons et al., 1999).

In the study of Olasupo et al. (2002), Enterobacteriaceae and TAC counts in *ogi* samples analysed were 5.6 and 6.5LogCFU/g, respectively, however, none of these samples was positive for *Vibrio* spp. This contrasts with the data obtained in our study wherein *Vibrio* spp. was recovered from some *ogi* samples analysed. Amongst the Enterobacteriaceae identified, *E. coli* was the most significant because its presence in samples such as *ogiri* and *iru* is an indication of faecal contamination. Previously, a variety of Enterobacteriaceae has been isolated and identified from foods during fermentation. For example, Nwamaka et al.,(2010) detected *E. coli* during *ugba* fermentation. In addition, *Escherichia coli* and *Serratia ficaria* were the enteric bacteria isolated from *mahewu* by Simango (2002), whereas *E. coli*, *Klebisiella pneumoniae*, *P. mirabilis* and *P. aeruginosa*, were the Gram-negative bacteria mainly recovered from *iru* by Ogunshe and Olasugba (2008).

It is important to note that most studies on fermented foods in Africa have mainly focused on microbiota involved in the fermentation process (Oguntoyinbo et al., 2007; Olasupo et al., 2016) using biochemical analytical methods and some (Okeke et al., 2015; Adedeji et al., 2017; Fugl et al., 2017) like ours, have studied the microbiological status of fermented foods using modern techniques such as DNA sequencing, which in our study, facilitated the establishment of previously unreported organisms of public health importance. For example, *P. canis* and *V. vulnificus* in *iru*, and *Aeromonas haemolyticus*, *Aeromonas iwoffii*, *A. salmonicida*, *Sphingomonas paucimobilim*, and *Rhizobium radiobacter* in *ugba* had not been previously reported.

Serratia marcescens was detected in 11% of *mahewu* and 33% of *ogi* samples obtained from Nigeria. This Gram-negative bacterium is associated with hospital-acquired infections (HAIs), such as urinary tract infections, and is abundantly found in the environment. Starchy foods have been reported to be an excellent substrate for their growth (Hejazi and Falkiner, 1997). While infections induced by *C. sakazakii* have principally been amongst infants and neonates, the occurrence of this causative agent in foods or beverages as noted in our study as for infant formula previously reported, raises new concerns particularly the risk it may pose amongst immunocompromised consumers (Beuchat et al., 2009). *C. sakazakii* was detected in 44% of *umqombothi* from South

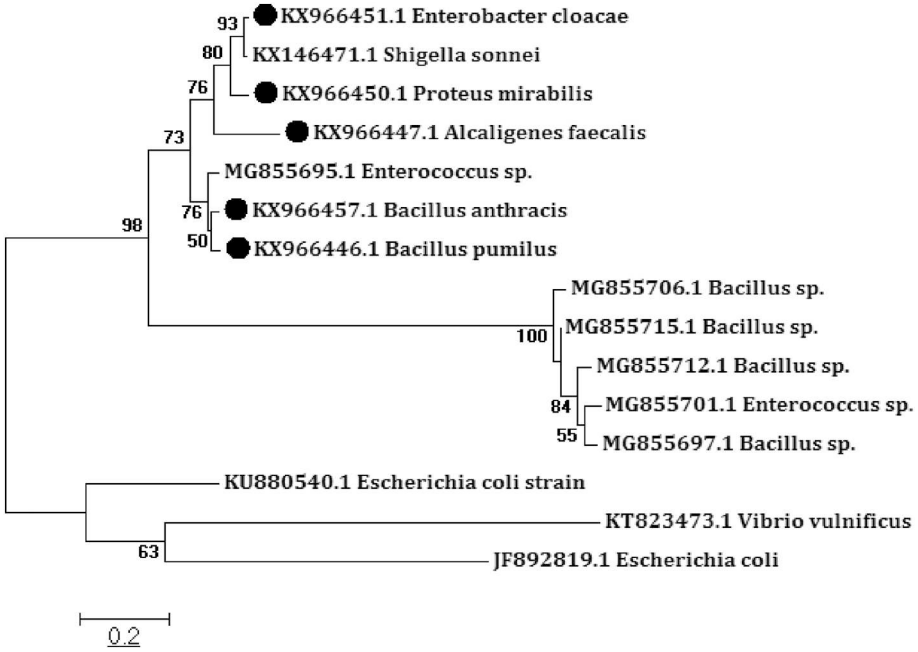

Fig. 1. Neighbour-joining phylogenetic tree of bacteria from *iru*. Taxa names with circles are related GenBank references. Bootstrap values are shown at internal nodes (bootstrap values < 50 are not shown). The scale bar below the tree indicates number of substitutions per site.

African markets.

This present study recorded the persistence of *Sphingomonas paucimobilis* in all the samples, which like *Serratia marcescens* is associated with HAIs and widely distributed in nature (Lugito et al., 2016). Further to that, two *Vibrio* spp. including *V. parahaemolyticus* and *V. vulnificus* were found in *ogiri*, which are pathogenic, causing acute gastroenteritis in humans (Newton et al., 2012). Since its recognition, *V. parahaemolyticus* has been found to be the leading cause of 20–30% of food poisoning cases reported in Asian countries (Alam et al., 2002) and gastroenteritis in humans, relating as well to seafood consumption in the United States (Newton et al., 2012).

The Gram-positive bacteria detected in the samples were primarily *Bacillus* spp. This is in excellent agreement with other reports that fermentation of vegetable proteins (*ugba*, *ogiri and iru*) is predominantly led by proteolytic *Bacillus* spp., particularly *B. subtilis* (Odunfa and Oyewole, 1998; Okorie and Olasupo, 2013; Eze et al., 2014). With respect to this bacteria genus, its incidence was typically high in *ugba* (67%) and *iru* (74%). *Bacillus altitudinis*, *B. pumilus*, *B. halotolerans*, *B. oleronius*, *B. safensis*, *B. xiamenensis* and *B. toyonensis* were members within the *Bacillus* genera isolated from the samples analysed, probably causing an increased level of hydrolytic enzyme production during fermentation due to their hydrolytic properties (Oguntoyinbo et al., 2007). However, when compared to other species, *B. subtilis* appears to be more adapted and dominant, because of its higher protease, amylase, polyglutamic, pyrazine, and subtiliosin production amongst other antimicrobials (Oguntoyinbo et al., 2007). The predominance of *B. cereus* in *ogi* and *umqombothi* with an incidence rate of 17% in each of the two sample types might be due to its wide distribution in the environment being spore formers (Okorie and Olasupo, 2013).

Enterococcus durans, *E. faecium*, *E. faecalis*, *E. gallinarum* and *E. casseliflavus* were also detected in the alkaline fermented foods but at varying levels. These findings corroborate those of Oladipo et al. (2013) with different *Enterococcus* spp. recovered from *ugba*, *iru* and *ogiri*. Generally, lactic acid fermented foods and beverages obtained from markets in both countries (*ogi*, *ogi baba*, *mahewu*, and *umqombothi*) had a better safety record in terms of their bacterial load and of the types of pathogenic microorganisms found than those alkaline fermented foods (*ugba*, *iru* and *ogiri*) under study. Alkaline fermentation involves the hydrolysis of protein and generation of ammonia and amino acids and its preservative action in condiments seems to be limited (Oguntoyinbo et al., 2007). On the other hand, lactic acid fermentation involves the conversion of carbohydrates to alcohol or organic acids, which creates an antagonistic environment for survival of pathogenic and spoilage organisms, enhancing, therefore, the safety of such foods and beverages.

The pathogenic bacteria present in these foods can be associated with several factors affecting the quality of these food products along the food chain. Poor food safety knowledge, use of contaminated raw materials, utilization of polluted water, inadequate hygienic practices, unstandardized production processes, mixed-culture processing, deplorable hygiene status of processing environments, poor packaging, inadequate preservation techniques, meagre storage habits, and unhygienic hawking activities, are some of the factors that provoke the presence of these pathogenic organisms in foods. When present, they are capable of directly causing a wide range of diseases in man. Aside from that, these microorganisms are capable of producing harmful toxins that indirectly cause ill-health, which prompted the need to assess the levels of endotoxins within the samples under study.

4.2. Endotoxin levels of fermented foods from Nigeria and South Africa markets

This study also established endotoxin levels, measured using the Limulus Amebocyte Lysate test within the range of 4.3×10 and 5.5×10^4 EU/g. Some of the inherent medical effects of the latter have been discussed and reviewed (Townsend et al., 2007; Wallace et al., 2016; Kalita et al., 2017). They are known to invade the gastrointestinal tract but do not enter the bloodstream of healthy individual's due to permeability barriers. With increased ingestion or collapse of the immune system for some apparent reasons, the permeability barriers may be disrupted, thereby offering entry of endotoxins into the bloodstream leading to serious health complications, even death in severe circumstances (Wallace et al., 2016). Endotoxins take a central stage in the pathogenesis of septic shock in man (Martich et al., 1993; Wallace et al., 2016) and induce a series of acute inflammatory responses including fever, fatigue, etc. (Porter et al., 2010). Being a heat stable toxin, endotoxin can persist in foods including fermented foods; other heat sensitive toxins can also persist in fermented foods as long as they are not heated.

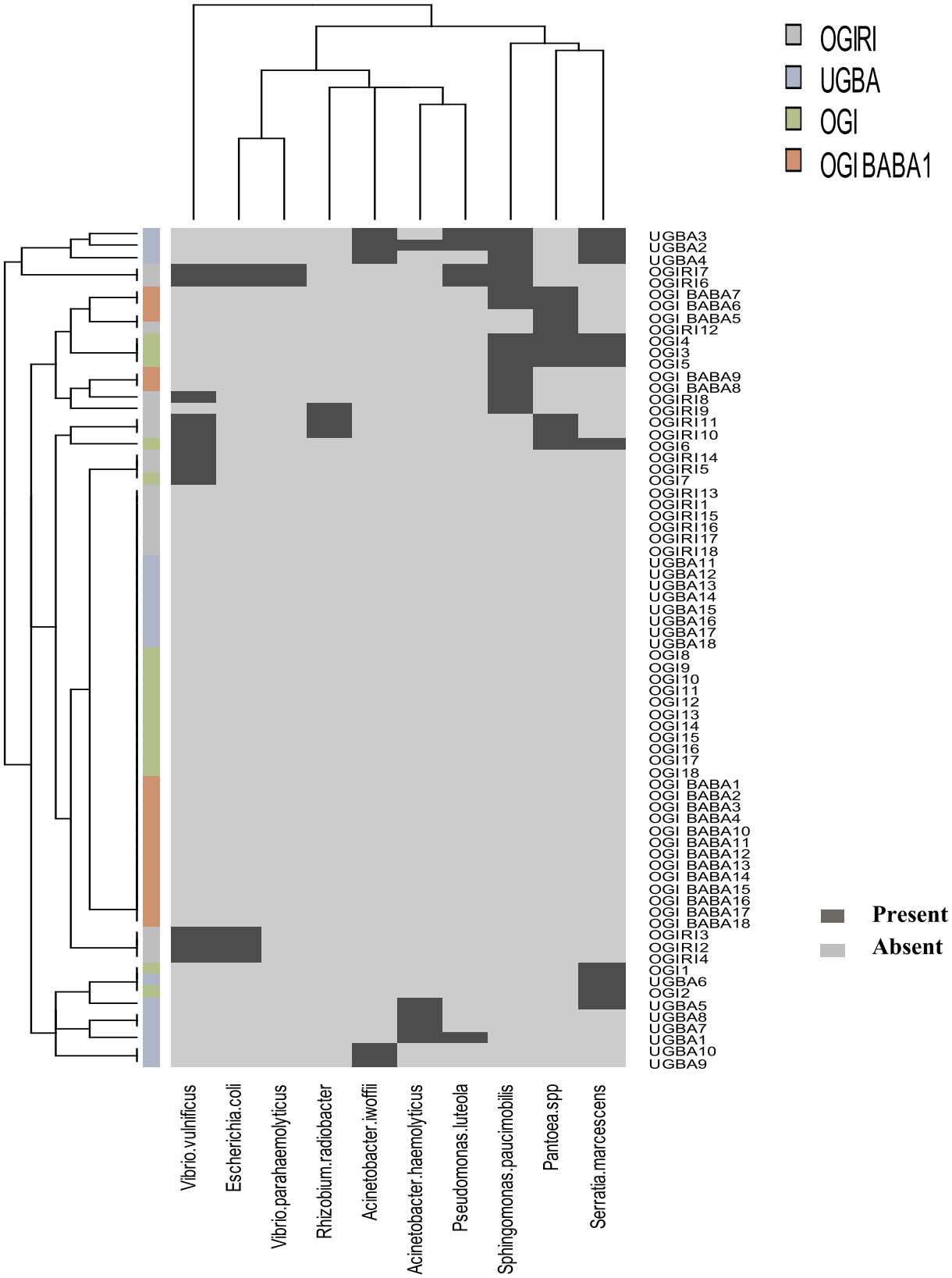

Fig. 2. Hierarchical clustering of the bacteria present in some Nigerian fermented foods.

In this study, endotoxin concentrations found ranged from 42.9 to 5.5×10^4 EU/g of sample. Their presence in the samples analysed can be accounted for by the series of Gram-negative bacteria earlier found in similar samples. This can be strengthened by the positive correlation that was observed amongst the samples which indicated that endotoxin level increased as bacterial load increased and vice versa Although, fermentation has been reported to inhibit the growth of pathogenic organisms, some studies have reported the persistence of pathogenic

Table 5
Levels (mean, range) and percentage contamination of endotoxins in fermented foods from Nigeria and South African markets.

Source	Fermented food samples	Mean[a] (range) of endotoxins in positive samples (EU/g of sample)	Percentage of samples positive for endotoxins (%)
Nigeria	*Ogiri*	[e]3.9×10^3 (8.5×10^2–3.8×10^4)	83
	Iru	[f]4.7×10^3 (9.4×10^2–5.5×10^4)	100
	Ugba	[ab]9.0×10^1 (4.4×10^1–2.3×10^2)	56
	Ogi	[a]4.8×10^1 (4.3×10^1–5.7×10^1)	17
	Ogi baba	[a]5.1×10^1 (4.3×10^1–6.6×10^1)	22
South Africa	*Ogiri*	[c]3.2×10^2 (1.3×10^2–2.0×10^3)	100
	Iru	[ef]4.1×10^3 (5.7×10^2–4.2×10^4)	92
	Ugba	[b]1.1×10^2 (5.9×10^1–1.7×10^2)	61
	Ogi	[a]4.6×10^1 (4.3×10^1–5.1×10^1)	39
	Mahewu	[b]1.0×10^2 (4.7×10^1–4.6×10^2)	28
	Umqombothi	[d]5.5×10^2 (6.9×10^1–9.8×10^2)	44

[a] Mean values with same superscripted letter do not differ significantly at 5% ($p < 0.05$) confidence level.

organisms during fermentation, therefore emphasizing the possible presence of their toxins in such foods. *Shigella* spp. and pathogenic *E. coli* were detected in *mahewu* after 24 h. fermentation (Shimago and Rukure, 1992), while Nwokoro and Chukwu (2012) detected *Serratia* sp., *E coli* and *E. aerogenes* in *ogi* after 72 h. fermentation. *Iru* samples had the highest mean endotoxin levels however, the highest contamination frequency (100%) occurred in *ogiri* (South Africa) and *iru* (Nigeria). This may be due to the presence of both viable and non-viable Gram-negative bacteria cells in the samples particularly *E. coli*, which is a known producer of LPS having extremely high endotoxin activity (Wallace et al., 2016). In addition, due to their alkaline state, they are more susceptible to microbial contamination than others including *ogi*, *ogi baba*, *mahewu* and *umqombothi* that have acidic pH. The physicochemical properties of these products including their pH have been reported in previous studies (Adekoya et al., 2017, 2018).

5. Conclusion

The study established the microbial profile of fermented foods and beverages from Nigeria and South Africa market outlets. The aerobic plate count of all samples tested exceeded the permissible levels for foodstuffs. Pathogenic microorganisms belonging to 14 genera recovered included *Escherichia coli*, *Vibrio vulnificus*, *Pseudomonas luteola*, *Bacillus cereus*, and *Serratia mercences*. Most of the Gram-negative bacteria present belonged to the Enterobacteriaceae family. Endotoxin, a heat stable component of the Gram-negative cell wall, was present in the samples but at varying concentrations. *Iru* samples had the highest mean endotoxin levels, while *ogi baba* was the least contaminated and therefore deduced to be of better microbial quality than any other sample type tested. To the best of our knowledge, this is the first study that assessed the presence of Gram-negative bacteria and their toxins in fermented foods and beverages from Nigerian and South African markets. It, therefore, follows that the presence of pathogenic bacteria and their toxins in these foods and beverages is undesirable and calls for the development and enactment of adaptable food safety measures. Further studies need to be conducted to establish the pathogenicity of some of the Gram-negative microorganisms.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108251.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgments

The authors are grateful for the financial support received from the Organization for Women in Science for Women in Science in for the Developing World, Italy and the University of Johannesburg, South Africa. The authors appreciate the input of Prof. M. Jakobsen in reviewing the manuscript and that of Prof N. Obinna in submitting the bacteria sequences to the GenBank.

References

Adams, P.G., Lamoureux, L., Swingle, K.L., Mukundan, H., Montano, G.A., 2014. Lipopolysaccharide-induced dynamic lipid membrane reorganization: Tubules, perforations, and stacks. Biophys. J. 106 (11), 2395–2407.
Adedeji, B.S., Ezeokoli, O.T., Ezekiel, C.N., Obadina, A.O., Somorin, Y.M., Sulyok, M., Adeleke, R.A., Warth, B., Nwangburuka, C.C., Omemu, A.M., Oyewole, O.B., Krska, R., 2017. Bacterial species and mycotoxin contamination associated with locust bean, melon and their fermented products in South-western Nigeria. Int. J. Food Microbiol. 258, 73–80.
Adekoya, I.O., Obadina, A.O., Phoku, J.Z., Nwinyi, O.A., Njobeh, P.B., 2017. Contamination of fermented foods in Nigeria with fungi. LWT-Food Sci. Technol. 86, 76–84.
Adekoya, I.O., De Saeger, S., Chilaka, A.C., De Boevre, S., Obadina, A.O., Njobeh, P.B., 2018. Mycobiota and co-occurrence of mycotoxins in South African traditional maize-based opaque beer. Int. J. Food Microbiol. 270, 22–30.
Alam, J.M., Tomochika, K.I., Miyoshi, S.I., Shinoda, S., 2002. Environmental investigation of potentially pathogenic *Vibrio parahaemolyticus* in the Seto-Inland Sea, Japan. FEMS Microbiol. Lett. 208, 83–87.
Aworh, O.C., 2008. The role of traditional food processing technologies in national development: the West African experience. In: Robertson, G.L., Lupien, J.R. (Eds.), Using Food Science and Technology to Improve Nutrition and Promote National Development. International Union of Food Science & Technology, Canada, pp. 1–18.
Beuchat, L.R., Kim, H., Gurtler, J.B., Lin, L.C., Ryu, J.H., Richards, G.M., 2009. *Cronobacter sakazakii* in foods and factors affecting its survival, growth, and inactivation. Int. J. Food Microbiol. 136, 204–213.
Blumberg, L., Jong, G., Thomas, J., Archer, B.N., Cengimbo, A., Cohen, C., 2011. Outbreaks in South Africa 2004–2011, the outbreak response unit of the NICD, and the vision of an inspired leader. South Afr J. Epidemiol. Infect. 26, 195–197.
Brenner, D., Krieg, N.R., Staley, J.T., Garrity, G.M., 2005. Bergey's manual of systematic bacteriology. Bergey's Manual Syst. Bacteriol. 333–379.
Cho, J.I., Lee, S.H., Lim, J.S., Koh, Y.J., Kwak, H.S., Hwang, I.G., 2011. Detection and distribution of food-borne bacteria in ready-to-eat foods in Korea. Food Sci. Biotechnol. 20, 525–529.
Choi, S., Cha, S., Tappert, C., 2009. A survey of binary similarity and distance measures. Syst. Cybern. Inf. 8, 1–6.
EC (European Commission), 2005. Commission regulation (EC) N0. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off. J. Eur. Comm. 338, 1–26.
Eze, V.C., Onwuakor, C.E., Ukeka, E., 2014. Proximate composition, biochemical and microbiological changes associated with fermenting African oil bean (*Pentaclethra macrophylla* Benth) seeds. Am. J. Microbiol. Res 2, 138–142.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Fugl, A., Berhe, T., Hussain, A.N.M., Laursen, M.F.M., Bahl, M.I., Hailu, Y., Sorensen, K.I., Guya, E., Egon, R., Hansen, B., 2017. Characterisation of lactic acid bacteria in spontaneously fermented camel milk and selection of strains for fermentation of camel milk. Int. Dairy J. 73, 19–24.
Hejazi, A., Falkiner, F.R., 1997. Serratia marcescens. J. Med. Microbiol. 46, 903–912.
Kalita, P., Chaturvedula, L.M., Sritharan, V., Gupta, S., 2017. *In vitro* flow-through assay for rapid detection of endotoxin in human sera: a proof-of-concept. Nanomedicine 13, 1483–1490.
Kimmons, J.E., Brown, K.H., Lartey, A., Collison, E., Mensah, P.P.A., Dewey, K.G., 1999. The effects of fermentation and/or vacuum flask storage on the presence of coliforms in complementary foods prepared for Ghanaian children. Int. J. Food Sci. Nutr. 50, 195–201.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley and Sons, New York, pp. 115–175.
Ligozzi, M., Bernini, C., Bonora, M.G., De Fatima, M., Zuliani, J., Fontana, R., 2002. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J. Clinic. Microbiol 40 (5), 1681–1686.
Lugito, H., Cucunawangsih, N.P., Kurniawan, A., 2016. A lethal case of *Sphingomonas paucimobilis* bacteremia in an immunocompromised patient. Case Rep. Infect. Dis. 2016, 1–4.
Malangu, N., 2014. Contribution of plants and traditional medicines to the disparities and similarities in acute poisoning incidents in Botswana, South Africa and Uganda. Afr. J. Tradit. Complement. Altern. Med. 11, 425–438.
Martich, G.D., Boujoukos, A.J., Suffredini, A.F., 1993. Response of man to endotoxin. Immunobiol 187, 403–416.
Neil, K.P., Sodha, S.V., Lukwago, L., O-Tipo, S., Mikoleit, M., Simington, S.D., Mukobi, P., Balinandi, S., Majalija, S., Ayers, J., Kagirita, A., Wefula, E., Asiimwe, F., Kweyamba, V., Talkington, D., Shieh, W.J., Adem, P., Batten, B.C., Zaki, S.R., Mintz, E., 2012. A large outbreak of typhoid fever associated with a high rate of intestinal perforation in Kasese district, Uganda, 2008–2009. Clin. Infect. Dis. 54, 1091–1099.
Newton, A., Kendall, M., Vugia, D.J., Henao, O.L., Mahon, B.E., 2012. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. Clin. Infect. Dis. 54, 391–395.
Nwachukwu, E., Orji, M., Abia, W., 2014. Pathogenic bacteria isolated from Nigerian fermented food ogiri made from melon. Int. Res. J. Sci. Find. 1, 4–6.
Nwamaka, N.T., Chike, A.O., Obiajulu, A., 2010. Role of bacteria isolates in the spoilage of fermented African oil bean seed ugba. Pak. J. Biol. Sci. 13, 497–503.
Nwokoro, O., Chukwu, B.C., 2012. Studies on Akamu, a traditional fermented maize food. Rev. Chil. Nutr. 39, 180–184.
Odunfa, S.A., Oyewole, O.B., 1998. African fermented foods. In: Wood, B.J.B. (Ed.), Microbiology of Fermented Foods. Springer, USA, pp. 713–752.
Ogunshe, A.O., Olasugba, K.O., 2008. Microbial loads and incidence of food-borne indicator bacteria in most popular indigenous fermented food condiments from middle-belt and southwestern Nigeria. J. Microbiol. 2, 332–339.
Ogunshe, A.A.O., Johnny, Z.M., Arinze, A.O., 2012. Effects of food spices on Gram-negative food indicator bacteria from some Nigerian ethnic fermented plant food condiments. African J. Plant Sci. 6, 8–14.
Oguntoyinbo, F.A., Sanni, A.I., Franz, C.M.A.P., Holzapfel, W.H., 2007. *In vitro* fermentation studies for selection and evaluation of *Bacillus* strains as starter cultures for the production of okpehe, a traditional African fermented condiment. Int. J. Food Microbiol. 113, 208–218.
Okeke, C.A., Ezekiel, C.N., Nwangburuka, C.C., Sulyok, M., Ezeamagu, C.O., Adeleke, R.A., Dike, S., Krska, R., 2015. Bacterial diversity and mycotoxin reduction during maize fermentation (steeping) for ogi production. Front. Microbiol. 6, 1–12.
Okorie, C., Olasupo, N., 2013. Controlled fermentation and preservation of ugba–an indigenous Nigerian fermented food. Springerplus 2, 470.
Oladipo, I.C., Sanni, A., Swarnakar, S., 2013. Phenotypic and genomic characterization of *Enterococcus* species from some Nigerian fermented foods. Food Biotechnol. 27, 39–53.
Olasupo, N.A., Smith, S.I., Akinsinde, K.A., 2002. Examination of the microbial status of selected indigenous fermented foods in Nigeria. J. Food Saf. 22, 85–93.
Olasupo, N.A., Okorie, C.P., Oguntoyinbo, F.A., 2016. The biotechnology of ugba, a Nigerian traditional fermented food condiment. Front. Microbiol. 7, 1153.
Pincus, D.H., 2006. Microbial identification using the bio Merieux VITEK® 2 system. In: Encyclopedia of Rapid Microbiological Methods. Parenteral Drug Association, Bethesda, MD.
Porter, K.J., Gonipeta, B., Parvataneni, S., Appledorn, D.M., Patial, S., Sharma, D., Gangur, V., Amalfitano, A., Parameswaran, N., 2010. Regulation of lipopolysaccharide-induced inflammatory response and endotoxemia by beta-arrestins. J. Cell. Physiol. 225, 406–416.
Quesada, M.D., Giménez, M., Molinos, S., Fernández, G., Sánchez, M.D., Rivelo, R., Ramírez, A., Banqué, G., Ausina, V., 2010. Performance of VITEK-2 Compact and overnight Micro Scan panels for direct identification and susceptibility testing of Gram-negative bacilli from positive FAN Bac T/ALERT blood culture bottles. Clin. Microbio. Infec. 16 (2), 137–140.
R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/, Accessed date: 20 July 2017.
Raetz, C.R.H., Whitfield, C., 2002. Lipopolysaccharide endotoxins. Annu. Rev. Biochem. 71, 635–700.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Simango, C., 2002. Lactic acid fermentation of sour porridge and mahewu, a non-alcoholic fermented cereal beverage. J. Appl. Sci. South. Afr. 8, 89–99.
Simango, C., Rukure, G., 1992. Survival of bacterial enteric pathogens in traditional fermented foods. J. Appl. Bacteriol 73 (1), 37–40.
Stephen, A., Thomas, L.M., Alejandto, A., Jinghui, Z., Zheng, Z., Webb, M., David, J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.
Sudershan, R.V., Naveen Kumar, R., Kashinath, L., Bhaskar, V., Polasa, K., 2014. Food-borne infections and intoxications in Hyderabad, India. Epidemiol. Res. Int. 942961, 1–5.
Tamura, K., Nei, M., Kumar, S., 2004. Prospects for inferring very large phylogenies by using the neighbour-joining method. Proceedings of the National Academy of Sciences (USA) 101, 11030–11035.
Townsend, S., Caubilla Barron, J., Loc-Carrillo, C., Forsythe, S., 2007. The presence of endotoxin in powdered infant formula milk and the influence of endotoxin and *Enterobacter sakazakii* on bacterial translocation in the infant rat. Food Microbiol. 24, 67–74.
Turner, S., Pryer, K.M., Miao, V.P.W., Palmer, J.D., 1999. Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. J. Eukaryot. Microbiol. 46, 327–338.
Wallace, R.J., Gropp, J., Dierick, N., Costa, L.G., Martelli, G., Brantom, P.G., Bampidis, V., Renshaw, D.W., Leng, L., 2016. Risks associated with endotoxins in feed additives produced by fermentation. Environ. Health 15, 5.