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Characterization and Evaluation of the Antibiotics Resistance of Bacterial Isolates from Locally Produced Tiger Nuts Drinks Commonly Marketed in Ado- Ekiti, Ekiti State, Nigeria

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ABSTRACT: Tiger nut drink, produced from *Cyperus esculentus L*., sugar, and water, is widely consumed across Nigeria for its nutritional and health benefits. However, contamination during processing and handling may compromise its safety, thus necessitating microbiological evaluation. Consequently, the objective of this paper is to characterize and evaluate the antibiotics resistance of bacterial isolates from locally produced tiger nut drinks commonly sold in Ado-Ekiti, Ekiti State, Nigeria using standard microbiological analysis. The bacterial counts varied among locations, with the lowest (2.4×10^4 CFU/ml) recorded in Adebayo market and the highest (8.1×10^4 CFU/ml) in Ijigbo market. Biochemical characterization identified several bacterial species including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp*., *Bacillus spp*., *Bacillus subtilis*, *Salmonella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Lactobacillus* spp. In addition, fungal contaminants such as *Fusarium* spp., *Aspergillus flavus*, and *Aspergillus niger* were detected. Antibiotic susceptibility tests showed high resistance rates to Tetracycline (61.54%), Cotrimoxazole (61.54%), Gentamicin (63.46%), Ampicillin (48.08%), and Erythromycin (40.39%), while lower resistance was observed against Amikacin (23.08%), Ciprofloxacin (36.54%), and Augmentin (36.54%). The presence of pathogenic bacteria and fungi highlights significant safety concerns, as these organisms are associated with foodborne infections that may compromise consumer health and reduce the nutritional quality of the drink. The contamination likely arises from poor hygiene practices, poor production processes, or exposure of raw materials to unsafe environments. To safeguard public health enforcement of strict hygienic measures are recommended. Such interventions will help reduce microbial loads and improve the safety of tiger nut drinks consumed in Ado-Ekiti, Ekiti State, Nigeria

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Keyword: Tigernut; tiger nut drink; microbial contamination, antibiotics, resistance

Tigernut milk (Kunun-aya) is a traditional non-alcoholic beverage commonly consumed in West-African countries, it is an aqueous extract of tigernut, coconut, date and spices blend (Ibrahim *et al*., 2016a; Kayode *et al*., 2017). The milk is also used as an ingredient in the production of Kunu, a well-known traditional beverage in West Africa (Ezekiel *et al*., 2019). Its low acid vegetable milk rich in protein and starch, also contains less energy when compared with dairy milk (Amponsah *et al*., 2017). Tigernut milk is a good substitute for vegetarians and persons with lactose intolerance (Amponsah *et al*., 2017).

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Beverages with acceptable sensory properties were developed by combining tigernut with other crops, these include tigernut and coconut (Echem and Torporo, 2018), tigernut and pineapple (Elizabeth and Tijesuni, 2020), tigernut and sweet potato (Idris *et al*., 2019), tigernut and cocoa (Oluwadunsin *et al*., 2021) and tigernut and baobab (Badejo *et al*., 2020). A probiotic beverage produced from tigernut possessed sensory qualities similar to that of dairy probiotic beverage (Amponsah *et al*., 2017). The findings of Wongnaa *et al*. (2019) showed that yogurt consumers in Kumasi, Ghana are positive about the nutritional and sensory qualities of tigernut yogurt and are willing to pay more for it. Optimum conditions for the production of tigernut yogurt are incubation for 3.12 h at 35 °C and a starter culture concentration of 2.74 %. In addition, tigernut milk was reported to improve the nutritional and sensory qualities of many foods. An increase in fat, calcium and potassium was reported in soy cheese containing 5 % tigernut (Balogun *et al*., 2019). Consequently, the objective of this paper is to characterize and evaluate the antibiotics resistance of bacterial isolates from locally produced tiger nut drinks commonly marketed Ado-Ekiti, Ekiti State, Nigeria.

MATERIALS AND METHODS

Sterilization of materials and disinfection of work bench: All glass wares were first washed with detergent and water and then rinsed thoroughly with distilled water, air dried after which they were sterilized by dry heat in a hot air oven at a temperature of 160ºC for 120 minutes. Micropipette and test tubes were corked with absorbent cotton wool and wrapped with aluminum foil paper, Sampling bottles were also wrapped with aluminum foil paper after drying and then sterilized to prevent external contamination. Working surfaces were disinfected by swabbing using cotton wool soaked with 75% ethanol before and after experiment.

Sample collection: The Tiger nuts drinks were aseptically obtained from five (5) different markets local sellers in Ado-Ekiti (Adebayo Market, Bawa Market, Ijigbo Market, Omisanjana Market, Fayose Market). The samples were collected in a sterile sampling bottles and were immediately transported to the Laboratory of the Department of Microbiology, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria at a cold temperature of about 6ºC,where the samples undergoes microbial analysis.

Preparation of culture media: The culture media used were prepared according to manufacturer's instructions, after which they were sterilized in an autoclave for 15 minutes at 121°C. The media used include nutrient agar (NA), potato dextrose agar (PDA).

Serial dilution and plating: For the analysis, 1 ml of each of the twenty (20) tiger nut drink samples was aseptically mixed with 9 ml of sterile distilled water at a temperature not exceeding 45°C, resulting in a 1:9 dilution. Serial dilutions were then prepared and labeled according to their respective 7 dilution factors, ranging from 10^{-1} to 10^{-9}. The diluted samples were incubated at room temperature for 2 to 3 hours to encourage microbial growth. Bacterial isolation was carried out using standard bacteriological procedures. Following serial dilution, 1 ml each from the 10^{-4} and 10^{-5} dilutions was aseptically transferred into pre-labeled sterile Petri dishes. Molten nutrient agar, cooled to approximately 45°C, was then poured into each plate. The pour plating was done in duplicates for each dilution. Plates were allowed to solidify, sealed with aluminum foil, and incubated in an inverted position at 37°C for 24 hours. After incubation, visible colonies were counted and recorded as colony-forming units per milliliter (CFU/ml). Representative colonies were sub-cultured on sterile nutrient agar to obtain pure isolates, which were then preserved at 4°C for further analysis.

Sub-Culturing and Isolation of Distinct Colony: Plates with an appropriate number of colonies were selected for further enumeration. Distinct colonies were identified based on observable characteristics and were sub-cultured onto fresh plates containing approximately 15 ml of sterile molten Nutrient Agar, Potato Dextrose Agar, and MacConkey Agar. Sub-culturing was performed using the streaking method under aseptic conditions, with proper flaming of the inoculating loop. The plates were then incubated aerobically at 35°C for 24 hours. From the sub-cultured plates, well-isolated colonies were carefully selected and transferred into McCartney bottles containing about 5 ml of sterile slanted nutrient agar. All transfers were done aseptically, with the wire loop, bottle cover, and bottle mouth properly flamed to maintain sterility.

Identification and Characterization of Isolated Organisms: Isolates were identified based on the physiological morphology and biochemical features they possess.

Isolation and Characterization of Fungal Isolates: The serially diluted samples were plated on Potato Dextrose Agar and incubated for 5 days at 28°C. The well-grown spread single colonies were picked up and further sub-cultured on potato dextrose agar

slants. The isolates were characterized based on their colonial morphology

Antibiotics susceptibility: The bacterial isolates from the tiger nut samples were subjected to antibiotic susceptibility testing using standard antibiotic discs. A loopful of each isolate grown on nutrient agar was suspended in sterile distilled water and serially diluted at a 1:10 ratio until the turbidity matched the 0.5 McFarland standard, equivalent to a cell density of 1×10^8 cells/ml. Mueller Hilton agar plates were then inoculated with 0.5 ml of the standardized suspension. Using sterile forceps, antibiotic discs were carefully placed on the surface of each inoculated plate. The plates were incubated at 37°C for 24 hours, and the zones of inhibition were later observed to determine antibiotic sensitivity (Benson, 2005).

Statistical Analyses: Statistical analysis was conducted through One-way analysis of variance (ANOVA) using the SPSS 20 software package for Windows. The means of the replicate determinations were computed and presented as ± standard error of the mean (SEM). Throughout the analysis, a significance level of $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The total bacterial count of tiger nut drink samples collected from five different markets in Ado-Ekiti revealed varying levels of microbial load. Adebayo Market recorded the lowest bacterial count at 2.4×10^4 CFU/ml, while the highest count, 8.1×10^4 CFU/ml, was observed in samples from Ijigbo Market (Table 1).

Table 1: Total Bacteria Count (CFU) of tiger nut sample gotten from five (5) different market in Ado- Ekiti

Isolates	Total Bacteria Count (Cfu/ml)
Adebayo Market	2.4×10^4
Bawa Market	3.9×10^4
Ijigbo Market	8.1×10^4
Omisanjana Market	4.7×10^4
Fayose Market	5.2×10^4

KEYS: AM-Adebayo Market; BM-Bawa Market; IM- Ijigbo Market; OM - Omisanjana Market, FM - Fayose Market.

Tiger nut (Cyperus esculentus L.) is a nutrient-rich tuber crop valued for its high iron and calcium content, supporting growth, digestion, and reducing the risk of diseases such as diabetes, cancer, and cardiovascular ailments. Despite its benefits, this study found that tiger nuts sold in Ado-Ekiti markets harbor high levels of microbial contamination. Bacterial counts ranged from 2.4×10^4 CFU/ml in Adebayo to 8.1×10^4 CFU/ml in Ijigbo, likely due to poor handling, environmental exposure, and unsanitary vendor practices (Akomolafe & Awe, 2017). Table 2 shows the biochemical test results which revealed the presence of several bacterial species in the tiger nut drink samples. Identified organisms includes *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Bacillus* spp., *Bacillus subtilis*, *Salmonella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, and *Lactobacillus* spp. Isolates included *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, and *Pseudomonas spp.*, suggesting faecal contamination and posing serious health risks (Ekeanyanwu *et al*., 2019; Oguike *et al*., 2018). Fungal species like *Aspergillus flavus* and *Fusarium spp.*—known producers of carcinogenic mycotoxins—were also identified. Table 3 shows the frequency and percentage occurrence of bacterial isolated from tigernut sample from different market in Ado metropolis. The highest bacterial occurrence was observed to be *Staphylococcus aureus* with the percentage of (28.85%) followed by *Enterobacter* spp (19.23%) while the least occurrence was observed to be *Bacillus* spp and *Klebsiella* sp (3.85%) 9. Table 4 shows the antimicrobial susceptibility patterns of bacteria from Tigernut drink and it revealed that the isolates exhibited high resistance to commonly used antibiotics, particularly gentamicin, tetracycline, cotrimoxazole, and ampicillin, with over 60% resistance to gentamicin and tetracycline. This is of serious concern given the widespread empirical use of these drugs in Nigeria. In contrast, amikacin showed the highest effectiveness, with only 23.08% resistance, while ciprofloxacin and augmentin demonstrated moderate activity. *Staphylococcus aureus* showed frequent resistance to multiple antibiotics, suggesting the presence of MRSA-like strains, while *Enterobacter* and *Klebsiella spp.* displayed high resistance to β-lactams, consistent with ESBL production. *Pseudomonas aeruginosa* exhibited multidrug resistance typical of its intrinsic mechanisms, and the detection of resistance in *Lactobacillus spp.* raises concerns about the potential for horizontal gene transfer within food systems. The high prevalence of resistance reflects extensive antibiotic exposure in the environment and supports growing evidence that foods act as reservoirs of resistant bacteria. Misuse of affordable antibiotics such as tetracycline and cotrimoxazole in low- and middle-income countries creates strong selective pressure that promotes resistance (O'Neill, 2020). ESBL-producing *Enterobacteriaceae* and MRSA in foods further highlight the public health risk of contaminated beverages (Founou *et al*., 2021; Adegoke *et al*., 2020).

Table 2: Biochemical characterization of bacterial isolated from tigernut sample from four different market in Ado Ekiti

Isolate Code	Gxn	Mot	Cat	Coag	Oxi	Man	Cit	Ind	Glu	Lact	MR	Organism
AM1	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
BM1	GNB	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	*E.coli*
IM1	GNB	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Klebsiella spp*
OM 1	GPB	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*Bacillus spp*
FM 1	GPB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Bacillus subtilis*
AM2	GNB	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	*E.coli*
BM2	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
IM2	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
OM 2	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
FM 2	GNB	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	*Pseudomonas aeroginosa*
AM3	GPB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Bacillus subtilis*
BM3	GNB	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Salmonella spp*
IM3	GNB	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Klebsiella spp*
OM 3	GNB	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	*E.coli*
FM 3	GPB	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*Bacillus spp*
AM4	GPB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Bacillus subtilis*
BM4	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
IM4	GNB	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	*Pseudomonas Aeroginosa*
OM 4	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
FM 4	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
AM5	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
BM5	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
IM5	GNB	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	*E.coli*
OM 5	GNB	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Salmonella spp*
FM 5	GPB	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	*Lactobacillus spp*
AM6	GPB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Bacillus subtilis*
BM6	GNB	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Salmonella spp*
IM6	GPB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Bacillus subtilis*
OM 6	GPB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Bacillus subtilis*
FM 6	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
AM7	GNB	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Salmonella spp*
BM7	GPB	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	*Lactobacillus spp*
IM7	GNB	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	*E.coli*
OM 7	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
FM 7	GNB	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	*Pseudomonas Aeroginosa*
AM8	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve	*Staphylococcus aureus*
BM8	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	*Enterobacter species*
IM8	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
OM 8	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
FM 8	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
AM9	GPB	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
BM9	GPB	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	*Lactobacillus spp*
IM9	GNB	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	*Salmonella spp*
OM 9	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
FM 9	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
AM10	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
FM 10	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
BM10	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
1M10	GNB	+ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	*E.coli*
OM 10	GNB	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Enterobacter species*
AM11	GPB	-ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	+ve	*Staphylococcus aureus*
BM11	GNB	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	*Pseudomonas Aeroginosa*

Keys: AM-Adebayo Market; BM-Bawa Market; IM - Ijigbo Market; OM - Omisanjana Market; FM - Fayose Market; GPB - Gram Positive Bacteria; GNB- Gram Negative Bacteria; -ve - Negative; +ve – Positive, GNX - Gram reaction; MOT- Motility, GLU-Glucose, CAT- Catalase, LACT-Lactose, COAG- Coagulase MR – Methyl red, OXI- Oxidase, MAN- Manose; CIT-Citrate, IND-Indole.

Table 3: Frequency and percentage occurrence of isolated organisms from tigernut

Microorganisms	Frequency (N)	Percentage (%)
Staphylococcus aureus	15	28.85
Bacillus subtilis	6	11.54
Salmonella spp	5	9.62
Escherichia coli	5	9.62
Pseudomonas aeroginosa	4	7.69
Enterobacter spp	10	19.23
Klebsiella sp	2	3.85
Lactobacillus spp	3	5.77
Bacillus spp	2	3.85
Total	52	100

Table 4: Antimicrobial susceptibility patterns of bacteria from tigernut drink

Organism	AMP	TET	COT	GEN	CIP	ERY	AMK	AUG
Staphylococcus aureus	R	R	R	R	R	R	R	I
E.coli	R	I	R	R	R	S	I	I
Klebsiella spp	R	R	R	R	R	S	S	S
Bacillus spp	R	S	S	R	S	S	I	I
Bacillus subtilis	1	S	R	S	R	R	R	R
E.coli	R	S	R	R	I	R	R	R
Enterobacter species	I	S	S	R	I	R	R	R
Staphylococcus aureus	I	S	R	R	I	S	I	I
Staphylococcus aureus	S	S	R	R	I	S	S	S
Pseudomonas aeroginosa	S	I	S	R	S	I	S	S
Bacillus subtilis	R	S	R	S	S	I	I	I
Salmonella spp	I	I	S	S	R	R	R	R
Klebsiella spp	R	R	R	R	R	S	S	R
E.coli	R	S	S	R	S	R	R	S
Bacillus spp	S	R	R	S	S	S	I	I
Bacillus subtilis	S	R	R	R	S	S	I	I
Enterobacter species	S	S	S	R	S	R	R	R
Pseudomonas aeroginosa	R	R	R	S	S	S	S	R
Enterobacter species	R	R	R	R	S	R	R	R
Enterobacter species	I	R	R	R	S	R	I	R
Staphylococcus aureus	I	S	S	S	S	S	I	S
Staphylococcus aureus	S	R	S	I	S	R	I	R
E.coli	S	R	R	R	S	S	I	I
Salmonella spp	R	R	S	S	S	S	I	R
Lactobacillus spp	R	R	I	I	S	S	I	S
Bacillus subtilis	R	R	R	R	S	R	I	R
Salmonella spp	R	S	S	S	S	S	R	R
Bacillus subtilis	S	S	R	R	S	R	S	S
Bacillus subtilis	R	R	R	R	R	R	I	I
Staphylococcus aureus	S	R	S	S	R	S	I	I
Salmonella spp	R	S	R	R	R	R	S	I
Lactobacillus spp	S	R	R	R	R	R	I	S
E.coli	R	R	S	S	R	S	S	I
Staphylococcus aureus	I	R	R	R	S	S	R	R
Pseudomonas aeroginosa	I	R	R	R	S	S	S	S
Staphylococcus aureus	I	R	R	R	R	I	I	I
Enterobacter species	R	S	S	S	R	I	I	I
Staphylococcus aureus	S	R	S	I	S	R	I	R
Staphylococcus aureus	S	R	R	R	S	S	I	I
Staphylococcus aureus	R	R	S	S	S	S	I	R
Staphylococcus aureus	R	R	I	I	S	S	I	S
Lactobacillus spp	R	R	R	R	S	R	I	R
Salmonella spp	R	S	S	S	S	S	R	R
Enterobacter species	S	S	R	R	S	R	S	S
Staphylococcus aureus	R	R	R	R	R	R	I	I
Staphylococcus aureus	S	R	S	S	R	S	I	I
Enterobacter species	R	S	R	R	R	R	S	I
Staphylococcus aureus	S	R	R	R	R	R	I	S
E.coli	R	R	S	S	R	S	S	I
Enterobacter species	I	R	R	R	S	S	R	R
Staphylococcus aureus	I	R	R	R	S	S	S	S
Pseudomonas aeroginosa	I	R	R	R	R	I	I	I
TOTAL	48.08%	61.54 %	61.54 %	63.46 %	36.5 4%	40.39%	23.08 %	36.54 %

Keys: AMP=Ampicillin, TET=Tetracycline, COT=Cotrimoxazole, GEN=Gentamicin, CIP=Ciprofloxacin, ERY=Erythromycin, AMK=Amikacin, AUG=Augumentin. R- Resistance, S- Susceptible, I- Intermediate

The relatively low resistance to amikacin aligns with its restricted clinical use (Tamma *et al*., 2021), while resistance in beneficial bacteria underscores the role of foods in disseminating resistance genes within the One Health framework (Zhang *et al*., 2020)

Table 5 shows the Morphology and cultural characteristics of the fungal isolated from tigernut sample were presented in table 5. Colonies on the plate shows white cotton and dark-purple under surface, and spores are oval when observed however,

it was suspected to be *Fusarium sp.* Colonies of yellowish-green coloration spores on the surface of the growth was observed, Septate hyphae, the spores are many.

However, it was suspected to be *Aspergillus flavus.* The identified fungi in the present study are; *Fusarium sp, Aspergillus flavus* and *Aspergillus niger*

Table 5: Morphology and *Cultural characteristics of the Fungal Isolates*

Colonies	Microscopy	Suspected Organism
White cotton and dark-purple under surface	The spores are oval tapering and septate	*Fusarium sp.*
Colonies of yellowish-green colouration spores on the surface of the growth	Septate hyphae, the spores are many,	*Aspergillus flavus*
Colonies start with white coloration which quickly becomes black with conidial production	Smooth coloured conidiophores and conidia	*Aspergillus niger*

Conclusion: In conclusion the practice of eating tiger nut improperly wash or unwashed at the point of buying is discouraged, since this practice can inoculate loads of pathogenic bacteria that can cause stomach cramping, diarrhea and possibly lead to death. There is serious need for quality of food items sold in the market and market by vendors to be monitored regularly by environmental health officers to avoid outbreak of epidemic as a result of contaminated food. We therefore, suggest molecular studies on bacteria isolate from tiger nuts to understand the possible antibiotics resistance genes and virulence genes their harbor. We also recommend further studies on isolates from tiger nut, human and environment to understand the clonal relationship, diversity of this multidrug resistant isolates and their role in one health.

DECLARATIONS

Ethical Approval: This study did not involve human participants or animal experimentation and therefore did not require formal ethical approval. All procedures involving data handling and analysis were conducted responsibly and in accordance with standard academic and institutional guidelines.

Disclaimer (Artificial Intelligence): Author(s) hereby declare that no generative Artificial Intelligence (AI) technologies such as Large Language Models (ChatGPT, COPILOT, XLNet, Ernie 3.0 Titan etc) and text-to-image generators have been used during writing or editing of this manuscript.

Declaration of Conflict of Interest: The authors declare that no known conflict of interest or personal relationships that have influence the work reported in this paper.

Declaration of Data Availability: Data are available upon request from the corresponding Author.

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