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Assessment of Bacterial and Fungal Pathogens Associated with Kolanuts and their Public Health Significance

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ABSTRACT
Kolanut (*Cola nitida*) is one of the economically and culturally important tree crops in Nigeria and is often traded in open-air markets where microbial contamination may be possible. In this study, the bacterial and fungal pathogens associated with apparently healthy and visibly deteriorated kolanuts purchased from six markets in Ibadan, Oyo State (Bodija, Oje, Oja-Oba, Sango, Aleshinloye and Agbeni) were evaluated. A total of 150 samples (90 healthy and 60 deteriorated) were processed using standard culture, biochemical and molecular identification techniques. Antibiotic susceptibility testing and mycotoxin screening of isolates of public health concern were performed. Deteriorated nuts had significantly greater bacterial load (59,002 ± 10,772 CFU/g against 22,167 ± 4,622 CFU/g) and fungal load (3,423 ± 682 CFU/g against 1,273 ± 294 CFU/g) than healthy nuts in all markets ($p < 0.001$), and *Escherichia coli*, *Salmonella species*, *Staphylococcus aureus* and toxigenic *Aspergillus species* were recovered significantly more often from deteriorated nuts, although 48.9% of healthy nuts also carried at least one of them. Multiple antibiotic resistance was widespread: 84.9% of Gram-negative isolates and 84.4% of *Staphylococcus aureus* isolates had a multiple antibiotic resistance index above 0.2, and 62.3% of Gram-negative isolates were multidrug-resistant. Aflatoxins were detected in 22 of 47 (46.8%) toxigenic *Aspergillus* isolates (4.2 to 52.6 µg/kg), 16 of which exceeded 10 µg/kg, and fumonisins were detected in 9 of 14 (64.3%) *Fusarium* isolates (110 to 1,480 µg/kg), with aflatoxin positivity unrelated to visible deterioration. These results support routine monitoring of the microbial and mycotoxin status of kolanuts in Nigerian markets and provide a basis for food safety measures for kolanut vendors and consumers.

Keywords: Kolanut, Cola Nitida, Food Safety, Market Contamination, Antimicrobial Resistance, Mycotoxins

INTRODUCTION

Kolanut (*Cola nitida* and *Cola acuminata*) is an important cultural tree crop, especially because of its stimulant properties, its central role in the traditional ceremonies of many ethnic groups in Nigeria, and its emerging importance in the country's agricultural research strategy for value added products. Nigeria is still one of the biggest producers of kolanut in the world and the crop sustains the livelihoods of people in the production, processing and marketing sectors, especially in Oyo State, located in South-West Nigeria, which is one of the major growing and trading areas of the crop.

Despite this economic and cultural significance, kolanuts are highly susceptible to microbial contamination at various stages of the value chain, such as during harvest, post-harvest handling, transportation and open market storage. Fungal genera like *Aspergillus*, *Penicillium* and *Fusarium* have been reported several times as the cause of post-harvest deterioration of kolanuts (Aduama-Larbi *et al.*, 2022), and pathogens like *Fusarium pallidoroseum* and *Lasiodiplodia theobromae* continue to pose a threat to the quality of stored kolanuts in West Africa (Imade *et al.*, 2021; Sulaimon *et al.*, 2024). In addition to crop level spoilage, the open market conditions in which kolanuts are normally sold and stored expose them to dust, handling by numerous people, other produce and the possibility of bacterial contamination, which poses a public health risk.

The presence of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella species* has been repeatedly reported at levels of concern in Nigerian studies of similar market vended foods, as well as in food handlers (Adenugba & Olusegun, 2024; Oyewole *et al.*, 2024), and antimicrobial resistance reviews of food-associated isolates continue to report alarming proportions of multidrug resistant organisms (Viana *et al.*, 2025). However, in contrast with staple grains, legumes and animal products, there has been comparatively little research interest in the food safety aspects of kolanut in the Nigerian food safety literature, while its trade is almost exclusively through informal open markets.

The present study therefore examines the bacterial and fungal pathogens isolated from kolanuts sold in six markets in Ibadan, Nigeria, compares apparently healthy and visibly deteriorated nuts, and investigates the public health significance of the isolates recovered using antibiotic sensitivity testing and screening for mycotoxins.

This study is aimed at evaluating the bacterial and fungal pathogens associated with the sale of kolanuts in the selected markets of Ibadan and their public health significance. The specific objectives were to identify the frequency and quantity of bacterial and fungal contamination of apparently healthy and visibly deteriorated kolanuts in six markets in Ibadan; to identify bacterial and fungal isolates of public health importance (*Escherichia coli*, *Salmonella species*,

Staphylococcus aureus and toxigenic *Aspergillus species*) by culture, biochemical and molecular techniques; to identify the antibiotic susceptibility profile of bacterial isolates of public health concern; to screen toxigenic fungal isolates for the production of aflatoxin and fumonisin; and to compare the level of contamination across markets and between healthy and deteriorated nuts using appropriate statistical tests.

MATERIALS AND METHODS

Study Area and Sampling Locations

The study was carried out in Ibadan, Oyo State, in south-western Nigeria, which is a large centre for trading agricultural produce. To capture the variation in the supply, storage and handling of kolanuts in the market, six markets (Bodija, Oje, Oja-Oba, Sango, Aleshinloye and Agbeni) were purposively selected. Oje and Oja-Oba have also been used in some previous microbiological studies of kolanuts (Garuba *et al.*, 2024).

From each market, 25 nuts were collected, 15 of which were apparently healthy nuts and 10 of which were visibly deteriorated nuts. This resulted in 90 healthy and 60 deteriorated nuts for a total of 150 samples.

Sample Collection and Sample Size

To reduce selection bias, vendors were randomly selected per market. Each sample was placed individually in a sterile polythene bag labelled with the market, vendor, nut condition and date of collection. Samples were brought to the laboratory in a cool box within 4 hours. The sample size was not based on an *a priori* power calculation because local prevalence data were unavailable. Instead, it was informed from other Nigerian market-food microbiology studies (Adenugba & Olusegun, 2024; Wakil *et al.*, n.d.) and was deemed appropriate for an exploratory survey.

Laboratory Facilities

The analyses were carried out at the Pathology Laboratory and Value Added Research Laboratory of the Cocoa Research Institute of Nigeria, Idi-Ayunre, Ibadan. Sample processing, microbial culture and isolation, biochemical and microscopic identification, and antibiotic susceptibility testing were done in the Pathology Laboratory. The Value Added Research Laboratory handled mycotoxin screening and molecular identification.

Isolation and Enumeration of Microorganisms

Kolanut samples were surface-sterilised, homogenised and serially diluted to 10^{-6} in sterile normal saline. Appropriate dilutions were plated in triplicate using the pour-plate method. Nutrient agar was used for general bacterial counts, MacConkey agar for Gram-negative enteric bacteria, mannitol salt agar for presumptive *Staphylococcus aureus*, *Salmonella-Shigella* agar for *Salmonella* and related organisms, and potato dextrose agar for fungi, including moulds and yeasts.

Bacterial plates were incubated for 24 to 48 hours at 37 °C and fungal plates were incubated for 3 to 7 days at 25 to 28 °C. The microbial load was expressed as colony forming units per gram (CFU/g) and calculated as follows:

$$\text{CFU/g} = \frac{\textit{Number of colonies X Dilution factor}}{\textit{Volume plated } (mL)}$$

The calculation was corrected, where necessary, for sample mass and volume of homogenate prepared.

Identification and Public Health Screening

Colony morphology, Gram staining and biochemical tests such as catalase, coagulase, indole, citrate utilisation, urease and triple sugar iron tests were used to identify bacterial isolates. Fungi were characterised on the basis of cultural characteristics and microscopic examination following lactophenol cotton blue staining of the colonies. Molecular confirmation was carried out by 16S ribosomal ribonucleic acid gene sequencing for bacteria and internal transcribed spacer sequencing for fungi (Garuba *et al.*, 2024).

Screening focused on organisms of public health concern, namely *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus* and potentially toxigenic *Aspergillus* species, as in other food safety studies (Adenugba & Olusegun, 2024; Viana *et al.*, 2025).

Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion method was used to test the potential bacterial pathogens. The panel for *Staphylococcus aureus* was 30 µg of cefoxitin, 10 units of penicillin, 15 µg of erythromycin, 2 µg of clindamycin, 10 µg of gentamicin, 30 µg of tetracycline, 30 µg of chloramphenicol and 5 µg of ciprofloxacin. Methicillin-resistant *Staphylococcus aureus* was screened for using cefoxitin.

Discs contained the following antibiotics for *Escherichia coli* and *Salmonella* species: ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg). Suspected extended-spectrum beta-lactamase activity was to be investigated using a double-disc synergy test where indicated. The Clinical and Laboratory Standards Institute M100, 35th edition (Clinical and Laboratory Standards Institute, 2025) was used for the interpretation of inhibition zones. The multiple antibiotic resistance index was determined as:

$$\textit{Multiple antibiotic resistance index} = \frac{a}{b}$$

where a represents the number of antibiotics the isolate was resistant to, and b represents the number of antibiotics tested.

Mycotoxin Screening

Aspergillus flavus and *Aspergillus parasiticus* isolates were screened for aflatoxins B_1, B_2, G_1 and G_2 using enzyme-linked immunosorbent assay. High-performance liquid chromatography was used for confirmation where available (Akinniyi, 2025). *Fusarium* isolates were screened for fumonisins using enzyme-linked immunosorbent assay (Anumudu *et al.*, 2025). Results were compared with applicable Codex Alimentarius and Nigerian Industrial Standards limits.

Ethical Considerations

Human beings and experimental animals were not subjected to the study, and market samples were purchased for the study. All vendors gave their respective verbal consent prior to purchase and no individual vendor was identified when reporting results.

Data Analysis

Microbial counts, prevalence, and antibiotic resistance were summarised using means, standard deviations, frequencies, and percentages. Differences in microbial loads among the six markets were analysed using one-way analysis of variance, followed by Tukey's post hoc test. Microbial loads between healthy and deteriorated nuts were compared using the independent-samples t-test. Pathogen prevalence was assessed using the chi-square test of independence. Statistical significance was set at $p \leq 0.05$. Data were analysed using Statistical Package for the Social Sciences, version 25.

Because the variance in the microbial counts of healthy and deteriorated nuts was not equal (Levene's test), Welch's correction was used in the independent-samples t-test and the results were confirmed using log10-transformed counts and the Mann-Whitney U test. Within each nut condition, pairwise comparisons were made using Tukey's honestly significant difference test, and means sharing a letter are considered homogeneous. To estimate the relative importance of each factor and their interaction (partial η^2), a two-way analysis of variance (market × nut condition) was fitted. Prevalence was reported using 95% Wilson confidence intervals, and odds ratios (deteriorated versus healthy) were calculated using Woolf 95% confidence intervals. Fisher's exact test was used for small cell counts, and the number of target organisms per sample was compared using the Mann-Whitney U test. Multidrug-resistant isolates were defined as those that were resistant to three or more antimicrobial classes (Magiorakos et al., 2012), and a high multiple antibiotic resistance index (>0.2) was used as an indicator of isolates from high-risk sources of contamination (Krumperman, 1983). Resistance to ceftazidime or cefotaxime was reported as third-generation cephalosporin resistance, and resistance to cefoxitin in *Staphylococcus aureus* was reported as presumptive methicillin-resistant *Staphylococcus aureus*. Aflatoxin levels were interpreted using the limits of 10 µg/kg and 15 µg/kg set for nuts in the surveillance reported by Abong et al. (2025), while fumonisin levels were not interpreted, as there are no limits applicable to kolanuts.

RESULTS AND DISCUSSION

Results

Bacterial and Fungal Load of Healthy and Deteriorated Kolanuts

A total of 150 samples of kolanuts (90 healthy and 60 deteriorated) were analysed (25 samples per market). Across all samples, the mean bacterial load was 36,901 ± 19,660 CFU/g and the mean fungal load was 2,133 ± 1,163 CFU/g. The nut condition had a significant effect on microbial load (Table 1). Deteriorated nuts carried a mean bacterial load of 59,002 ± 10,772 CFU/g, which was 2.66 times the 22,167 ± 4,622 CFU/g recorded in healthy nuts (mean difference 36,835 CFU/g; 95% confidence interval, 33,899 to 39,771 CFU/g; Welch's $t(73.6) = 25.00$, $p < 0.001$; Cohen's $d = 4.79$). Fungal load followed the same pattern, with 3,423 ± 682 CFU/g in deteriorated nuts against 1,273 ± 294 CFU/g in healthy nuts, a 2.69-fold increase (mean difference 2,150 CFU/g; 95% confidence interval, 1,964 to 2,336 CFU/g; Welch's $t(73.7) = 23.02$, $p < 0.001$; Cohen's $d = 4.41$). The variances were different between the two groups (Levene's test, $p < 0.001$ for both variables) so the data were log10-transformed and tested for differences by the Mann-Whitney U test, which yielded similar results ($p < 0.001$ for both variables).

Table 1: Bacterial and Fungal Load of Healthy and Deteriorated Kolanuts (All Six Markets Pooled)

Parameter	Nut condition	*n*	Mean ± standard deviation (CFU/g)	Median (CFU/g)	Range (CFU/g)	Welch's *t* (degrees of freedom)	*p*
Bacterial load	Healthy	90	22,167 ± 4,622	22,000	12,700–38,800	25.00 (73.6)	< 0.001
	Deteriorated	60	59,002 ± 10,772	58,350	37,900–86,100		
Fungal load	Healthy	90	1,273 ± 294	1,255	750–2,210	23.02 (73.7)	< 0.001
	Deteriorated	60	3,423 ± 682	3,395	1,760–5,220		

Note: Loads are Colony-Forming Units per Gram of Kolanut (CFU/g), Presented as Full Numbers. *p*-values are from WELCH'S Independent-Samples *t*-test (Deteriorated Versus Healthy); the Mann-Whitney *U* test gave $p < 0.001$ for Both Variables. Standard Deviation, Standard Deviation.

Microbial Load Across Markets

Table 2 details the mean loads in each market and Figure 1 depicts them graphically. The bacterial load (2.48- to 2.82-fold) and fungal load (2.38- to 3.03-fold) were significantly higher in deteriorated nuts than in healthy nuts in all 12 comparisons, one for each market and each load (Welch's t-test, $p < 0.001$). Among healthy nuts, bacterial load differed between markets (one-way analysis of variance, $F(5, 84) = 4.01$, $p = 0.003$, $\eta^2 = 0.19$); Tukey's test showed that nuts from Sango (25,847 CFU/g) carried more bacteria than those from Oja-Oba ($p < 0.001$) and Agbeni ($p = 0.023$). There were no significant differences between markets in bacterial load of the deteriorated nuts ($F(5, 54) = 1.91$, $p = 0.108$). Fungal load differed between markets in both groups: in healthy nuts ($F(5, 84) = 5.41$, $p < 0.001$, $\eta^2 = 0.24$), Oje and Sango exceeded Bodija and Oja-Oba (Tukey $p = 0.002$ to 0.029), and in deteriorated nuts ($F(5, 54) = 4.28$, $p = 0.002$, $\eta^2 = 0.28$), Sango (4,150 CFU/g) exceeded Oja-Oba, Aleshinloye and Agbeni (Tukey $p = 0.002$ to 0.039).

Table 2: Bacterial and Fungal Load (CFU/g) of Healthy and Deteriorated Kolanuts by Market

Market	Healthy (*n* = 15)	Deteriorated (*n* = 10)	Fold increase	Welch's *t* (degrees of freedom)	*p*
Bacterial load (CFU/g)					
Bodija	21,920 ± 2,976^{ab}	60,750 ± 8,057^{a}	2.77	14.59 (10.7)	< 0.001
Oje	22,353 ± 3,991^{ab}	58,550 ± 8,450^{a}	2.62	12.64 (11.7)	< 0.001
Oja-Oba	19,220 ± 3,420^{b}	54,200 ± 9,846^{a}	2.82	10.81 (10.5)	< 0.001
Sango	25,847 ± 6,197^{a}	67,120 ± 10,771^{a}	2.60	10.97 (13.0)	< 0.001
Aleshinloye	22,800 ± 5,049^{ab}	56,580 ± 15,030^{a}	2.48	6.85 (10.4)	< 0.001
Agbeni	20,860 ± 3,043^{b}	56,810 ± 8,435^{a}	2.72	12.93 (10.6)	< 0.001
One-way analysis of variance across markets	$F(5, 84) = 4.01$; $p = 0.003$	$F(5, 54) = 1.91$; $p = 0.108$			
Fungal load (CFU/g)					

Market	Healthy (n = 15)	Deteriorated (n = 10)	Fold increase	Welch's t (degrees of freedom)	p
Bodija	1,141 ± 232^{b}	3,461 ± 378^{ab}	3.03	17.33 (13.5)	< 0.001
Oje	1,449 ± 215^{a}	3,446 ± 443^{ab}	2.38	13.25 (11.9)	< 0.001
Oja-Oba	1,067 ± 185^{b}	3,101 ± 612^{b}	2.91	10.21 (10.1)	< 0.001
Sango	1,441 ± 345^{a}	4,150 ± 846^{a}	2.88	9.61 (11.0)	< 0.001
Aleshinloye	1,323 ± 301^{ab}	3,325 ± 639^{b}	2.51	9.25 (11.7)	< 0.001
Agbeni	1,217 ± 267^{ab}	3,055 ± 591^{b}	2.51	9.23 (11.5)	< 0.001
One-way analysis of variance across markets	$F(5, 84) = 5.41$; $p < 0.001$	$F(5, 54) = 4.28$; $p = 0.002$			

Note: Values are Mean ± Standard Deviation (CFU/g). Within a Column, Means Sharing a Superscript Letter do not Differ Significantly (Tukey's Test, $p > 0.05$). p-values Refer to Welch's t-test Between Nut Conditions Within Each Market. Fold increase is the Ratio of the Deteriorated Mean to the Healthy Mean.

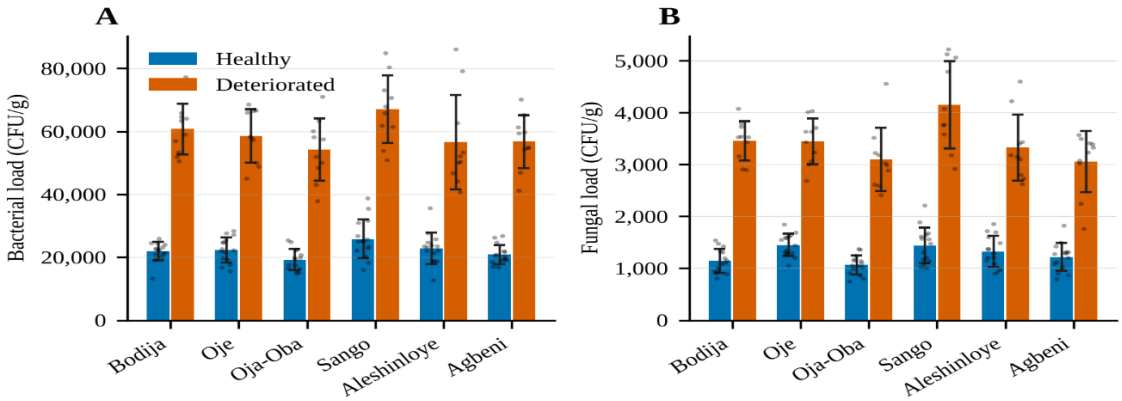


Figure 1: Mean (A) Bacterial Load and (B) Fungal Load of Healthy and Deteriorated Kolanuts in the Six Markets. Bars Show the Mean, Error Bars Show ± 1 Standard Deviation, and Dots are Individual Samples (healthy, n = 15; Deteriorated, n = 10 per Market)

The two-way analysis of variance (market × nut condition) confirmed that the difference between markets accounted for a much smaller but significant amount of variation in bacterial load (partial $\eta^2 = 0.14$) and fungal load (partial $\eta^2 = 0.21$), while nut condition alone accounted for an overwhelming amount of variation in both bacterial load (partial $\eta^2 = 0.87$) and fungal load (partial $\eta^2 = 0.87$). The interaction between market and nut condition did not influence bacterial load (p = 0.518) but was significant for fungal load ($F(5, 138) = 3.24$, p = 0.009); this means that there were greater between-market variations in fungal load in deteriorated nuts, largely due to the high fungal load values observed in Sango.

Prevalence of Organisms of Public Health Concern

Ninety-seven of the 150 samples (64.7%; 95% confidence interval, 56.7% to 71.9%) were positive for any of the four target organisms, and 79 samples (52.7%) were positive for any of the three bacterial pathogens (Table 3; Figure 2). All isolates of these organisms were identified by culture, biochemical and molecular techniques. Toxigenic *Aspergillus* species (47 samples; 31.3%) were the most common, followed by *Staphylococcus aureus* (45; 30.0%), *Escherichia coli* (36; 24.0%) and *Salmonella* species (17; 11.3%), while *Fusarium* species were found in 14 samples (9.3%). All organisms were significantly more common in the deteriorated nuts than in the healthy nuts, with odds ratios between 2.86 (*Staphylococcus aureus*) and 4.94 (toxigenic *Aspergillus* species). At least one target organism was recovered from 88.3% of deteriorated nuts and 48.9% of healthy nuts (odds ratio, 7.92; 95% confidence interval, 3.25 to 19.28; $\chi^2(1) = 24.51$, $p < 0.001$). Importantly, 44 out of the 90 apparently healthy nuts (48.9%) contained at least one target organism with 16 (17.8%) containing toxigenic *Aspergillus* species, showing that the absence of visible deterioration was not a guarantee of microbiological safety.

Table 3: Prevalence of Bacterial and Fungal Organisms of Public Health Concern in Healthy and Deteriorated Kolanuts

Organism	Healthy (n = 90)	Deteriorated (n = 60)	All samples (n = 150)	χ^2 (degrees of freedom = 1)	p	Odds ratio (95% confidence interval)
Escherichia coli	13 (14.4)	23 (38.3)	36 (24.0)	11.26	< 0.001	3.68 (1.68–8.07)
Salmonella species.	5 (5.6)	12 (20.0)	17 (11.3)	7.47	0.006	4.25 (1.41–12.79)
Staphylococcus aureus	19 (21.1)	26 (43.3)	45 (30.0)	8.47	0.004	2.86 (1.39–5.87)
Toxigenic *Aspergillus species.*	16 (17.8)	31 (51.7)	47 (31.3)	19.22	< 0.001	4.94 (2.36–10.37)
Fusarium species.	4 (4.4)	10 (16.7)	14 (9.3)	6.36	0.012	4.30 (1.28–14.43)

Organism	Healthy (*n* = 90)	Deteriorated (*n* = 60)	All samples (*n* = 150)	χ^2 (degrees of freedom = 1)	*p*	Odds ratio (95% confidence interval)
Any of the four target organisms	44 (48.9)	53 (88.3)	97 (64.7)	24.51	< 0.001	7.92 (3.25–19.28)

Note: Values are Number of Positive Samples (Percentage of Samples). *p*-Values are from Pearson's Chi-Square Test of Independence (All Expected Counts ≥ 5); Fisher's Exact Test Gave the same Conclusions. Odds Ratios Compare Deteriorated with Healthy Nuts, with Woolf 95% Confidence Intervals. *Fusarium species* were Screened for Fumonisin Production, and Toxigenic *Aspergillus species* for Aflatoxin Production.

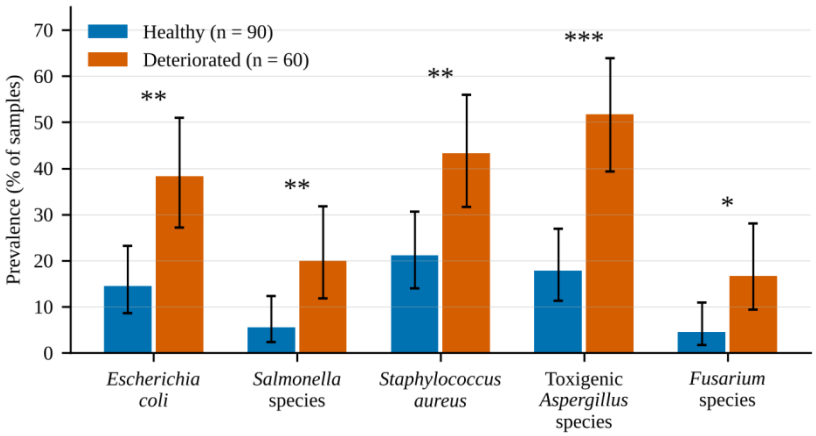


Figure 2: Prevalence of the Target Organisms in Healthy (*n* = 90) and Deteriorated (*n* = 60) Kolanuts. Error Bars Show Wilson 95% Confidence Intervals. Asterisks Denote Significant Differences Between Nut Conditions (Fisher's Exact Test: ∗ $p < 0.05$, ∗∗ $p < 0.01$, ∗∗∗ $p < 0.001$)

The prevalence of each organism did not differ significantly among the markets (Table 4; chi-square test, $p = 0.654$ to 0.997) and the proportion of samples that carried at least one of the target organisms varied only from 52.0% in Oje market to 72.0% in Oja-Oba and Agbeni ($p = 0.654$). Contamination was not confined to a single market but was distributed throughout all six markets.

Table 4: Number and Percentage of Samples (*n* = 25 Per Market) Positive for Each Organism, by Market

Organism	Bodija	Oje	Oja-Oba	Sango	Aleshinloye	Agbeni	*p*
Escherichia coli	8 (32.0)	4 (16.0)	7 (28.0)	5 (20.0)	5 (20.0)	7 (28.0)	0.757
Salmonella species.	3 (12.0)	3 (12.0)	2 (8.0)	3 (12.0)	3 (12.0)	3 (12.0)	0.997
Staphylococcus aureus	8 (32.0)	6 (24.0)	10 (40.0)	5 (20.0)	8 (32.0)	8 (32.0)	0.707
Toxigenic *Aspergillus species.*	8 (32.0)	6 (24.0)	11 (44.0)	8 (32.0)	6 (24.0)	8 (32.0)	0.680
Fusarium species.	1 (4.0)	2 (8.0)	4 (16.0)	3 (12.0)	2 (8.0)	2 (8.0)	0.773
Any target organism	16 (64.0)	13 (52.0)	18 (72.0)	17 (68.0)	15 (60.0)	18 (72.0)	0.654

Note: Values are Number of Positive Samples (Percentage). *p*-values are from the Chi-Square Test of Independence Across the Six Markets (Degrees of Freedom = 5); Expected Counts were Below 5 for *Salmonella species* and *Fusarium species.* Co-contamination was Common in Deteriorated Nuts: only 7 of 60 (11.7%) Carried None of the Four target Organisms, Whereas 26 (43.3%), 16 (26.7%), 10 (16.7%) and 1 (1.7%) carried ONE, Two, Three and Four, Respectively; the Corresponding Figures for Healthy Nuts were 46 (51.1%), 36 (40.0%), 7 (7.8%), 1 (1.1%) and None. The Mean Number of Target Organisms per Nut was 1.53 ± 0.96 in Deteriorated Nuts and 0.59 ± 0.69 in Healthy Nuts (Mann-Whitney $U = 4{,}178$, $p < 0.001$). Two or more target Organisms were Present in 27 of 60 (45.0%) Deteriorated Nuts and 8 of 90 (8.9%) Healthy Nuts (Fisher's exact test, $p < 0.001$), and One Deteriorated Nut Contained All Four Organisms. Among Organism Pairs, only *Escherichia coli* and Toxigenic *Aspergillus* Species were Significantly Associated with Each Other (17 of 36 *Escherichia coli*-positive Samples [47.2%] were Also Positive for Toxigenic *Aspergillus* Species Versus 30 of 114 *Escherichia coli*-Negative Samples [26.3%]; Fisher's Exact test, $p = 0.024$; $p \geq 0.17$ for All Other Pairs).

Antibiotic Susceptibility of Bacterial Isolates

The 45 *Staphylococcus aureus* and 53 Gram-negative (36 *Escherichia coli* and 17 *Salmonella* species) isolates were subjected to susceptibility testing. Among the *Staphylococcus aureus* isolates, the highest rates of resistance were observed to penicillin (71.1%) and erythromycin (42.2%) and the lowest rate to gentamicin (8.9%) (Table 5). Eleven isolates (24.4%; 95% confidence interval, 14.2% to 38.7%) were resistant to cefoxitin and were considered presumptive methicillin-resistant *Staphylococcus aureus*. For Gram-negative isolates (Table 6; Figure 3), resistance was most common to ampicillin (64.2%), followed by trimethoprim-sulfamethoxazole (50.9%) and tetracycline (45.3%), and least common to gentamicin (13.2%). Of the 53 Gram-negative isolates, 26 (49.1%) were resistant to at least one of the third-generation cephalosporins (ceftazidime or cefotaxime); 19 of

36 *Escherichia coli* (52.8%) and 7 of 17 *Salmonella* species (41.2%) were resistant, with only two resistant to both. Of the 53 Gram-negative isolates, 9 (17.0%) were resistant to ciprofloxacin. The resistance rates of *Escherichia coli* and *Salmonella* species were not significantly different for any antibiotic (Fisher's exact test, $p \geq 0.07$); *Escherichia coli* had numerically higher resistance to amoxicillin-clavulanate (44.4% versus 17.6%) and cefotaxime (36.1% versus 11.8%), and *Salmonella* species had numerically higher resistance to ceftazidime (35.3% versus 19.4%) and gentamicin (23.5% versus 8.3%).

Table 5: Antibiotic Resistance Among *Staphylococcus aureus* Isolates from Kolanuts (*n* = 45)

Antibiotic	Disc content	Antimicrobial class	Resistant, *n* (%)	95% confidence interval (%)
Penicillin	10 units	β-lactam	32 (71.1)	56.6–82.3
Erythromycin	15 µg	Macrolide	19 (42.2)	29.0–56.7
Tetracycline	30 µg	Tetracycline	17 (37.8)	25.1–52.4
Cefoxitin (presumptive methicillin-resistant *Staphylococcus aureus*)	30 µg	β-lactam (cephamycin)	11 (24.4)	14.2–38.7
Clindamycin	2 µg	Lincosamide	9 (20.0)	10.9–33.8
Chloramphenicol	30 µg	Phenicol	7 (15.6)	7.7–28.8
Ciprofloxacin	5 µg	Fluoroquinolone	6 (13.3)	6.3–26.2
Gentamicin	10 µg	Aminoglycoside	4 (8.9)	3.5–20.7

Note: Interpreted Using Clinical and Laboratory Standards Institute M100 (35th edition) Breakpoints. The 95% Confidence Interval is the Wilson Interval.

Table 6: Antibiotic Resistance Among Gram-negative Isolates from Kolanuts

Antibiotic	Disc content	*Escherichia coli* (*n* = 36)	*Salmonella species* (*n* = 17)	All Gram-negative (*n* = 53)	95% confidence interval (%)	*p*
Ampicillin	10 µg	21 (58.3)	13 (76.5)	34 (64.2)	50.7–75.7	0.235
Trimethoprim-sulfamethoxazole	1.25/23.75 µg	19 (52.8)	8 (47.1)	27 (50.9)	37.9–63.9	0.773
Tetracycline	30 µg	16 (44.4)	8 (47.1)	24 (45.3)	32.7–58.5	1.000
Amoxicillin-clavulanate	20/10 µg	16 (44.4)	3 (17.6)	19 (35.8)	24.3–49.3	0.072
Cefotaxime	30 µg	13 (36.1)	2 (11.8)	15 (28.3)	18.0–41.6	0.103
Ceftazidime	30 µg	7 (19.4)	6 (35.3)	13 (24.5)	14.9–37.6	0.306
Ciprofloxacin	5 µg	7 (19.4)	2 (11.8)	9 (17.0)	9.2–29.2	0.701
Gentamicin	10 µg	3 (8.3)	4 (23.5)	7 (13.2)	6.5–24.8	0.193

Note: Values are Number of Resistant Isolates (percentage). Interpreted using Clinical and Laboratory Standards Institute M100 (35th Edition) Breakpoints. The 95% confidence interval is the Wilson Interval for all Gram-negative Isolates; *p*, Fisher's Exact Test Comparing *Escherichia coli* with *Salmonella species*.

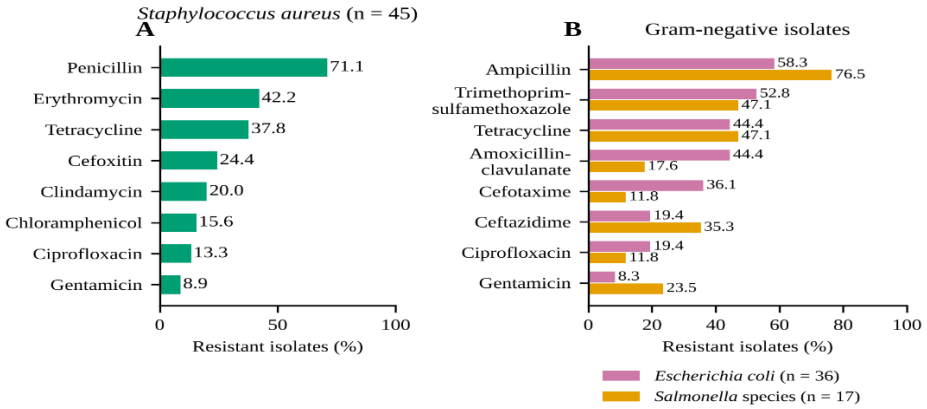


Figure 3: Percentage of (A) *Staphylococcus aureus* Isolates and (B) Gram-Negative Isolates (*Escherichia coli* and *Salmonella species*) Resistant to Each Antibiotic Tested

Multiple antibiotic resistance was common (Table 7; Figure 4). The mean multiple antibiotic resistance index was 0.292 ± 0.136 for *Staphylococcus aureus*, 0.354 ± 0.151 for *Escherichia coli* and 0.338 ± 0.152 for *Salmonella* species, and 38 of 45 *Staphylococcus aureus* isolates (84.4%) and 45 of 53 Gram-negative isolates (84.9%) had a multiple antibiotic resistance index above 0.2. The frequency of multidrug resistance and the multiple antibiotic resistance index were greater for Gram-negative isolates than for *Staphylococcus aureus* isolates (33 [62.3%] versus 15 [33.3%]; Fisher's exact test, $p = 0.005$; and Mann-Whitney U test, $p = 0.036$, respectively), while *Escherichia coli* and *Salmonella* species did not differ in multidrug resistance frequency ($p = 0.375$) or in multiple antibiotic resistance

index (p = 0.595). The mean multiple antibiotic resistance index for *Staphylococcus aureus* isolates from deteriorated nuts was higher than that for *Staphylococcus aureus* from healthy nuts (0.332 versus 0.237; p = 0.021), and the rate of multidrug resistance was also higher for *Staphylococcus aureus* from deteriorated nuts than for those from healthy nuts (12 of 26, 46.2%, versus 3 of 19, 15.8%, respectively; p = 0.054); there was no such difference among the Gram-negative isolates (0.346 versus 0.354; p = 0.900).

Table 7: Multiple Antibiotic Resistance Index and Multidrug Resistance Among Bacterial Isolates

Organism	*n*	multiple antibiotic resistance index, mean ± standard deviation	multiple antibiotic resistance index, median (range)	multiple antibiotic resistance > 0.2, *n* (%)	multidrug resistance, *n* (%)	Susceptible to all agents, *n* (%)
Staphylococcus aureus	45	0.292 ± 0.136	0.250 (0.000–0.625)	38 (84.4)	15 (33.3)	3 (6.7)
Escherichia coli	36	0.354 ± 0.151	0.375 (0.000–0.625)	31 (86.1)	24 (66.7)	2 (5.6)
Salmonella species.	17	0.338 ± 0.152	0.375 (0.125–0.625)	14 (82.4)	9 (52.9)	0 (0.0)
All Gram-negative	53	0.349 ± 0.150	0.375 (0.000–0.625)	45 (84.9)	33 (62.3)	2 (3.8)

Note: Multiple Antibiotic Resistance Index = Number of Antibiotics to which an Isolate was Resistant Divided by the Number Tested (eight per panel). Multidrug Resistance is Resistance to Three or More Antimicrobial Classes. Both the Gram-Positive and the Gram-Negative Panels Covered Seven Antimicrobial Classes.

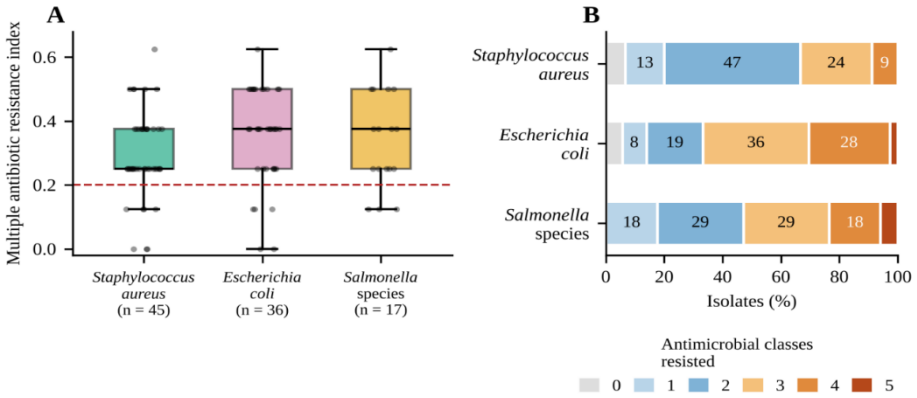


Figure 4: (A) Distribution of the Multiple Antibiotic Resistance Index Among *Staphylococcus aureus*, *Escherichia coli* and *Salmonella species* isolates; the Dashed Line Marks the Multiple Antibiotic Resistance Threshold of 0.2. (B) Percentage of Isolates Resistant to 0 to 5 Antimicrobial Classes; Isolates Resistant to Three or More Classes are Multidrug-Resistant

Mycotoxin Screening

Aflatoxins were detected in 22 of 47 toxigenic *Aspergillus* isolates (46.8%; 95% confidence interval, 33.3% to 60.8%), at levels from 4.2 to 52.6 µg/kg (mean 19.7 ± 12.8 µg/kg; median 18.9 µg/kg; Table 8; Figure 5). Sixteen positive samples (72.7% of positives; 34.0% of those screened) exceeded 10 µg/kg and 14 (63.6% of positives; 29.8% of those screened) exceeded 15 µg/kg, while three exceeded 30 µg/kg (36.9, 39.0 and 52.6 µg/kg). At least 10.7% of the 150 samples (16 of 150) contained more than 10 µg/kg of aflatoxins, with no difference in aflatoxin positivity between nut conditions (Fisher's exact test, p = 1.000), and the concentrations of aflatoxins were not significantly different (healthy, 21.0 ± 17.5 µg/kg; deteriorated, 19.1 ± 10.7 µg/kg; Mann-Whitney U test, p = 0.860). Five of the seven healthy nuts with positive results for aflatoxin had levels greater than 10 µg/kg, while four had levels greater than 15 µg/kg; there was no correlation between fungal load and level of aflatoxin (Spearman's ρ = 0.02, p = 0.912). The number of positive isolates differed among markets (permutation chi-square test, p = 0.008): none of the 8 isolates from Bodija was positive, while 8 of the 11 isolates from Oja-Oba were positive (72.7%).

Fumonisins were detected in 9 of 14 *Fusarium* isolates (64.3%; 95% confidence interval, 38.8% to 83.7%), at levels from 110 to 1,480 µg/kg (mean 670 ± 395 µg/kg; median 701 µg/kg). The positivity rates for isolates from healthy and deteriorated nuts were 50.0% (2 of 4) and 70.0% (7 of 10) respectively (Fisher's exact test, p = 0.580). The largest amount of fumonisin (1,480 µg/kg) was found in a healthy nut. Three samples were positive for both aflatoxins and fumonisins, and 28 of the 150 samples (18.7%) were positive for at least one toxin: 20 of 60 deteriorated nuts (33.3%) and 8 of 90 healthy nuts (8.9%) (Fisher's exact test, p < 0.001; Table 8).

Table 8: Aflatoxin and Fumonisin Levels in Kolanut Samples Positive for Toxigenic *Aspergillus species* and *Fusarium species*, by Nut Condition

Toxin (µg/kg)	Source nut	Isolates screened	Positive, *n* (%)	Mean ± standard deviation (µg/kg)	Median (µg/kg)	Range (µg/kg)
Aflatoxins (B_1 + B_2 + G_1 + G_2)	Healthy	16	7 (43.8)	21.0 ± 17.5	15.8	4.2–52.6
	Deteriorated	31	15 (48.4)	19.1 ± 10.7	20.0	4.2–39.0
	All isolates	47	22 (46.8)	19.7 ± 12.8	18.9	4.2–52.6
Fumonisins (B_1 + B_2)	Healthy	4	2 (50.0)	1,101 ± 536	1,101	722–1,480
	Deteriorated	10	7 (70.0)	547 ± 283	687	110–910
	All isolates	14	9 (64.3)	670 ± 395	701	110–1,480

Note: Levels were Measured by Enzyme-Linked Immunosorbent Assay; Mean, Median and Range are Calculated from Positive Isolates Only. Aflatoxins were Screened in Isolates of *Aspergillus flavus* and *Aspergillus parasiticus*, and fumonisins in *Fusarium* Isolates.

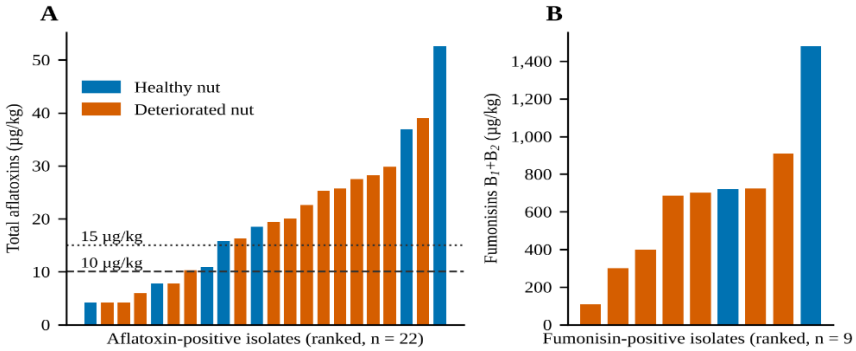


Figure 5: Enzyme-Linked Immunosorbent Assay-Reported Levels of (A) Total Aflatoxins and (B) Fumonisins B_1 + B_2 in Positive Isolates, Ranked from Lowest to Highest and Coloured by Nut Condition. Horizontal Lines in (A) Mark the 10 µg/kg and 15 µg/kg Limits used as Benchmarks

Discussion

Principal Findings

Deteriorated nuts contained, on average, 2.7 times more bacteria and fungi than healthy nuts and there was a greater prevalence of *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus* and toxigenic *Aspergillus* species in deteriorated nuts, and almost half of the apparently healthy nuts contained at least one of these organisms. Most of the bacterial isolates were resistant to multiple antibiotics, and aflatoxins and fumonisins were detected in 46.8% and 64.3% of the fungal isolates screened, respectively, with aflatoxin detection unrelated to visible deterioration. As a result, pathogenic, drug-resistant or toxigenic organisms may be present in kolanuts sold in open markets, and this cannot be determined by visual grading.

Microbial Load and Nut Condition

The higher microbial loads in deteriorated nuts (2.66-fold and 2.69-fold higher bacterial and fungal loads, respectively) are consistent with the lesions serving as sites of multiplication of microorganisms. Garuba et al. (2024) sampled microbes from kolanuts with lesions and found *Aspergillus wentii*, *Bacillus cereus* and *Bacillus subtilis*, implying that the lesions contain a complex microbial community comprising both fungi and bacteria. Aduama-Larbi et al. (2022) obtained 30 mould species from 14 genera from fresh and cured kolanuts from three regions in Ghana and observed that most of the genera isolated that are known mycotoxin producers were *Aspergillus*, *Fusarium* and *Penicillium* species, while Sulaimon et al. (2024) highlighted *Fusarium pallidoroseum* as a post-harvest threat to kolanut in Nigeria. The moisture content of the kolanuts (53.70% to 59.42%) reported by Aduama-Larbi et al. (2022) creates a favourable environment for microbial survival, and physical injury is likely to increase the opportunity for microbial colonisation. Moshood (2022) reported weevil attack on 30 to 100% of kolanuts and explained how fruit fly oviposition holes allow the entry of pathogens that cause rotting in kolanuts. Insect damage was not recorded in the present study and should be added to sampling in the future.

The loads varied only marginally between markets, with Sango having the highest, while the interaction between market and nut condition (fungal load) indicated that the market effect was more pronounced in deteriorated nuts. Turnover, storage time, ventilation and vendor practices are possible factors affecting the situation but were not measured. The contribution of nut condition (partial η^2 of 0.87 for both loads) was much greater than the contribution of market (0.14 and 0.21).

Pathogen Prevalence and Hygiene Implications

Overall, 24.0% of samples were positive for *Escherichia coli*, 11.3% for *Salmonella* species and 30.0% for *Staphylococcus aureus*. These proportions in kolanuts are within or below the ranges outlined in the summary of ready-to-eat street foods in Africa. In a scoping review of 40 studies, Onohuean et al. (2025) found antibiotic-resistant pathogenic bacteria in 5,338 of 11,653 samples (45.80%), with study-level prevalence ranging from 33.33% to 86.41% for *Escherichia coli*, 1.12% to 20.83% for *Salmonella* and 9.41% to 72.86% for *Staphylococcus aureus*. The majority of the foods studied in those experiments were either meat or mixed dishes, so only

a comparison of the order of magnitude is possible. Cowhide sold in a market in south-western Nigeria has been found to contain *Escherichia coli* (34.21%) and *Staphylococcus aureus* (26.31%) as the dominant organisms (Mensah-Agyei et al., 2024). Other local studies in Ibadan, of tiger nuts (Adenugba & Olusegun, 2024) and of *suya* meat (Oyewole et al., 2024), likewise found foods carrying bacteria of public health concern. The six markets had a nearly uniform distribution of contamination, indicating that the sources of contamination are shared along the supply chain and may include handling, packaging, contaminated water used to moisten nuts, or exposure to other produce. Food handlers are known carriers of resistant *Staphylococcus*, *Escherichia coli* and *Salmonella* (Viana et al., 2025), and kolanuts are handled by numerous people from harvest until they are sold.

One key finding was that a large proportion (48.9%) of apparently healthy nuts had at least one of the target organisms present, and 17.8% of these nuts contained toxigenic *Aspergillus* species. Healthy nuts were, however, less contaminated than deteriorated nuts, with odds ratios of 2.86 to 4.94 for each organism and 7.92 for any organism. Discarding nuts that appear spoilt therefore reduces, but does not eliminate, the hazard. Co-contamination was also significantly higher in the deteriorated nuts (45.0% of nuts had two or more organisms versus 8.9% of healthy nuts).

Antimicrobial Resistance

The resistance of Gram-negative isolates to ampicillin, trimethoprim-sulfamethoxazole and tetracycline, and *Staphylococcus aureus* to penicillin, erythromycin and tetracycline reflects the use of older, widely available and over-the-counter antibiotics, which is recognised as a source of resistance in Nigeria. In Nigeria, Awulu et al. (2025) reported that *Salmonella* species and *Escherichia coli* were the most critical pathogens posing a threat to human and animal health because of antimicrobial resistance, with easy access to antimicrobials and lack of infection prevention and control identified as the biggest challenges. The resistance levels observed in the present study resemble those of cowhide isolates from Ilishan-Remo with trimethoprim-sulfamethoxazole (as Septrin) having the highest resistance level (86.84%) and gentamicin the lowest (7.89%) (Mensah-Agyei et al., 2024), as gentamicin was also the least affected antibiotic in both of our panels (8.9% and 13.2%). Multidrug-resistant *Escherichia coli* with high-level resistance and virulence gene content has also been found in ready-to-eat food items in Lagos (Makinde et al., 2026); and reviews of African street foods have reported ampicillin, ciprofloxacin, chloramphenicol, tetracycline, gentamicin as frequently tested drugs, while *tet*, *blaCTX-M*, *blaTEM*, *sul1/sul2* and *mecA* were among the reported resistance genes (Onohuean et al., 2025).

Three observations are of special interest. First, 84.4% of *Staphylococcus aureus* and 84.9% of Gram-negative isolates had multiple antibiotic resistance indices above 0.2, a value traditionally considered the threshold for a high-risk source (where antibiotics are heavily used), and 62.3% of Gram-negative isolates met the definition of multidrug resistance (Magiorakos et al., 2012). Second, almost half of the Gram-negative isolates (49.1%) were resistant to third-generation cephalosporins, which is the phenotype of extended-spectrum β-lactamase producers, although this was not confirmed because synergy testing was not done. Third, 24.4% of *Staphylococcus aureus* isolates were resistant to cefoxitin and are presumptive methicillin-resistant *Staphylococcus aureus*, which should be confirmed by detection of the *mecA* gene or of penicillin-binding protein 2a. The higher frequency of multidrug resistance and higher multiple antibiotic resistance index of *Staphylococcus aureus* isolates from deteriorated nuts, compared with the similar distribution of both among Gram-negative isolates across nut conditions, indicates that the association of *Staphylococcus aureus* with the nuts may be due to longer exposure or poor handling of deteriorated nuts, while that of the Gram-negative isolates may be due to handlers, water or the market environment.

Mycotoxin Contamination

Aflatoxins were detected in almost half of the toxigenic *Aspergillus* isolates (46.8%), with 16 isolates having concentrations above 10 µg/kg. Zakari et al. (2025) found that fungal infection reduced the saponin level of kolanut from 0.836% to less than 0.046%, indicating that aflatoxin production and the loss of phytochemicals may be linked. Contamination of food with mycotoxins is a common occurrence in Nigeria (Imade et al., 2021), while enzyme-linked immunosorbent assay and high-performance liquid chromatography are the two major methods used in the detection of mycotoxins in Africa (Akinniyi, 2025; Akullo et al., 2025). The present study presents quantitative enzyme-linked immunosorbent assay values for kolanut, and thereby confirms that the levels of contamination could reach 52.6 µg/kg. For comparison, 100% of macadamia nuts and over 95% of cashew nuts met the limit of 10 µg/kg while the compliance of the groundnut kernels was 50% to 62% during 13 years of surveillance (Abong et al., 2025). The proportion of samples showing >10 µg/kg is closer to the proportion of non-compliant groundnut kernels (38 to 50%) than cashew and macadamia nuts (Abong et al., 2025). The comparison is only indicative, as there is no limit for kolanut and the commodities and denominators vary.

In practice, the results showed that aflatoxin positivity was comparable in healthy (43.8%) and deteriorated nuts (48.4%) and that the level of aflatoxin did not correlate with the amount of fungi present in the nuts ($\rho = 0.02$). This suggests that visual examination and total fungal count are not good indicators of toxicity and that toxigenic *Aspergillus* species may be present in nuts that appear sound. Aflatoxins are classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (International Agency for Research on Cancer, 2012) and therefore low-dose chronic exposure should be considered. Kolanut is used as a stimulant in ceremonies and trade in West Africa and is consumed raw, so stored nuts should not be chemically treated because of the unacceptable residues (George, 2025; Moshood, 2022). Prevention must take place before storage as well as during storage. Akullo et al. (2025) proposed biological control using atoxigenic *Aspergillus flavus* strains and decontamination using plant extracts as viable tools for aflatoxin control in Africa, and these should be explored for kolanut.

Fumonisins are considered possibly carcinogenic to humans (Group 2B; International Agency for Research on Cancer, 2002) and their biosynthesis, metabolism and effects on humans and animals have been reviewed by Anumudu et al. (2025). Two cautions apply. First, the sample size was small (14 isolates), the confidence interval was wide (38.8% to 83.7%) and there was little power to compare nut conditions. Second, fumonisin production is more commonly associated with other *Fusarium* species than *Fusarium pallidoroseum*, so some of the positive enzyme-linked immunosorbent assay results could be due to co-occurring producers or antibody cross-reactivity. Confirmation by liquid chromatography-mass spectrometry is therefore required before any exposure assessment is attempted. Nevertheless, 18.7% of all samples

(28 of 150) contained at least one toxin, and three samples contained both toxins, suggesting an additive hazard. The presence of the two types of hazards has also been observed in ready-to-eat food items sold in Lagos (Makinde et al., 2023), which supports the assessment of the two hazards in the same sample.

Comparison with Earlier Reports

Most of the reports on kolanut reviewed here have focused on the identification of fungi and provided little information on enteric bacterial pathogens, antibiotic resistance or quantified mycotoxins and the present study extends this work by covering all these hazards in a single sample set and by comparing healthy and visibly deteriorated nuts.

Public Health Significance

Kolanut is traded, given away and chewed at marriage ceremonies, religious ceremonies and social events, and it is often broken and shared with bare hands (George, 2025). Bacteria, resistant strains and toxins from contaminated nuts can then be passed on to consumers and vendors alike. Additionally, the use of kolanut and related species as feed additives (Ojediran & Ojediran, 2024) implies that the use of contaminated material in animal feed could be a concern, supporting the One Health approach advocated for by Awulu et al. (2025).

Strengths and Limitations

A strength of the study is that it combined culture, biochemical and molecular identification, standardised resistance panels and quantitative mycotoxin analysis in the same six markets with equal sampling effort. Some limitations also exist. Nuts were deliberately chosen, with a fixed number of healthy nuts (15) and deteriorated nuts (10) per market, so the comparison of prevalence between conditions is fair; however, the overall prevalence estimates reflect the sample design and should not be generalised to all of the kolanuts sold in Ibadan. No a priori power calculation was made for the sample size, which restricts the power of market-level comparisons, especially for *Salmonella* species and *Fusarium* species. Resistance was determined phenotypically and was not confirmed, and neither *mecA* nor any other resistance genes were tested for. The number of toxin-positive nuts in the total sample may be underestimated because only samples positive for toxigenic fungi were screened for mycotoxins, and confirmation by high-performance liquid chromatography was done only where available.

CONCLUSION

The aim of this study, to evaluate the bacterial and fungal pathogens associated with the sale of kolanuts in the selected markets of Ibadan and their public health significance, was achieved, and each of the specific objectives was met. The bacterial and fungal loads on kolanuts in the six markets were significantly higher in deteriorated kolanuts (59,002 ± 10,772 CFU/g and 3,423 ± 682 CFU/g, respectively) than in healthy kolanuts (22,167 ± 4,622 CFU/g and 1,273 ± 294 CFU/g, respectively). *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus* and toxigenic *Aspergillus* species were identified by culture, biochemical and molecular techniques and were significantly more likely to be present in the deteriorated nuts, while 48.9% of nuts that looked healthy contained at least one of the four target organisms. The multiple antibiotic resistance indices of most of the bacterial isolates were greater than 0.2, 62.3% of the Gram-negative isolates were multidrug-resistant, 49.1% of the Gram-negative isolates were resistant to third-generation cephalosporins, and 24.4% of the *Staphylococcus aureus* isolates were presumptive methicillin-resistant *Staphylococcus aureus*. Aflatoxins and fumonisins were found in 46.8% of the toxigenic *Aspergillus* isolates and 64.3% of the *Fusarium* isolates tested, respectively, and there was no correlation between aflatoxin positivity and visible deterioration or fungal load. Therefore, the consumption of kolanuts from open markets is an under-recognised source of exposure to pathogenic, antibiotic-resistant and toxigenic organisms, and visual sorting does not protect consumers from exposure.

Recommendations

i. Vendors and traders should immediately remove nuts that appear to be deteriorated, should not soak the nuts in unclean water, should wash their hands before touching the nuts, and should keep the nuts off the ground in clean, dry, ventilated storage containers.
ii. Consumers should not judge nuts by their appearance, should discard nuts with mould, rot or insect damage, and should wash nuts and hands before eating.
iii. National food safety authorities should consider adding kolanut to their routine microbiological and mycotoxin monitoring efforts, establish limits for these toxins in kolanut, and offer training on proper hygiene and storage practices for kolanut vendors.
iv. Post-harvest measures to minimise spoilage and toxigenic fungi should be explored and evaluated, such as biological control with atoxigenic strains of *Aspergillus*, improved drying and curing, and control of weevils and fruit flies, with care taken over chemical residues in an unprocessed commodity.
v. Further studies should investigate the genetic basis of antibiotic resistance, toxin presence, insect damage, storage conditions, seasonal variations, and dietary exposure to aflatoxins and fumonisins.
vi. Antimicrobial stewardship, such as limiting the over-the-counter sale of antibiotics, needs to be improved.

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