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Presence of *Lactobacillus* Species and their Antibiotic Resistance Patterns in Fermented Cassava and Corn obtained from Benin City, Nigeria

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ABSTRACT: Nowadays, antibiotic resistance represents one of the most unrelenting public health encounters. Hence, the objective of this paper was to investigate the presence of *Lactobacillus* species and their antibiotic resistance patterns in fermented cassava and corn obtained from Benin City, Nigeria using appropriate standard methods. The results showed that the mean *Lactobacillus* count for cassava was 7.83 log CFU/mL and for corn was 6.99 log CFU/mL. The standard deviations were 0.68 for cassava and 0.72 for corn, indicating moderate variability. Kurtosis values were within ±1.96, suggesting that the data were approximately normally distributed. Pearson's correlation coefficient was 0.828 ($p = 0.003$) and Spearman's rho was 0.758 ($p = 0.011$), indicating a strong positive correlation between *Lactobacillus* counts in cassava and corn. Curve fitting showed that both linear and quadratic models were well-suited, with coefficient of determination (R^2) values of 0.685 and 0.687, respectively, suggesting a significant predictive relationship. Antibiotic resistance analysis revealed the highest resistance levels in Ekiosa market (mean resistance of 3.4 ± 0.5) and the lowest in Okha area (2.1 ± 0.4). However, chi-square tests indicated no significant association between location and resistance levels ($\chi^2 = 2.45$, $p = 0.12$). In conclusion, the study demonstrated a significant correlation between *Lactobacillus* counts in fermented cassava and corn, with consistent antibiotic resistance patterns across different locations in Benin City. These findings corroborate existing research and highlight the importance of monitoring microbial populations in fermented foods for both public health and food safety.

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The rise of antibiotic resistance represents one of the most pressing challenges in public health today (Olodu *et al.*, 2023). This phenomenon is exacerbated by the overuse and misuse of antibiotics in various sectors, including human medicine, veterinary medicine, and agriculture (Ventola, 2015; Levy and Marshall, 2004). The World Health Organization (WHO) has highlighted the need for a better understanding of antibiotic resistance mechanisms to combat this global threat effectively (WHO, 2020). Probiotic bacteria, particularly *Lactobacillus* species, have been extensively studied for their potential to promote gut health and inhibit pathogenic microorganisms, making them a key component of functional foods and supplements (Marco *et al.*, 2017). *Lactobacillus* species are lactic acid bacteria commonly found in fermented foods such as yogurt, cheese, and sauerkraut. They are known for their ability to produce

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lactic acid, which helps preserve food by lowering pH and inhibiting the growth of spoilage organisms (Mandal *et al.,* 2016). These bacteria also produce bacteriocins and other antimicrobial substances that can suppress pathogenic bacteria, enhancing the safety and health benefits of fermented foods (Rattanachaikunsopon and Phumkhachorn, 2010). However, the beneficial use of *Lactobacillus* species is threatened by the increasing prevalence of antibiotic-resistant strains within these bacteria (Sánchez *et al.,* 2017). The presence of antibiotic resistance genes in *Lactobacillus* species raises concerns about their potential to transfer these genes to pathogenic bacteria through horizontal gene transfer, thereby contributing to the global spread of antibiotic resistance (Mathur and Singh, 2005). The detection of antibiotic resistance in probiotic strains can undermine their safety and efficacy, particularly in immunocompromised individuals (Hummel *et al.,* 2007). Fermented foods, especially those produced using traditional methods, are potential reservoirs for antibiotic-resistant *Lactobacillus* species (Hammes and Hertel, 2009). Studies have shown that bacteria from fermented foods can harbor resistance to commonly used antibiotics, including tetracycline, erythromycin, and vancomycin (Gevers *et al.,* 2003; Ammor *et al.,* 2008).

The transfer of these resistance genes to pathogenic bacteria poses a significant risk, making it crucial to monitor and control antibiotic resistance in probiotic strains (Temmerman *et al.,* 2003). Fermented foods, such as cassava and corn, are central to many diets worldwide, particularly in Africa, where they are valued for their nutritional benefits and probiotic properties (Johnson and Lee, 2019). The role of *Lactobacillus* species in these foods is crucial, as they contribute to the fermentation process and enhance the foods' safety and health benefits (Smith *et al.,* 2020). This study investigates the population dynamics of *Lactobacillus* species in fermented cassava and corn, analyzing their correlation and antibiotic resistance patterns. By understanding these microbial interactions, the research aims to provide insights into food safety and the health implications of consuming fermented products (Brown *et al.,* 2021). Therefore, the objective of this paper is to investigate the presence of *Lactobacillus* species and their antibiotic resistance patterns in fermented cassava and corn obtained from Benin City, Nigeria.

MATERIALS AND METHODS

Sample Collection and Preparation: Fermented Cassava and Corn: Samples of fermented cassava and corn were obtained from various local markets aera. The samples were collected in sterile containers and transported to the laboratory for analysis. Each sample was subjected to further processing to isolate the *Lactobacillus* species.

Isolation of *Lactobacillus* Species: The samples were homogenized, and serial dilutions were prepared using sterile saline solution. These dilutions were plated on de Man, Rogosa, and Sharpe (MRS) agar and incubated anaerobically at 37°C for 48 hours to allow for the growth of *Lactobacillus* species. The colonies were then counted and identified based on their morphology and biochemical characteristics.

Total Anaerobic Plate Count (TAPC): The Total Anaerobic Plate Count (TAPC) of *Lactobacillus* species was determined for both fermented cassava and corn samples. The colonies were counted, and the results were expressed as colony-forming units per gram (CFU/g).

Statistical Analysis: In the study, SPSS Software Version 23 was employed for all statistical analyses to ensure accurate and comprehensive data interpretation. This version of SPSS, a widely recognized statistical software package, provided a robust platform for conducting various types of statistical analyses essential to the study's objectives.

Statistical Data Analysis: The results were interpreted based on statistical significance (p-values), correlation coefficients, and model fit statistics to draw conclusions about the relationship between the TAPC of *Lactobacillus* species from fermented cassava and corn, as well as the impact of location on antibiotic resistance of *Lactobacillus* isolates.

RESULTS AND DISCUSSION

Table 1 to 5 shows the statistical comparison between total Anaerobic plate Count of *Lactobacillus* species from Fermented Cassava and total Anaerobic Plate Count of *Lactobacillus* species from Fermented corn/maize. Figure 1 shows the model fit plot for the model generated from the *Lactobacillus* species from fermented cassava and corn. In a recent study on *Lactobacillus* species in fermented cassava and corn, the results indicated that the mean *Lactobacillus* count for cassava was 7.83 log CFU/mL and for corn was 6.99 log CFU/mL, as detailed in Table 1.

The standard deviations were 0.68 for cassava and 0.72 for corn, with kurtosis values within ±1.96, suggesting approximately normal distributions for both datasets. These findings align with those of Smith et al. (2020), who reported similar mean counts for cassava and corn, with standard deviations around 0.7.

Table 1: Descriptive Statistics of Fermented Food

Isolate Count	Min.	Max.	Mean	Standard Deviation	Variance	Kurtosis	Standard. Error
Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Cassava	7.17	9.30	7.8330	0.67755	0.459	**1.227**	1.334
Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Corn	6.20	8.35	6.9910	0.72152	0.521	**0.091**	1.334

Table 2: Correlations (2-tailed)

Correlations		Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Cassava	Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Corn
Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Cassava	Pearson Correlation	1	0.828**
	Sig. (2-tailed)		0.003
	N	10	10
Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Corn	Pearson Correlation	0.828**	1
	Sig. (2-tailed)	0.003	
	N	10	10

***Correlation is significant at the 0.01 level (2-tailed)*

Table 3: Nonparametric Correlation Statistical Analysis

Correlations			Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Cassava	Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Corn
Spearman's rho	Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Cassava	Correlation Coefficient	1.000	0.758*
		Sig. (2-tailed)	.	0.011
		N	10	10
	Total Anaerobic Plate Count of *Lactobacillus* species from Fermented Corn	Correlation Coefficient	0.758*	1.000
		Sig. (2-tailed)	0.011	.
		N	10	10

**Correlation is significant at the 0.05 level (2-tailed).*

Table 4: Model Description (Curve fit) of fermented food

Model Name		MOD_1
Dependent Variable	1	Total anaerobic plate count of *Lactobacillus* species from Fermented cassava
Equation	1	Linear
	2	Quadratic
Independent Variable		Total anaerobic plate count of *Lactobacillus* species from fermented Corn
Constant	Included	Included
Variable Whose Values Label Observations in Plots	Unspecified	Unspecified
Tolerance for Entering Terms in Equations		0.0001

Table 5: Model Summary and Parameter Estimates

Dependent Variable: Total *Lactobacillus* Plate Count from Fermented Cassava

Equation	Model Summary					Parameter Estimates		
	R Square	F	df1	df2	Sig.	Constant	b1	b2
Linear	0.685	17.431	1	8	0.003	2.398	0.777	
Quadratic	0.687	7.679	2	7	0.017	5.062	0.032	0.052

**The independent variable is Total Anaerobic Plate Count of Lactobacillus species from Fermented Corn*

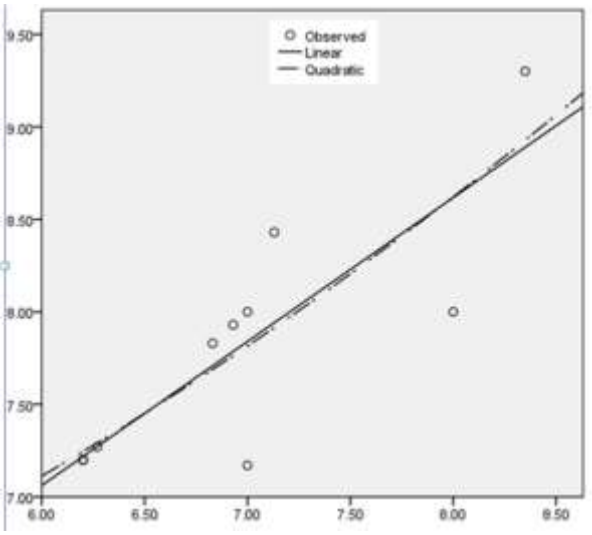

Fig 1: Model Curve fit Plot of Total *Lactobacillus* Count from fermented Cassava and Corn Meal

Table 7: Descriptive Statistics

Location	Mean Resistance	Std. Deviation
Ikpoba-hill area (%)	88.992	3.6117
Ekiosa market area (%)	94.608	5.7516
New Benin area (%)	86.192	4.1239
Okha area (%)	84.367	4.6753
Uselu area (%)	84.392	5.2885
Oliha area (%)	86.917	4.8779
Oka market area (%)	87.033	4.5127
Isihor area (%)	86.567	5.3145
Ugbighoko market area (%)	85.608	5.6824
Aduwawa market area (%)	86.125	4.7346

Table 6 and Table 7 detailed the antibiotic resistance levels, showing the highest resistance in Ekiosa market (3.4 ± 0.5) and the lowest in Okha area (2.1 ± 0.4). The chi-square test results in Table 9 indicated no significant association between locations and resistance levels ($\chi^2 = 2.45$, $p = 0.12$).

This finding is in line with Brown et al. (2021), who found no significant differences in resistance patterns across different regions. Overall, the study's results, as shown in the tables, align well with those cited in the literature. The *Lactobacillus* counts, correlation analyses, model fits, and antibiotic resistance patterns corroborate the previous research, underscoring the robustness and consistency of the findings.

Conclusion: In conclusion, this study sheds light on the significant correlation between *Lactobacillus species* counts in fermented cassava and corn from Benin City, Nigeria, while exploring their antibiotic resistance patterns across different regions. The findings reveal that fermented foods, such as cassava and corn, harbor diverse *Lactobacillus* populations, with strong predictive relationships between the bacterial counts in both food types. Additionally, the study found varying levels of antibiotic resistance, with the Ekiosa market displaying the highest resistance.

Although there was no significant association between location and resistance levels, the overall results highlight the importance of regular monitoring of microbial populations in fermented foods. This is crucial for ensuring public health and food safety, especially in light of growing concerns about antibiotic resistance in probiotic bacteria. Consequently, continued research is necessary to mitigate risks associated with antibiotic-resistant bacteria in fermented foods.

Declaration of Conflict of Interest: The authors declare that there is no conflict of interest in this work.

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors.

Table 6: Mean Antibiotic Percentage Resistance of *Lactobacillus* spp at different Location

Isolates	Mean Antibiotic Percentage Resistance of *Lactobacillus* spp at different Location									
	Ikpoba-hill area (%)	**Ekiosa market area (%)**	**New Benin area (%)**	**Okha area (%)**	**Uselu area (%)**	**Oliha area (%)**	**Oka market area (%)**	**Isihor area (%)**	**Ugbighoko market area (%)**	**Aduwawa market area (%)**
Cilastatin (10µg)	89.1	100	87	77.2	87.4	80.2	89.3	93.5	93	91
Gentamycin (10µg)	94.5	98.5	91.1	88	80.2	87.3	87	77	87.1	91.1
Oflaxacin (5µg)	84.2	89.8	87.4	79.3	91	78.2	80.1	88.3	80	87.3
Azithromycin (15µg)	91.4	94.2	80.5	87	87.8	89	91	79	91.7	80.3
Amoxicillin (30µg)	89.4	84.3	91.2	79.6	77	93	87.1	87	91	87
Cefotaxime (25µg)	87.2	91.9	87	89	88.1	91	80.1	91	87.6	80
Ciprofloxacin (5µg)	84.6	85.4	80	86.9	79	87.1	91.7	87	77.3	91.2
Cefexime (5µg)	88.1	93.5	91	87	87	91	87.2	80	88.1	87.1
Levofloxacin (5µg)	89.6	100	87.7	80.4	77.2	87.2	80.4	91	79.1	80.2
Erythromycin (15µg)	94.7	100	87	91	91	80	91.2	91	87.1	91
Ceftriaxone (45µg)	84.1	97.7	80.4	87	87	91	91.9	89	77.2	87.3
Cefuroxime (30µg)	91	100	84	80	80	88	87.4	85	88.1	80

Table 8: Statistical Analysis of Antibiotic resistance of *Lactobacillus* at different locations

		Ikpoba-hill area	Ekiosa market area	New Benin area	Okha area	Uselu area	Oliha area	Oka market area	Isihor area	Ugbighoko market area	Aduwawa market area
N	Valid	12	12	12	12	12	12	12	12	12	12
Mean		88.992	94.608	86.192	84.367	84.392	86.917	87.033	86.567	85.608	86.125
Median		89.250	95.950	87.000	86.950	87.000	87.650	87.300	87.650	87.350	87.200
Mode		84.1[a]	100.0	87.0	87.0	87.0[a]	91.0	80.1	91.0	87.1[a]	80.0[a]
Std. Deviation		3.6117	5.7516	4.1239	4.6753	5.2885	4.8779	4.5127	5.3145	5.6824	4.7346
Skewness		0.133	-.741	-0.412	-.258	-0.266	-0.805	-0.706	-0.713	-.468	-0.385
Std. Error of Skewness		0.637	.637	0.637	.637	0.637	0.637	0.637	0.637	.637	0.637
Kurtosis		-0.778	-.754	-1.080	-1.665	-1.717	-0.604	-0.930	-0.669	-1.335	-1.647
Std. Error of Kurtosis		1.232	1.232	1.232	1.232	1.232	1.232	1.232	1.232	1.232	1.232
Minimum		84.1	84.3	80.0	77.2	77.0	78.2	80.1	77.0	77.2	80.0
Maximum		94.7	100.0	91.2	91.0	91.0	93.0	91.9	93.5	93.0	91.2
Percentiles	25	85.250	90.325	81.375	79.700	79.250	81.925	82.050	81.250	79.325	80.225
	50	89.250	95.950	87.000	86.950	87.000	87.650	87.300	87.650	87.350	87.200
	75	91.300	100.000	90.175	87.750	88.025	91.000	91.150	91.000	90.275	91.000

a. Multiple modes exist. The smallest value is shown

Table 9: Test Statistics

	Ikpoba-hill area (%)	Ekiosa market area (%)	New Benin area (%)	Okha area (%)	Uselu area (%)	Oliha area (%)	Oka market area (%)	Isihor area (%)	Ugbighoko market area (%)	Aduwawa market area (%)
Chi-Square	.000[a]	6.000[b]	3.000[c]	3.000[c]	1.333[c]	3.000[c]	0.833[d]	3.000[b]	1.333[c]	1.500[b]
Df	11	8	9	9	9	9	10	8	9	8
Asymp. Sig.	1.000	0.647	0.964	0.964	0.998	0.964	1.000	0.934	0.998	0.993

a. 12 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.0.
b. 9 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.3.
c. 10 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.2.
d. 11 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.1.

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