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Antibacterial Susceptibility Pattern of Bacteria Isolated from Ready-to-Eat Lettuce and Gurasa Sold within Kaduna State University (Main Campus), Kaduna State, Nigeria

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Abstract

The consumption of ready-to-eat lettuce and gurasa has gained acceptance *due to its appealing taste and nutritional value. However, these foods can serve as a vehicle for transmission of pathogens. This study was carried out to assess the antibacterial susceptibility pattern of pathogens isolated from lettuce and gurasa sold at Kaduna State University. Samples were collected from five vendors of gurasa and lettuce. Standard microbiological methods were carried out using a pour plate and a modified Kirby-Bauer disc diffusion method to determine the antibacterial susceptibility pattern. The isolates obtained were examined for morphological and biochemical characteristics. Escherichia coli, Bacillus spp, Salmonella spp, Staphylococcus aureus, and Klebsiella spp. were isolated and identified. Staphylococcus aureus was the most prevalent bacterium in this study, having 27.7% in lettuce and 38.4% in gurasa, and Escherichia coli, having 16.6% in lettuce and 30.7% in gurasa. There was no significant difference (P > 0.05) in the total colony counts of bacteria among the samples. Lettuce had the least bacteria count (1.48 x 10^6 CFU/g), while gurasa had the highest (1.55 x 10^6 CFU/g). Antimicrobial sensitivity test results showed that 10 S. aureus isolates were resistant to Rocephin (100%), 7 E. coli isolates were resistant to Septrin, Amoxicillin, and Augmentin (100%), 6 Klebsiella spp isolates were resistant to Amoxicillin and Augmentin(100%), 5 Salmonella spp isolates were resistant to Septrin, Amoxicillin and Augmentin (100%) and 3 Bacillus spp were resistant to Rocephin (100%). The high bacterial resistance to antibiotics is of great concern as infections with these organisms could be lethal.*

Keywords: Ready-to-Eat, Lettuce, Gurasa, Kaduna State University, Resistance, Pathogens.

INTRODUCTION

Ready-to-eat food is ready for immediate consumption at the point of sale (Omoloya and Adeleke, 2013). Ready-to-eat food could be raw or cooked, hot or chilled, and consumed without further heat treatment (Clarence *et al.*, 2009). Different terms have been used to describe such ready-to-eat food; these include convenient, ready, instant and fast foods. Such ready-to-eat foods include moi-moi, jollof-rice, lettuce salad, pastries, meat pie, and *gurasa*. Self-service restaurants where food is served ready to be consumed are liable to have some products contaminated by pathogenic microorganisms causing foodborne diseases (Her *et al.*, 2019). Food safety is a growing concern for consumers and professionals in the food and food service industry (Ruqaya *et al.*, 2016).

Gurasa is a delicacy introduced to Kano by settlers from the Kingdom of Saudi Arabia, who settled around the ancient Dala Hills Kano (Idoko *et al.*, 2022). Flatbread is made from flour, yeast, baking powder, and egg. It can be made with wheat flour or a combination of the two. It is similar to making bread; however, the dough for *gurasa* is lighter than that of bread. *Gurasa* can be fried or baked using a locally made oval earthenware pot known as tanderu (Idoko *et al.*, 2022). According to Gocmen (2009), flatbreads are usually made with high-extraction flour, usually of low specific volume with a high crust-to-crumb ratio. When fried, it becomes circular while the edges become brownish. It becomes tough if exposed to air or kept for over three days after production. It can be consumed in contrast to regular consumption patterns. Like, "You can eat *Gurasa* with tea if you like with vegetable soup, it can also be eaten with pepper soup, even with suya meat, you will realize that every Suya seller sells *Gurasa* (Marcano *et al.*, 2010). Some *Gurasa* can be eaten with fried eggs (Idoko *et al.*, 2022).

According to Nafisat and Mustapha (2018), *Gurasa* is locally made bread. Leavened bread is made with aerated yeasted viscosity dough, which expands by the action of gas produced by the yeast fermentation process to gain volume and decrease its density. *Gurasa's* top crust has many small blisters. It was formally adjudged the rich man's specialty and known to be found on the dining tables of the royals and the elites in society in the early 80s (Nafisat *et al.*, 2015). *Gurasa* sold by vendors are likely to harbor some microbial contamination, such as bacteria constituting of cause of chronic or life-threatening illness. Some microbial contamination responsible for food-borne diseases include cholera, gastroenteritis, salmonellosis, shigellosis, and typhoid fever (Ruqayya *et al.*, 2016).

Lettuce (*Lactuca sativa*) is consumed worldwide and is important in the Nigerian market. This leafy vegetable has beneficent qualities for health due to its fiber rates and antioxidant properties (Pereira *et al.*, 2013). Since the lettuce is consumed raw, adequate hygiene processes should be undertaken to eliminate pathogen microorganisms (Bennett *et al.*, 2013). The lettuce has been associated with contamination by certain pathogenic microorganisms such as *Salmonella spp.*, *Escherichia coli*, and *Listeria monocytogenes* (Jeddi *et al.*, 2014). Lettuce is one of the most widely used vegetable crops in our diets. We consume lettuce as a base for salads, sandwiches, and burgers to add texture and even as a garnish to decorate food trays at parties. In addition to salad greens, lettuce has multiple purposes in the kitchen. It can be used to roll appetizers, such as a cabbage roll. Similarly, it can be rolled like sushi in place of nori. Even more, although most people don't consider lettuce as an ingredient in soup, cream of lettuce soup is a tasty and refreshing dish (Pereira *et al.*, 2013).

Pathogens resistant to multiple classes of antibiotics are considered multidrug-resistant (MDR) or, more colloquially, superbugs. Microbes, rather than people, develop resistance to antibiotics (CDC, 2009). According to D'Costa *et al.* 2011, Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21st Century, in particular as it pertains to pathogenic organisms (the term is especially relevant to organisms that cause disease in humans). In the simplest cases, drug-resistant organisms may have acquired resistance to first-line antibiotics, necessitating second-line agents. Typically, a first-line agent is selected based on several factors, including safety, availability, and cost; a second-line agent is usually broader in the spectrum, has a less favourable risk-benefit profile, and is more expensive or maybe locally unavailable. In the case of some MDR pathogens, resistance to second and even third-line antibiotics is thus sequentially acquired, a case quintessentially illustrated by *Staphylococcus aureus* in some nosocomial settings. Many antibiotic resistance genes reside on transmissible plasmids, facilitating their transfer. Exposure to an antibiotic naturally selects for the survival of the organisms with the genes for resistance. In this way, a gene for antibiotic resistance may readily spread through an ecosystem of bacteria. Antibiotic-resistance plasmids frequently contain genes conferring resistance to several different antibiotics. However, the increasing prevalence of antibiotic-resistant bacterial infections in clinical practice stems from antibiotic use within human and veterinary medicine. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off. As antibiotic resistance becomes more common, a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies, there has been a continued decline in the number of newly approved drugs. Antibiotic resistance, therefore, poses a significant problem (Donadio *et al.*, 2010).

MATERIALS AND METHODS

Sample Collection

Samples were collected from five gurasa vendors and five ready-to-eat lettuce vendors, making ten samples. The sample was collected aseptically and immediately transported to Kaduna State University Microbiology Department Laboratory for analysis.

Microbiological Assessment

Total bacterial aerobic plate count

From each dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, 1.0mL was dispensed into a sterile Petri dish, and 25mls of nutrient agar was added, which was allowed to gel. The plates were incubated at 37^{0}C for 24 hours. All colonies that grew on the agar are considered, then counted, and expressed as colony-forming units per gram (cfu/g) (Adesiyun *et al.*, 2005).

Isolation of Bacteria from Ready-to-eat Lettuce and *Gurasa*

Each set of samples for the serial dilution was prepared by introducing 1.0g each of ready-to-eat lettuce and *gurasa* into a test tube containing 9.0 mL of sterilized distilled water to form a stock solution. The test tubes were labeled 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4,}$ and $l0^{-5}$ for samples A, B, and C, respectively. Nutrient agar was prepared according to the manufacturer's instructions. Using the pour plating technique, 1.0 mL of each labeled sample was aseptically pipetted onto Petri dishes containing the prepared culture media and were incubated at 37ºC for 24 hours.(Adesiyun*et al.*, 2005).

Identification of the Isolates

The pure colonies obtained in the bijou slant bottles were subjected to biochemical tests to identify the isolates. The biochemical tests carried out include Catalase, Coagulase Indole, Methyl-red, and Citrate utilization, as described by (Oyeleke and Manga, 2008).

Antimicrobial Susceptibility Testing

Mueller Hinton Agar was prepared according to the manufacturer's instructions. Susceptibility testing was conducted using the disk diffusion technique on Muller Hilton Agar (MHA) using a standard method by the Clinical and Laboratory Standard Institute (CLSI, 2020). Inoculation was carried out by dipping a sterile swab into the inoculum suspension adjusted to a turbidity of 0.5 McFarland standards (10^8 cells/mL), and the agar surface was streaked across in four directions. Antibiotic discs were placed on the streaked media, after which the plate was incubated at 37ºC for 24 hours (Asthana *et al.*, 2014). The gram-positive antimicrobial agents to be tested are composed of antimicrobial disc: ampicillin (10 µg), penicillin (10 units), oxacillin (1 µg), clindamycin (2 µg), sulfamethoxazole/trimethoprim (23.75/1.25 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), tetracycline (30 µg), vancomycin (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), rifampicin (5 µg).While the Gram negative antimicrobial agents tested composed of ampicillin (10 µg), penicillin (10 units), ofloxacin (5 µg), cefazolin (30 µg), ceftazidime (30 µg), cefdinir (5 µg), cefuroxime (30 µg), gatifloxacin (5 µg), cotrimoxazole (25 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), linezolid (30 µg), meropenem (10 µg), (CLSI, 2020).

Disk Diffusion Method

Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets, or strips into the solid culture Muller Hinton Agar that was seeded with the selected inoculum isolated in a pure culture and incubated (Jorgensen and Turnidge, 2015). The diffusion of the antimicrobial agent into the seeded culture media resulted in a gradient of the antimicrobial disk diffusion, which was based on the determination of an inhibition zone proportional to the bacterial susceptibility of the antimicrobial present in the disk after incubation for 24 hours. It was observed using the Clinical and Laboratory Standard Institute (CLSI) as a guideline.

RESULTS

Bacterial counts of the isolates.

Ready-to-eat *gurasa* had the highest count (1.55 x 10^6 CFU/g), while lettuce had the lowest (1.48 x 10^6 CFU/g). Statistical analysis shows no significant difference ($P > 0.05$) in the total colony count of bacteria between the samples. The results are expressed as the mean of duplicate samples (Table 1).

Biochemical characterization of the bacterial isolates.

The biochemical results of bacterial isolates from ready-to-eat lettuce and *gurasa* are shown in Table 2. *Escherichia coli* was reactive to methyl red, catalase, and citrate positive. *Bacillus spp* was reactive to methyl red and indole test. *Staphylococcus aureus* was reactive to methyl red, citrate, urease, catalase, and coagulase test. *Salmonella spp* were reactive to methyl red and catalase, and *Klebsiella spp* were reactive to citrate, urease, and catalase test.

Frequency occurrence of the isolates

The percentage occurrence of bacteria isolates in ready-to-eat lettuce and *gurasa* is presented in Table 3. The result shows that *Staphylococcus aureus* was the most prevalent bacterium, accounting for 100% of lettuce and 100% of *gurasa*, followed by *Escherichia coli,* accounting for 160% of lettuce and 80% of *gurasa*. *Bacillus spp* had the lowest prevalence among the isolates accounting for 40% in lettuce and 20% in *gurasa*.

Antibiotic Susceptibility Pattern of Gram-Negative and Gram-Positive Bacteria

The antibiotic susceptibility of Gram-negative bacteria is shown in Table 4. *Escherichia coli* was 100% resistant to Septrin, Amoxicillin and Augmentin. *Klebsiella spp* was 100% resistant to Amoxicillin and Augmentin. *Salmonella spp* was 100% resistant to Septrin, Amoxicillin and Augmentin.The antibiotic susceptibility of the Gram-positive bacteria is in Table 5. *Bacillus spp* and *Staphylococcus aureus* were 100% resistant to Rocephin.

Table 1: Total bacterial counts of ready-to-eat lettuce and *gurasa*

Sample	1	2	3	4	5	Mean (CFU/g)
Lettuce	78	102	218	244	98	1.48×10^6
Gurasa	240	32	176	53	276	1.55×10^6

P=0.905

Table 2: Biochemical Characteristics of the bacterial isolates from ready-to-eat lettuce and *gurasa*

Methyl Red	Citrate	Urease	Indole	Catalase	Coagulase	Suspected organism
+	-	+	-	+	-	*Escherichia coli*
+	-	-	+	-	-	*Bacillus spp*
+	+	+	-	+	+	*Staphylococcus aureus*
+	-	-	-	+	-	*Salmonella spp*
-	+	+	-	+	-	*Klebsiella spp*

Keys: - Negative
+ Positive

Table 3: Percentage occurrence of bacteria isolated from ready-to-eat lettuce and *gurasa*

Bacteria Isolate	**Positive Isolates**		**Occurrence (%)**	
	Lettuce	***Gurasa***	**Lettuce**	***Gurasa***
Escherichia coli	3	4	60	80
Salmonella spp	4	1	80	20
Staphylococcus spp	5	5	100	100
Klebsiella spp	4	2	80	40
Bacillus spp	2	1	40	20

Table 4: Antibiotic susceptibility of the Gram-negative isolates

Antibiotics	Discs conc.(µg)	Zone of inhibition (mm)								
		Escherichia coli			*Klebsiella spp*			*Salmonella spp*		
		n = 7			n = 6			n = 5		
		R	I	S	R	I	S	R	I	S
Septrin	3	7(100%)	0(0.00)	0(0.00)	3(50%)	1(16.67%)	2(33.33%)	5(100%)	0(0.00)	0(0.00)
Chloramphenicol	30	3(42.86%)	4(57.14%)	0(0.00)	0(0.00)	4(66.67%)	2(33.33%)	4(80%)	1(20%)	0(0.00)
Sparfloxacin	10	6(85.71%)	1(14.29%)	0(0.00)	1(16.67%)	3(50%)	2(33.33%)	3(60%)	2(40%)	0(0.00)
Ciprofloxacin	30	1(14.29%)	3(42.85%)	3(42.85%)	2(33.33%)	2(33.33%)	2(33.33%)	1(20%)	1(20%)	3(50%)
Amoxicillin	30	7(100%)	0(0.00)	0(0.00)	6(100%)	0(0.00)	0(0.00)	5(100%)	0(0.00)	0(0.00)
Augmentin	10	7(100%)	0(0.00)	0(0.00)	6(100%)	0(0.00)	0(0.00)	5(100%)	0(0.00)	0(0.00)
Gentamycin	30	5(71.43%)	2(28.57%)	0(0.00)	1(16.67%)	5(83.33%)	0(0.00)	0(0.00)	5(100%)	0(0.00)
Pefloxacin	30	3(42.86%)	4(57.14%)	0(0.00)	0(0.00)	3(50%)	3(50%)	2(40%)	2(40%)	1(20%)
Tariuid	10	5(71.43%)	1(14.28%)	1(14.28%)	2(33.33%)	2(33.33%)	2(33.33%)	2(40%)	3(60%)	0(0.00)
Streptomycin	30	5(71.43%)	2(28.57%)	0(0.00)	3(50%)	3(50%)	0(0.00)	1(20%)	4(80%)	0(0.00)

Table 5: Antibiotic susceptibility of the Gram-positive isolates

Antibiotics	Discs conc. (µ g)	Zone of inhibition (mm)					
		Bacillus spp.			*Staphylococcus spp*		
		n = 3			n = 10		
		R	I	S	R	I	S
Pefloxacin	10	1(33.33%)	2(66.67%)	0(0.00)	0(0.00)	5(50%)	5(50%)
Gentamycin	10	0(0.00)	1(33.33%)	2(66.67%)	2(20%)	5(50%)	3(30%)
Ampiclox	30	2(66.67%)	0(0.00)	1(33.33%)	7(70%)	3(30%)	0(0.00)
Amoxicillin	30	0(0.00)	1(33.33%)	2(66.67%)	7(70%)	3(30%)	0(0.00)
Ciprofloxacin	10	0(0.00)	1(33.33%)	2(66.67%)	4(40%)	4(40%)	2(20%)
Streptomycin	30	1(33.33%)	2(66.67%)	0(0.00)	5(50%)	5(50%)	0(0.00)
Septrin	30	0(0.00)	1(33.33%)	2(66.67%)	3(30%)	4(40%)	3(30%)
Erythromycin	10	0(0.00)	1(33.33%)	2(66.67%)	6(60%)	4(40%)	0(0.00)
Rocephin	25	3(100%)	0(0.00)	0(0.00)	10(100%)	0(0.00)	0(0.00)
Zinnacef	20	0(0.00)	2(66.67%)	1(33.33%)	4(40%)	1(10%)	4(50%)

DISCUSSION

The consumption of ready-to-eat *lettuce and gurasa* is increasing daily due to their appealing taste and nutritional value. However, ready-to-eat *lettuce and gurasa* can serve as a vehicle for transmitting pathogens when contaminated. In the findings of this study, the bacteria associated with ready-to-eat lettuce and *gurasa* were *Escherichia coli*, *Bacillus spp.*, *Salmonella spp*, *Staphylococcus spp.*, *and Klebsiella spp.* The results obtained from this study conform to the findings of Oluwasanmi *et al.* (2019), who isolated similar microorganisms in ready-to-eat lettuce and salads.

In this study, statistical data analysis shows no significant difference (P > 0.05) in the total colony counts of bacteria among the samples. Ready-to-eat *gurasa* had the highest bacteria colony counts (1.55 x 10^6 CFU/g), while lettuce had the least (1.48 x 10^6 CFU/g). According to the International Commission for Microbiological Specification for Foods, the acceptable plate count of ready-to-eat foods is between 0-103 (ICMSF, 2006). Therefore, ready-to-eat gurasa in this study are unfit or unacceptable for humans, while lettuce is tolerable. The high microbial load in ready-to-eat *gurasa* and lettuce could be associated with inadequate handling and processing by vendors, contamination caused by storage facilities, poor hygiene, and water used. Similarly, the extensive mixing during processing could have introduced contaminants through food handlers, utensils, and the environment.

Staphylococcus aureus was the most prevalent bacterium in this study. This result aligns with the study of Osamwonyi *et al.* (2013), who reported a higher prevalence of *Staphylococcus spp* and *Escherichia coli* in ready-to-eat vegetable salad. The high prevalence of *Staphylococcus spp* may be because these organisms are normal inhabitant of the human skin, nasal passage, throat and hair, and could easily contaminate food products during handling and preparation. Outside the body, *Staphylococcus spp* can survive in a dry state for long periods, making it one of the most resistant non-spore-forming pathogens. *Staphylococcus spp* is regarded as the main source of food contamination through direct contact or respiratory secretions (Bennett *et al.*, 2013). Some *Staphylococcus* strains are enterotoxigenic; ingesting food contaminated with the toxin is one of the leading causes of global food poisoning (Bennett *et al.*, 2013).

Escherichia coli may indicate unsanitary conditions and a dirty environment where these ready-to-eat lettuce and *gurasa* are processed and hawked. *Escherichia coli*, *Salmonella spp,* and *Klebsiella spp* indicate fecal contamination during processing. Vendors mostly use untreated tap and well water to prepare lettuce and *gurasa*. This untreated water can serve as a means of contamination of these food products during preparation (Amusa and Ashaye, 2019).

The antibiotic susceptibility pattern of the bacterial isolates in the study showed multi-antibiotic resistance, as all the isolates were resistant to more than two classes of antibiotics. These could be attributed, amongst other factors, to possessing resistance genes. *Escherichia coli* and *Klebsiellaspp* were the most resistant Gram-negative bacteria, while *Bacillus spp* was the most resistant Gram-positive. These bacteria evolved different mechanisms that confer resistance to antibiotics. *Escherichia coli* can produce extended-spectrum beta-lactamase (ESBL), which makes it resistant to antibiotics that contain beta-lactams (e.g., cephalosporins, monobactams, etc.) (Boyko *et al.,* 2015). *Klebsiella* strains, on the other hand, have genes that confer carbapenem resistance (e.g., imipenem, ertapenem, and meropenem). *Bacillus* are a rapidly evolving group of β-lactamases. These enzymes can break down the active ingredients by cleaving the beta-lactam ring of penicillins and cephalosporin antibiotics, resulting in the inactivation of these drugs (Geiser *et al.,* 2021).

CONCLUSION

This study has indicated that ready-to-eat lettuce and *gurasa* contain bacteria. Ready-to-eat *gurasa* had the highest bacteria colony counts (1.55 x 10^6 CFU/g). The bacteria isolated and identified include *Escherichia coli*, *Bacillusspp*, *Salmonella spp*, *Klebsiella spp,* and *Staphylococcus spp*. *Staphylococcus aureus* was the most prevalent bacterial isolate, accounting for 27.7% in lettuce and 38.4% in *gurasa*, followed by *Escherichia coli*, accounting for 16.6% in lettuce and 30.7% in *gurasa*. The antibiotic susceptibility pattern of the bacterial isolates in this study showed multi-antibiotic resistance, as all the isolates were resistant to more than two classes of antibiotics.

RECOMMENDATION

In line with the findings of this study, Ready-to-eat food vendors on the campus should be educated on food safety principles and the provision of basic facilities such as running water, toilets, proper storage, and waste disposal facilities at preparation and service points.

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