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Isolation, Identification, and Characterization of Bacterial Isolates in Garlic, Ginger, Pepper, and Turmeric Commonly Traded Within Ilorin, Kwara State, Nigeria

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ABSTRACT: Food condiments/spices consisting of leaves, flowers, seeds or stems of plants are food additives that add special aromas and flavors to food thereby increasing its taste but could harbor an array of microbes. Hence, the objective of this paper was to isolate, identify, and characterize the bacterial isolates in food condiments (Garlic, Ginger, Pepper, and Turmeric) commonly traded within Ilorin, Kwara State, Nigeria using various standard microbial techniques. The highest total heterotrophic count (THC) was 21.52 ± 5.31 Cfu/ml obtained from the samples at location A. The highest total coliform count (TCC) was found in garlic sample (6.67 ± 4.93 Cfu/ml) obtained from location B. The highest total *Staphylococcus* count (TSC) was found in garlic sample (4.00 ± 1.00 Cfu/ml) obtained from location C. The highest Total *Salmonella-Shigella* Count of (4.67 ± 3.06 Cfu/ml) was found in garlic sample obtained from location A. The highest total fungi count (TFC) was found in pepper sample (4.13 ± 2.06 Cfu/ml) obtained from location A. The presence of various pathogenic bacteria in the food condiments analyzed raises concern on their impact to the health of the consumers and these food spices could also serve as a vehicle for the transmission of disease causing bacteria.

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Condiments are food additives that add special aromas and flavors to food thereby increasing its taste. They are widely used for culinary purposes, medicinal purposes, and to ease digestion. Condiment consists of leaves, flowers, seeds or stems of plants. Food condiments give pleasant aroma to soups, sauces and other prepared dishes worldwide, especially in most African countries and India where protein calorie malnutrition is a major problem (Jiang, 2019). The present study is based on four type of condiments; pepper, ginger, garlic, and turmeric. Pepper is grown throughout Nigeria, there are different varieties and their level of spiciness differs. Pepper could be used as it is, in a powder or paste form. Dry peppers are added to various dishes giving the dish a red hue thus making it more attractive (Nadeem *et al.,* 2019). Garlic (*Allium sativum*) is a member of the family Liliaceae is used as both a condiment and as a medication for more than 4000 years (Agarwal, 2017). Probably originating in Central Asia, it was used by Sumerians, Egyptians, Greeks, and Romans, reaching modern Europe and the United States, and is widely used today (Shang *et al.,* 2019). Besides its taste and aroma, garlic is also associated with inhibition of lipid peroxidation and improvements in the functioning of the

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cardiovascular system, and is thought to have antiviral, antifungal, antimicrobial, and anti-tumorigenic properties (Melguizo-Rodriguez *et al.,* 2022). Turmeric is the dried rhizome of an herbaceous plant. It is mainly used for making the color of the dishes (Chattopadhyay *et al.,* 2019). Ginger (*Zingiber officinale*), originally from Southeast Asia, has been introduced to many parts of the world and is one of the most used spices, including for medicinal purposes (Gamage *et al.,* 2022). Ginger use has been widespread around the world since the Medieval period, and ginger is regularly consumed in other tropical countries, such as Nigeria, Sierra Leone, Indonesia, Bangladesh, Australia, Fiji, Jamaica, Nepal, Haiti, Mexico, and Hawaii (Abbasi *et al.,* 2019). Ginger rhizome is typically consumed as a fresh paste, dried powder, condiments preserved in syrup, candy (crystallized ginger), or as tea flavoring (Chrubasik *et al.,* 2018; Shahrajabian *et al.,* 2019). During cleaning and processing procedure of condiment, there is progressive reduction in the number and types of microorganisms; those remaining are usually aerobic spore-forming bacteria and common moulds (Lee *et al.,* 2018). In addition to the contamination of raw food supplies that occurs during growing, shipping and processing, there is the problem of food contamination caused by people who are carriers of pathogens such as *Escherichia coli* and *Staphylococcus aureus*. Coliform bacteria occur sporadically and usually in small populations in condiments and are associated with fecal contamination (Afrin *et al.,* 2019). Yeast and mould population differ with respect to the individual condiments, but are usually quite low (Ogur, 2022). An array of pathogens has been found in food condiments in the market, especially *Salmonella* species, *Bacillus cereus* and *Clostridium perfringens*. There have also been several disease outbreaks associated with food condiments with infants and children being the primary population impacted by 33 percent of the food condiments attributed outbreaks, including the largest (~1 000 illnesses) outbreak (Parveen *et al.,* 2014). The outbreaks attributed to food condiments are likely underreported (WHO, 2022). Condiments are known to harbour a vast reservoir of drug resistant enteric bacteria (Ogur, 2022), and infections/diseases that arise from the consumption of these Multidrug Resistance (MDR) bacteria-laden condiments can lead to failure of conventional treatments, longer treatments and death. They may as well serve as a potential transfer route of the antibiotic resistant bacteria and resistant genes into human food-chain and environment. Considering their fast emergence in recent times, resulting in both community-acquired and nosocomial infections (Bakobie *et al.,* 2017), and the paucity of information about their activity in condiments with respect to Ilorin, Kwara State, Nigeria, it therefore becomes imperative to investigate and report this for public health enlightenment. Therefore, the objective of this paper is to isolate, identify, and characterize the bacterial isolates in food condiments (Garlic, Ginger, Pepper, and Turmeric) commonly traded within Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS

Sample Collection: Samples of condiments (ginger, garlic, turmeric and dried pepper) were collected from three different markets in Kwara State, Nigeria. The markets were Oja-oba market, Ganmo market and Yoruba market. The samples were collected into sterile container and transported immediately to the Microbiology laboratory of the Department of Biological Sciences (SCHOOL) for analysis (Cicero *et al*., 2022).

Isolation of Bacteria: The method used for isolation of bacteria was pour plate. Serial 10 fold (decimal) dilutions of sample suspension of up to 10^{-9} were made i.e. one gram of each sample was dissolved in 9 ml of sterile distilled water. It was agitated for 2 minutes. This form 10^{-1} dilution, from this dilution, 1ml of the Aliquot using a sterile pipette was transferred into fresh 9ml of distilled water in sterile test-tube. Subsequent dilutions were made up to 10^{-9}. This procedure was repeated for other samples (Tasnia and Aftab, 2020).

Plating Out a Sample Dilution Series by the Pour Plate Method: Sterile Petri-dishes (in duplicates) were labelled with the dilution 10^{-2}, 10^{-4}, 10^{-5} and 1ml of the diluent was aseptically withdrawn from the series of dilutions and transferred into appropriate labelled sterile plate. Then 19ml of the molten Nutrient Agar (NA) was aseptically poured on it and rocked gently to allow even distribution of the dilution and the medium were allowed to set on the bench and the plates were incubated in inverted position at 37°C for 24hours. This procedure was repeated for other samples (Tasnia and Aftab, 2020).

Enumeration of Bacteria Colonies: After the incubation period, the numbers of bacteria colonies was counted using a colony counter. The number of colonies on a plate was multiplied by the dilution factor to give the plate count per ml of the sample (Ijeoma and Chika, 2024).

Sub-Culturing: After the incubation period of 24 hours, the Petri-dishes were brought out and were

observed for growth of bacteria with the aid of sterile wire loop, a small portion was picked and streaked onto a freshly prepared culture media to get a pure culture (Bruslind, 2021).

Identification of Bacteria Cells: This was done using the classical method i.e. cultural characterization of bacteria cells, Gram staining, motility test as well as biochemical characterization (such as methyl red test, indole test, oxidase test, starch hydrolysis test, glucose test, urease test (Ijeoma and Chika, 2024).

Antibiotic Susceptibility Test of Isolates: The bacterial isolates were tested for antibiotic susceptibility against ten antibacterial drugs by disc diffusion assay on Mueller-Hinton agar plates. The antibiotic paper disc includes pefloxacin, cefalexin, ampiclox, zinnacef, amoxacillin, rifampin, ciproflaxacin, streptomycin, trimethoprim-sulfamethoxazole and erythromycin. Muller-Hinton agar was prepared and distributed into McCartney bottles and sterilized in an autoclave at 121ºC for 30 minutes. The sterile medium was allowed to cool at 45ºC, and was then mixed uniformly with 1 ml standardized isolates; the mixture was then introduced into sterile Petri-dishes. Each plate was allowed to stand in a horizontal position until the agar solidified. Five antibiotic paper discs were placed in each Petri-dish. Within 15 minutes of application of the disc, the plates were inverted and incubated at 37ºC for 18-24 hours. The zone of inhibition for individual antibiotics was recorded (Gajic *et al*., 2022).

RESULTS AND DISCUSSION

Bacteria Count and Fungi Count on Sample of Location A, B, C and D: The total heterotrophic count (THC) obtained from the samples at location A, B and C ranged from 1.38 ± 0.38 to 21.52 ± 5.31 Cfu/ml; with the highest total heterotrophic count (THC) observed in garlic (21.52 ± 5.31 Cfu/ml) obtained from location A. The total coliform count (TCC) obtained from the samples of location A, B and C ranged from 1.33 ± 0.52 to 6.67 ± 4.93 Cfu/ml.

The highest total coliform count (TCC) was found in garlic sample (6.67 ± 4.93 Cfu/ml) obtained from location B. The total *Staphylococcus* count (TSC) obtained from the samples at location A, B and C ranged from 1.00 ± 1.00 to 4.00 ± 1.00 Cfu/ml. The total *Staphylococcus* count (TSC) was found to be highest in garlic sample (4.00 ± 1.00 Cfu/ml) obtained from location C.

The total *Salmonella-Shigella* count (TSSC) obtained from the samples of location A, B and C ranged from 1.33 ± 0.58 to 4.67 ± 3.06 Cfu/ml. The total *Salmonella-Shigella* count (TSSC) was highest in garlic sample (4.67 ± 3.06 Cfu/ml) obtained from location A. There was no total feacal count (TFeC) in all four food condiment samples (dried ginger, turmeric, garlic and pepper) obtained from location A, B and C. The total fungi count (TFC) obtained from the samples at location A, B and C ranged from 1.33 ± 0.57 to 4.13 ± 2.06 Cfu/ml.

The total fungi count (TFC) was highest in pepper sample (4.13 ± 2.06 Cfu/ml) obtained from location A.

Bacteria Isolated from Food Condiment Samples at Location A, B and C: The bacteria isolated from food condiment at location A was *Bacillus subtilis, B. cereus, Pseudomonas aeruginosa, B. licheniformis, C. perfringens, B. coagulase* and *P. florenscence* (Table 2A). Those obtained from location B was *Salmonella enterica, B. subtilis, B. licheniformis*, *P. putida, B. cereus, B. coagulans* (Table 2B) and *P. aeruginosa* and from location C was *S. enterica, B. subtilis, C. septicum, B. licheniformis*, *P. aeruginosa, C. perfringens* and *B. cereus* (Table 2C).

Table 1: Microorganisms in Food Samples Obtained From Location, A, B and C

		Means Population x 10^5 cfu/ml					
Location	**Sample**	**THC**	**TCC**	**TSC**	**TSSC**	**TFeC**	**TfC**
A	A	21.52±5.31^b	3.53±1.53^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
	B	6.22±2.05^a	1.66±0.43^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
	C	12.44±2.55^a	5.76±1.42^b	3.36±1.52^a	0.00±0.00^a	0.00±0.00^a	4.13±2.06^a
	D	5.03±2.01^a	3.25±0.42^a	2.15±1.03^a	0.00±0.00^a	0.00±0.00^a	1.53±0.35^a
B	A	1.38±0.38^a	6.67±4.93^a	0.00±0.00^a	4.67±3.06^b	0.00±0.00^a	0.00±0.00^a
	B	3.00±1.00^a	1.33±0.52^a	1.00±1.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
	C	8.00±4.00^b	4.33±3.21^a	0.00±0.00^a	1.33±0.58^a	0.00±0.00^a	1.63±0.44^b
	D	3.00±1.00^a	1.39±0.58^a	1.00±1.00^a	0.00±0.00^a	0.00±0.00^a	1.43±1.53^a
C	A	13.04±2.64^a	6.63±1.62^a	4.00±1.00^b	0.00±0.00^a	0.00±0.00^a	2.69±1.62^b
	B	8.04±2.00^a	3.66±1.17^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a	0.00±0.00^a
	C	9.58±5.63^a	2.36±0.53^a	0.00±0,00^a	3.55±2.65^a	0.00±0.00^a	0.00±0.00^a
	D	4.00±2.00^a	2.07±0.33^a	2.01±1.00^a	0.00±0.00^a	0.00±0.00^a	2.44±0.23^b

Values are means ± SD of replicate (n =4). Values with different alphabets are significantly different at (α < 0.05)

Key: *THC: - Total Heterotrophic count, TCC: - Total coliform count TSC: - Total Staphylococcus count, TSSC: - Total Salmonella shigella count, TFeC: - Total Feacal count TFC: - Total Fungi count, A = Garlic, B = Ginger, C = Pepper, D = Turmeric*

Table 2A: Biochemical Characterization of Bacteria Isolate from Sample of Location A

Sample	Bacterial Isolate	Ca	O	I	Mr	U	Co	Mt	Gl	Gr	Probable Microorganism
A	A1	+	+	-	-	-	-	+	+	+	*B. subtilis*
	A2	+	+	-	-	-	-	+	+	+	*B. cereus*
B	B1	+	+	-	+	-	-	+	+	+	*P. aeruginosa*
	B2	+	+	-	-	+	-	+	+	+	*B. subtilis*
	B3	+	-	-	-	-	-	+	+	+	*B. licheniformis*
	B4	+	+	-	-	-	-	+	+	+	*B. cereus*
C	C1	+	-	-	-	-	-	+	+	+	*C. perfringens*
	C2	+	+	-	-	-	-	+	+	+	*B. coagulase*
	C3	+	+	-	-	-	-	+	+	-	*P. florenscence*
D	D1	+	+	-	-	-	-	+	+	+	*B. cereus*
	D2	+	-	-	+	-	-	-	+	+	*C. perfringens*
	D3	+	+	-	+	-	-	-	+	+	*B. coagulase*

Key: *Ca = Catalase, O = Oxidase, I = Indole, Mr = Methyl red, U = Urease, Co = Coagulase; Mt = Motility, Gl = Glucose, Gr = Gram reaction*

Table 2B: Biochemical Characterization of Bacteria Isolate from Sample of Location B

Sample	Bacterial Isolate	Ca	O	I	Mr	U	Co	Mt	Gl	Gr	Probable Microorganism
A	A1	+	-	-	-	-	-	+	-	+	*S. enterica*
	A2	+	+	-	-	-	-	+	+	+	*B. subtilis*
	A3	+	-	-	-	-	-	+	+	+	*C. perfringens*
B	B1	+	+	-	-	+	-	+	+	+	*B. subtilis*
	B2	+	-	-	-	-	-	+	+	-	*B. licheniformis*
	B3	+	+	-	+	-	-	+	+	+	*P. putida*
C	C1	+	-	-	-	-	-	+	-	+	*S. enterica*
	C2	+	+	-	-	-	-	+	+	+	*B. cereus*
D	D1	+	+	-	-	-	-	+	+	+	*B. coagulans*
	D2	+	+	-	+	-	-	+	+	-	*P. aeruginosa*

Table 2C: Biochemical Characterization of Bacteria Isolate from Sample of Location C

Sample	Bacterial Isolate	Ca	O	I	Mr	U	Co	Mt	Gl	Gr	Probable Microorganism
A	A1	+	-	-	-	-	-	+	-	+	*S. enterica*
	A2	+	+	-	-	-	-	+	+	+	*B. subtilis*
	A3	+	+	-	-	-	-	+	+	+	*C. septicum*
B	B1	+	-	-	-	-	-	+	+	+	*B. licheniformis*
	B2	+	+	-	-	-	-	+	+	+	*B. subtilis*
C	C1	+	-	-	-	-	-	+	-	+	*S. enterica*
	C2	+	+	-	+	-	-	+	+	+	*P. aeruginosa*
D	D1	+	-	-	+	-	-	-	+	+	*C. perfringens*
	D2	+	+	-	-	-	-	+	+	+	*B. cereus*

Antibiotic Susceptibility Test on Bacteria Isolated from Food Condiment Samples at Location A, B and C: The results of antibiotic susceptibility test on isolated bacteria from location A, B and C is shown in Table 3. *Bacillus subtilis, B. licheniformis, C. perferinges, B. coagulans, P. putida, C. septidium* and *S. enterica* were susceptible to pefloxacin however *B. cereus, P. aerugenosa* and *P. florescens* were resistant to pefloxacin. *B. subtilis, B. cereus P. aerugenosa* and *P. florescens* were resistant to cefalexin. All isolated bacteria were susceptible to ampiclox except *P. putida* and *C. septidium* which were resistant ampiclox. *P. aerugenosa*, *P. florescens*, *P. putida* and *S. enterica* were resistant to amoxacillin. All isolated bacteria were resistant to Zinnacef and Rifampin. All isolated bacteria are susceptible to ciprofloxacin. *P. aerugenosa*, *C. perferinges*, *B. coagulans* and *S. enterica* are resistant to streptomycin. *B. subtilis*, *P. aerugenosa*, *C. perferinges*, *B. coagulans*, *P. putida*, *C. septidium* and *S. enterica* were resistant to trimethoprim-sulfamethoxazole. All isolated bacteria were resistant to erythromycin except *B. coagulans.* It was observed from this study that the sampled food spices contained an array of microorganisms. The total heterotrophic, total coliform, total *Salmonella-Shigella* and total *Staphylococcus* counts was highest in the garlic sample. This is contrary to the report of Sospedra *et al.* (2010) who reported zero count of bacteria in food spices such as thyme, basil, cinnamom, clove, turmeric, ginger, aniseed and dry parsley. The total fungal count was highest in pepper sample, this is similar to the findings of Ogur (2022) who reported high counts (3.82 ± 0.30 log cfu/g) of molds and yeasts in red pepper samples and contrary to the report of Khan *et al*. (2020) who reported high incidence of mold and yeast counts in chopped parsley (5.24 $\log_{10}$ cfu/g) and less than 4.0 $\log_{10}$ cfu/g in red and black pepper.

The predominant bacteria isolated from this study was *Bacillus* spp, this is similar to the study of Omorodion (2020), who reported high counts of *Bacillus* (38.4%). However, the high counts of *Pseudomonas* spp and *Clostridium* spp observed in this study is contrary to the findings of Omorodion (2020) who reported a count of 24.0% and 23.07% of *Micrococcus* and *Staphylococcus* respectively. The findings of this study is similar to that of Nwobi *et al.* (2024) who reported the predominance of *Bacillus* spp, *Pseudomonas* spp and *Micrococcus* spp in the food condiments (ogiri, ukpaka and okpei) sold in selected markets in Enugu state. The predominant bacteria observed in this study have been reported to be major cause of diseases in humans as such their presence in the food spices pose serious health risk to the consumer.

Table 3: Antibiotic Sensitivity Test on Bacteria Isolated From Samples at Location A, B and C

	Antibiotics/ Zone of Inhibition(mm)									
Bacterial isolates	**PEF**	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**S**	**SXT**	**E**
B. subtilis	10	-	8	-	10	-	14	8	-	-
B. cereus	-	-	15	-	4	-	10	6	2	-
P. aerugenosa	-	-	6	-	-	-	12	-	-	-
B. licheniformis	8	12	12	-	14	-	8	2	4	-
C. perfringens	6	10	14	-	10	-	4	-	-	-
B. coagulans	12	8	12	-	10	-	2	-	-	8
P. florescens	-	-	5	-	-	-	6	6	4	-
P. putida	5	6	-	-	-	-	8	4	-	-
C. septidium	4	10	-	-	6	-	10	6	-	-
S. enterica	7	3	4	-	-	-	4	-	-	-

Keys: PEF: Pefloxacin, CN: Cefalexin, APX: Ampiclox, Z: Zinnacef, AM: Amoxacillin, R: Rifampin, CPX: Ciproflaxacin, S: Streptomycin, SXT: Trimethoprim-sulfamethoxazole; E: Erythromycin; Zones of inhibition ≤ 15 = Resistant (CLSI, 2015)

The antibiogram result shows that the bacteria isolated from this study were all resistant to the various antibiotics according to the standard provided in CLSI (2015). The resistance of *Bacillus* spp to all the antibiotics disagrees with the findings of Banerjee and Sakar (2004) who reported that 8% of the total strains of *B. cereus* tested were susceptible to Polymyxin B. However, similar to this finding is the report of the same author on 26 strains of *C. perfringens* resistant to 11 antibiotics among which is glycoside, lactone, quinolone, gentamycin, kanamycin and streptomycin. The findings here was also contrary to the report of Khan *et al.* (2019) who reported that the Gram-positive bacteria were relatively susceptible to Levofloxacin and Ceftriaxone, however Vancomycin and Methicillin were relatively less active against the Gram positive bacteria and Ciprofloxacin, Cefoxitin, Choramphenicol and Ceclor had highest activity against the Gram-negative bacteria.

Conclusion: This study isolated bacteria from garlic, ginger, pepper, and turmeric sold in markets. The presence of bacteria highlights the risk of microbial contamination. Proper handling, storage, and processing are crucial to ensure safety and quality.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

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