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ASSESSMENT OF THE MICROBIAL PROFILE OF SELECTED COMMERCIALLY PREPARED FOOD SPICES IN NIGERIA

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ABSTRACT: *The microbial profiles and antibiogram patterns of some commercially prepared food spices in Nigeria were evaluated in this study. Ten selected food spices (cinnamon, coriander, black pepper, chilli pepper, suya spice, salad cream, paprika, parsley, mint and basil) were purchased from the popular Oja-Oba market in Akure, Nigeria. Standard microbiological assays were used to identify and quantify microorganisms in the spices. The antibiotic sensitivity of bacterial and fungal isolates was tested using the disk diffusion method. Twelve bacterial species (Staphylococcus aureus, Enterococcus faecalis, Lactobacillus plantarum, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus mirabilis, Enterobacter aerogenes, Bacillus cereus, Clostridium perfringens, Klebsiella pneumoniae and Citrobacter freundii) were isolated from the food spices. Bacillus cereus and Enterobacter aerogenes were the most prevalent, while Proteus mirabilis, Pseudomonas aeruginosa, and Clostridium perfringens were the least frequently encountered bacterial species. Four Fungal species (Aspergillus niger, Aspergillus fumigatus, Saccharomyces cerevisiae, and Fusarium oxysporum) were also isolated from the food spices, with Aspergillus niger and Saccharomyces cerevisiae the most frequently encountered and Aspergillus fumigatus the least regularly encountered fungal species. The bacterial load of the food spices ranged from 8.0 x 103 to 9.0 x 105 CFU/ml, while the mean fungal count ranged from 2.0 x 103 to 1.2 x 105 SFU/ml. Antibiogram analysis revealed that Pefloxacin had the highest efficacy and Zinacef the least in all isolated bacterial species. At the same time, Ketoconazole exhibited the highest effectiveness in all isolated fungal species, and Nystatin showed the least effectiveness. The high population of pathogenic microorganisms coupled with the presence of Salmonella typhimurium and other enteric microorganisms in the food spices can cause severe foodborne illness to the consumers of such food spices and may lead to foodborne illness outbreaks.*

KEYWORDS: Food spices, microbial profiles, antibiograms, foodborne illness, uncooked food.

INTRODUCTION

Spices are aromatic plant materials widely used across the globe to enhance the flavour and palatability of food (La Torre *et al*., 2015). While primarily appreciated for their culinary properties, spices possess inherent antimicrobial activity due to various bioactive compounds like essential oils, phenolics, and alkaloids (Rahman *et al*., 2021). This natural antimicrobial potential has long been recognised, contributing to the preservation of food in ancient times before the advent of modern refrigeration (Kristina, 2023).

However, despite their inherent antimicrobial properties, spices are not sterile and can harbour diverse microbial communities (György *et al*., 2021). Spices are known to be heavily contaminated with microorganisms. For example, Ground black pepper was reported by El-Rahman (2019) to have a bacterial load of 107 cfu/g. Spices may be contaminated with enterotoxigenic *Bacillus cereus*, often at counts below 10^3 cfu/g but capable of multiplying to high quantities (10^5–10^6 cfu/g) in foods to which they are added. This may be enough to cause food poisoning if the food is handled or stored incorrectly (Tirloni *et al*., 2022).

Factors such as contamination during harvesting, storage, production, and processing due to poor hygiene practices, inadequate storage conditions, and a lack of food safety management systems (Karam *et al*., 2021) can contribute to microbial presence in spices. For example, unhygienic conditions during the collection and handling of powdered plant parts have resulted in high bacterial counts and coliform bacteria in crude spice products (Di Bella *et al*., 2019).

While cooking usually eliminates most vegetative bacteria, spore-forming bacteria and thermotolerant fungi can survive the cooking process. These surviving microbes can potentially multiply in cooked food under favourable conditions leading to foodborne illness outbreaks. Identifying the dominant microbial communities in commonly used spices can help to assess potential food safety risks associated with their addition to cooked dishes. Also, the potential misuse of antibiotics in agricultural practices can lead to the selection and proliferation of antibiotic-resistant bacteria in spices. By investigating the antibiogram profiles of isolated microorganisms, we can gain insights into the prevalence of antibiotic resistance among spice-associated microbes. This information can inform public health interventions and antimicrobial stewardship strategies to combat the growing threat of antibiotic resistance.

In evaluating the microbial profile of a food product, however, it is also necessary to determine the antibiogram profile of the microorganisms to know the danger such foods pose to consumers' health. This study, therefore, evaluates the types of microorganisms present in common commercially prepared spices sprinkled or added to cooked foods or salads and the antibiogram of these microorganisms to know whether they are safe for consumption.

MATERIALS AND METHODS

Collection of Samples

The commercially prepared spice samples were bought from the Oja-oba market in Akure, Ondo State, and taken to the Microbiology laboratory at the Federal University of Technology, Akure, School of Life Sciences (SLS) for analyses.

Isolation of Bacteria and Fungi

To isolate microbes, spice samples were homogenized (1g) and weighed before being transferred to a stock solution of 10 mL sterile water and serially diluted to 10^{-3}. The serially diluted sample (0.1mL) was transferred to a Petri dish with Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. Differential media such as Mannitol Salt Agar (MSA), MacConkey Agar (MCA), Eosin Methylene Blue (EMB), and de Mann Rogosa Sharpe Agar (MRS) were also used. Plates were incubated at 37°C for 24 and 48 hours for bacteria and fungi, respectively. Anaerobically, MRS plates were incubated at 30°C for 48–72 hours for lactic acid bacteria (LAB) count. After incubation, colonies were counted using a colony counter (TT20, Techmel and Techmel, USA). Results were represented as CFU/g for bacteria and SFU/g for fungus. Pure cultures were created by subculturing discrete colonies.

Identification of Bacteria and Fungi

According to Cheesbrough (2006), Varghese and Joy (2014), and Sapkota (2022), bacterial isolates were identified using gram staining and a variety of biochemical processes, such as IMViC, Urease, Coagulase, Catalase, H_2S production, sugar fermentation assays, and so on. Results were interpreted using Bergey’s Manual of Systematic Bacteriology (Krieg *et al.*, 2010). Fungal isolates were identified using their cultural and microscopic characteristics, according to Varghese and Joy (2014).

Antibiotic Sensitivity Test

The inoculum was standardised using Cheesbrough's (2006) methodology and the antibiotic sensitivity test was carried out using the disc diffusion method, as detailed by Prescott *et al.* (2005). The diameter of the zones of inhibition was measured with a calibrated ruler. A zone of inhibition $\leq$ 17.00mm was recorded as resistant, according to Hudzicki (2009). The data generated was then subjected to statistical analysis using SPSS version 26.

Antifungal Sensitivity Test

The fungal isolates were inoculated into 10 ml of potato dextrose broth and incubated at 25°C for 72 hours (CLSI, 2017). The antifungal susceptibility assay was performed using the agar well diffusion method (Varghese & Joy, 2014), A calibrated ruler measured the zone of inhibition's diameter and recorded it in millimetres (CLSI, 2017). The data generated was then subjected to statistical analysis using SPSS version 26.

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RESULTS

Microbial Loads of Spice Samples

The total viable counts of microbes isolated from each sample with the dilution factor (10^{-3}) on Nutrient agar, MacConkey agar, Mannitol salt agar, Eosin Methylene Blue agar, and Potato dextrose agar are shown in Table 1. On Nutrient Agar (NA), chilli pepper showed the highest total viable bacterial count of 9.0 x 10^5 cfu/ml, while paprika exhibited the lowest total viable bacterial count of 8.0 x 10^3 cfu/ml.

On MacConkey Agar (MCA), mint recorded the highest total viable coliform count at 9.0 x 10^4 cfu/ml, while paprika showed the least viable coliform count at 2.0 x 10^3 cfu/ml.

On Mannitol Salt Agar (MSA), the highest total viable staphylococcal count of 5.2 x 10^5 cfu/ml was observed in black pepper. In comparison, parsley displayed the least total viable staphylococcal count of 1.0 x 10^4 cfu/ml. On Eosin Methylene Blue Agar (EMB) and De Mann Rogosa Sharpe Agar (MRS), mint exhibited the highest total viable *Escherichia coli* count, with 3.2 x 10^5 cfu/ml and *Lactobacilli* count of 2.0 x 10^5 cfu/ml, respectively. In contrast, both paprika and coriander had the least total viable *E. coli* count of 6.0 x 10^3 cfu/ml on EMB, while paprika, cinnamon, chilli pepper, salad cream, and basil had the lowest total viable *Lactobacillus* count of 2.0 x 10^3 cfu/ml.

On Potato Dextrose Agar (PDA), the highest total viable fungal count of 1.16 x 105 sfu/ml was observed in chilli pepper. At the same time, paprika and cinnamon exhibited the least total viable fungal count of 2.0 x 10^3 sfu/ml each.

Table 1: Total Microbial Count of Isolates in Spices

SPICE	NA	MCA	MSA (Cfu/ml)	EMB	MRS	PDA Sfu/ml
Paprika	8.0 x 10^3	2.0 x 10^3	6.6 x 10^4	6.0 x 10^3	2.0 x 10^3	2.0 x 10^3
Cinnamon	6.6 x 10^4	7.4 x 10^4	6.0 x 10^4	6.0 x 10^4	2.0 x 10^3	2.0 x 10^3
Chili pepper	9.0 x 10^5	5.0 x 10^4	5.0 x10^5	4.8 x10^4	2.0 x10^3	1.2 x 10^5
Mint	2.1 x 10^5	9.0 x 10^4	9.8 x 10^4	3.2 x 10^5	2.0 x 10^5	5.2 x 10^4
Basil	1.2 x 10^5	8.6 x 10^4	4.8 x 10^4	1.2 x 10^5	2.0 x 10^3	1.6 x 10^4
Salad cream	2.0 x 10^4	No growth	4.0 x 10^4	6.8 x 10^4	2.0 x 10^3	8.0 x 10^3
Parsley	2.0 x 10^4	6.0 x 10^3	1.0 x 10^4	1.6 x 10^5	6.0 x 10^3	6.0 x 10^3
Coriander	1.4 x 10^4	No growth	2.9 x 10^5	6.0 x 10^3	8.4 x 10^4	1.0 x 10^5
Suya spice	3.2 x 10^5	No growth	2.4 x 10^5	No growth	1.8 x 10^5	1.0 x 10^4
Black pepper	8.8 x 10^4	No growth	5.2 x 10^5	No growth	1.6 x 10^4	9.6 x 10^4

Key: NA = Nutrient Agar (for less fastidious organisms' isolation), MCA = MacConkey Agar (for Gram-negative enteric bacteria isolation), MSA = Mannitol Salt Agar (for *Staphylococcus* isolation), EMB = Eosin Methylene Blue Agar (for Gram-negative Bacilli and enteric Bacilli isolation, MRS = de Mann Rogosa Sharpe Agar (for *Lactobacillus* isolation)

Types of Microbes Isolated from Spices

Bacterial Species

A total of 12 bacterial isolates were observed in the spices worked on. These are Staphylococcus aureus, Enterococcus faecalis, Lactobacillus plantarum, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus mirabilis, Enterobacter aerogenes, Bacillus cereus, Clostridium perfringens, Klebsiella pneumoniae and Citrobacter freundii.

The results of characterizing the bacterial isolates are shown in Tables 2 and 3. The most frequently encountered bacterial species in the spices are *Bacillus cereus* (16%) and *Enterobacter aerogenes* (16%). In contrast, the least frequently encountered bacterial species are *Proteus mirabilis, Pseudomonas aeruginosa, and Clostridium perfringens, with a* percentage occurrence of 2% each (Table 4). Coriander has the highest level of bacterial contamination in which 7 different bacterial species (*Staphylococcus aureus*, *Lactobacillus plantarum, Salmonella typhimurium, Enterobacter aerogenes, Bacillus cereus, Clostridium perfringens*, and *Citrobacter freundii*) were isolated while the least contaminated is salad cream in which only 1 bacterial specie *Bacillus cereus* was isolated.

Table 2: Morphological Characteristics of Bacteria Isolated from Spices

Isolates	Color	Size	Elevation	Form	Margin	Surface
BIS1	Golden yellow	Small	Convex	Circular	Entire	Smooth
BIS2	Grey	Small	Convex	Spherical	Entire	Smooth
BIS3	White	Moderate	Flat	Circular	Entire	Mucoid
BIS4	Pink	Small	Slightly raised	Round	Entire	Smooth
BIS5	Green	Large	Flat	Irregular	Irregular	Smooth
BIS6	Off-white	Large	Low convex	Circular	Entire	Smooth
BIS7	White	Small	Flat	Circular	Entire	Smooth
BIS8	Grey-white	Large	Convex	Circular	Entire	Not smooth
BIS9	Whitish	Large	Flat	Circular	Oval	Wrinkled
BIS10	Gray	Small	Flat	Round	Irregular	Smooth and Shiny
BIS11	Cream	Small	Flat	Circular	Lobate	Slimy
BIS12	Grey	Small	Convex	Round	Entire	Shiny

Key: BIS = Bacterial Isolate

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Table 3: Biochemical Characteristics of Bacterial Isolated from Spices

Isolates	**Gram reaction**	**Shape**	**Catalase**	**Coagulase**	**H_2S**	**Indole**	**Motility**	**Mannitol**	**Maltose**	**Lactose**	**Sucrose**	**Glucose**	**Citrate**	**Urease**	**Methyl red**	**Voges Proskauer**	**Gas**	**Probable Identity**
BIS1	+	Cocci	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	Staphylococcus aureus
BIS2	+	Rod	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	Lactobacillus plantarum
BIS3	-	Rod	+	-	-	+	+	+	+	+	-	+	-	-	+	-	+	Escherichia coli
BIS4	-	Rod	+	-	+	-	+	+	+	-	-	+	-	-	+	-	-	Salmonella typhimurium
BIS5	-	Rod	+	-	+	-	+	-	-	-	-	+	+	+	+	-	+	Proteus mirabilis
BIS6	+	Rod	+	-	-	-	+	-	+	-	-	+	+	+	-	+	-	Bacillus cereus
BIS7	-	Rod	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	Pseudomonas aeruginosa
BIS8	-	Rod	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	Citrobacter freundii
BIS9	-	Rod	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+	Enterobacter aerogenes
BIS10	-	Rod	+	-	-	-	-	+	+	+	+	+	+	+	-	+	+	Klebsiella pneumoniae
BIS11	+	Rod	-	-	+	-	-	+	+	+	+	+	+	-	-	+	+	Clostridium perfringens
BIS12	+	Cocci	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	Enterococcus faecalis

Key: BIS = Bacterial Isolate

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Table 4: Frequency of Occurrence of Bacterial Species from Spices Examined

Organism Isolated	Black pepper	Cinnamon	Mint	Coriander	Salad cream	Chili pepper	Basil	Paprika	Parsley	Suya spice	Frequency of Occurrence	% Occurrence
St Staphylococcus aureus	+	+	+	+	-	-	-	+	-	+	6	14%
Lactobacillus plantarum	+	-	+	+	-	-	-	-	+	+	5	12%
Escherichia coli	-	+	+	-	-	+	-	-	+	-	4	9%
Salmonella typhimurium	-	-	-	+	-	-	-	-	+	-	2	5%
Proteus mirabilis	-	-	-	-	-	+	-	-	-	-	1	2%
Bacillus cereus	+	+	+	+	+	-	+	+	-	-	7	16%
Pseudomonas aeruginosa	-	-	-	-	-	-	-	+	-	-	1	2%
Citrobacter freundii	+	-	-	+	-	+	-	-	+	-	4	9%
Enterobacter aerogenes	+	-	+	+	-	+	+	-	+	+	7	16%
Klebsiella pneumonia	+	+	-	-	-	-	-	-	+	-	3	7%
Clostridium perfringens	-	-	-	+	-	-	-	-	-	-	1	2%
Enterococcus faecalis	-	-	+	-	-	+	-	-	-	-	2	5%
TOTAL	**6**	**4**	**6**	**7**	**1**	**5**	**2**	**3**	**6**	**3**	**43**	

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Fungal Species

Four different species of fungi were isolated from the spices worked on. These are *Aspergillus niger*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, and *Fusarium oxysporum.* The results of the characterization of the fungal isolates are shown in Table 5. The most frequently encountered fungi are *Aspergillus niger* and *Saccharomyces cerevisiae*, with a percentage occurrence of 31% each, while the least encountered was *Aspergillus fumigatus* (14%) (Table 6).

All fungal species isolated were found in Coriander and Paprika. Black pepper, Basil, and Parsley contained all except *Aspergillus fumigatus. Fusarium oxysporum* and *Aspergillus fumigatus* were not observed in Mint and Chili pepper. Cinnamon and Salad cream contained all organisms isolated except *Fusarium oxysporum* and *Aspergillus niger*, respectively; Suya spice was observed to contain all isolated fungi species except *Fusarium oxysporum* and *Saccharomyces cerevisiae* (Table 6).

Table 5: Cultural and Microscopic Characteristics of Fungi Isolated from Spices

Isolates	Colony color	Texture	Pigmentation	Reverse Colour	Microscopic Characteristics	Isolates
FIS1	White with green at the centre	Powdery	Flat	Tan	Conidiophores with smooth walls and enlarged flask-shaped vesicles	Aspergillus fumigatus
FIS2	White with black center	Velvety	Raised	Pale yellow	Vesicle on conidiophore	Aspergillus niger
FIS3	White	Wooly	Raised	Lavender	Septate macroconidia and randomly distributed microconidia	Fusarium oxysporium
FIS4	Cream	Smooth & Creamy	Flat	Red or White	oval (egg-shaped) cells	Saccharomyces Cerevisiae

Key: FIS = Fungal Isolate

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Table 6: Frequency of Occurrence of Fungal Species from Spices Examined

Organism Isolated	Black pepper	Cinnamon	Mint	Coriander	Salad cream	Chilli pepper	Basil	Paprika	Parsley	Suya spice	Frequency of Occurrence	%Percentage of Occurrence
Aspergillus fumigatus	-	+	-	+	+	-	-	+	-	-	4	14%
Aspergillus niger	+	+	+	+	-	+	+	+	+	+	9	31%
Fusarium oxysporum	+	-	-	+	+	-	+	+	+	+	7	24%
Saccharomyces cerevisiae	+	+	+	+	+	+	+	+	+	-	9	31%
TOTAL	**3**	**3**	**2**	**4**	**3**	**2**	**3**	**4**	**3**	**2**	**29**	

Antibiotic Susceptibility Patterns of Isolated Microorganisms from Spices

The antibiotic susceptibility pattern of the bacteria isolated showed pefloxacin as the most effective antibiotic on all the Gram-positive bacterial isolates except for *Staphylococcus aureus,* which had the most significant growth inhibition by ciprofloxacin (26.17±0.17mm) (Table 7). Pefloxacin was also the most effective antibiotic on almost all the isolated Gram-negative bacterial species (Table 8). All the fungal isolates were susceptible to growth inhibition by ketoconazole with zone diameters ranging from 18.00±0.00 – 35.33±0.17 mm (Table 9).

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Table 7: Antibiotic Susceptibility Patterns of Gram-Positive Bacteria

Isolates	**PEF**	**CN**	**APX**	**Z**	**AM**	**R**	**S**	**SXT**	**E**	**MAR Index**
Staphylococcus aureus	19.33 ± 0.17^{f}	18.33 ± 0.17^{e}	17.00 ± 0.00^{d}	0.00 ± 0.00^{a}	15.00 ± 0.00^{b}	16.00 ± 0.00^{c}	15.17 ± 0.17^{b}	25.33 ± 0.17^{g}	16.17 ± 0.17^{c}	0.6
Lactobacillus plantarum	22.33 ± 0.17^{f}	20.33 ± 0.33^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	16.83 ± 0.44^{c}	19.00 ± 0.00^{d}	25.33 ± 0.17^{g}	25.50 ± 0.00^{g}	25.00 ± 0.00^{g}	0.4
Bacillus cereus	25.33 ± 0.17^{f}	25.50 ± 0.00^{f}	11.33 ± 0.17^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	13.33 ± 0.17^{d}	15.33 ± 0.17^{e}	12.33 ± 0.17^{c}	25.33 ± 0.17^{f}	0.6
Clostridium perfringens	15.00 ± 0.00^{c}	15.50 ± 0.00^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00±0.00a	16.33 ± 0.17^{e}	19.00 ± 0.00^{f}	16.33 ± 0.17^{e}	4.33 ± 0.17^{b}	0.8
Enterococcus faecalis	20.67 ± 0.33^{g}	9.33 ± 0.17^{b}	13.33 ± 0.17^{d}	0.00 ± 0.00^{a}	21.67 ± 0.33^{g}	9.33 ± 0.17^{b}	12.00 ± 0.00^{c}	16.67 ± 0.33^{f}	14.33 ± 0.17^{e}	0.8

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the duplicate rows are not significantly different ($p<0.05$) according to Duncan’s New Multiple Range Test.

Key: PEF = Pefloxacin, GN = Gentamicin, APX = Ampiclox, Z = Zinacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin, MAR = Multiple Antibiotic Resistance.

A zone of inhibition ≤ 17.00 mm is resistant, according to Hudzicki (2009).

Table 8: Antibiotic Susceptibility Patterns of Gram-Negative Bacteria Isolated from Spices

Isolates	**PEF**	**OFX**	**S**	**SXT**	**CH**	**SP**	**CPX**	**AM**	**AU**	**CN**	**MAR Index**
			Diameter of zone of inhibition (mm)								
Escherichia coli	24.83 ± 0.44^{f}	25.50 ± 0.00^{g}	4.50 ± 0.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	25.33 ± 0.17^{fg}	16.67 ± 0.33^{e}	9.33 ± 0.17^{c}	10.33 ± 0.17^{d}	0.7
Salmonella typhimurium	25.33 ± 0.17^{g}	25.50 ± 0.00^{g}	11.33 ± 0.17^{b}	10.17 ± 0.17^{a}	11.00 ± 0.00^{b}	14.50 ± 0.00^{d}	25.33 ± 0.17^{g}	14.00 ± 0.00^{c}	15.00 ± 0.00^{e}	16.33 ± 0.17^{f}	0.7
Proteus mirabilis	25.17 ± 0.17^{g}	25.50 ± 0.00^{g}	16.33 ± 0.33^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	17.50 ± 0.00^{f}	15.33 ± 0.17^{d}	12.00 ± 0.00^{b}	14.67 ± 0.00^{c}	25.33 ± 0.17^{g}	0.6
Pseudomonas aeruginosa	25.50 ± 0.00^{e}	25.50 ± 0.00^{e}	15.33 ± 0.33^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	20.33 ± 0.33^{d}	25.50 ± 0.00^{e}	20.00 ± 0.00^{d}	13.67 ± 0.33^{b}	15.17 ± 0.17^{c}	0.5
Enterobacter aerogenes	25.50 ± 0.00^{f}	24.73 ± 0.37^{e}	19.17 ± 0.17^{d}	14.33 ± 0.17^{a}	15.33 ± 0.17^{b}	25.33 ± 0.17^{ef}	25.17 ± 0.17^{ef}	17.50 ± 0.29^{c}	14.67 ± 0.33^{a}	25.33 ± 0.17^{ef}	0.5
Klebsiella pneumoniae	19.17 ± 0.17^{f}	12.33 ± 0.17^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	15.33 ± 0.17^{e}	25.33 ± 0.17^{g}	0.00 ± 0.00^{a}	13.00 ± 0.00^{c}	15.00 ± 0.00^{d}	0.8
Citrobacter freundii	11.33 ± 0.17^{c}	0.00 ± 0.00^{a}	18.33 ± 0.17^{g}	0.00 ± 0.00^{a}	17.00 ± 0.00^{f}	25.50 ± 0.00^{h}	0.00 ± 0.00^{a}	13.33 ± 0.17^{d}	14.00 ± 0.00^{e}	9.33 ± 0.17^{b}	0.7

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the duplicate rows are not significantly different ($p<0.05$) according to Duncan's New Multiple Range Test.

Key: SXT = Septrin, CH = Chloramphenicol, SP = Sparfloxacin, CPX = Ciprofloxacin, AM = Amoxicillin, AU = Augmentin, GN = Gentamicin, PEF = Pefloxacin, OFX = Tarivid, S = Streptomycin, MAR = Multiple Antibiotic Resistant.

A zone of inhibition ≤ 17.00mm is resistant, according to Hudzicki (2009).

Table 9: Antifungal Susceptibility Patterns of Fungi Isolated from Spices

Isolates	KETO	CLO	NYS	FLU
	Diameter of zone of inhibition (mm)			
Aspergillus fumigatus	35.33 ± 0.17^{c}	13.67 ± 0.17^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Aspergillus niger	18.00 ± 0.00^{c}	21.67 ± 0.33^{d}	0.00 ± 0.00^{a}	16.67 ± 0.33^{b}
Fusarium oxysporium	33.33 ± 0.17^{d}	0.00 ± 0.00^{a}	21.67 ± 0.33^{c}	17.33 ± 0.33^{b}
Saccharomyces cerevisiae	28.33 ± 0.33^{c}	23.33 ± 0.33^{b}	0.00 ± 0.00^{a}	31.33 ± 0.17^{d}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the duplicate rows are not significantly different ($p<0.05$) according to Duncan's New Multiple Range Test.

Key: Keto = Ketoconazole, Clo = Clotrimazole, Nys = Nystatin, Flu = Fluconazole.

DISCUSSION

The results of the study showed that the spices evaluated were contaminated with various microorganisms, including *Aspergillus niger*, *Fusarium oxysporum*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Bacillus cereus* which have been reported in previous works on spices while some (*Klebsiella pneumoniae, Enterococcus faecalis)* were not frequently encountered. The isolation of these pathogens from the spices indicates the potential of these spices to serve as vehicles for foodborne illnesses to susceptible consumers. *Staphylococcus aureus* is known to cause Staphylococcal food-borne disease (SFD) (Hart, 2015). The majority of *E. coli* strains are benign or only temporarily induce diarrhoea. However, some strains, including *E. coli* O157:H7, can result in vomiting, violent stomach pains, and bloody diarrhoea (Gambushe *et al.*, 2022). Species of *Salmonella* are known to cause salmonellosis characterized by gastrointestinal illness and fever (Bakobie *et al.*, 2017). *Enterococci* can produce biogenic amines, causing food intoxication (Giraffa, 2002). *Pseudomonas aeruginosa* can cause gastrointestinal infections (Fakhkhari *et al.*, 2020). Though less common in spices, *Proteus mirabilis* can still lead to gastrointestinal issues and food poisoning (Gong *et al.*, 2019). *Clostridium perfringens* and *Bacillus cereus* are common causes of foodborne illnesses, producing toxins that cause illness (Grass *et al.*, 2013; Nutrition, 2021). *Klebsiella pneumoniae,* while not traditionally a foodborne pathogen, is linked to gut-related diseases (Karlowsky et al., 2003; Siu *et al.*, 2011). *Citrobacter freundii* causes gastroenteritis from parsley-associated food (Tschäpe *et al.*, 1995). Some strains of *Lactobacillus plantarum* produce bacteriocins, a natural antibiotic that inhibits other bacteria; therefore, they are considered safe (Liu *et al.*, 2018). However, they can cause food spoilage. According to Smittle (1977), the predominant spoiling organisms in defined salad dressings and mayonnaise were identified as *Lactobacilli* and yeasts. *Aspergillus* species produce mycotoxins (e.g., aflatoxin, ochratoxin A, patulin). Some food-borne mycotoxins have immediate impacts, with severe disease symptoms developing shortly after consuming contaminated food. Mycotoxins produced by toxin-producing *Fusarium* species can cause mycotoxicosis following ingestion of contaminated food (Nelson *et al*., 1994). *Saccharomyces cerevisiae* is considered safe for human health. However, some individuals with pre-existing allergies to yeast or mould may experience allergic reactions upon consuming spices contaminated with *S. cerevisiae* (Xing *et al.,* 2022).

The microbiological loads in most spices examined in this study do not meet the acceptable standards set by the Centre for Food Safety (2007); this contrasts with Solomon et al. (2013), who found that microbial loads in examined spices were within acceptable limits. Chilli pepper showed the highest bacterial and fungal counts at 9.0 x 10^5 cfu/ml and 1.16 x 10^5 sfu/ml, respectively, followed by suya spice and coriander with 3.2 x 10^5 cfu/ml and 1.0 x 10^5 sfu/ml, respectively. These findings align with previous research indicating that food spices can harbour pathogenic microorganisms (Odongo *et al*., 2013; Saxena *et al*., 2016; Fernández & Vazquez, 2019). Microbial contamination in spices results from harvesting and processing practices, storage conditions, and transportation, which provide favourable conditions for microbial growth and proliferation. The analysis of antibiograms disclosed diverse reactions among bacterial isolates to antibiotics, with Pefloxacin demonstrating the highest efficacy and Zinnacef displaying the least. Regarding antifungal drugs, Ketoconazole exerted the highest effectiveness against the isolated fungi, while Nystatin was the least effective. This finding is consistent with previous research highlighting the increasing prevalence of antibiotic-resistant microorganisms in food products (Morales *et al*., 2018; KuKanich *et al*., 2020). Evaluating the antibiograms of microorganisms in food is becoming increasingly important for monitoring and controlling antibiotic resistance in foodborne pathogens. Unit-specific antibiograms are particularly useful for selecting appropriate empiric therapy for infections caused by bacteria, such as *Salmonella* and *Escherichia* (Pogue *et al*., 2011).

This study examines the microbial profiles and antibiograms of selected spices but has limitations: It only covers specific spices and one geographic area, uses culture-dependent techniques that may miss microbial diversity, focuses on identification without exploring resistance mechanisms, and has a small sample size. Despite these issues, it highlights the need for regulations to ensure spices are free from pathogens and antibiotic resistance. Future research should broaden the scope, investigate resistance across regions, and explore resistance mechanisms.

CONCLUSION

This study shows that most commercially prepared spices examined are loaded with a high population of pathogenic microorganisms that threaten human health and can lead to severe epidemiological outbreaks of foodborne illnesses.

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None.

Conflicts Of Interest

The authors report no conflicts of interest.

Abbreviations

CFU - colony-forming unit per mil;

SFU - spore-forming unit per mil;

SPP - species;

SP - specie;

SLS - school of life sciences;

IMViC - indole methyl red Voges Prauskeur;

NA - nutrient agar;

PDA - potato dextrose agar;

MSA - mannitol salt agar;

MCA - macConkey agar;

EMB - eosin methylene blue;

MRS - de Mann Rogosa Sharpe agar;

BIS - Bacterial Isolate;

FIS - Fungal Isolate.

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