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Isolation and Identification of Microbes Associated with Some Meat Seasoning in Egypt

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Abstract

THIS study aimed to assess the microbiological quality and safety of various dried household spices collected from spice shops, with a particular focus on identifying any pathogenic bacteria present. A series of tests was conducted to determine whether these spices, which are commonly used for seasoning meat, serve as a source of microbes. During this study, common microorganisms from seasoning meat were successfully isolated and identified.Various methods for identifying isolated microorganisms include cultural characterization, conventional biochemical processes, and the Vitek 2 compact system. The microorganisms isolated from seasoning meat consisted of five Gram-negative bacteria ,and all isolated Gram-negative microorganisms are Pathogens: *Escherichia coli* (n=20, 22.22%), *Citrobacter freundii* (n=20, 22.22%), *Citrobacter braakii* (n=15, 16.67%), *Raoultella planticola* (n=16.67%), and *Enterobacter aerogenes* (n=5.56%), along with one Gram-positive bacterium (*Leuconostoc citreum* (n=16.67%). Thus, *Escherichia coli* and *Citrobacter freundii* were the most predominant Gram-negative isolates, while *Leuconostoc citreum* was the predominant Gram-positive microorganism found in seasoned meat. All isolated microorganisms exhibited resistance to all commercial antibiotics.

Keywords: Seasoning Meat, Microorganisms, Isolation, Identification, Vitek, Gram Negative, Gram Positive, Conventional Biochemical, Commercial Antibiotics.

Introduction

The abundance of high biological value proteins, B complex vitamins-particularly B12, iron, selenium, phosphorus, and zinc makes meat a vital component of a balanced and healthful diet [1]. Recently, the safety of meat has gained significant attention from society, highlighting ongoing problems and challenges related to meat safety that may persist in the future. Among these difficulties is the necessity to reduce the prevalence and concentration of both established and newly discovered foodborne pathogens, some of which may have higher virulence and be linked to food-related stressors [2]. [3] recorded that the production of biogenic amines (BAs), which are associated with contaminating microorganisms, is regarded as one of the bacteriological quality indicators of meat and meat products. These low-molecular-weight compounds are primarily produced by microbial enzyme activity during storage, facilitating the enzymatic decarboxylation of specific amino acids present in meat [4] Various groups of microorganisms, particularly those in the *Enterobacteriaceae*, *Micrococcaceae*, and *Pseudomonadaceae* families, have been shown to possess these enzymatic activities [5]. Tyramine, cadaverine, putrescine, and histamine are the most prevalent biogenic amines in meat and meat products [6]. Since these quantities rise during microbial spoilage even during chilled storage, their amounts and ratios have been suggested to indicate the hygienic conditions of raw materials and manufacturing processes [7].

In addition to serving as a sign of bacterial contamination, BAs may be harmful to the consumer [8]. For example, eating too much tyramine or histamine can result in food poisoning or migraine headaches, respectively [9]. Despite not directly causing harm, putrescine and cadaverine increase the

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toxicity of histamines and tyramines by blocking the enzymes that detoxify them[10]. Additionally, when biogenic polyamines like putrescine and cadaverine occur together, they can have additional detrimental physiological effects like carcinogenicity when they transform into sTable carcinogenic N-nitroso compounds [11].

The physicochemical characteristics of biogenic amines make their identification time-consuming and intricate. Over the past ten years, many quick and accurate analytical techniques for identifying BAs in meat and meat products have been developed and compiled in several reviews [12,13,14]. Furthermore, several studies have created extraction methods that are safer for the environment by combining less hazardous organic solvents with analytical instruments that are sensitive and selective, such as mass spectrometers and tandem mass spectrometer detectors [15, 16,17,18].

The meat industry faces the challenge of developing effective manufacturing technologies to produce products that are free or nearly free of BAs. The primary method for preventing the formation of BAs in food is temperature control, which effectively manages biogenic amines by inhibiting microbial growth [19].Because of their thermal stability, BAs are difficult to remove with high-temperature treatment once they have formed [20]. An alternative approach involves inhibiting microbial growth and decarboxylase activity. Newer strategies include microbial modeling [21], high hydrostatic pressures [22], packaging techniques [23], and the use of natural coatings and preservatives [24, 25, 26].

Over the past 30 years, it has been shown that using seasonings to improve meat products' technological and microbiological properties is an effective processing and preservation method. Recently, acidic seasonings have become popular as antimicrobial agents. Lactic acid, for example, can specifically inhibit the growth of many food-spoilage bacteria by reducing microbial loads on meat products, including gram-negative bacteria from the *Enterobacteriaceae* and *Pseudomonadaceae* families [27, 28]. This antimicrobial effect attributed to decreasing the pH below levels necessary for microbial growth and inhibiting bacterial metabolism due to undissociated acid molecules [29]. Many Middle Eastern meat dishes often use spices and edible plants such as garlic, onion, and black pepper as seasonings [30]. These seasonings show antimicrobial activity because they contain specific compounds, like allicin and organ sulfur compounds in garlic and onion [31, 32] and piperine in black pepper. Additionally, in the raw meatball dish called cig kofte, lemon juice, and black pepper have demonstrated synergistic antimicrobial effects [33 , 34]. Furthermore, soy sauce seasoning has shown antimicrobial activity against foodborne pathogens and bacteria responsible for food spoilage [35].

For centuries, spices and herbs were used for a wide variety of purposes. Spices and herbs were so valuable in ancient times that they were used in profiTable business dealings. However, for many years, they were so costly that only the wealthy could afford them. They served a variety of purposes, primarily as cosmetics, perfumes, and sexual aphrodisiacs, in addition to seasoning food with spices. Roman, Greek, and ancient Chinese texts all mention the frequent use of spices and herbs for therapeutic purposes [36]. Since ancient times, people have utilized spices and herbs for their flavour and aroma as well as for other culinary qualities. Since it was difficult to preserve food in the past, spices were used to hide food corruption. However, this is no longer a valid use of spices and herbs, but the growing demand for spicy foods and the desire to consume delecTable foods low in fat and sodium have led to an increase in their use for those purposes [37].

Regretfully, herbs and spices play a significant role in the sensory and sensational qualities of food. In regions with warm temperatures and high humidity, many spices are cultivated and harvested in unsanitary conditions. These circumstances promote development and possible microbial contamination [38]. Numerous agricultural products met a variety of environmental microbiological contaminants during assembly, processing, and retail, including dust, sewage, animal waste, and even human waste. According to [39] this also occurs with herbs and spices.

Since spices are frequently added to foods that are not prepared or consumed raw, eating certain foods that contain them can be harmful to the general public's health. To reduce the microbial load, spices are often sold untreated after drying in open fields or on open roads in many countries that produce them, including India. It is therefore anticipated that the spices offered for sale in these regions will include some micro flora from production and agricultural regions. Comparing these spices to international standards, they were of low quality [40; 41, 42, 43].

Spices are valuable agricultural products that are almost essential for food preparation. They can be used in many forms, including fresh, mature, dried, broken, powder, and more, all of which help add flavor, color, sharpness, aroma, and taste to food. According to [44], spices may include bark, buds, flowers, fruits, roots, leaves, seeds, stigma, rhizomes, patterns, or entire plant tops. Certain diseases and contamination spread by specific foods have been linked to contaminated spices [45, 46]. Although the

amounts of fungi and yeasts vary greatly among spices, they are usually present at very low levels. Besides enhancing our taste buds, spice is also beneficial to our health [47].Because of the widespread use of heavy metals in technology, industry, medicine, and agriculture, these metals are now common in nature, raising concerns about their potential harmful effects on human health and the environment. Unfortunately, these metallic ions can interact or combine with cellular particles. As a result, heavy metal poisoning can cause serious damage to some body organs; therefore, it is important to study and develop treatments for heavy metal poisoning [48].

Although some spices have well-known antimicrobial properties, their effectiveness in inhibiting bacterial growth and preventing pantothenic acid formation in chilled meat has not been determined. This study conducted to investigate the microbiological content and heavy metal presence in spices and herbs available in the local market that directly consumed by humans. A group of food-contaminating microorganisms was isolated from a batch of meat marinated with various spices collected from this market. These spices can contaminate meat when used during the marinating process. Ninety spice mixtures purchased from the Egyptian market and used to marinate boneless chicken and white meat. A variety of microorganisms were isolated, including *Citrobacter freundii*, *Citrobacter braakii*, *Escherichia coli*, *Enterobacter aerogenes*, *Raoultella planticola*, and *Leuconostoc citreum*, after two days of storage at 5°C.A series of chemical tests was then conducted to assess the antimicrobial activity of various solvent extracts of various medicinal plants that were chosen and identified from Egypt.

Material and Methods

Collection area

Ninety samples used in this study were collected from home-made marinades for red and white meat, and others from processed meat products. Samples were taken from the home and included chicken and meat slices marinated with a variety of spices bought from a spice store in the Egyptian market. The following spice group was used during the marinating process: ground black pepper, paprika, ginger, rosemary, onion powder, cumin, red chilli, bay leaf, and cinnamon. Twenty g of spices were given to each group. The isolates were extracted from the samples after they were kept at a standard refrigeration temperature of about 4°C. 24 hours, 36 hours, and 48 hours.

Bacteria were isolated using swabs to prepare bacterial cultures under suiTable temperature conditions. The different bacterial species that formed were identified through various tests and classified, each containing a specific type of bacteria.

Sample inoculation and purification

The samples were inoculated onto the surface of Petri dishes, which contained different media (Nutrient agar, MRS, XLD agar, and MacConkey agar) usinga sterilized loop for the isolation of microorganisms. All plates were incubated at 37^{o}C for 18-24 hours. Then, purified colonies were obtained by repeated streaking of the single colony on fresh nutrient agar plates. Pure culture colonies were subcultured on agar medium. Colonies' parameters: size, shape, texture, odor, pigmentation. Gram staining to differentiate the bacterial isolates into Gram-positive and Gram-negative groups was carried out [49]. Nutrient agar,MRS, XLD agar, Peptone broth,Citrateagar, Bile esculin agar,Mueller-Hinton agar, and MacConkey agarmedia were used for isolation, purification, and differentiation. For the isolation process, we used types of media: MacConkey agar (MA), Xylose-Lysine-Deoxycholate agar (XLD), De Man, Rogosa, and Sharpe agar (MRS), as well as Peptone broth, Citrate agar, Bile Esculin agar, Mueller-Hinton agar, and nutrient agar.

Identification of bacteria

The isolated microorganisms were differentiated and identified by various conventional biochemical methods (biochemical analysis) such as **(**MIO) Motility Indole Ornithine tests, citrate utilization, urease, and oxidase [49]. Catalase activity analysis to distinguish among *Streptococcus* and *Staphylococcus* species [50], a deoxyribonuclease (DNase) activity test to differentiate between *Staphylococcus aureus and Staphylococcus epidermidis*, and a bile-esculintestto distinguish between *Streptococcus* and *Enterococcus* were performed. Then Analytical (Vitek 2 compact system) was used as well.

Sensitivity test for antibiotics

The Clinical and Laboratory Standards Institute (CLSI) claims that the main organisation in Egypt that sets standards for antimicrobial susceptibility testing (AST) is the Performance Standards for Antimicrobial Susceptibility Testing. The disc diffusion method used to assess the isolates' antibiotic resistance. This technique involves spreading a bacterial culture on an agar plate and covering it with discs impregnated with commercial antibiotics. A concentration gradient produced as the antibiotic seeps through the agar. Following incubation, the diameter of the zone of inhibition—the area where bacterial growth suppressed—is measured. To ascertain whether the isolate is

susceptible, intermediate, or resistant to the antibiotic, this zone size compared with predetermined criteria.

Different antibiotic groups with distinct mechanisms (disruption of cell wall, disruption of cell membrane, disruption of DNA, etc.) were employed. These classes include penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, and fluoroquinolones. Because the highest dose of antibiotics was used, which spreads throughout the environment, all bacteria were resistant to the known antibiotic responses.

Results

Sample collection and preparation

From seasoned meat and chicken, 90 specimens were gathered. To the separation of harmful bacteria. Use a range of spices that you bought from an Egyptian market spice store to marinate meat at home. samples were taken at different times (24, 48 hours), which is considered the maximum period for keeping food under normal temperatures in the refrigerator. Bacteria were isolated using swabs to make and make bacterial cultures under suiTable conditions of temperature, and the different bacterial species formed were detected using a different set of tests and divided into groups, each group containing a specific type of bacteria, and the percentage of each group was determined as shown in Table 1,2.We used four types of media for isolation:MA (MacConkey agar), XLD Agar (Xylose-Lysine-Deoxycholate),MRS agar (DeMan, Rogosa, and Sharpe), Peptone broth, Citrate agar, Bile esculin agar, Mueller-Hinton agar, and nutrient agar media.

Identification of isolated bacteria

Identification of isolates that belong to G1

These isolates were found to be gram-negative, rod-shaped, non-spor-forming, anaerobic, motile, citrate-utilizing, and Catalase are positive while, H_2S production, urease, indole test, and oxidase are negative, as shown in Table 2. Identification with citrate utilization test, with a very good identification as *Citrobacter freundii*. As illustrated in Fig.1 and Table (3, 5).

Identification of isolates that belong to G2

These isolates were found to be gram negative, rod-shaped, non-Spor forming, anaerobic, motile, citrate-utilizing, and Catalase positive while H_2S production, urease, Indole test, and oxidase are negative as shown in Table 3. Identification with the Vitek 2 compact system, with a very good identification as *Citrobacter braakii*. As shown in Fig.2 and Table (3, 6).

Identification of isolates which belong to G3

The isolates were found to be gram-negative, rod-shaped, and facultative anaerobes. Oxidase, Voges-Proskauer, and H2S production were negative, while catalase, methyl red, and indole formation were positive as shown in Table (3). Identification with the indole formation test, with a very good identification as *Escherichia coli.* As shown in Fig.3 and Table (3, 7).

Identification of isolates, which belong to G4

These isolates were found to be gram-negative, non-motile, encapsulated, rod-shaped bacteria and facultative anaerobic. It was positive for citrate utilization and urease, while indole production and oxidase were negative. Other criteria are presented in Table 3. Identification with Gram Stain test, with a very good identification as *Raoultella planticola*. As shown in Fig.4 and Tables (3, 8).

Identification of isolates which belonging to G5

These isolates found to be gram-negative, rod-shaped. Indole formation and oxidase test negative, while catalase test and citrate utilization are positive as shown in Table 3. Identification with Vitek 2 compact system, with a very good identification as *Enterobacter aerogenes*. As shown in tables (3, 9).

Identification of isolates, which belong to G6

These isolates were found to be gram-positive, cocci-shaped. Bile Esculin, Hemolysis Gamma (γ), and Growth w/ Tellurite hydrolyze PYR (pyrolidonyl-β-naphthylamide) positive, while catalase and oxidase testsare negative, with a very good identification as *Leuconostoc citreum.* As shown in Fig. 5 and Tables (4, 10).

Sensitivity test for antibiotics

All microbialisolateswere resistance to all examined all commercial antibiotics as shown in tables (12, 13). The isolated strains of bacteria have shown resistance to several antibiotics, including ceftazidime, amoxicillin/clavulanic acid, penicillin, and amoxicillin, based on susceptibility tests. Consequently, certain strains of Gram-negative bacteria, such as *Citrobacter freundii, Escherichia coli*, and *Raoultella planticola*, which are resistant to antibiotics, are classified as pathogens. These bacteria can be transmitted through marinated meat and pose a risk to public health. Additionally, susceptibility tests indicate that the isolated Gram-positive strain, *Leuconostoc citreum*, is also resistant to several antibiotics, including vancomycin, ciprofloxacin, and doxycycline. Interestingly, this strain displayed sensitivity to gentamicin. As shown in Fig.6.

Discussion

The public's awareness of food poisoning and its grave health risks has increased as a result of food consumption, especially of marinated meats. Many attempts have been made in recent years to create high-quality foods that are safe for human consumption by eliminating these microbial hazards. As shown in Table 1. 90 meat samples were collected and seasoned with a variety of spices. A variety of microbes were isolated that contaminated the meat [51].The results in Table 2 indicated that the number of Gram-negative bacteria in the isolated samples was about 75 samples, representing (83.3%), and the number of Gram-positive bacteria was about 15 samples, representing (16.66%) [52].

Table 3 also shows the characteristics of Gram-negative bacteria isolated using biochemical methods such as Gram Staining, Indole, Citrate utilization, Catalase, and Oxidase [53], and [54]. Table 4 shows the characteristics of Gram-positive bacteria and some biochemical reactions, such as the Bile-esculin reaction, which is characteristic of them [55]. The Vitek 2 compact system was one of the techniques used to verify the kinds of bacteria that were isolated and identified in the lab using conventional biochemical techniques. The results are displayed in Tables 5, 6, 7, 8, 9, and 10 [56].

Table 11 shows the proportion of each bacterium that found in marinated meat. Twenty *Citrobacter freundii* samples in all, or 22.22% of the samples, were isolated. *Citrobacter braakii* was also isolated from 15 samples at a rate of 16.67% [57]. Additionally, there were 20 *Escherichia coli* samples, or 22.22% [58]. Additionally, 15 *Raoultella planticola* samples were isolated at a rate of 16.67% [59]. Furthermore, 5 samples (5.56%) of *Enterobacter aerogenes* [60]. Lastly, 15 samples of *Leuconostoc citreum* were isolated at a rate of 16.67% [61].

About 90 samples in all, or 100% of the study, were isolated from marinated meat, a possible source of food contamination. Tables 12, 13 presents the test for antibiotic sensitivity. Using the disc diffusion method, the isolates were assessed for antibiotic resistance. Eight different antibiotics were tested on the isolates: Ceftazidime (CAZ), Amoxicillin/Clavulanic acid (AMC), Amoxicillin (AX), Penicillin (P), Vancomycin (VA), Ciprofloxacin (CIP), Gentamicin (CN), and Doxycycline (DO). The discs were incubated for twenty-four hours on Mueller-Hinton agar plates. Based on the diameter of the inhibition zone, isolates were classified as sensitive, intermediate, or resistant after measuring the halo zone surrounding the disc [62].

Conclusion

Food poisoning is one of the most common and important problemsin Egypt and most African countries. The control of Food poisoning infectionsis frequently difficult because of the problem connected with the identification of the etiological agents and frequent cases of self-medication. Identification of microorganisms associated with Food poisoning by more than one method is very important in order to determine the appropriate therapy based on the testing of antibiotic sensitivity for the true causative agents. Therefore, in this study, isolated samples from marinated meat and chicken slices were examined microbiologically in a lab setting using biochemical tests. Gram-positive bacteria accounted for 16.66% of the isolated bacteria, while Gram-negative bacteria made up 83.3%. Furthermore, these pathogenic microbes were confirmed and identified using the Vitek device, and to find out how resistant the isolated bacteria were to commercial antibiotics, their sensitivity to antibiotics was also investigated.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; 49/11/2023).

TABLE 1. Samples of marinated chicken and meat collected, and 90 bacteria were isolated and identified using a variety of tests

Isolation Number	Type	The age of isolation
E1	Chicken seasoning	After 24 hours
E2	Meat seasoning	After 24 hours
E3	Chicken seasoning	After 36 hours
E4	Chicken seasoning	After 36 hours
E5	Meat seasoning	After 36 hours
E6	Chicken seasoning	After 48 hours
E7	Meat seasoning	After 48 hours
E8	Chicken seasoning	After 48 hours
E9	Meat seasoning	After 48 hours

E= group, and each group contains several samples

TABLE 2. Occurrence of microorganisms isolated from seasoned meat and Chicken

Number of isolations isolated bacteria	Number of Gram-negative bacteria	Number of Gram-positive bacteria
90	75(83.3 %)	15 (16.66%)

TABLE 3. Characteristics of isolated Gram-negative bacteria by conventional biochemical methods

Characteristics	Groups of isolates				
	GN1 (E1, E5)	**GN2 (E7)**	**GN3 (E2, E3)**	**GN4 (E4,E6)**	**GN5 (E8)**
Cell shape	Rod	Rod	Rod	Rod	Rod
Spore	Non- Sporing	Non-Sporing	Non-Sporing	Non-Sporing	Non-Sporing
Gram Staining	-ve	-ve	-ve	-ve	-ve
Indole	-ve	-ve	+ve	-ve	-ve
Citrate utilization	+ve	-ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve	-ve	-ve

TABLE 4. Characteristics of isolated Gram-positive bacteria by biochemical methods

Characteristics	Groups of isolates
	GP6 (E9)
Gram stain	+ve
Sporeformation	Non-Sporing
Cell shape	Spherical
Catalase	-ve
URE	-ve
Bile-esculin	+ve
Citrate utilization	-ve
Oxidase	-ve

+indicates positive reaction results; -indicates negative reaction results.

TABLE 5. Identification of *Citrobacter freundii* with the Vitek test 2 compact System

Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result
APPA	-	ADO	-	PyrA	+	lARL	-	dCEL	-	BGAL	+
H2S	+	BNAG	-	AGLTp	-	dGLU	+	GGT	-	OFF	+
BGLU	-	dMAL	+	dMAN	+	dMNE	+	PXYL	-	BAlap	-
ProA	-	LIP	-	PLE	-	TyrA	-	URE	+	dSOR	+
SAC	+	dTAG\	-	dTRE	+	CIT	+	MNT	-	5KG	+
lLATk	-	AGLU	-	SUCT	+	NAGA	-	AGAL	+	PHOS	+
GlyA	-	ODC	+	LDC	+	lHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	-	lMLTa	-	ELLM	-	lLATa	-		

TABLE 6. Identification of *Citrobacter braakii* with the Vitek test

Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result
APPA	-	ADO	-	PyrA	+	lARL	-	dCEL	+	BGAL	+
H2S	+	BNAG	-	AGLTp	-	dGLU	-	GGT	-	OFF	+
BGLU	-	dMAL	+	dMAN	+	dMNE	+	PXYL	-	BAlap	-
ProA	+	LIP	-	PLE	+	TyrA	+	URE	-	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	+	MNT	-	5KG	+
lLATK	-	AGLU	-	SUCT	+	NAGA	-	AGAL	+	PHOS	-
GlyA	-	ODC	+	LDC	-	lHISa	-	CMT	-	BGUR	-
O129R	+	GGAA	-	lMLTa	-	ELLM	-	lLATa	-		

TABLE 7. Identification of *E. coli* with the Vitek test

Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result
APPA	-	ADO	-	PyrA	-	lARL	-	dCEL	-	BGAL	+
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	-	OFF	+
BGLU	-	dMAL	+	dMAN	+	dMNE	+	PXYL	-	BAlap	-
ProA	-	LIP	-	PLE	-	TyrA	+	URE	-	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	-	MNT	-	5KG	-
lLATK	-	AGLU	-	SUCT	-	NAGA	-	AGAL	+	PHOS	-
GlyA	-	ODC	+	LDC	+	lHISa	-	CMT	+	BGUR	+
O129R	+	GGAA	-	lMLTa	-	ELLM	-	lLATa	-		

TABLE 8. Identification of *Raoultella planticola* with the Vitek test

Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result
APPA	-	ADO	+	PyrA	+	lARL	-	dCEL	+	BGAL	+
H2S	-	BNAG	+	AGLTp	-	dGLU	+	GGT	+	OFF	+
BGLU	+	dMAL	+	dMAN	+	dMNE	-	BXYL	+	BAlap	-
ProA	-	LIP	-	PLE	+	TyrA	+	URE	+	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	+	MNT	+	5KG	+
lLATK	+	AGLU	-	SUCT	-	NAGA	+	AGAL	+	PHOS	+
GlyA	+	ODC	-	LDC	+	lHISa	-	CMT	-	BGUR	-
O129R	+	GGAA	-	lMLTa	-	ELLM	-	lLATa	-		

TABLE 9. Identification of *Enterobacter aerogenes* with the Vitek test

Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result
APPA	-	ADO	+	PyrA	+	lARL	-	dCEL	+	BGAL	+
H2S	+	BNAG	+	AGLTp	-	dGLU	+	GGT	+	OFF	+
BGLU	+	dMAL	+	dMAN	+	dMNE	+	PXYL	+	BAlap	-
ProA	+	LIP	-	PLE	+	TyrA	+	URE	+	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	+	MNT	+	5KG	-
lLATK	+	AGLU	-	SUCT	+	NAGA	+	AGAL	+	PHOS	+
GlyA	+	ODC	+	LDC	+	lHISa	+	CMT	+	BGUR	+
O129R	+	GGAA	-	lMLTa	+	ELLM	-	lLATa	+		

TABLE 10. Identification of *Leuconostoc citreum* with the Vitek test

Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result	Tests	Result
AMY	-	PIPLC	-	dXYL	+	ADH1	+	BGAL	-	AGLU	-
APPA	-	CDEX	-	AspA	-	BGAR	-	AMAN	-	PHOS	-
LeuA	-	ProA	-	BGURr	-	AGAL	-	PyrA	-	BGUR	-
AIaA	-	TyrA	-	dSOR	-	URE	-	POLYB	-	dGAL	-
dRIB	-	lLATk	+	LAC	+	NAG	+	dMAL	+	BACI	+
NOVO	+	NC6.5	+	dMAN	+	dMNE	+	MBdG	+	PUL	+
dRAF	-	O129R	-	SAL	+	SAC	+	dTRE	-	ADH2s	+
OPTO	+										

Distribution of microbial isolates: The selected 6 bacterial species were chosen because they are similar to the charter isolates groups. The representative bacteria would be *Escherichia coli, Citrobacter freundii, Citrobacter braakii, Leuconostoc citreum, Raoultella planticola, and Enterobacter aerogenes.*

TABLE 11. Distribution of total microorganisms isolated from Seasoning Meat

No	Species	Number of isolates	Percentage
1	*Citrobacter freundii*	20	22.22%
2	*citrobacter braakii*	15	16.67%
3	*Escherichia coli*	20	22.22%
4	*Raoultella planticola*	15	16.67%
5	*Enterobacter aerogenes*	5	5.56%
6	*Leuconostoc citreum*	15	16.67%
Total	Total Species	90	100

TABLE 12. Effect of Antibiotic on the growth of all isolates of Gram-negative microorganisms from Seasoning Meat

No	Antibiotic	Inhibition zone mm		
		Citrobacter Freundii	***E. coli***	***Raoultella planticola***
1	(Penicillin) P 10 IU	R (0)	R (0)	R (0)
2	(Amoxicillin) AX 25 µg	R (7±0)	R (0)	R (10±0.57)
3	(Ceftazidime) CAZ 30 µg	R (15±0)	R(15±0)	R(13±0.57)
4	(Amoxicillin / Clavulanic acid) AMC 30 µg	R (0)	R(0)	R(0)

TABLE 13. Effect of Antibiotic on the growth of 1 isolate of Gram-positive bacteria from Seasoning Meat

No	Antibiotic	Inhibition zone mm
		Leuconostoc citreum
1	(Vancomycin) VA 30 µg	R (0)
2	(Ciprofloxacin) CIP 5 µg	R (0)
3	(Gentamicin) CN 10 µg	S (35±0)
4	(Doxycycline) DO 30 µg	R (0)

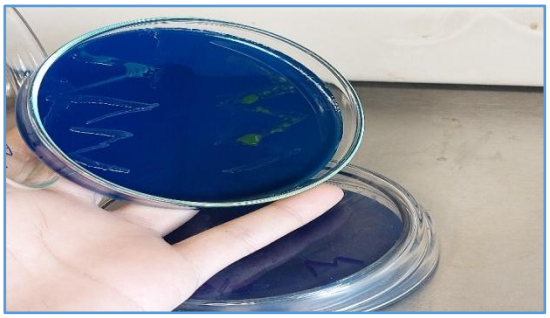

Fig.1. Citrate utilization test of *Citrobacter freundii*

Fig. 2. The Vitek 2 compact system (https://www.gu.edu.eg/product/microbial-identification-by-vitek/)

Fig. 3. Indole formation test of *Escherichia coli*

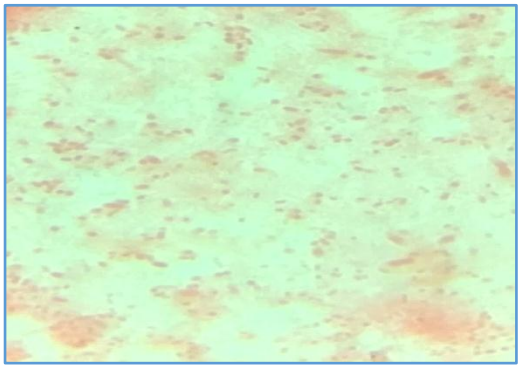

Fig. 4. Gram Stain test of *Raoultella planticola*.

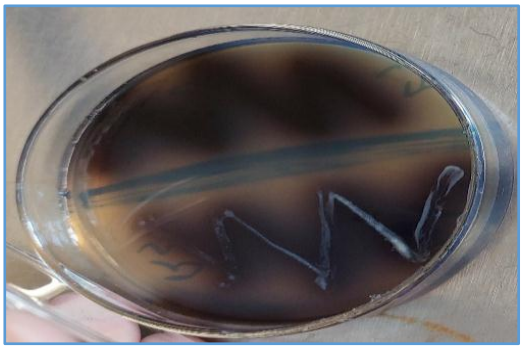

Fig. 5. Bile Esculin test of *Leuconostoc citreum*.

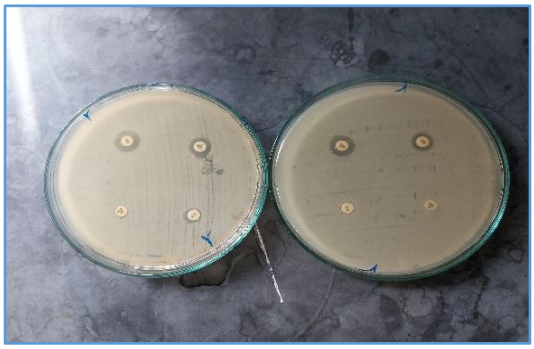

Fig.6. Antibiotic sensitivity testing shows inhibition zone.

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عزل وتحديد الميكروبات المرتبطة ببعض توابل اللحوم في مصر

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الملخص

هدفت هذه الدراسة إلى تقييم الجودة الميكروبيولوجية وسلامة مختلف التوابل المنزلية المجففة التي جُمعت من محلات التوابل، مع التركيز بشكل خاص على تحديد وجود أي بكتيريا ممرضة. أُجريت سلسلة من الاختبارات لتحديد ما إذا كانت هذه التوابل، المستخدمة عادةً لتتبيل اللحوم، تُعدّ مصدرًا للميكروبات. خلال هذه الدراسة، تم بنجاح عزل وتحديد الكائنات الدقيقة الشائعة من تتبيل اللحوم. تشمل الطرق المختلفة لتحديد الكائنات الدقيقة المعزولة التوصيف الزراعي، والعمليات الكيميائية الحيوية التقليدية،ونظام the Vitek 2 compact system . تتكون الكائنات الدقيقة المعزولة من لحوم التتبيل من خمس بكتيريا سالبة الجرام، وجميعها ممرضة: الإشريكية القولونية (ن = 20، 22.22%)، وسيتروباكتر فريوندي (ن = 20، 22.22%)، وسيتروباكتر براكاي (ن = 15، 16.67%)، وراؤولتيلا بلانتيكولا (ن = 16.67%)، وإنتروباكتر أيروجينس (ن = 5.56%)، بالإضافة إلى بكتيريا موجبة الجرام واحدة: ليوكونوستوك سيتريوم (ن = 16.67%). وبالتالي، كانت الإشريكية القولونية وسيتروباكتر فريوندي أكثر عزلات سالبة الجرام انتشارًا، بينما كانت ليوكونوستوك سيتريوم أكثر الكائنات الدقيقة موجبة الجرام انتشارًا في اللحوم المتبلة. أظهرت جميع الكائنات الدقيقة المعزولة مقاومة لجميع المضادات الحيوية التجارية.

الكلمات الدالة: تتبيل اللحوم، سلبية وإيجابية الجرام، الكيمياء الحيوية التقليدية، المضادات الحيوية التجارية، Vitek.