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Mohammed Mahdi Shadhan Hamidi
Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Al Diwaniyah, Iraq

Kadhim Hassan Abbas
Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Al Diwaniyah, Iraq

Correspondence Author:
Mohammed Mahdi Shadhan Hamidi
Department of Public Health, College of Veterinary Medicine, University of Al-Qadisiyah, Al Diwaniyah, Iraq

Antifungal activity of *Lactobacillus* spp against antifungal resistance of *Candida albicans*

Mohammed Mahdi Shadhan Hamidi and Kadhim Hassan Abbas

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Abstract
Meat borne diseases are a significant public health concern, and the presence of pathogenic yeasts such as *Candida albicans* in meat poses a potential risk to consumers. This study aimed to investigate the prevalence and antifungal resistance profiles of *C. albicans* isolated from meat samples collected from various markets in Diwaniyah, lraq. A total of 300 meat samples, including beef, mutton, and chicken, were collected and subjected to microbiological analysis. *C. albicans* isolates were identified using conventional methods and further characterized by molecular techniques. Antifungal susceptibility testing was performed to determine the resistance patterns of the isolates against commonly used antifungal agents. The results revealed a significant prevalence of *C. albicans* in meat samples, with varying rates observed among different meat types. Molecular characterization confirmed the identity of the isolates and provided insights into their genetic diversity. Antifungal susceptibility testing showed concerning levels of resistance among *C. albicans* isolates, particularly to azole antifungal agents. This highlights the potential challenges in treating *C. albicans* infections associated with contaminated meat consumption. The findings of this study underscore the importance of implementing effective surveillance and control measures to mitigate the risks associated with *C. albicans* in the meat supply chain. Regular monitoring of antifungal resistance and the promotion of hygienic practices in meat production and handling are crucial for ensuring food safety and protecting public health. Further research is needed to explore the underlying mechanisms of antifungal resistance in *C. albicans* and to develop strategies for combating this emerging issue. This study contributes to the existing knowledge on the prevalence and antifungal resistance of *C. albicans* in raw meat, providing valuable information for policymakers, food safety authorities, and healthcare professionals in lraq and beyond.

Keywords: *Candida albicans*, meat, foodborne pathogen, lraq

Introduction
Meat microbiology is the study of microorganisms that are present in meat and meat products. These microorganisms can be either beneficial or harmful to humans. Beneficial microorganisms can help to preserve meat by inhibiting the growth of harmful bacteria. Harmful microorganisms can cause foodborne illnesses, such as *Candida* spp [1].

Candida spp is one of the fungal spores that can be found in meat, and previous research has demonstrated that the presence of this type of spores can have a significant impact on the safety of meat products. ln this context, this research seeks to understand the prevalence of *Candida* species in raw meat and how it can be controlled to ensure the quality and safety of the product [2].

Candida spp is the most common species causing human infections [3]. *Candida* spp are often considered a reliable source of diarrhea, but are rarely documented [4]. Meat is generally eaten cooked, but in some traditional recipes of but meat is eaten raw or is partially cooked. Meat tissues get contamination during the various stages of slaughter and transportatio. Contaminated raw meat is one of the main sources of food borne illness [5].

Antibiotic resistance of fungi is a serious health problem that requires a deep understanding of the mechanisms behind it and its implications for public health. Antibiotic resistance mechanisms of fungi compromise the effectiveness of treatment and challenge infection control, exposing individuals to the risk of contracting and exacerbating fungal diseases [6].

The development of antibiotic resistance in fungi depends on multiple factors, including repeated exposure to and suboptimal use of antibiotics, as well as genetic multifunctionality that contributes to resistance formation. These functions include active transport pathways for antigens, physiological modifications in cells, and the production of antigen-degrading enzymes [7].

Mechanisms of fungal antibiotic resistance are diverse and involve gene conversions that lead to changes in structural components of cells, such as modifications in antigen target proteins and drug skipping. ln addition, fungi can generate antibiotic-degrading enzymes or modify transport channels affected by antibiotics [6].

This study investigated antifungal resistance of *C. albicans* in raw meat in markets in the city of Diwaniyah, lraq.

Lactobacillus spp

Lactobacillus spp. are a genus of Gram-positive, rod-shaped bacteria. They are a part of the lactic acid bacteria (LAB) group and are used in the production of fermented foods such as yogurt, cheese, and sauerkraut. *Lactobacillus* spp. are also used in the production of probiotics Figure 3, which are live microorganisms that are beneficial to human health [8].

Lactobacillus spp. are found in a variety of environments, including the human body, soil, and water. They are typically found in the intestines of humans and animals, where they play an important role in digestion. *Lactobacillus* spp. also produce lactic acid, which helps to preserve food and prevent the growth of harmful bacteria [9].

Lactobacillus spp. are a diverse group of bacteria, and there are over 200 different species. Some of the most common species include *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus bulgaricus*. These species are all used in the production of fermented foods, and they are also available as probiotics [10].

Lactobacillus spp. are generally considered to be safe for human consumption. However, some people may experience side effects such as bloating, gas, and diarrhea after consuming *Lactobacillus* spp. These side effects are typically mild and go away on their own [11].

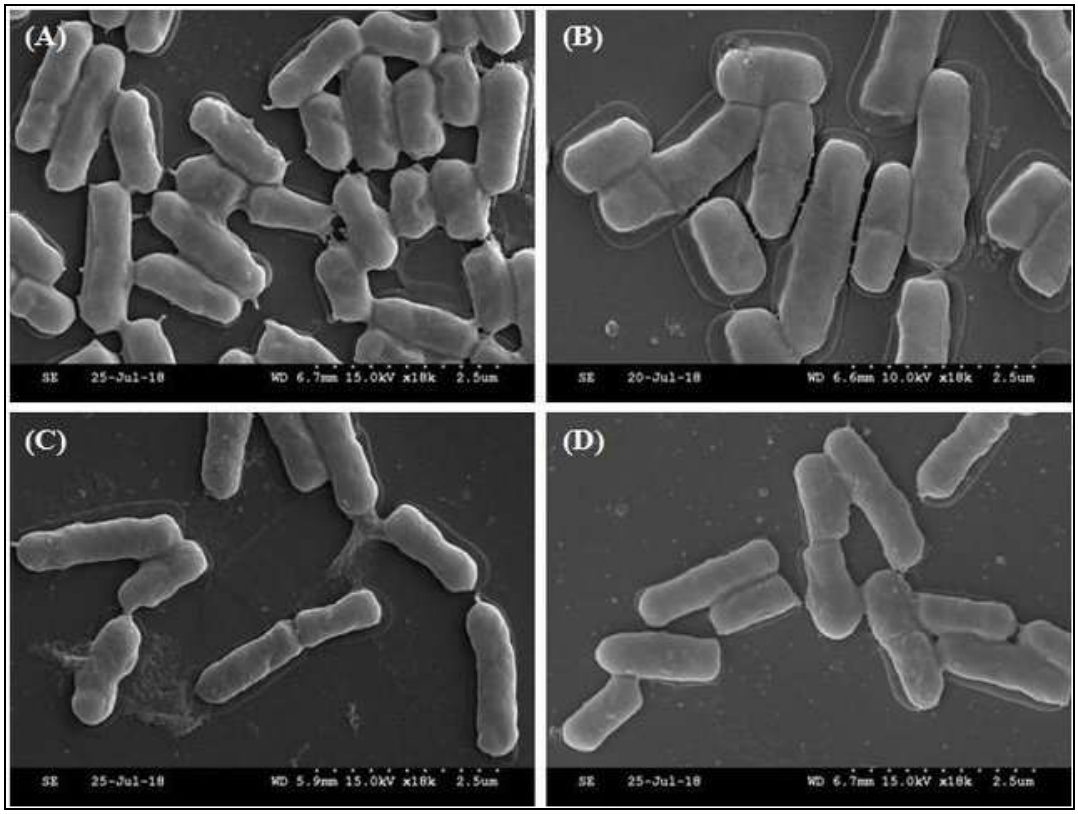

Fig 1: The scanning electron microscope (SEM)images of selected strains:(A) *Lactobacillus* plantarum (B) L. fermentum (C) L. fermentum and(D) L. plantarum

Antifungal Activity of *Lactobacillus* spp

For the food industry, food safety and hygiene are some of the key issues in its development process. Fungal contamination, which causes the physical and chemical deterioration of food, is often accompanied by detrimental sensory changes, including changes in taste, odor, color and texture, in meat, seafood, dairy products and grains [12].

Sadiq et al. reviewed the research progress of lactic acid bacteria (LAB) as antifungal and antimycotoxin agents [13]. Rodriguez Assaf et al. isolated several fungi, including Aureobasidium pullulans, Cryptococcus magnus, Metschnikowia pulcherrima and Rhodotorula glutinis, with antifungal activity from fermentation microenvironments and the surfaces of refrigerated grapes [14]. Recent trends in detecting, controlling and detoxifying the patulin mycotoxin were observed using biotechnology methods [15].

Lactobacillus spp. lt is a genus of bacteria in the Lactobacillaceae family. lt is a lactic acid bacteria that lives naturally in the digestive system of humans and animals [16].

Lactobacillus spp. lts prominent importance in the fermentation process, as it helps transform milk into wonderful products such as yogurt and cheese. Additionally, they are used as nutritional supplements to promote digestive health and improve the balance of bacteria in the digestive tract [17].

Lactobacillus spp. An essential part of normal human intestinal exhalation, playing a role in maintaining the balance of bacteria in the digestive system and providing health benefits. Recent research indicates that these bacteria may have a positive effect on the health of the immune system and controlling intestinal irritations [18].

Lactobacillus spp. lt has antifungal mechanisms that help it prevent the growth of harmful fungal species. This includes the secretion of substances such as lactic acid and antimicrobial peptides, which create an unfavorable environment for harmful fungi [19].
Lactobacillus spp. By inhibiting the growth of harmful fungi by producing compounds that inhibit their vital enzymes and hinder their reproduction processes. This effect is due to the strong ability to produce compounds such as hydrogen peroxide and lactic acid, which are effective against harmful fungi [20].
There are a number of LAB bacteria strains of different origins whose antibiotic activities have been demonstrated For fungi and the possibility of biological preservation, of which the species of the genus Lactobacillus are the most isolated
Bacterial bacteria associated with antifungal activity, which plays an important role in inhibiting the growth of a variety of species From fungi [21].

Materials and Methods

Samples Collection: In the current study 50 samples were collected of dairy products, including 25 samples of Activia yogurt and 25 samples of local dairy (Taj al-Nahrain products) products available in the markets. After confirming their morphological characteristics, 3 samples were sent for sequencing, as shown in Table (1).

Table 1: Distribution of *Lactobacillus acidophilus* ısolates among activia yogurt and local products

Species	Total No.	Activia Yogurt No=25	Local Products (Taj al-Nahrain products) No=25
Lactobacillus acidophilus	50	6(24%)	4(16%)
Total		10	
X^2		0.500	
P value		0.450(NS)	

NS: No significant difference at $p<0.05$

Bactrial Diagnosis

1 ml of the collected curd samples was taken and spread on bacillus cereus agar base medium It was incubated under aerobic conditions at 37 °C For 48 hours [22].
The bacterial isolates were stained with Gram stain and examined microscopically to identify the shape of the cells and their interaction with the Gram stain.

Molecular Study

DNA Extraction: Genomic DNA extraction from Lactobacillus was carried out in accordance with [23].

DNA Amplification

To confirm Candida presence, PCR amplifies a specific DNA region (16s RNA) . A pre-mixed solution (25 µl) containing essential ingredients and specific primers (3 µl each) targeting *Lactobacillus* DNA are combined with the isolated bacteria DNA (5 µl). The PCR thermocycler then orchestrates a thermal dance: high temperatures (95 °C) separate DNA strands for primer binding (repeated 35 times), followed by a lower temperature (48 °C) for specific primer attachment. Finally, an enzyme extends these primers (72 °C, repeated 35 times) creating new DNA copies with each cycle this step was done according to (Foongladda et al., 2014). The primers that using in current study was forward Primer: (5'-AGAGTTTGATCCTGGCTCAG -3') and reverse Primer: (5'- GGTTACCTTGTTACGACTT -3').

Gel Electrophoresis

A 1.5% agarose gel, stained with Ethidium Bromide for DNA visualization .

Statistical analysis

The statistical analysis was carried out with Excel. UPGMA relied on [24] for the genetic tree that was generated by the NCBI website and the analysis of that tree.

Results

The current study obtained a total of 10 *lactobacillus acidophilius* as shown in Fig 2.

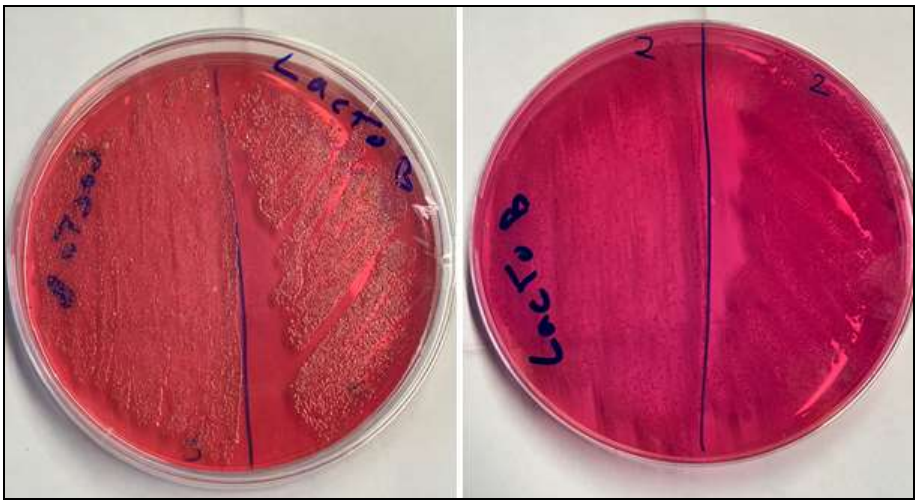

Fig 2: *Lactobacillus acidophilus* on bacillius cerus agar antifungal activity of *Lactobacillus acidophilus*

Table (1) illustrates the antifungal activity of *Lactobacillus acidophilus* against 12 isolates of *Candida albicans*. The table presents the results of three different treatments across five wells:

Supernatant (Plate A): This treatment involved using the supernatant of *L. acidophilus* culture, which contains secreted metabolites.

Sonicator (Plate B): This treatment utilized a Sonicator of *L. acidophilus* cells, which releases intracellular components.

Supernatant + Sonicator (Plate C): This treatment combined both the supernatant and Sonicator.

Control: Wells 3 in all plates served as controls, likely without any treatment, and did not exhibit inhibition.
The results suggest that both the supernatant and Sonicator of *L. acidophilus* possess antifungal activity against *C. albicans*. The combined treatment demonstrated slightly reduced activity compared to individual treatments, possibly due to interactions between the components.

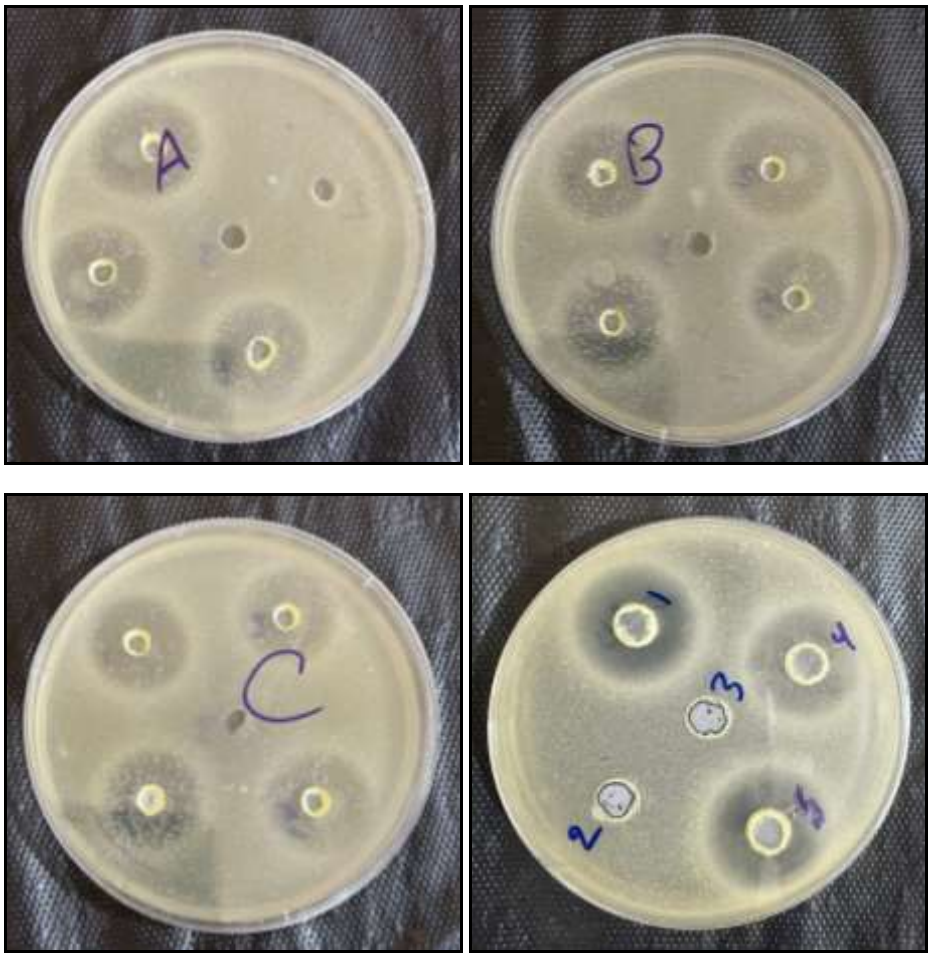

Fig 3: Shows fungi cultured on nutrient media for antifungal activity of *Lactobacillus acidophilus*

Table 2: Zone inhibition diameters (mm) of the sensitivity of *C. albicans* (N=12) to the cell free supernatant (CFS) of probiotic *Lactobacillus* spp, Sonicator and both Supernatant + Sonicator

Lactobacillus pattern	Concentration of lactobacillus				
	10^4	10^5	10^6	10^7	Control
L. acidophilus	15.6±0.44	16.2±0.81	0±0	18.5±1.1	0±0
Sonicator	14.2±0.2	14.8±0.41	16.4±0.62	18.1±0.81	0±0
Supernatant and Sonicator	15.2±0.5	15.2±0.52	16.4±0.6	16.6±0.22	0±0
LSD (p<0.05)	0.542				

Values represent mean ±SE for 12 isolates

Phylogenetic tree
The phylogenetic tree provided displays the genetic relationships between various *Lactobacillus acidophilus* strains based on their 16S ribosomal RNA gene sequences.

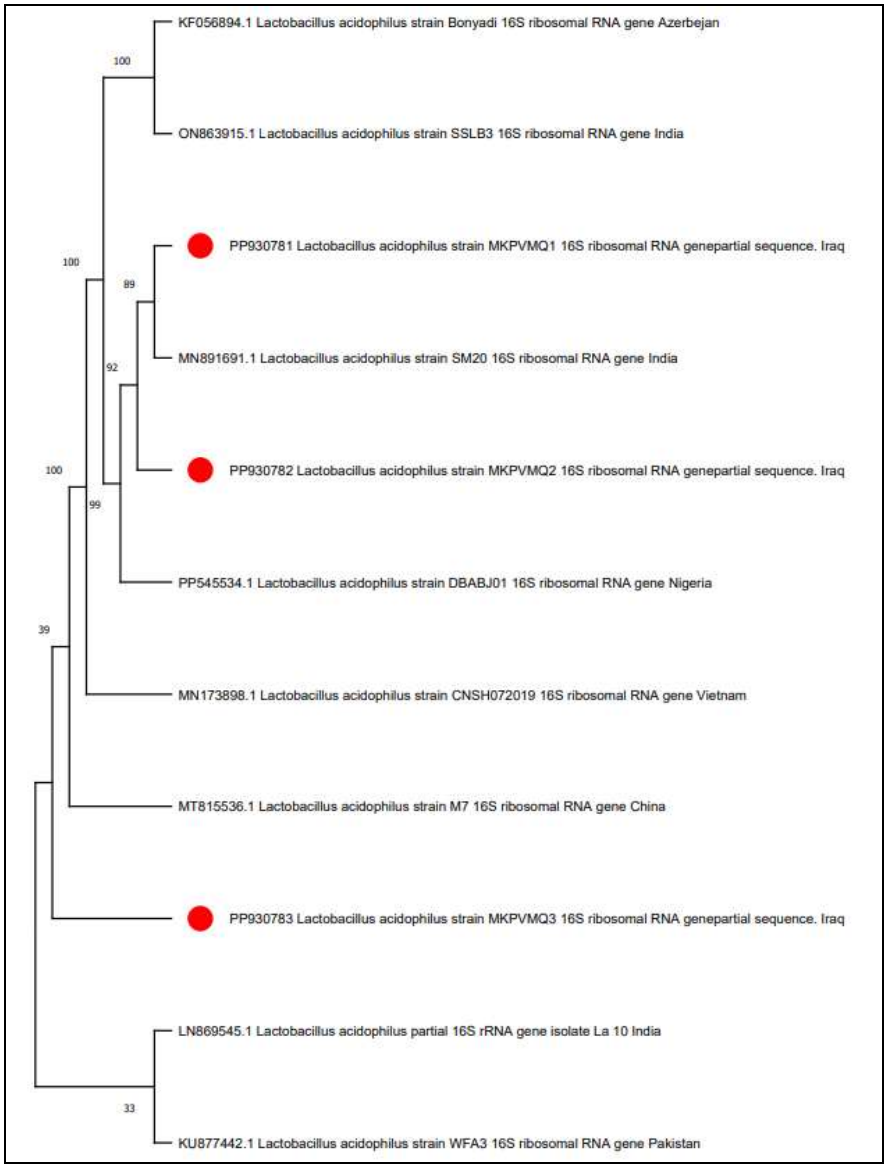

Fig 4: The tree represents the genetic relationships between different isolates of lactobacillus species, as shown

Conclusions

This study investigated antifungal resistance of *C. albicans* in meat in markets in the city of Diwaniyah, lraq. The research aimed to understand the prevalence of *Candida* species in meat and how it can be controlled to ensure product quality and safety. The study also explored the mechanisms behind antifungal resistance and its implications for public health, emphasizing the need for a deep understanding of these factors to address the growing problem of resistance.

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