

Research article



The Antibacterial Effect of Chitosan against Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Beef Meat

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Abstract | The effectiveness of chitosan as an antibacterial agent against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from frozen and fresh beef was evaluated. Bacterial isolates were confirmed using cultural, biochemical, and the VITEK 2 system. All suspected MRSA isolates tested positive for MRSA. In frozen meat samples (n=50) collected from different markets in Baghdad city, the number and isolation percentage (%) of *S. aureus* and MRSA were 11/50 (22%) and 4/50 (8%), respectively. In fresh meat samples (n=50), these figures were 16/50 (32%) and 3/50 (6%), respectively. Chitosan solutions at different concentrations (0.5%, 1%, 1.5%, and 2%) were prepared by dissolving food-grade chitosan in 1% acetic acid. Evaluation of bacterial reduction in log units was recorded. The bacterial counts before dipping meat samples in 2% chitosan solution showed initial counts of 5-6 log₁₀ CFU/g. After treatment at both ambient and refrigeration (4°C) temperatures for 4 hours, counts were reduced to 2-3 log₁₀ CFU/g. The number of MRSA isolated from frozen meat decreased significantly more when treated with 2% chitosan at refrigeration temperature compared to ambient temperature. The antibacterial activity was also evaluated by measuring the zone of inhibition (mm). Results indicated that 2% chitosan was significantly effective ($P \leq 0.05$), showing the highest zone of inhibition (38 mm) against MRSA. In conclusion, the application of chitosan as a natural food-grade antibacterial agent against MRSA effectively reduced bacterial counts and maintained the bacteriological quality of beef sold in local markets.

Keywords: Methicillin-resistant *Staphylococcus aureus*, MRSA, Chitosan, Frozen, Fresh, Beef meat

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INTRODUCTION

Meat is ideal medium for the growth and multiplications of both spoilage and pathogenic microorganisms specially the high pathogens such as *Bacillus cereus*, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* (Nungesser and Winter, 2021; Ajaycoba, et al., 2023). Meat, meat products, bovine milk and raw milk products can be contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria through the different handling steps, slaughtering, processing and marketing. *Staphylococcus aureus* widely distributed in a broad range of hosts, including humans and animals (Khudhir and Ahmed, 2017; Al-Rudha, et al., 2021; Al-Rasheed, et al., 2022).

Chitosan is a nontoxic edible substance that is easily biodegradable and possesses characteristics such as antioxidant properties and enzyme inhibition (Liu, 2022). It is a biopolymer with antimicrobial and anti-biofilm activities, utilizing nitric oxide (NO) as a donor to prevent infection from biofilm-producing microorganisms (Choi et al., 2020; Muñoz-Tebar et al., 2023). Chitosan is uniquely beneficial in meat and meat products due to its ability to act as a barrier to oxygen and moisture, as well as being cost-effective (Yu et al., 2019). Its derivatives are very useful in food products and have applications in cosmetics, pharmaceuticals, agriculture, and biomedicine due to their biological activity, antimicrobial properties, and ability to inhibit film formation by pathogenic bacteria (Kumar et al., 2020;

Ahmed et al., 2020). It is listed as a generally recognized as safe (GRAS) substance, commercially produced from crab and shrimp shell wastes, characterized by differences in deacetylation degree, molecular weights, and antibacterial efficacy against both Gram-positive and Gram-negative bacteria (Egorov et al., 2023). The antibacterial activity of chitosan compounds is less effective against Gram-positive bacteria compared to Gram-negative bacteria (Umoren et al., 2020; Ardean et al., 2021). The antibacterial effectiveness of chitosan against *S. aureus* increases with molecular weight (MW) up to < 300 kDa, whereas its effectiveness against *E. coli* decreases with increasing MW (Zheng et al., 2022; Román-Doval et al., 2023). The main goal of this study was to evaluate the effectiveness of chitosan as a natural antibacterial against MRSA isolated from two types of beef meat.

MATERIALS AND METHODS

SAMPLES COLLECTION

A total of 100 meat samples (50 fresh and 50 frozen) were obtained from local butchers and supermarkets in Baghdad city over a 10-month period from March 2022 to January 2023. Each meat sample was cultured directly on chromagar, mannitol salt agar, and Baird–Parker agar medium. All culture and biochemical media were prepared according to the Manufacturer Company. Suspected colonies appeared as mucoid, smooth, and green to greenish-blue on chromagar™ (MeReSa Agar Base), yellow (golden) on mannitol salt agar, and dark gray to black on Baird–Parker agar, with clear zones around the colonies. Biochemical tests including oxidase, Dry spot (Staphytest Plus), latex agglutination, DNase, catalase, and coagulase tests were conducted for MRSA identification.

PROCESSING OF FRESH MEAT SAMPLES

Bacterial contamination was identified using standard microbiological methods. Twenty-five grams of boneless, fat-trimmed fresh beef meat were homogenized in 225 ml of sterile 0.1% peptone water for 2–5 minutes. The homogenate was then serially diluted (10^4 , 10^5 , up to 10^6) with sterile 0.1% peptone water, inoculated onto mannitol salt agar, and incubated at 37°C for 24 hours. Isolates were purified and characterized based on morphology, Gram staining, and additional biochemical properties. Positive *S. aureus* isolates were confirmed using the VITEK-2 Compact System following the manufacturer's instructions (Patricia, 2014; Al-Dujaily and Mahmood, 2022).

PROCESSING OF FROZEN MEAT SAMPLES

Frozen minced beef samples vacuum-packaged in bulk were obtained from supermarkets in Baghdad city, with each sample weighing 500 g. All samples were transferred in their original sealed packaging. The meat cuts measured

2 cm in thickness, 2 cm in width, and 5 cm in length. After holding, the samples were thawed under aseptic conditions and vigorously homogenized for 2–5 minutes inside sterile plastic bags using a lab stomacher. One milliliter of the homogenized meat mixture was transferred to a universal bottle containing 9 ml of sterile 0.1% peptone water for a series of dilutions ranging from 10^1 to 10^6 . This was done to detect and enumerate *S. aureus* and MRSA bacteria before and after subjecting them to various treatments (Hafez et al., 2019).

PREPARATION OF STOCK CHITOSAN SOLUTION

Food-grade chitosan prepared from shrimp shells with molecular weight of 3700–20000Da, degree of deacetylation 90%, >300 cP dissolved in 1% (v/v) glacial acetic acid for preparation of stock solutions (0.5%, 1%, 1.5% and 2% (w/v). Stirring was done at 55°C overnight for ensure the complete particles dissolution. The solutions were sterilized by Millipore filters (0.45 µm), and pH of solution was adjusted to 5.4 with 10 M NaOH and stored in dark container at 4°C until use (Kibar and Sabir, 2018).

INOCULUM PREPARATION

Brain heart infusion broth was inoculated with MRSA isolates and after 6–8 hours of incubation it was adjusted with PBS with McFarland standard 0.5 (Umerska et al., 2017).

ANTIBACTERIAL EFFICACY OF CHITOSAN AGAINST MRSA

Sterile plates of Mueller Hinton agar were inoculated by swabbing with 100 µl of a suspension containing 5×10^8 CFU/mL, prepared from overnight cultures. Three to five colonies were collected using a sterile wire loop and suspended in sterile peptone water. The turbidity of the suspension was adjusted to 0.5 McFarland standard (equivalent to 5×10^5 CFU/mL). The tested bacteria were swabbed onto the agar surface. Seven-millimeter wells were created on the seeded agar using a sterile cork borer, and 100 µL of each concentration of chitosan was placed inside the wells. Levofloxacin at a concentration of 250 µg/ml served as the positive control, and sterile distilled water was used as the negative control. The assays were conducted in triplicate. The plates were then incubated for 24 hours at 37°C, and the activity was assessed by measuring the diameter (mm) of the inhibition zones (Manilal et al., 2020).

THE EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF CHITOSAN

Meat cubes divided into two groups, the first group was kept without dipping in the chitosan solution, kept in the sterile distal water as (control), second group was dipped in the chitosan containing the best concentration (2%) for 4 hrs as contact time at ambient and refrigeration tempera

Table 1: Number and percentage (%) of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from frozen and local fresh meat

Source of meat sample	Total number and percentage of Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)		
	No. of samples	No. and percentage of <i>S. aureus</i>	No. and percentage of MRSA
Imported meat	50	11/50 (22.00)	4 / 50 (8.00%)
Local meat	50	16/50 (32.00%)	3 / 50 (6.00%)
Total	100	27/100 (27.00%)	7 / 100 (7.00%)
P-value		0.26	0.69

Table 2: The antibacterial activity of chitosan against Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from frozen meat.

	Zone of inhibition (mm)					
	Chitosan concentrations %				Control positive levofloxacin 250mg/ml	Control negative (distal water)
	(0.5%)	(1%)	(1.5%)	(2%)		
Means±SE	9.00±0.57e	17.00±1.15d	23.00±2.30c	38.00±2.30b	47.00±0.00a	0.00±0.f
LSD	3.97					

Means with different letter are significantly different ($P \leq 0.05$)

Table 3: The antibacterial activity of chitosan against Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from fresh meat.

	Zone of inhibition (mm)				Control positive levofloxacin 250mg/ml	Control negative (distal water)
	Chitosan concentrations %					
	(0.5%)	(1%)	(1.5%)	(2%)		
Means ±SE	10.00±0.91e	17.00±0.91d	23.00±91c	38.00±1029b	47.00±0.00A	0.00±0.00f
LSD	2.47					

Means with different letter are significantly different ($P \leq 0.05$)

ture (Khudhir, 2021a).

STATISTICAL ANALYSIS

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Least-significant difference, post hoc test was performed to assess significant differences among means whereas dependent and independent t tests were used in this current study.

RESULTS

LABORATORY IDENTIFICATION OF MRSA BY CULTURAL AND BIOCHEMICAL CHARACTERISTICS AND VITEK 2 SYSTEM

Meat samples were cultured directly on chromagar, mannitol salt agar, and Baird–Parker agar medium. Suspected colonies appeared as mucoid, smooth, and green to greenish-blue on chromagar™ (MeReSa Agar Base), yellow (golden) on mannitol salt agar, and dark gray to black on Baird–Parker agar, with clear zones around the colonies. In frozen meat samples ($n=50$) collected from different markets in Baghdad city, the number and isolation percentage (%) of *S. aureus* and MRSA were 11/50 (22%) and 4/50 (8%), respectively. In fresh meat samples ($n=50$), these fig-

ures were 16/50 (32%) and 3/50 (6%), respectively (Table 1).

Biochemical tests for MRSA identification showed negative results for oxidase, and positive results for Dry spot (Staphytest Plus), latex agglutination, DNase, catalase, and coagulase tests. Morphological characteristics of MRSA on Baird–Parker agar included black colonies surrounded by clear zones after 24 hours. Isolates were further purified on Baird–Parker agar supplemented with egg yolk and potassium tellurite, due to the action of lecithinase which breaks down egg yolk and reduces tellurite. Biochemical test results indicated mannitol fermentation (positive), Gram-positive staining (due to thick peptidoglycan in the bacterial cell wall), oxidase-negative, catalase-positive (breaks down hydrogen peroxide into water and oxygen gas), coagulase-positive (clots blood plasma), and DNase-positive (hydrolyzes DNA with a clear zone around the growth). The Dry spot test (Staphytest Plus) was used for detecting clumping factor, Protein A, and certain polysaccharides in MRSA (Kadhum and Abood, 2022; Abbas and Rady, 2023).

ANTIBACTERIAL EFFICIENCY OF CHITOSAN SOLUTION

Chitosan from shrimp shells was used in the current study

to assess its effectiveness as a natural antibacterial solution against MRSA at different concentrations (0.5%, 1%, 1.5%, and 2%). The results indicated that the most effective concentration of chitosan as an antibacterial solution against MRSA isolated from frozen and fresh meat was 2%, showing the highest zone of inhibition (38 mm), as shown in Figures 1 and Tables 2–3.

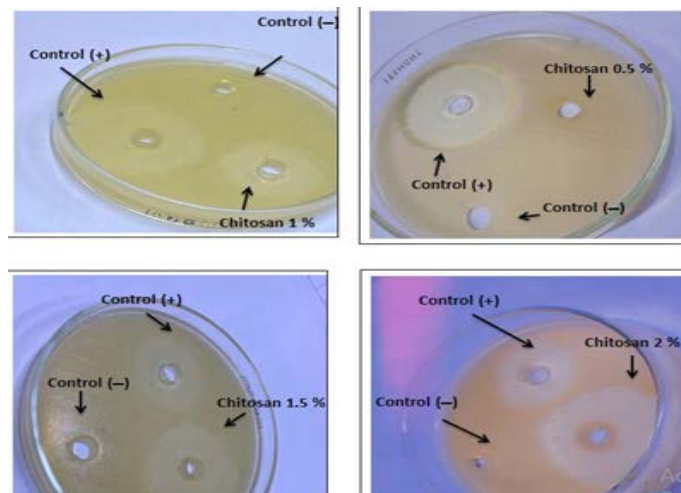


Figure 1: zone of inhibition of different concentration of chitosan used against MRSA isolated from frozen and fresh meat

ENUMERATION OF MRSA AFTER ANTIBACTERIAL ACTIVITY OF CHITOSAN SOLUTION:

In current study, after confirmatory diagnosis of MRSA, the isolates were subjected to antibacterial solutions (2%) at two different temperatures, (ambient and refrigerator temperature). Meat samples were immersed in 2% chitosan solution, with contact time for 4 hours to determine the antibacterial effect of the treatment solution against MRSA as shown in Table 4 and 5. The bacterial counts before dipping meat samples in 2% chitosan solution showed initial counts of 5–6 log₁₀ CFU/g. After treatment at both ambient and refrigeration (4°C) temperatures for 4 hours, counts were reduced to 2–3 log₁₀ CFU/g. The reduction in CFU log₁₀/g of bacterial counts varied with temperature, as shown in Table 6.

Table 4: Number of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from frozen meat after subjecting to chitosan activity (2 %) for 4hrs at refrigeration temperature.

frozen MRSA isolates	Count of MRSA (CFU/g) before treatment (control)	Count of MRSA (CFU/g) after treatment
Mean ± SE	6.37±0.29 a	2.73±0.09 b

*Dependent test (P<0.05)

Table 5: Number of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from fresh meat after subjecting to chitosan treatment (2 %) for 4hrs at ambient temperature.

Fresh MRSA isolates	Count of MRSA (CFU/g) before treatment (control)	Count of MRSA (CFU/g) after treatment
Mean ± SE	5.56±0.28 a	2.74±0.07 b

*Dependent test (P<0.05)

Table 6: Means of reduction CFU.log₁₀/g of Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from local and imported (frozen) meat after subjecting to action of chitosan

Treatments / Temperature	Means±SE
Reduction CFU.log ₁₀ /g in imported (frozen) meat after subjecting to chitosan (2%) treatment at refrigeration temperature.	3.64±0.20 a
Reduction CFU.log ₁₀ /g in local (fresh) meat after subjecting to chitosan (2%) treatment at ambient temperature.	2.82±0.31 b

DISCUSSION

Food contamination by *S. aureus* bacteria can be influenced by factors such as carriers, poor hygiene practices, mishandling by infected workers, and inadequate transport systems. These findings are consistent with Porpino et al. (2015), who reported that meat contamination can occur at various stages—from preparation and production to distribution and storage in retail supermarkets—due to improper refrigeration temperatures that can increase bacterial loads and lead to the production of dangerous enterotoxins (Rani et al., 2023). Mohammed and Alwan (2017) isolated MRSA from meat collected in Karbala city markets and highlighted bovine meat as a major source of foodborne diseases in Iraq. Aziz and Lafta (2021) found that *Staphylococcus* accounted for 27% of isolates from both healthy and infected sheep.

The results of the hygienic assessment of examined frozen meat samples showed that imported meat had higher counts and percentages (%) than local meat. This poses a significant public health hazard compared to local meat. Frozen imported meat samples were found to harbor MRSA. The contamination of these meats, sold in Baghdad markets, can be attributed to inadequate hygienic practices during handling, contaminated equipment, and unsanitary conditions in local market processing facilities. Poor personal hygiene, such as frequent thawing and refreezing of frozen meat, can decrease meat quality and render it unfit for human consumption. Local authorities should educate the public about the health risks associated with consuming such meats and ensure proper cooking methods are employed. The presence of bacterial isolates in

frozen meats indicates that imported bovine meats could serve as vehicles for MRSA infections. Recent reports have shown an increase in MRSA infections in calves, sheep, horses, and poultry (Guo et al., 2022).

Chitosan dissolved in the acid solutions used as edible for keeping quality and shelf-life extension of many foods products as meat, meat products and fish (Karsli et al., 2018). The results of this study showed that MRSA isolates were greater sensitive to chitosan at concentration (2 %) which gave highest zone of inhibition (38 mm). This result in agreement with Eldaly et al. (2018) who recorded that coated the chicken fillets samples with chitosan at different concentrations (1.0%, 0.5%, and 2.0%) caused a significant reduction in bacterial load total aerobic bacteria, total Enterobacteriaceae, and Staphylococcus spp. according the storage period. Chitosan have been widely studied in last years in which used as antimicrobial agents and polymer substrates at the same time (Amankwaah et al., 2020). Chitosan activity against *S. aureus* was promoted positively with the increase of Mw, but the antibacterial effect against *E. coli* was reduced with the elevated of the Mw (Liu, et al., 2020).

The DD (degree of de-acetylation) of chitosan which used in conducting study was 90 %. The DD must not exceed 95% in order to dissolve with the glacial acetic acid 1%. Byun, et al. (2013) reported that the DD interfered with antibacterial properties of chitosan, the higher DD of chitosan, the stronger of its antibacterial activity. Chitosan with a DD of 81.56% against *Salmonella Enteritidis*, *L. monocytogenes* and *S. aureus* was higher than chitosan with DD of 62.71%. The antimicrobial activity of chitosan is due to binding to the teichoic acids that found in the Gram-positive bacterial cell wall, coupled with lipids membrane that finally resulted death of the bacterial cell (Zaghoul et al., 2019).

The relationship of pH with chitosan is influenced not only by its solubility in acidic conditions but also by the poly-cationic nature of chitosan molecules at pH < 6.5. Chitosan's antibacterial activity decreases with increasing pH and is completely lost at pH 7.0, when the amino groups of chitosan are no longer significantly charged (Ardean et al., 2021). Both natural chitosan extracted from shrimp shells (food-grade chitosan) and commercially synthesized chitosan exhibit pH-dependent antibacterial activities against *S. aureus*. The results indicate that natural chitosan shows greater antibacterial activity against both Gram-positive and Gram-negative bacteria compared to commercial chitosan (Chang et al., 2019). Dipping meat pieces in antibacterial solutions for 4 hours at room temperature and refrigeration temperature (4°C) resulted in a reduction of bacterial counts by 2-3 log₁₀ CFU/g (Tables 5 and 6). These findings are consistent with Fujimoto et

al. (2006), who reported that chitosan coating reduced viable bacterial counts by 2-3 log cycles in *S. aureus* and *B. cereus*, and by 1-2 log cycles in *P. fluorescens* and *E. coli*. The current study's results are also aligned with Amato et al. (2018), who demonstrated that chitosan applied as a coating significantly reduced the adhesion of *S. epidermidis* by 3 logs compared to uncoated dressing tissues. The antibacterial activity of chitosan is attributed to its ability to lyse bacterial cell membranes, leading to increased membrane permeability and structural alterations that result in leakage of cellular contents such as enzymes, nucleotides, ions, and proteins (Tao et al., 2011).

The results of the present study are consistent with Davis et al. (2012), who investigated the inhibition of five bacterial species including *S. aureus*, *E. coli*, *Yersinia enterocolitica*, *L. monocytogenes*, and *S. typhimurium*. The study utilized six concentrations of chitosan ranging from 0.5% to 2.5% (Kaloti and Bohidar, 2010). Pathogenic bacterial growth was assessed after 8 days of storage at pH 6.5; chitosan concentrations of 2.0% and 2.5% showed a reduction of 2-3 log in *S. aureus* counts after one day of incubation at pH 5.5 (do Amaral Sobral et al., 2022). Various levels of chitosan (0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) completely prevented the growth of *S. aureus* after 1 day. The findings of the current study are consistent with several researchers who have reported the antibacterial effects of chitosan against *S. aureus* and other Gram-positive bacteria (Soni et al., 2018; Abd El-Hack et al., 2020). Knowles and Roller (2001) discussed the effects of chitosan at concentrations of 0.5%, 1.0%, and 2.0% against *L. monocytogenes*, *S. enterica* serovar Typhimurium, *S. aureus*, and *S. cerevisiae*; chitosan showed varying reductions in *S. aureus* counts, up to 0.8 log CFU/ml.

According to Firdaus et al. (2024), water-soluble chitosan exhibits no antimicrobial activity compared to chitosan dissolved in acidic solutions. Acidic chitosan forms an amino group that attaches to the phosphoryl group of bacterial cell phospholipids, creating pores in the cell membrane that lead to leakage of cellular substances and bacterial cell death (Sukmark et al., 2011). In another study, Khudhir (2021b) reported on the antimicrobial activities of chitosan against coliforms, *E. coli* O157, yeasts, and molds in local Iraqi cheese products. Chitosan (CH) exhibited the best antimicrobial activity at a concentration of 5% with a contact time of 6 hours at refrigeration temperature (4°C). Our findings regarding the logarithmic reduction (log₁₀ CFU/g) indicate significant differences, with the highest reduction achieved using 2% chitosan under refrigerated conditions, resulting in a 3 log₁₀ CFU/g reduction. This result can be explained by the storage temperature of chitosan, which greatly influences its antibacterial efficacy. Temperature not only affects the antibacterial activity of chitosan but also its viscosity or molecular weight, which

may vary at different temperatures (Kong et al., 2010). Temperature has a significant impact on the antibacterial activity of chitosan due to the physiological properties of the tested bacteria. For instance, the optimal temperature for the antibacterial activity of water-soluble chitosan against oral bacteria is 37°C (Chen and Chung, 2012). Chitosan stored at 4°C exhibits stronger antibacterial activity compared to chitosan stored at 25°C (No et al., 2006). These findings are consistent with and support the results obtained in this study.

Reducing microbial contamination and ensuring food safety often require employing multiple traditional preservation methods. The concept of hurdles combines various methods such as high and low temperatures, water activity (aw), synthetic preservatives (e.g., nitrite, sorbate), lactic acid bacteria and their bacteriocins, and pH reduction, which are utilized to stabilize various food products including meat, fish, milk, and vegetables (Leistner, 2000; Ibrahim et al., 2020). Alam et al. (2017) reported that chitosan inhibits the growth and multiplication of spoilage bacteria in beef meat stored at 4°C for 12 days.

CONCLUSION

The application of chitosan as a natural food-grade antibacterial against MRSA was effective in reducing bacterial counts and maintaining the bacteriological quality of beef meat sold at different temperatures in public markets in Baghdad, Iraq.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. Authors declaration: We hereby confirm that all the Tables in the manuscript are ours work.

NOVELTY STATEMENT

This study indicated that meat serves as a reservoir for the highly dangerous MRSA, posing risks to human health. Natural food-grade chitosan plays a crucial role as an antibacterial substance in preserving the quality of meat.

AUTHORS' CONTRIBUTION

Research is extracted from a doctoral Desertion of the Ph.

D Student Qais Abdulrahman, University of Baghdad, Baghdad, Iraq. Dr zina Saab Khudhir the adviser was designed all the experiments. Qais Abdulrahman performed all experiments, collected the samples and data, and wrote the draft of the research. Dr zina contributed to checked the analyses of the data to the finalize the manuscript for journal submission. Final version of the current manuscript wrote by both authors for publishing in the respected Journal of Animal and Health Production.

REFERENCES

- Abbas L. A., Rady A. M. (2023). Isolation and molecular detection of *Staphylococcus aureus* from feline otitis in Baghdad. *Iraqi J. Vet. Sci.*, 37: 141-146. <https://doi.org/10.33899/ijvs.2023.1375560.2696>
- Abd El-Hack M. E., El-Saadony M. T., Shafi M. E., Zabermaawi N. M., Arif M., Batiha G. E., Al-Sagheer A. A. (2020). Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review. *International J. Biolog. Macromol.*, 164: 2726-2744. <https://doi.org/10.1016/j.ijbiomac.2020.08.153>
- Ahmed B. J., Saleem M. H., Tomma J. H. (2020). New Derivative of Chitosan Based on Epichlorohydrine, Synthesis, Characterization, Antibacterial Activity and Swelling Study. *Biochem. Cellul. Archiv.*, 20(1). <https://doi.org/10.35124/bca.2020.20.1.1851>.
- Ahmed Z. A., Yousif A. A. (2021). Molecular and phylogenetic analysis of methicillin resistant *Staphylococcus aureus* isolated from subclinical mastitis in lactating ewes. *Iraqi J. Vet. Sci.*, 35(Supplement I-III): 121-126. <https://doi.org/10.33899/IJVS.2021.131854.2013>
- Al-Dujaily A. H., Mahmood A. K. (2022). Evaluation of Antibacterial and antibiofilm activity of biogenic silver nanoparticles and gentamicin against *Staphylococcus aureus* isolated from caprine mastitis. *Iraqi J. Vet. Med.*, 46(1): 10-16. <https://doi.org/10.30539/ijvm.v46i1.1309>
- Ajayeoba T. A., Kaka O. M., Ajibade O. A. (2023). 7 Microbial food spoilage of selected food and food products. *Food Sci. Technol. Fundament. Innovat*, 173.
- Al-Rasheed A. A., Sana'a S. A., Al-Jashamy K. A., Garba B. (2022). Immunopathological Responses to the Bovine Mastitis Associated with *Staphylococcus* Species Infection. *Iraqi J. Vet. Med.*, 46(2): 7-11. <https://orcid.org/0000-0001-6551-1045>
- Alam J., Murshed H. M., Rahman S. M. E., Oh D. H. (2017). Effect of chitosan on quality and shelf life of beef at refrigerated storage. <https://doi.org/10.3329/bjas.v46i4.36963>
- Al-Rudha A. M., Khalil N. K., Altaai N. A. (2021). Evaluation of bacterial contaminants and heavy metals in cow and buffalo raw milk sold in Baghdad governorate. *Iraqi J. Vet. Sci.*, 35: 101-105. <https://doi.org/10.33899/ijvs.2021.131744.1999>
- Amankwaah C., Li J., Lee J., Pascall M. A. (2020). Antimicrobial activity of chitosan-based films enriched with green tea extracts on murine norovirus, *Escherichia coli*, and *Listeria innocua*. *Int. J. Food Sci.* 2020:1-9. <https://doi.org/10.1155/2020/3941924>
- Amato A., Migneco L. M., Martinelli A., Pietrelli L., Piozzi A., Francolini I. (2018). Antimicrobial activity of catechol functionalized-chitosan versus *Staphylococcus epidermidis*. *Carbohydrate polymers*, 179: 273-281. <https://doi.org/10.1016/j.carbpol.2018.05.055>

- doi.org/10.1016/j.carbpol.2017.09.073
- Ardean C., Davidescu C. M., Nemeş N. S., Negrea A., Ciopec M., Duteanu N., Muşta V. (2021). Factors influencing the antibacterial activity of chitosan and chitosan modified by functionalization. *Int. J. Molec. Sci.*, 22(14): 7449. <https://doi.org/10.3390/ijms22147449>
- Aziz T. A., Lafta I. J. (2021). Isolation and antimicrobial resistance of *Staphylococcus* spp., enteric bacteria and *Pseudomonas* spp. associated with respiratory tract infections of sheep. *Iraqi J. Vet. Sci.*, 35: 53-58. <https://doi.org/10.3389/ijvs.2021.131098.1917>
- Byun S. M., No H. K., Hong J. H., Lee S. I., Prinyawiwatkul W. (2013). Comparison of physicochemical, binding, antioxidant and antibacterial properties of chitosans prepared from ground and entire crab leg shells. *Int. Food Sci. Technol.*, 48(1): 136-142. <https://doi.org/10.1111/j.1365-2621.2012.03169>
- Chang A. K. T., Frias Jr, R. R., Alvarez L. V., Bigol U. G., Guzman J. P. M. D. (2019). Comparative antibacterial activity of commercial chitosan and chitosan extracted from *Auricularia* sp. *Biocatal. Agricult. Biotechnol.*, 17: 189-195. <https://doi.org/10.1016/j.bcab.2018.11.016>
- Chen C. Y., Chung Y. C. (2012). Antibacterial effect of water-soluble chitosan on representative dental pathogens *Streptococcus mutans* and *Lactobacilli brevis*. *J. Appl. Oral Sci.*, 20: 620-627. <https://doi.org/10.1590/s1678-77572012000600006>
- Choi M., Hasan N., Cao J., Lee J., Hlaing S. P., Yoo J. W. (2020). Chitosan-based nitric oxide-releasing dressing for anti-biofilm and in vivo healing activities in MRSA biofilm-infected wounds. *Int. J. Biology. Macromolecule.*, 142: 680-692. <https://doi.org/10.1016/j.jbiomac.2019.10.009>
- Davis R., Zivanovic S., D'Souza D. H., Davidson P. M. (2012). Effectiveness of chitosan on the inactivation of enteric viral surrogates. *Food Microbiol.*, 32(1): 57-62. <https://doi.org/10.1016/j.fm.2012.04.008>
- Dickson J. S., Cutter C. N., Siragusa G. R. (1994). Antimicrobial effects of trisodium phosphate against bacteria attached to beef tissue. *J. Food Protect.*, 57(11): 952-955. <https://doi.org/10.4315/0362-028X-57.11.952>
- do Amaral Sobral P. J., Gebremariam G., Drudi F., De Aguiar Saldanha Pinheiro A. C., Romani S., Rocculi P., Dalla Rosa M. (2022). Rheological and viscoelastic properties of chitosan solutions prepared with different chitosan or acetic acid concentrations. *Foods.*, 11(17): 2692. <https://doi.org/10.3390/foods11172692>
- Egorov A. R., Kirichuk A. A., Rubanik V. V., Rubanik Jr V. V., Tskhovrebov A. G., Kritchenkov A. S. (2023). Chitosan and Its Derivatives: Preparation and Antibacterial Properties. *Materials.*, 16(18): 6076. <https://doi.org/10.3390/ma16186076>
- Eldaly A. E., Mahmoud F. A., Abobakr H. M. (2018). Preservative effect of chitosan coating on shelf life and sensory properties of chicken fillets during chilled storage. *J. Nutrit. Food Secur.*, 3(3): 139-148. <https://doi.org/10.1001.1.24767417.2018.3.3.5.0>
- Firdaus S., Ahmad F., Zaidi S. (2024). Preparation and characterization of biodegradable food packaging films using lemon peel pectin and chitosan incorporated with neem leaf extract and its application on apricot fruit. *Int. J. Biolog. Macromol.*, 130358. <https://doi.org/10.1016/j.jbiomac.2024.130358>
- Fujimoto T., Tsuchiya Y., Terao M., Nakamura N., Yamamoto M. (2006). Antibacterial effects of chitosan solution against *Legionella pneumophila*, *Escherichia coli*, and *Staphylococcus aureus*. *Int. J. Food Microbiol.*, 112, 96e101. <https://doi.org/10.1016/j.ijfoodmicro.2006.06.003>
- Guo Q., Ren C. W., Cai J. H., Zhang C. Y., Li Y. T., Xu B., Farooq M. A. (2022). The synergistic inhibition and mechanism of epicatechin gallate and chitosan against methicillin-resistant *Staphylococcus aureus* and the application in pork preservation. *LWT.*, 163: 113575. <https://doi.org/10.1016/j.lwt.2022.113575>
- Hafez M., Ibrahim H., Amin R. (2019). Assessment of bacterial evaluation of imported frozen meat. *Benha Vet. Med. J.*, 37(2): 42-45. <https://doi.org/10.21608/bvmj.2019.18547.1123>
- Ibrahim M., Hegazy A. I., Osheba A. S., Nageib A. M. (2020). Using of hurdles technology to improve the quality characteristics of chicken fillets during storage. *Al-Azhar J. Agricult. Res.*, 45(1): 56-74. <https://doi.org/10.21608/ajar.2020.126634>
- Kadhum H. H., Abood Z. H. (2022). *Staphylococcus aureus* Incidence in Some Patients with a Topic Dermatitis in Baghdad City. *Iraqi J. Biotechnol.*, 21(2): 13-20. <http://jige.uobaghdad.edu.iq/index.php/IJB>
- Kaloti M., Bohidar H. B. (2010). Kinetics of coacervation transition versus nanoparticle formation in chitosan-sodium tripolyphosphate solutions. *Colloids Surf. B: Biointerf.*, 81(1): 165-173. <https://doi.org/10.1016/j.colsurfb.2010.07.006>
- Karsli B., Caglak E., Li D., Rubio N. K., Janes M., Prinyawiwatkul W. (2018). Inhibition of selected pathogens inoculated on the surface of catfish fillets by high molecular weight chitosan coating. *Int. J. Food Sci. Technol.*, <https://doi.org/10.1111/ijfs.13897>
- Khudhir Z. S. (2021a). Evaluation the Antibacterial Activity of the Brine, Nisin Solution, and Ozonated Water Against *E. coli* O157: H7 in the Experimentally Local Produced Soft Cheese. *Iraqi J. Vet. Med.*, 45(1): 17-21. <https://doi.org/10.30539/ijvm.v45i1.1035>
- Khudhir Z. S. (2021b). Antimicrobial activity of chitosan and/or Gum Arabic in the local produce soft and hard cheese in Baghdad city. *Biochem. Cellul. Archiv.*, Apr 2;21. <https://doi.org/10.37506/ijfnt.v16i1.17563>
- Khudhir Z. S., Ahmed A. J. (2017). Evaluation of the bactericidal effect of Nisin and/or Potassium sorbate and Sodium chloride on the viability of *Staphylococcus aureus* in soft cheese. *Iraqi J. Vet. Med.*, 41(1): 55-59. <https://doi.org/10.30539/iraqijvm.v41i1.79>
- Kibar H. F., Sabir F. K. (2018). Chitosan coating for extending postharvest quality of tomatoes (*Lycopersicon esculentum* Mill.) maintained at different storage temperatures. *AIMS Agricult. Food*, 3(2). <https://doi.org/10.3934/agrfood.2018.2.97>
- Knowles J., Roller S. (2001). Efficacy of chitosan, carvacrol, and a hydrogen peroxide-based biocide against foodborne microorganisms in suspension and adhered to stainless steel. *J. Food Protect.*, 64(10): 1542-1548. <https://doi.org/10.4315/0362-028x-64.10.1542>
- Kong M., Chen X. G., Xing K., Park H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state-of-the-art review. *Int. J. Food Microbiol.*, 144(1): 51-63 <https://doi.org/10.1016/j.ijfoodmicro.2010.09.012>
- Kumar S., Mukherjee A., Dutta J. (2020). Chitosan based nano composite films and coatings: emerging antimicrobial food packaging alternatives. *Trends Food Sci. Technol* 97:196-209. <https://doi.org/10.1016/j.tifs.2020.01.002>

- Leistner L. (2000). Hurdle technology in the design of minimally processed foods. In: Alzamora, S.M., Tapia, M.S., López-Malo, A., Ed., Minimally Processing Fruits and Vegetables Aspen Publishers, Inc., Gaithersburg, MD: pp. 13–27.
- Liu S., Cai X., Xue W., Ma D., Zhang W. (2020). Chitosan derivatives co-delivering nitric oxide and methicillin for the effective therapy to the methicillin-resistant *S. aureus* infection. Carbohydr. Polymers., 234: 115928. <https://doi.org/10.1016/j.carbpol.2020.115928>
- Liu T., Li J., Tang Q., Qiu P., Gou D., Zhao J. (2022). Chitosan-based materials: An overview of potential applications in food packaging. Foods., 11(10): 1490. <https://doi.org/10.3390/foods11101490>
- Manilal A., Sabu K. R., Shewangizaw et al. (2020). In vitro antibacterial activity of medicinal plants against biofilm-forming methicillin-resistant *Staphylococcus aureus*: efficacy of *Moringa stenopetala* and *Rosmarinus officinalis* extracts. Heliyon, vol. 6, no. 1, p. e 03303 <https://doi.org/10.1016/j.heliyon.2020.e03303>
- Mohammed N. I., Mohammad J. Alwan (2017): Isolation and Identification of *Staphylococcus aureus* strain from fresh and frozen meat in karbala province. Int. J. Sci. Nat., 8(3): 704–709. : <https://www.researchgate.net/publication/327976973>
- Munoz-Tebar N., Perez-Alvarez J. A., Fernandez-Lopez J., Viuda-Martos M. (2023). Chitosan Edible Films and Coatings with Added Bioactive Compounds: Antibacterial and Antioxidant Properties and Their Application to Food Products: A Review. Polymers., 15(2): 396. <https://doi.org/10.3390/polym15020396>
- No H. K., Kim S. H., Lee S. H., Park N. Y., Prinyawiwatkul W. (2006). Stability and antibacterial activity of chitosan solutions affected by storage temperature and time. Carbohydr. Poly., 65(2): 174–178. <https://doi.org/10.1016/j.carbpol.2005.12.036>
- Nungesser F., Winter M. (2021). Meat and social change. Osterr. Z. Soziologie 46: 109–124. <https://doi.org/10.1007/s11614-021-00453-0>
- Patricia M. T. (2014). Bailey & Scott's Diagnostic Microbiology, 13th Mosby, Inc., an affiliate of Elsevier Inc. Riverport Lane, St. Louis, MI, USA. ISBN: 9780323681056
- Porpino G., Parente J., Wansink B. (2015). Food waste paradox: antecedents of food disposal in low-income households. Int. J. Consum. Stud., 39(6): 619–629. <https://doi.org/10.1111/ijcs.12207>
- Qin C., Li H., Xiao Q., Liu Y., Zhu J., Du Y. (2006). Water-solubility of chitosan and its antimicrobial activity. Carbohydr. Poly., 63(3): 367–374. <https://doi.org/10.1016/j.carbpol.2005.09.023>
- Rani Z. T., Mhlongo L. C., Hugo A. (2023). Microbial profiles of meat at different stages of the distribution chain from the abattoir to retail outlets. Int. J. Environ. Res. Pub. Health., 20(3): 1986. <https://doi.org/10.3390/ijerph20031986>
- Román-Doval R., Torres-Arellanes S. P., Tenorio-Barajas A. Y., Gómez-Sánchez A., Valencia-Lazcano A. A. (2023). Chitosan: Properties and its application in agriculture in context of molecular weight. Polymers., 15(13): 2867. <https://doi.org/10.3390/polym15132867>
- Soni B., Mahmoud B., Chang S., El-Giar E. M. (2018). Physicochemical, antimicrobial and antioxidant properties of chitosan/TEMPO biocomposite packaging films. Food packaging and shelf life., 17: 73–79. <https://doi.org/10.1016/j.fpsl.2018.06.001>
- Sukmark T., Rachtanapun P., Rachtanapun C. (2011). Antimicrobial activity of oligomer and polymer chitosan from different sources against foodborne pathogenic bacteria. Kasetsart J. (Nat. Sci.). 45:636–643. <https://li01.tci-thaijo.org/index.php/anres/article/view/245342>.
- Tao Y., Qian L. H., Xie J. (2011). Effect of chitosan on membrane permeability and cell morphology of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Carbohydr. Poly., 86(2): 969–974. <https://doi.org/10.1016/j.carbpol.2011.05.054>
- Umerska A., Cassisa V., Bastiat G., Matougui N., Nehme H., Manero F., Saulnier P. (2017). Synergistic interactions between antimicrobial peptides derived from plectasin and lipid nanocapsules containing monolaurin as a cosurfactant against *Staphylococcus aureus*. Int. J. Nanomed., 5687–5699. <https://doi.org/10.2147/IJN.S139625>
- Umoren S. A., Solomon M. M., Nzila. A., Obot I. B. (2020). Preparation of silver/chitosan nanofluids using selected plant extracts: Characterization and antimicrobial studies against gram-positive and gram-negative bacteria. Materials., 13(7): 1629. <https://doi.org/10.3390/ma13071629>
- Weißpflog J., Vehlou D., Müller M., Kohn B., Scheler U., Boye S., Schwarz S. (2021). Characterization of chitosan with different degree of deacetylation and equal viscosity in dissolved and solid state—Insights by various complimentary methods. Int. J. Biolog. Macromol., 171: 242–261. <https://doi.org/10.1016/j.ijbiomac.2021.01.010>.
- Yu D., Regenstein J.M., Xia W. (2019). Bio-based edible coatings for the preservation of fishery products: A review. Crit. Rev. Food Sci. Nutr. 59: 2481–2493. <https://doi.org/10.1080/10408398.2018.1457623>.
- Zaghloul H. E., Ibrahim A. H. (2019). Comparative study on antimicrobial activity of commercial and extracted chitin and chitosan from *Marsupenaeus japonicus* shells. Egyptian J. Aquat. Biol. Fisher., 23(2): 291–302. <https://doi.org/10.21608/ejabf.2019.31536>
- Zheng T., Tang P., Li G. (2022). Effects of chitosan molecular weight and deacetylation degree on the properties of collagen-chitosan composite films for food packaging. J. Appl. Polymer Sci., 139(41): e52995. <https://doi.org/10.1002/app.52995>