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Original Article

ASSESSMENT OF BACTERIAL CONTAMINATION IN CHICKEN MEAT SHAWARMA IN ERBIL CITY AND ANTIBACTERIAL EFFECT OF ZnO–CHITOSAN NANOPARTICLES

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ABSTRACT

This study investigated the microbiological safety of chicken shawarma sold in Erbil, Kurdistan Region, Iraq, by considering its contamination level and studying the antibacterial action of ZnO-chitosan nanoparticles. Thirty chicken shawarma samples were collected and screened for bacterial contamination using standard microbiological methods. It was revealed that 70% of the samples had high total viable counts. *Salmonella* (26%), *Escherichia coli* O157 (16.6%), and some coliforms (10%), as well as *Klebsiella* (10%), *Proteus* (6.6%), and *Staphylococcus aureus* (6.6%) were also isolated, indicating that the sample had poor hygiene and the associated practices were unsafe. It was observed that ZnO-chitosan nanoparticles showed remarkable inhibition zones against all the bacteria tested, with the highest zones at 10 mg/mL. These results raise serious concerns about the public's health concerning chicken shawarma and suggest that natural antimicrobial nanocomposites could be effective as preservatives for enhancing shelf life and food safety.

Keywords: Chicken meat shawarma, bacterial contamination, ZnO-chitosan nanoparticles, antimicrobial activity, food safety.

1. INTRODUCTION

Shawarma is a Levantine Arab dish, consisting of lamb (red meat), chicken, or other species such as turkey and beef. Shawarma meal may be presented on a plate or as a wrap or sandwich, but its meat is roasted only from the outside. Hence, the meat inside remains uncooked, despite the shavings being cut off part of the meat for serving, and the rest meat is kept roasted on the rotating skewer (Ahmed *et al*., 2015).

Chicken shawarma indeed comprises one of the famed and delicious treats of the Middle East. The chicken is usually marinated in selected aromatic spices and grilled or roasted thin, served within a warm pita or flatbread (Ehsanur Rahman *et al*., 2023). The marinade is typically made with a mixture of yoghurt, lemon juice, crushed garlic, cumin, paprika, turmeric, and a pinch of cinnamon, resulting in a truly flavorful and intoxicating scent. When cooked to tender perfection, they are placed in wraps with pickles and fresh vegetable dressing, or sometimes they are crushed with garlic toum or tahini. The chicken shawarma can be enjoyed in various ways, such as in wraps, rice bowls, or as a salad, making it loved all around the world for its intense and satisfying flavours. Its versatility and rich taste have helped it gain popularity far beyond its Middle Eastern roots, becoming a favourite street food and fast-casual meal in many countries.

Hygiene practices during the preparation of shawarma play a decisive role in determining its microbial quality and overall safety. Shawarma is typically prepared under conditions that involve prolonged exposure of meat to ambient temperature, repeated handling, and continuous slicing from rotating spits, all of which increase the risk of microbial contamination. Poor hygiene practices, such as inadequate hand washing, cross-contamination from raw to cooked meat, and improper cleaning of utensils or cutting surfaces, can accelerate bacterial growth and survival. Pathogenic microorganisms such as *Salmonella*, *Escherichia coli* (*E. coli*), *Listeria monocytogenes*, and *Staphylococcus aureus* (*S. aureus*) have frequently been reported in shawarma samples, highlighting the vulnerability of this food product to contamination when hygiene standards are neglected (Ibrahim, 2024). Furthermore, lapses in maintaining appropriate cooking and holding temperatures create an environment that favors the proliferation of foodborne pathogens, thereby elevating public health risks (Ayaz *et al*., 1985). To mitigate these risks, interventions must focus on strengthening food safety protocols at both the vendor and regulatory levels. This includes strict enforcement of personal hygiene practices, proper thermal processing, regular sanitization of preparation equipment, and the application of Hazard Analysis and Critical Control Points (HACCP) systems to monitor critical steps in shawarma preparation. Beyond conventional hygienic measures, recent advances suggest that natural nanocomposites hold promise as sustainable preservatives capable of enhancing microbial safety. For instance, nanocomposites based on zinc oxide, chitosan, and silver nanoparticles have demonstrated potent antimicrobial activity against a broad spectrum of foodborne pathogens. Their incorporation into edible coatings or packaging materials can create a protective barrier that suppresses microbial growth, extends shelf life, and reduces reliance on synthetic preservatives. These bio-based nanomaterials not only address food safety concerns but also align with consumer demands for environmentally friendly and health-conscious preservation methods (Kumar *et al*., 2020). Chicken shawarma establishments can be fined if food safety protocols are not correctly implemented during meat preparation, cooking, or storage. Once marinated, the chicken is skewered vertically on a rotisserie, allowing an undercooked portion in the Middle to become a breeding ground for dangerous bacteria such as *Salmonella*, *E. coli*, *Proteus*, *Klebsiella*,

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or *S. aureus*. More than 250 different foodborne diseases have been described; most of these diseases are caused by a variety of pathogenic bacteria, parasites, and viruses that can be transmitted through food and cause food poisoning (Nimri *et al*., 2014). Cross-contamination can spread bacteria from raw chicken to other surfaces, utensils, or foods, such as raw vegetables, that may not have been cleaned thoroughly (Carrasco *et al*., 2012). Additionally, based on the sauces the chicken may be dressed with, such as garlic mayonnaise or yoghurt-based dressings, bacterial growth could be possible if they are not stored at the right temperature. Foodborne illnesses become more likely when food is left out for an extended period and is not refrigerated or stored properly (Malak & Soliman, 2021). Good sanitary measures and cooking principles, along with proper handling, will eliminate any possibility of contamination that would render the shawarma unsafe to consume (Terpstra *et al*., 2005).

Chitin, a naturally abundant substance, transforms into chitosan, a specific type of linear cationic polysaccharide. Chitosan, a naturally occurring substance with an alkaline nature, is sourced from the exoskeletons of marine crustaceans, such as prawns and crabs. It has been empirically substantiated to manifest diverse biological functionalities (Dong *et al*., 2022, Gao & Wu, 2022, Hamodi & Younis, 2023).

ZnO-chitosan nanoparticles have been integrated into a single system to create a potent natural antimicrobial system, ensuring the safety and extending the shelf life of easily perishable food items, such as chicken shawarma. As a polymeric biopolymer, chitosan, derived from chitin, exhibits antimicrobial properties and versatility in film formation, making it suitable for food protective coatings and preservation (Munoz-Tebar *et al*., 2023). Bacteria are known to have a broad spectrum and high stability, and ZnO nanoparticles have been observed to exhibit vigorous synergistic antibacterial activity with chitosan against spoilage bacteria (Mohammed, 2023), including *E. coli*, *Salmonella*, and *S. aureus* (Al-Nabulsi *et al*., 2020). ZnO-chitosan nanoparticles are utilised in chicken shawarma to reduce the microbial load and extend shelf life, reflecting the growing interest in natural food preservation methods that avoid chemical preservatives (Sasidharan *et al*., 2024).

In response to foodborne contamination risks, natural nanocomposite-based preservatives show significant promise as sustainable interventions. Chitosan—a biodegradable biopolymer derived from crustacean waste—exhibits intrinsic antimicrobial properties and film-forming capabilities, making it suitable for edible coatings and packaging systems. When combined with zinc oxide nanoparticles (ZnO NPs), the resulting nanocomposites substantially enhance antimicrobial efficacy and shelf life across various foods (Al-Naamani *et al*., 2018). For example, chitosan-ZnO nanocomposite films reduced bacterial and fungal growth in okra by approximately 63% and two-fold, respectively, without compromising key quality attributes such as moisture, pH, or soluble solids over 12 days of storage (Novikov *et al*., 2023). Similarly, chitosan-ZnO coatings applied to fresh-cut papaya and strawberries effectively delayed microbial growth and maintained texture and firmness (Al-Naamani *et al*., 2018, Anugrah *et al*., 2020). Broader reviews also confirm that incorporating ZnONPs into chitosan or related biopolymer matrices significantly improves mechanical properties, barrier functions, and antimicrobial activity, particularly against *E. coli* and *S. aureus*, thereby extending shelf life across produce, meat, and dairy products (Babaei-Ghazvini *et al*., 2021).

The primary aim of isolating and identifying bacteria from chicken meat shawarma is to evaluate its microbiological safety and assess potential public health risks associated with its consumption. Specifically, the study seeks aims to determine the prevalence of bacterial contaminants, characterise the types of bacterial isolates, isolate and accurately identify the bacterial species using conventional culture techniques and biochemical or molecular methods, focusing on common foodborne pathogens such as Salmonella*Salmonella* spp., S. aureus*S. aureus*, E. coli*E. coli* (including E. coli*E. coli* O157:H7) (Issa, 2024), and Proteus*Proteus* spp. Evaluate the hygiene practices and risk factors, support public health and food safety policies, and guide future antimicrobial resistance monitoring.

2. MATERIALS AND METHODS

Study Area:

A cross-sectional investigation was conducted, and samples were collected from various restaurants in Erbil city between October 15, 2024, and January 15, 2025, and subsequently screened. This study was conducted in Erbil, Kurdistan Region, Iraq. Shawarma restaurants were selected from various districts to ensure a representative sample. During the sampling period, several suspected indicators of poor hygienic practices were observed, which likely contributed to the high microbial loads detected in the shawarma samples. One of the most prominent concerns was inadequate handling procedures by food handlers. In many cases, gloves or protective coverings were either not worn or not changed frequently, increasing the risk of cross-contamination between raw and cooked meat.

Sample Collection:

Samples were collected directly from various shawarma restaurants in Erbil city and placed in sterilised containers, then transferred to the lab for culture on an appropriate medium. The samples consisting of chicken meat shawarma comprised a total of thirty samples of chicken shawarma used in its preparation at a restaurant. The collected shawarma samples were examined in triplicate for the presence of bacteria (Eglezos *et al*., 2010).

Preparation of Shawarma Samples and Isolation of Bacteria:

Each shawarma sample was homogenised by blending 10 g of the sample with 90 mL of sterile peptone water (pre-enrichment step) (HiMedia, India) in a stomacher bag using a stomacher blender for 1-2 minutes to ensure uniform microbial distribution. The homogenate served as the initial 10^{-1} dilution. A series of tenfold serial dilutions (up to 10^{-6}) was then prepared using sterile normal saline as diluent. From appropriate dilutions, 0.1 mL or 1.0 mL aliquots were aseptically inoculated onto various selective and differential culture media using the spread plate or pour plate technique, depending on the medium: Nutrient agar (NA) (HiMedia, India) for total viable bacterial count, MacConkey agar (HiMedia, India) for isolation of Gram-negative, lactose-fermenting and non-fermenting Enterobacteriaceae, Mannitol Salt agar (HiMedia, India) for selective isolation of *Staphylococcus* spp., Xylose Lysine Deoxycholate (XLD) agar (HiMedia, India) for identification of *Salmonella* and *Shigella* spp., and Eosin Methylene Blue (EMB) agar (HiMedia, India) for isolation and differentiation of coliforms, particularly *E. coli*. All plates were incubated aerobically at $37 \pm 2^{\circ}$C for 24-48 hours. Following incubation, colonies were examined for their morphology (size, shape, colour, edge, and elevation), pigmentation, and lactose fermentation characteristics (where applicable). Colony-forming units (CFU) were calculated and recorded as CFU/g of the original sample. Distinct colonies were picked and sub-cultured onto fresh media to obtain pure isolates. These purified isolates were then subjected to Gram staining for preliminary classification as Gram-positive or Gram-negative bacteria.

Subsequently, biochemical methods were performed using standard tests, including the Catalase and oxidase test for preliminary grouping, Triple Sugar Iron (TSI) agar slants to assess sugar fermentation and hydrogen sulfide production, the Indole production test, Simmons' Citrate test for citrate utilisation, the Urease test, and the Motility test. All procedures were performed following aseptic techniques and standard microbiological protocols. Confirmed isolates were preserved in brain heart infusion broth with 20% glycerol at -20°C for further analysis.

Identification using the VITEK 2 Compact System:

Following primary bacterial isolation from the samples, purified colonies were subjected to preliminary identification using conventional biochemical tests. These classical methods provided presumptive identification and classification of the isolates based on standard microbiological criteria.

For confirmation and species-level identification, the selected isolates were further analyzed using the VITEK® 2 Compact System (bioMérieux, France), an automated bacterial identification system that utilises advanced colourimetric and fluorometric biochemical assays. Fresh, pure cultures of the isolates were obtained by subculturing onto Nutrient Agar and incubating at $35 \pm 2°C$ for 18-24 hours. A sterile swab was used to prepare a bacterial suspension in sterile 0.45% sodium chloride (NaCl) solution, and the turbidity was adjusted to match the 0.5 McFarland standard using a DensiCHEK™ turbidity meter (Nimer *et al*., 2016). The prepared suspension was loaded into the VITEK 2 cassette along with the corresponding identification card. The VITEK 2 Compact system automatically fills, seals, and incubates the cards at 35–37°C , with results generated within 3 to 7 hours, depending on the bacterial species. Identification results were interpreted by the VITEK 2 software, which compared the biochemical profile of each isolate to its extensive database and provided species-level identification with a confidence level percentage. Only identifications with ≥95% confidence and acceptable test quality control were considered valid.

Preparation of Zinc Oxide Nanoparticles:

Zinc oxide nanoparticles were synthesised by dissolving 0.1 M zinc acetate dihydrate in 100 mL of deionised water under continuous stirring at 60°C . To this solution, 1.0 M NaOH was added dropwise under vigorous magnetic stirring until the pH reached approximately 10. A white precipitate began to form, indicating the formation of zinc hydroxide, which was then converted to ZnO upon heating. The reaction mixture was maintained at $60 - 70°C$ for 2 hours, followed by cooling to room temperature. The precipitate was collected by centrifugation at 10,000 rpm for 15 minutes and washed several times with distilled water and ethanol to remove any residual ions or impurities. The final product was dried at 60°C in a hot-air oven for 12 hours and ground into fine ZnO nano powder.

Preparation of Chitosan Solution:

A chitosan solution was prepared by dissolving 0.5 g of chitosan in 100 mL of 1% (v/v) glacial acetic acid under magnetic stirring for 3-4 hours at room temperature, until a clear, homogeneous solution was obtained. The pH of the chitosan solution was adjusted to 5.5 using 1 M NaOH (Bhaskaran *et al*., 2023).

Synthesis of ZnO–chitosan Nanoparticles:

ZnO-chitosan nanoparticles were synthesised by slowly adding a known amount of ZnO nano powder (e.g., 0.1–0.3 g) to 100 mL of the prepared chitosan solution under magnetic stirring. The suspension was continuously stirred at room temperature for 2-4 hours to ensure uniform dispersion and interaction between ZnO and chitosan molecules. The resulting colloidal solution was then subjected to ultrasonication (20 kHz) for 30 minutes to enhance nanoparticle stabilisation and prevent agglomeration (Ali *et al*., 2024). The final suspension was centrifuged at 10,000 rpm for 20 minutes, and the obtained pellet was washed with distilled water, followed by ethanol. The ZnO-chitosan nanoparticles were dried at $50 - 60°C$ and stored in airtight containers for further characterisation and application (Alhujaily *et al*., 2022).

3. RESULTS

Bacterial Isolation and Identification from Chicken Meat Shawarma:

A total of 30 chicken meat shawarma samples were analysed for bacterial contamination, and showed dangerous levels of contamination. Contamination was as high as 70% (21 samples), with apparently high total viability counts. Among the bacterial isolates recovered from the chicken shawarma samples, *Salmonella* spp. was detected in 26% of the cases. Additionally, *E. coli* O157 was identified in 16.6% of the samples. Total coliforms were also detected in 10% of the samples,. Other opportunistic pathogens, including *Klebsiella* spp. (10%), Staphylococcus spp. (6.6%), and *Proteus* spp. (6.6%), were also isolated. The most frequently isolated bacteria from the chicken meat shawarma are revealed in Table 1.

Table 1: Percentages of bacterial contamination in thirty samples of chicken meat shawarma.

Bacterial species	Frequency of isolation	Percentage %
Total viable count	21	70.0
Total coliform	3	10.0
Staphylococcus	2	6.6
***E. coli* 0157**	5	16.6
Salmonella	6	26.0
Proteas	2	6.6
Klebsiella	3	10

Figure 1 presents the total viable bacterial counts identified in thirty chicken meat shawarma samples, evaluated across four treatment concentrations: 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL, and 10 mg/mL. The quantitative analysis demonstrated a varied distribution of bacterial contamination levels among the isolates. Notably, *Salmonella* spp. exhibited the highest mean viable counts across all concentrations. In contrast, *Proteus* spp. and Staphylococcus spp. showed the lowest levels of bacterial load among the identified pathogens, with comparatively fewer CFUs recorded per Gram of sample.

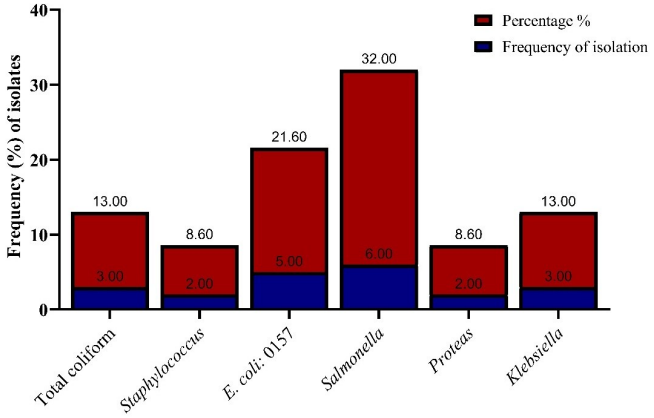


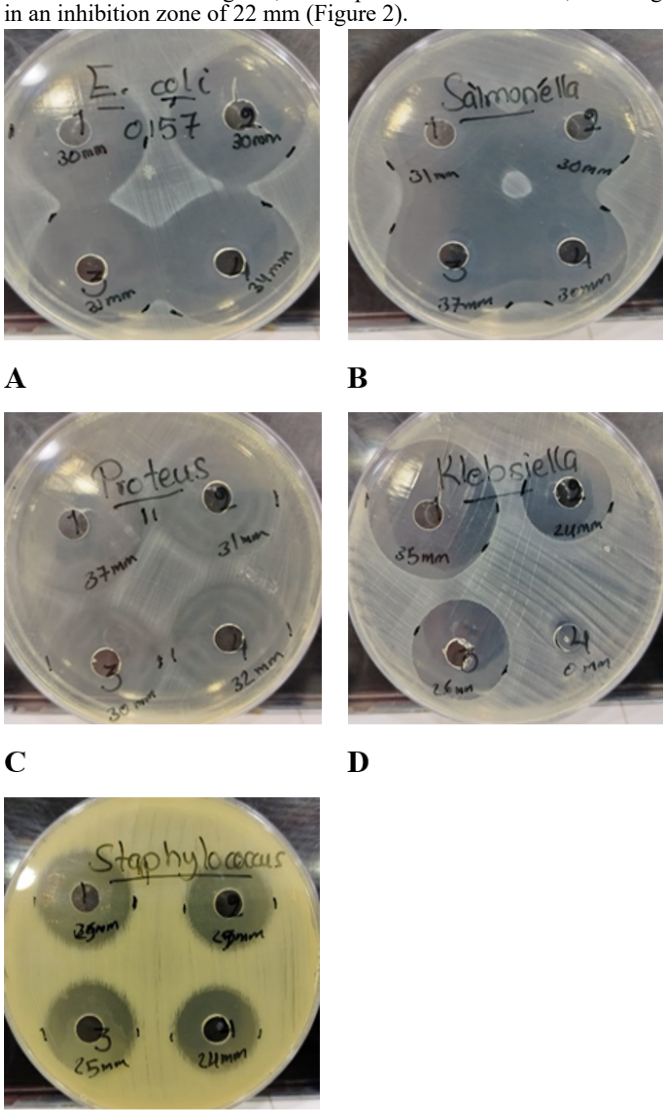
Figure 1: Total viable count of bacterial contamination in chicken meat shawarm

The inhibition zone diameters of bacterial isolates treated with ZnO-chitosan nanoparticles at varying concentrations demonstrated differential antimicrobial effects across species. At the highest tested concentration (10 mg/mL), *Proteus* spp. exhibited the largest inhibition zone, while *E. coli* O157 displayed the smallest inhibition zone, sparkly lower susceptibility. When the concentration was reduced to 5 mg/mL, both *E. coli* O157 and *Salmonella* spp. showed similar inhibition zone sizes, whereas *Klebsiella* spp. recorded the smallest zone,. Interestingly, at 2.5 mg/mL, *Salmonella* spp. demonstrated the greatest inhibition zone,. At the lowest concentration of 1.5 mg/mL, *Klebsiella* spp. showed no inhibition zone, (Table 2).

Table 2: Inhibition zone diameter (mm) of ZnO-chitosan nanoparticles toward bacterial strains isolated for this study.

Concentration (mg/mL)	***E. coli***	***Klebsiell***	***Proteus***	***Salmonella***	***Staphylococcus***
10.0	30	35	37	31	25
5.0	30	24	31	30	25
2.5	31	26	30	37	25
1.5	34	0	32	30	24

The ZnO-chitosan nanoparticles demonstrated significant antibacterial effects against the tested bacterial isolates, with notable variations depending on both concentration and bacterial species. The most substantial inhibition against *E. coli* O157 was observed when ZnO-chitosan nanoparticles were applied at a concentration of 1.5 mg/mL with a nanoparticle size of 31 nm, resulting in an inhibition zone measuring 34 mm. In contrast, *Klebsiella* spp. showed complete resistance to the same treatment conditions, with no detectable inhibition zone (0 mm). For *Salmonella* spp., the highest antibacterial activity was observed when ZnO and chitosan were applied at a concentration of 2.5 mg/mL, with a particle size of 37 nm, resulting in an inhibition zone of 22 mm (Figure 2).



A **B**

C **D**

E

Figure 2: The inhibition zone diameters (mm) of ZnO-chitosan nanoparticles at different concentrations toward various bacteria isolates. Using different concentrations of ZnO-chitosan nanoparticles, no. 1 = 1000 µg/mL, no. 2 = 500 µg/mL, no. 3 = 250 µg/mL, no. 4 = 125 µg/mL. Inhibition zone diameter of ZnO-chitosan nanopartilces against (A). *E. coli* O157. (B) *Salmonella* group. (C). P. mirabilis. (D) *Klebsiella* sp., and (E). *S. aureus*.

4. DISCUSSION

The microbiological assessment of 30 chicken shawarma samples revealed a high prevalence of bacterial contamination, with 70% of the samples exhibiting elevated viable counts. This level of microbial burden is consistent with previous findings in similar food products, where poor hygiene during handling, preparation, and storage plays a central role in microbial proliferation (Saeed & Mohammad, 2021). The detection of foodborne pathogens such as *E. coli* O157, *Proteus* spp., *Salmonella* spp., and *S. aureus* underscores the significant public health risks associated with the consumption of inadequately prepared or stored shawarma (Viana *et al*., 2025). The high contamination rates found in this study reflect substantial deficiencies in food hygiene practices, including improper handling, inadequate cleaning of utensils, and insufficient cooking or storage temperatures. These findings are supported by similar studies in Iraq and Egypt that reported high microbial loads in fast food due to lapses in food safety controls (Ahmed *et al*., 2015; Mohamed & El-Zahaby, 2024).

The antimicrobial activity assay revealed a strong dose-dependent inhibitory effect of ZnO nanoparticles and chitosan on the tested bacterial isolates. At 10 mg/mL, the largest zones of inhibition were recorded against *Proteus* spp. and *Salmonella* spp., measuring 35 mm and 31 mm, respectively. These findings are in agreement with previous studies that demonstrated the potent bactericidal effects of ZnO nanoparticles, particularly at nanoscale sizes (22-40 nm), due to their capacity to generate reactive oxygen species and disrupt bacterial membranes (Murali *et al*., 2023; Nimri *et al*., 2014).

Chitosan, a biopolymer known for its biocompatibility and antimicrobial potential, likely enhances the efficacy of ZnO by destabilising bacterial cell membranes and interfering with biofilm formation (Shakir *et al*., 2021). The synergistic effect of ZnO and chitosan observed in the current study supports their combined use as a promising antimicrobial system. The synergistic effect of ZnO and chitosan against bacterial pathogens arises from their complementary mechanisms of action. ZnO nanoparticles disrupt bacterial cell membranes and generate reactive oxygen species (ROS), causing oxidative stress and DNA damage, while chitosan, a natural biopolymer, binds to negatively charged bacterial cell walls, inhibiting nutrient uptake and promoting membrane permeability. Together, they enhance antibacterial efficacy by combining physical disruption (ZnO) with electrostatic interference (chitosan), reducing the likelihood of bacterial resistance. Additionally, chitosan can improve the dispersion and stability of ZnO nanoparticles, ensuring sustained antimicrobial activity. This synergy allows for lower doses of each component, minimizing potential toxicity while maximizing antibacterial performance against a broad spectrum of pathogens (Scolari *et al*., 2024).

Interestingly, *Klebsiella* spp. exhibited complete resistance at the lowest tested concentration (1.25 mg/mL), with no observable inhibition zone. This may indicate intrinsic or acquired resistance mechanisms such as capsular polysaccharide production, biofilm formation, or efflux pump expression (Kudaer *et al*., 2022). Subtherapeutic concentrations of antimicrobial agents can contribute to the development of resistance and should be avoided, highlighting the need to optimise dosage in any food preservation system (Rawson *et al*., 2021).

The chitosan metal complexes enhance the stability of the coating and increase antimicrobial activity 10- to 20-fold (Kong *et al*., 2010). For example, a thiourea chitosan-silver ion complex overcame the instability of Ag+ and increased antibacterial activity by 20-fold compared to chitosan alone (Al-Naamani *et al*., 2016). While chitosan ZnO nanoparticle nanocomposites are slightly less effective than silver nanoparticle composites (Dhillon *et al*., 2014), they are less toxic to humans and environmentally friendly (Reddy *et al*., 2007). Haldorai and Shim (2013) found that 99.92% of viable bacteria of *E. coli* are inactivated after 24 hour incubation with chitosan-ZnO nanocomposite. Chitosan ZnO composite films showed enhanced antibacterial activities against B. subtilis, *E. coli* and *S. aureus* (Li *et al*., 2010). Similar observations for *S. aureus* and Micrococcus luteus were reported in a study by Dhillon et al. (2014). The enhanced antibacterial activity of nanocomposite coatings is possibly due to synergistic antibacterial activities of chitosan and ZnO nanoparticles. The antimicrobial activity of ZnO nanoparticles is most likely due to disruption of the bacterial cell membranes by zin ions and oxidative stress because of photocatalytic production of reactive oxygen species (ROS). ROS eventually leads to the damage of bacterial proteins, DNA and lipids leading to cell death (Shi *et al*., 2014).

5. CONCLUSION

The results of this study suggest that the chicken shawarma sold in Erbil, Kurdistan Region, may pose potential health risks due to microbiological contamination. The identified ready-eat food, along with the presence of human pathogenic bacteria such as, *Salmonella*, *S. aureus*, and coliforms, indicate unsatisfactory and inadequate food preparation, cleaning, and storage practices. The presence of *Salmonella*, *S. aureus*, and coliforms suggests that these bacteria are present in high concentrations and can cause foodborne diseases, which raises the question of whether some of these places are observing proper food safety measures. The presence of *Klebsiella* sp, a bacterium that causes spoilage, raises doubts regarding the storage conditions. The results suggest that numerous steps, including changes in hygiene policies, regular monitoring, staff training sessions, and effective rule enforcement, must be taken to safeguard public health and ensure safe shawarma consumption within the region.

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Ethical Statment:

This study design was approved by ethical code SUE2025AREC in the Research Center at Salahaddin University-Erbil. Also, consent forms were completed for all participants.

Conflict of Interests:

The authors declare no competing interests.

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Author Contributions:

Z.A.A. performed formal analysis and was responsible for writing the original draft, as well as review and editing. K.I.A. contributed to the conceptualization and visualization of the study, and was responsible for supervision, resources, data curation, statistics, validation, and software. Both authors have read and agreed to the published version of the manuscript.

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