Original Research

Evaluation of Zinc Oxide Nanoparticles to Control Some Pathogenic Bacteria Isolated from Minced Meat

Ahmed A. Maarouf [1], Suzan F. Elsisy [2], Nariman H. Abd Elhady [2], Zeinab A. Ahmed [3], Mohamed E. Nabil [2]*

1. Bacteriology Dept., Animal Health Research Institute (Benha Lab.), ARC, Egypt.
2. Food Hygiene Dept., Animal Health Research Institute (Benha Lab.), ARC, Egypt.
3. Food Hygiene Dept., Animal Health Research Institute (Luxor Lab.), ARC, Egypt.

Correspondence*
Mohamed Nabil Elsayed Abdelqader, Researcher of Food Hygiene, Animal Health Research Institute (Benha Lab.), ARC, Egypt.
Tel. +201228589655

Email: mhmdvet2010@gmail.com

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ABSTRACT

The safety and shelf life of meat products are among the most important points of concern to consumers and meat manufacturers worldwide. So, the current study investigated the prevalence of some foodborne bacteria in a total of 125 random samples of raw minced meat collected from various butchers in the Qalubiya Governorate. Results revealed detection of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in the examined samples, with a total prevalence of 18.4%, 23.2% and 1.6%, respectively, while *Shigella* species were not detected in any of the examined samples. The isolated *E. coli* and *S. aureus* strains were subjected to an antibacterial sensitivity test, where they showed multidrug resistance to the various antibacterial discs used. In addition, molecular detection of some antibiotic resistance genes represented by the blaSHV gene against *E. coli* and the mecA gene against *S. aureus* strains was investigated, and both genes were positively detected in all the examined isolates. Also, the addition of zinc oxide nanoparticles (ZnO NPs) with the concentrations of 6 mmol and 12 mmol was assessed on experimentally injected minced meat with about 106 CFU/g of *E. coli* and *Salmonella* Typhimurium reference strains in chilling condition (4±1OC). Results revealed significant improvement of sensory characters with reduction percent of 99.6% and 99.9% for *E. coli*, and 99.6% and 99.9% for the tested *S. Typhimurium* in the treated groups with 6 and 12 mmol ZnO NPs up to fifteen days of cold storage, respectively. So, it can be concluded that *E. coli* and *S. aureus* are one of the main pathogenic bacteria in raw minced meat; moreover, ZnO NPs have a potential strong antibacterial effect, which may be used for minced meat preservation in chilling conditions.

Keywords: Minced meat, Food poisoning bacteria, Zinc oxide nanoparticle, Qalubiya governorate.

INTRODUCTION

As key suppliers of protein, fat, vital amino acids, minerals, vitamins, and other nutrients, meat and meat products are among the most appealing and highly desirable diets for humans **(Zafar et al. 2016).** However, due to their high moisture content, high proportion of nitrogenous compounds, abundant supply of minerals, some fermentable carbohydrates (glycogen), and favorable pH for most microorganisms, they have been thought to be an ideal culture medium for the growth of many microorganisms, which results in spoilage, financial losses, foodborne infections in humans, and health risks **(Komba et al. 2012).**

The meat production is negatively impacted by the high sensitivity of minced meat to spoilage during all stages of processing, which is primarily caused by the microbial breakdown of protein and lipids **(Lorenzo et al. 2018).** Globally rising meat consumption raises questions and presents difficulties related to meat safety and quality. Most of these worries are biological in nature and involve bacterial infections **(Sofos and Georgnaras 2010)**.

Escherichia coli and *Salmonella* are usually indicating a possible fecal contamination, with *Staphylococcus aureus* that indicates poor personal hygiene, have been reported as the causative agents of about two thirds of foodborne disease

outbreaks causing gastroenteritis, even those acquired directly or indirectly from raw meat, but they are mostly due to excessive handling or contamination during or after cooking of meat and meat products **(Carstens et al. 2019)**. Additionally, the development of antibiotic resistance among microorganisms that cause food poisoning has significant public health ramifications. Numerous investigations revealed that animal-derived strains of *E. coli, Salmonella*, and *S. aureus* were the root cause of drug-resistant infections in humans and that these infectious organisms carried the same mobile resistance genes as various bacterial species derived from a range of animal sources **(Rasheed et al. 2014).** So, since the shelf life of perishable foods may be shortened without the addition of chemical preservatives, which have many safety limitations and drive food technology to seek out other safer additives, nanotechnology offers the food sector a variety of new opportunities to improve the quality, safety, shelf life, and nutritional value of meat and meat products **(Huang et al. 2017).**

The recent technological revolution may be starting with the relatively new field of nanotechnology in order to create better, tastier, safer, and more acceptable products for consumers, some large food companies experiment with nanotechnologies; additionally, it offers the possibility of reducing preservatives and other undesirable or potentially harmful substances in food **(Singh et al. 2017).** So, the advantage of nanotechnology has recently been applied to overcome these challenges of food and environmental issues **(Jaiswal et al. 2019).**

Nanomaterials are currently used for meat applications generally; including the use of nanomaterials as food ingredients or additives that are used directly into food, or as part of packaging materials **(Rhim et al. 2013)**. Today, it is generally recognized that a variety of metals, including silver, gold, aluminum, titanium, and zinc, have antibacterial properties **(Turner, 2017).** The enzymatic inhibition of metal ions by enzymes **(Stadtman and Levine, 2003),** the facilitation of reactive oxygen species generation (the Fenton reaction) **(Valko et al. 2005)**, cell membrane damage **(Li et al. 2009),** and the inhibition of vitally important microelements uptake by microbes are a few examples of the many factors that affect the antimicrobial activity of metals **(Pereira et al. 2008).** Additionally, certain metals have direct genotoxic effects **(Wong, 1988).**

Nano-size materials with appropriate properties, as food preservatives, result in better performance and a longer food shelf-life. There is a wide range of nanomaterials used in industry, which ZnO NPs are considered a multifunctional one because ZnO NPs exhibit high antimicrobial efficacy, near-UV emission **(Mihindukulasuriya and Lim, 2014).** ZnO has been considered as a GRAS (generally recognized as safe) material by the USFDA (21 CFR 182.8991). As such, ZnO NPs are frequently used in food industry as an active antimicrobial agent **(Sharma et al. 2017)**. The mechanisms of action of zinc oxide nanoparticles can be demonstrated as cell membrane disruption **(Liu et al. 2009)**, proteins and DNA binding, reactive oxygen species generation (ROS) **(Saha et al. 2020)**, the processes of bacterial DNA amplification disturbance, alteration (more often, downregulation) of expression in a wide range of genes **(Xie et al. 2011).** Zinc oxide NPs elevate the level of reactive oxygen species (ROS) and malondialdehyde in bacterial cells due to membrane lipid peroxidation **(Rajapandiyan et al. 2022).** The peculiarities of the gram-negative bacteria's cell wall structure are the main cause of their higher resistance to ZnO nanoparticles than the gram-positive bacteria **(Zhong et al. 2018).**

As a result, the current study sought to investigate the bacteriological quality of minced meat samples collected from Qalubiya Governorate, as well as to conduct an experimental evaluation of zinc oxide nanoparticles on the sensory and bacteriological quality of minced meat.

MATERIALS AND METHODS

❖ **Survey part:**

Collection of minced meat samples: A total of 125 samples of raw fresh minced meat (beef meat), weighted about 250 g for each sample, were purchased randomly from different butchers located in five cities in the Qalubiya Governorate (Benha, Toukh, Kafr-Shokr, Kaha, and Qalub cities) (25 samples / each city) during the spring season (from March, 2022 to June, 2022). Each sample was kept a plastic, and transferred, in an ice box, to the laboratory as soon as possible. Samples were subjected to bacteriological evaluation for detection of some food poisoning bacteria. **Bacteriological evaluation: 1-Prevalence of Enteropathogenic *Escherichia coli*** was performed according to **ISO 16649-2 (2001)**. Twenty-five gm of each sample were incubated in MacConkey broth at 37°C for 24h as an enrichment step, followed by plating of 1 ml of the previously enrichment broth on Tryptone Bile X-glucoronide agar (TBX agar) by pour-plate technique. Plates were incubated at 44°C for 18-24h. Suspected *E. coli* isolates (bluish-green colonies) were subjected to biochemical identification. 2-**Prevalence of *salmonellae* was performed according to ISO 6579 (2017)** Twenty-five gm of each sample were incubated in buffered peptone water broth at 37°C ± 1°C for 18 ± 2h, then transferred to Rappaport Vassilidis broth (RV broth) and incubated at 43°C\ 24h. One ml of the enriched sample was plated on selective XLD agar and Brilliant Green agar and incubated at 37 °C for 24h. Plates were examined for suspected *Salmonella* colonies appeared as red colonies with black center on XLD agar, while appeared as pinkish-white or red colonies surrounded by red halo in the medium, which were then isolated for biochemical confirmation. **3-Prevalence of *Staphylococcus aureus*** included enrichment on tryptic soy broth for 24h at 37 °C, and plating on Baird Parker agar. Suspected colonies appeared as black, shiny, convex colonies surrounded by a lightening halo of the egg yolk, which were purified and subjected to further biochemical identification following ISO 6888- 1 (2021). 4- **Prevalence of *Shigella* species included** enrichment on *Shigella* broth with

novobiocin for 18h at 41.5^{O}C in anaerobic condition, then plating on Hekton-Enteric agar and incubating at 37 ^{O}C for 20-24h. Suspected colonies appeared as moist green colonies, which were purified and subjected to further biochemical identification following **ISO 21567 (2004).**

In-Vitro anti-microbial susceptibility test for the isolated food poisoning bacteria: *In-vitro* sensitivity testing was done on each isolated strain to study its antibiotic sensitivity according to **NCCLS (2007)** on Muller Hinton agar plates using Oxoid standardized single discs. The plates were read after incubation at 37 °C for 24 hrs.

Table (1): Antimicrobial standardized discs, concentrations and interpretation of their effect:

Antimicrobial disks		Disk concentration	Zone of inhibition (mm)		
			Resistant <mm (R)	Intermediate mm range (IS)	Sensitive ≥ mm (S)
Amoxicillin/Clavulinic acid	AMX/25	25µg	14	15-19	20
Cefotaxime	CTX/30	30 µg	14	15-22	23
Ciprofloxacin	CIP/5	5 µg	15	16-21	22
Erythromycin	E/15	15 µg	13	14-22	23
Gentamicin	CN/10	10 µg	12	13-14	15
Lomefloxacin	LOM10	10 µg	18	19-21	22
Neomycin	N30	30 µg	13	14-17	18
Norfloxacin	NOR/10	10 µg	12	13-16	17
Streptomycin	S/10	10 µg	11	12-14	15
Trimethoprim/Sulphamethoxazol	SXT/25	(1.25/23.75) µg	10	11-19	19

Molecular detection of some virulence genes and antibiotic resistance genes: Oligonucleotide primers used in PCR were obtained from **Metabion (Germany), with specific sequences as in Table (2).**

Table (2): Oligonucleotide primers sequences source:

Target	Primer	Sequence	Amplified product	Reference
E. coli	bla_{SHV}	(F) AGGATTGACTGCCTTTTTG (R) ATTTGCTGATTTCGCTCG	392 bp	Colom *et al.* 2003
	eaeA	(F) ATG CTT AGT GCT GGT TTA GG (R) GCC TTC ATC ATT TCG CTT TC	248 bp	Bisi-Johnson *et al.* 2011
S. aureus	*clfA*	(F) GCAAAATCCAGCACAACAGGAAACGA (R) CTTGATCTCCAGCCATAATTGGTGG	638 bp	Mason *et al.* 2001
	mecA	(F) GTA GAA ATG ACT GAA CGT CCG ATA A (R) CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	McClure *et al.* 2006

Extraction of DNA: PCR amplification kit the **QIAamp® DNA Mini Kit** (Catalogue no. 51304) used according to QIAamp DNA mini kit instructions.

PCR Master Mix: was prepared according to **Emerald Amp GT PCR master mix (Takara)** Code No. RR310A kit.

Cycling conditions: Temperature and time conditions of the primers during PCR were adjusted according to Emerald Amp GT PCR master mix (Takara) kit. After amplification process, products were inoculated in agarose gel for electrophoreses reading according to **(Sambrook *et al.* 1989).**

❖ **Experimental part:**

The experiment was conducted in the Animal Health Research Institute (Benha branch), ARC, Egypt.

1-Minced beef: One kg and eight-hundred g of raw minced meat were purchased, kept in sterile plastic bags, and stored at 4 °C until use.

2-Bacterial strain: *Escherichia coli* (ATCC® 25922TM) ~ 8 log CFU/ ml and *S. Typhimurium* (ATCC®14028TM) were obtained from Food Hygiene Department, Animal Health Research Institute, ARC, Egypt.

3-Synthesis and preparation of zinc oxide nanoparticles: Zinc oxide nanoparticles were prepared by dissolving 11 g zinc acetate hydrate with 99.9% purity (Zn (Ac)2•2H2O, Sigma-Aldrich) in 500 ml ethanol. Then, 2.9 g sodium hydroxide was added into the solution through ultra-sonication, and a transparent solution was obtained. The conical flask containing the transparent solution was put into a water tank with a constant temperature of 60 °C. After that, 10 ml of distilled water was added to the solution into the conical flask. The solution was stirred for 30 min at 60 °C. The prepared ZnO nanoparticles were collected by centrifuging and drying at 60 °C (Wang et al. 2007). From which, 6 and 12 mM were prepared by suspension of 488.3 and 976.6 g of ZnO NP powder in double-distilled water and constantly stirred until a uniform colloidal suspension was formed to yield solutions of 6 and 12 mM, respectively. Zinc oxide nanoparticles were characterized using transmission electron microscopy (TEM) techniques (JEOL JEM 1400). TEM was conducted in Cairo University Research Park, Egypt.

Zinc Oxide NPs Characterized by white colored powder of 20 ±5 nm of 99.9% Purity. It appeared spherical shaped under TEM. In addition, it formed a stable colloid in mixture of ethanol and chloroform and water

4-Preparation of the bacterial strain: Previously enriched reference strain count of *E. coli* and *S. Typhimurium* were determined by serial dilution method, followed by plating on nutrient agar for counting of the original culture, from which, certain working culture count was adjusted by serial dilution technique on a sterile normal saline (0.9% NaCl)

5-Assessment of the antibacterial activity of zinc oxide nanomaterials on aerobic plate count and anaerobic bacteria count: Six-hundred grams of minced meat were equally divided into three groups as follow:

1st group: Control untreated minced meat without treatment

2nd group: 200 g minced meat + 6 mM ZnO NPs.

3rd group: 200 g minced meat + 12 mM ZnO Nps.

After preparation of samples according to **ISO 6887-2 (2017),** samples were examined for APC according to **ISO 4833-1 (2013)** and anaerobic plate count **NMKL (2017)** before treatment, and then were kept in refrigerator (4±1°C) for the next two hours after treatment, and then examined as zero time, followed by their examination each three days. Reduction percent was calculated according the formula **Reduction rate (%) =** $\frac{A-B}{A}x100$ where B = mean value of the next level, A = mean value of the previous level.

The experiment was repeated in triplicate for each group and mean values were calculated.

6-Assessment of the antibacterial activity of zinc oxide nanomaterials on *E. coli* and *S. Typhimurium* counts:

N.B. The used minced meat in the current part of the study was divided into six equal groups in the form of thin films, and were treated with ultraviolet light (wavelength 385 nm) for 30 min to eliminate background microflora before addition of ZnO NP suspension or inoculation of the test strain **(Morsy *et al.* 2018).**

In a sterile bag, minced meat was inoculated with *E. coli* and *S. Typhimurium* (in separate groups) (~ 8 log CFU/ml) to achieve final concentration ~ 6 log CFU/g of minced meat. Then, they were mixed thoroughly by gently squeezing till even distribution of microbe occurred. The initial load of the inoculated microbes was determined before the addition of nanomaterials. Minced meat sample was divided into six groups (200 g each); Group 1 (minced meat without treatment, for *E. coli* part), Group 2 (6mM ZnO + *E. coli*), Group 3 (12mM ZnO + *E. coli*), Group 4 (minced meat without treatment for *Salmonella* part), Group 5 (6 mM ZnO + *S. Typhimurium*), and Group 6 (12 mM ZnO + *S. Typhimurium*). Nanomaterials were mixed thoroughly, then all labeled samples were packed and kept at 4±1°C till spoilage of minced meat. Counting of *E. coli* and *S. Typhimurium* in the control and treated and sensory evaluation were performed on days zero and each of three days.

The experiment was repeated in triplicate for each group and mean values were calculated.

After preparation of samples according to **ISO 6887-2 (2017),** enumeration of *E. coli* was performed according to **ISO 16649-2 (2001)** on TBX agar, whereas, *Salmonella* was counted on XLD agar according to **ISO 6579 (2017).** Reduction percent was calculated according the formula **Reduction rate (%) =** $\frac{A-B}{A}x100$ where B = mean value of the next level, A = mean value of the previous level.

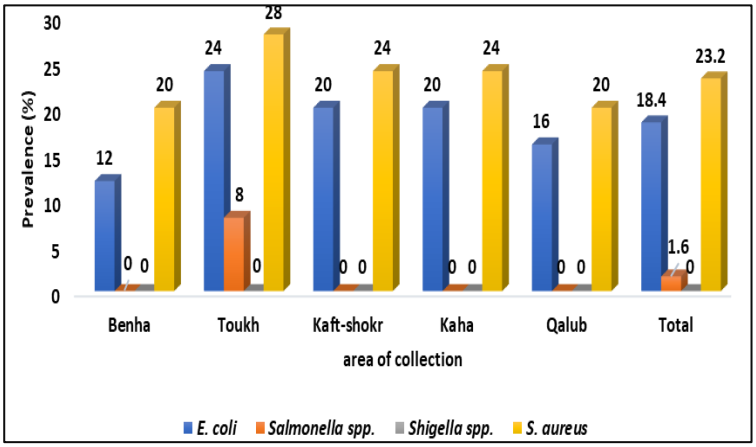
7-Sensory evaluation: Average of color, odor and texture scores were recorded as overall sensory scores following **Mörlein (2019**) in scores (1 to 5), where ≤1- represented the worst while 5- represented the excellent mark.

Statistical analysis (SPSS, 2018): Data were coded then entered and analyzed using the SPSS version 26-2018 (statistical package for the social science; IBM Corp, Armonk, NY, USA) for Ms Win. 10.

RESULTS

Prevalence of some food poisoning bacteria in the examined minced meat samples

The obtained results in **Fig. (1)** revealed that the total prevalence of *E. coli, Salmonella* spp. and *S. aureus* in the examined samples were 18.4%, 1.6% and 23.2%, respectively, while *Shigella* species was not detected in any of the examined sample. In addition, regarding to the area of collection, *Salmonella* species was only detected in the collected samples from Toukh city with the prevalence of 8%. the recorded results of the prevalence of *E. coli* and *S. aureus* revealed their detection in the prevalence of 12% and 20% from the collected samples from Benha city, 24% and 28% from the collected samples from Toukh city, 20% and 24% from the collected samples from both Kafr-shokr and Kaha cities, respectively; moreover, they were detected in 16% and 20% of the collected samples from Qalub city, which indicated that the highest contamination levels were detected in the collected samples from Toukh city).



In-vitro antibacterial sensitivity

Referring to the obtained findings of the antibiotic sensitivity of twenty-three *E. coli* and twenty-nine *S. aureus* isolates, multidrug resistant strains were detected as was recorded in Tables (3) and (4), respectively.

Escherichia coli showed the highest resistance to oxytetracycline, streptomycin and ampicillin in the percent of 78.3%, 74.0% and 73.9%, respectively; while, were mostly sensitive to gentamicin and enrofloxacin with the incidence of 91.3% and 87.0%, respectively. Moreover, 75.9% and 82.8% of the examined *S. aureus* isolates were resistant to methicillin and oxacillin, respectively; so, they could be considered as MRSA strains.

Table (3): In-Vitro anti-microbial Sensitivity test for isolated *E. coli* (23)

Antimicrobial agents	Sensitive		Intermediate		Resistant		AA
	No.	%	No.	%	No.	%	
Amoxicillin	0	0.0	7	30.4	16	69.6	R
Ampicillin	2	8.7	4	17.4	17	73.9	R
Cefotaxime	18	78.3	2	8.7	3	13.0	S
Ciprofloxacin	15	65.2	4	17.4	4	17.4	S
Enrofloxacin	20	87.0	2	8.7	1	4.3	S
Erythromycin	7	30.4	5	21.8	11	47.8	R
Gentamicin	21	91.3	2	8.7	0	0.0	S
Methicillin	2	8.7	5	21.7	16	69.6	R
Norfloxacin	19	82.6	2	8.7	1	4.3	S
Oxacillin	1	4.3	8	34.8	14	60.9	R
Oxytetracycline	1	4.3	4	17.4	18	78.3	R
Streptomycin	1	4.3	5	21.7	17	74.0	R
Trimethoprim/ Sulphamethoxazol	5	21.7	6	26.1	12	52.2	R

No.: Number of isolates AA: Antibiogram activity %Percentage in relation to total number of isolated *E. coli* (23)

Table (4): In-Vitro anti-microbial Sensitivity test for isolated *S. aureus* strains (n=29)

Antimicrobial agents	Sensitive		Intermediate		Resistant		AA
	No.	%	No.	%	No.	%	
Amoxicillin	3	10.3	5	17.2	21	72.4	R
Ampicillin	5	17.2	5	17.2	19	65.4	R
Cefotaxime	5	17.2	10	34.5	14	48.3	R
Ciprofloxacin	23	79.4	3	10.3	3	10.3	S
Enrofloxacin	24	82.8	3	10.3	2	6.9	S
Erythromycin	8	27.6	17	58.6	4	13.8	IS
Gentamicin	25	86.2	3	10.3	1	3.4	S
Methicillin	1	3.4	6	20.7	22	75.9	R
Norfloxacin	22	75.9	4	13.8	3	10.3	S
Oxacillin	2	6.9	3	10.3	24	82.8	R
Oxytetracycline	4	13.8	9	31.0	16	55.2	R
Streptomycin	4	13.8	15	51.7	10	34.5	IS
Trimethoprim/ Sulphamethoxazol	10	34.5	14	48.3	5	17.2	IS

No.: Number of isolates AA: Antibiogram activity %: Percentage in relation to total number of isolates (29).

Results of PCR amplification of some virulence and antibiotic resistance gene

Regarding with the examined two isolates of *E. coli* for *eae*A and *bla*$_{SHV}$ genes, the *eae*A gene was amplified in both *E. coli* strains giving product of 248 bp as shown in Fig. (2). In addition, the *bla*$_{SHV}$ gene was amplified in both *E. coli* strains giving product of 392 bp as shown in Fig. (3). Moreover, two isolates of *S. aureus* were examined for clumping factor (*clf*A) and methicillin resistant (*mec*A) genes. Results showed that *clfA* and *mec*A genes were detected in both studied strains (Figs. 4 and 5, respectively)

❖ **Experimental part:**

1- Effect of ZnO NP on the sensory quality of the treated minced meat

The overall acceptability of minced meat groups during cold storage at 4±1°c is shown in Fig. (6). The results showed that obvious significant improvement in the treated samples when compared with the control group as control sample spoiled after the 6th day of storage and continue decrease during all storage period, which usually occurs due to microbial lipolysis and protein degradation.

The data also reveals that the shelf life of the treated minced meat groups extended to the 15th day of storage. Higher ZnO NP concentration gave greater enhancement in the sensory quality.

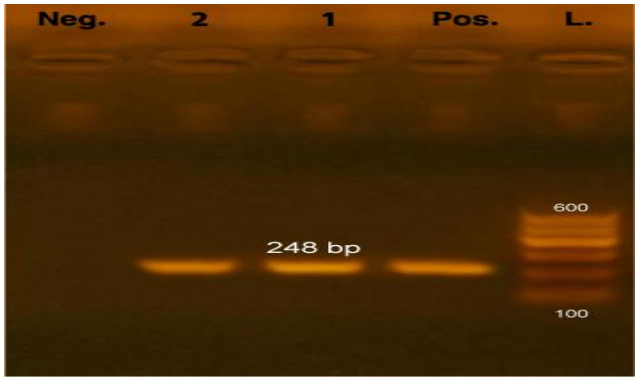


Fig. (2): Gel electrophoresis of Intiman of *E. coli* attaching and effacing (*eae*A) gene at 248 bp.
Lane L: 100-600 bpDNA Ladder.
Lane 1 and 2: *E. coli* isolates (Positive).
1st strain was isolated from minced meat from Benha city
2nd strain was isolated from minced meat from Toukh city
Neg.: Negative control (*S. aureus,* ATCC25923)
Pos.: Positive control (*E. coli*, AJ413986).

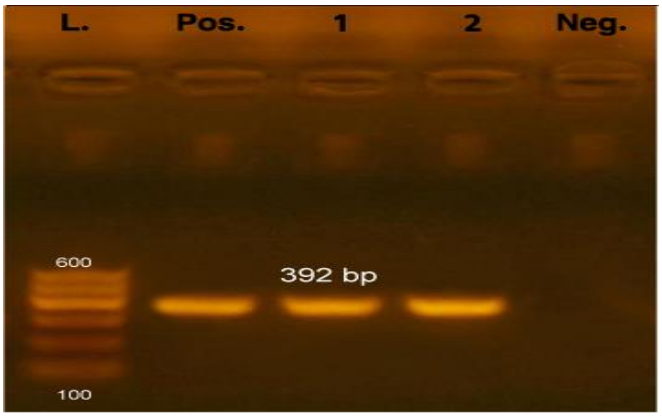


Fig (3): Gel electrophoresis of *bla*$_{SHV}$ gene at 392bp
Lane L: 100-600 bp DNA Ladder.
Lane 1 and 2: *E. coli* isolates (Positive).
1st strain was isolated from minced meat from Benha city
2nd strain was isolated from minced meat from Toukh city
Neg.: Negative control (*S. aureus,* ATCC25923).
Pos.: Positive control (*E. coli*, AJ413986).

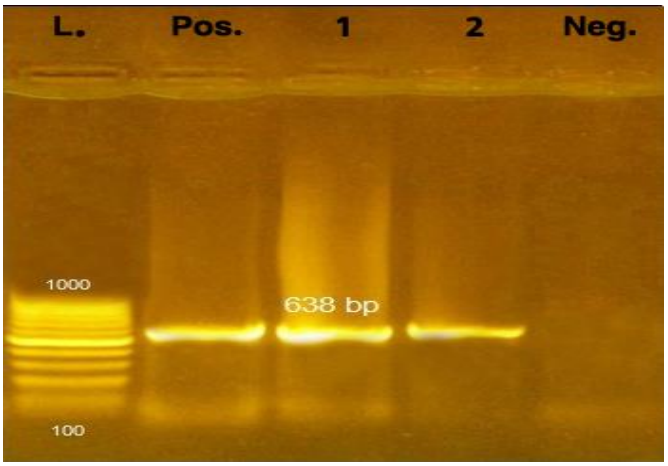


Fig. (4): Gel electrophoresis of clumping factor (*clf*A) gene at 638 bp
Lane L: 100-1000 bpDNA Ladder**.**
Lane 1 and 2: *S. aureus* isolates (Positive).
1st strain was isolated from minced meat from Benha city
2nd strain was isolated from minced meat from Toukh city
Neg.: Negative control (*E. coli*, AJ413986)
Pos.: Positive control (*S. aureus,* ATCC25923) (at 638 bp).

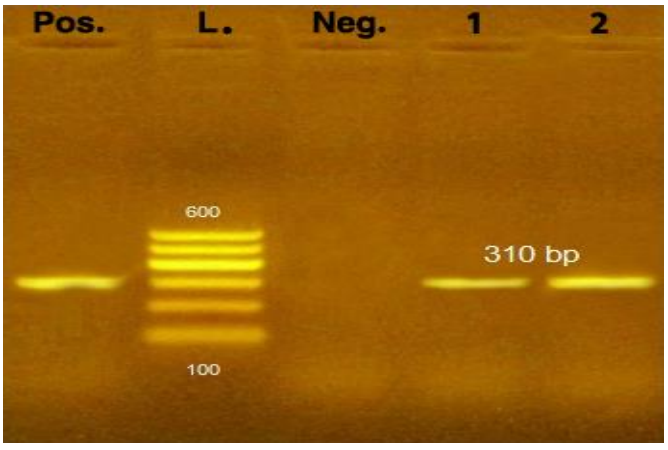


Fig. (5): Gel electrophoresis of methicillin resistant gene (*mec*A) at 310bp
Lane L: 100-600 bpDNA Ladder.
Lane 1 and 2: *S. aureus* isolates (Positive).
1st strain was isolated from minced meat from Benha city
2nd strain was isolated from minced meat from Toukh city
Neg.: Negative control (*E. coli*, AJ413986).
Pos.: Positive control (*S. aureus,* ATCC25923) (at 310 bp).

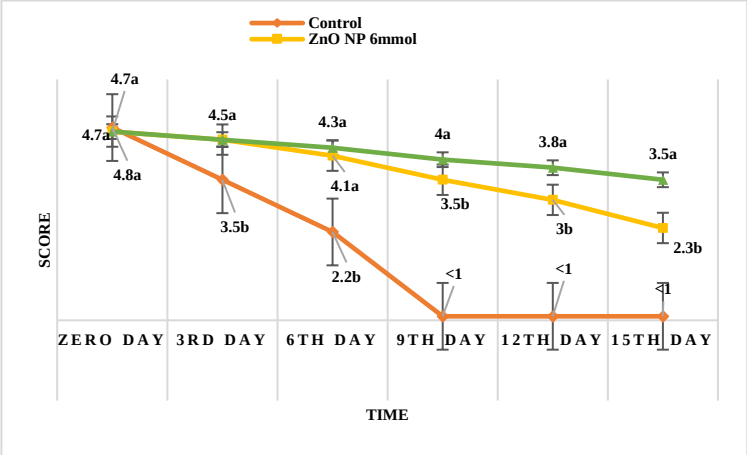


Fig. (6): Sensory quality scores of the control and ZnO NP treated minced meat

2- Bacteriological profile of the control and ZnO NP treated minced meat groups

Referring to the recorded results in Figs. (7 to 10), treatment with ZnO NPs showed significant reductions in the bacterial count when compared with the control untreated group that showed signs of spoilage after the 6th day of cold storage. The recorded results of the bacteriological quality came in agreement with the improvement in the sensory quality of the treated groups.

The next figures indicate that greater reduction and more powerful antibacterial effect of ZnO NPs was recorded with increasing its concentration. Fig. (7) shows significant reduction in the aerobic plate count (log_{10} CFU/g) in the treated minced, which started from the zero day from 4.5 log_{10} to 2.55 log_{10} and 1.98 log_{10} with reduction percent of 98.9% and 99.7% for the treated groups with 6 and 12 mM ZnO NPs, respectively. In addition, Fig. (8) shows significant reduction in the anaerobic bacterial count in the treated minced, in which it recorded <1 log_{10} CFU/g in the 15th day of the experiment. Moreover, Fig. (9) shows significant reduction in *E. coli* count (log_{10} CFU/g) in the treated minced, which started from the zero day from 6.1 log_{10} to 3.72 log_{10} and 2.24 log_{10} with reduction percent of 99.6% and 99.9% for the treated groups with 6 and 12 mM ZnO NPs, respectively. Furthermore, *S. Typhimurium* count declined from 5.98 log_{10} to 3.53 log_{10} and 2.1 log_{10} with reduction percent of 99.5% and 99.9% for the treated groups with 6 and 12 mM ZnO NPs, respectively.

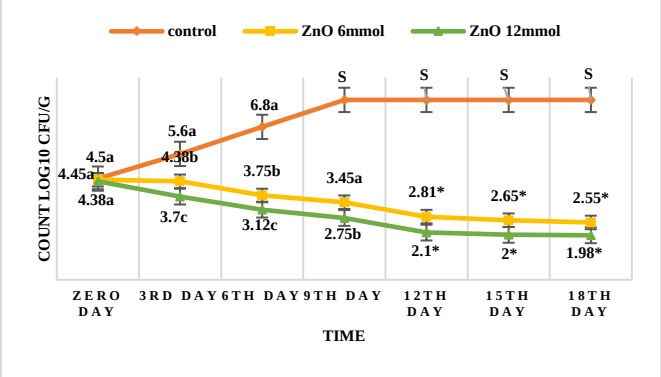


Fig (7): Effect of ZnO nanoparticles on aerobic bacterial count in control and treated minced meat during chilling storage

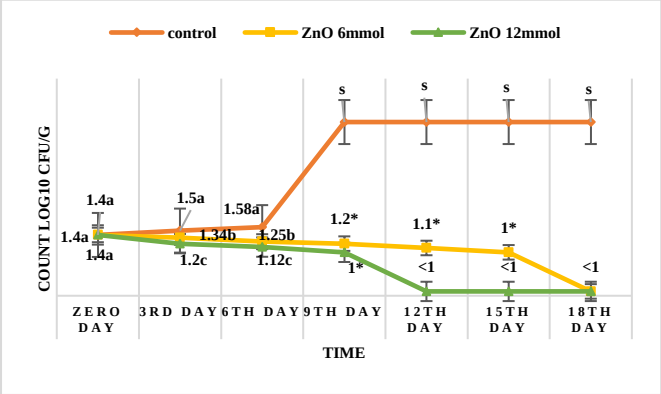


Fig (8): Effect of ZnO nanoparticles on anaerobic bacterial count in control and treated minced meat during chilling storage

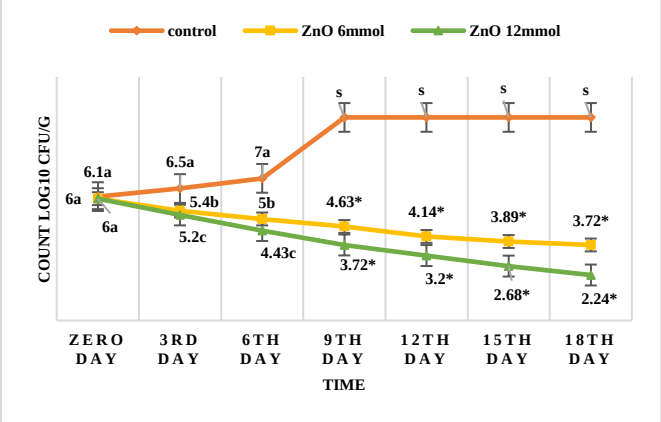


Fig. (9): Effect of ZnO nanoparticles on *E. coli* count in control and treated minced meat during chilling storage

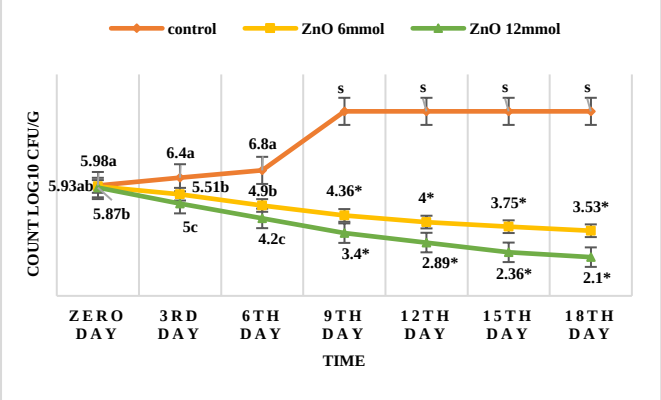


Fig. (10): Effect of ZnO nanoparticles on *Salmonella* count in control and treated minced meat during chilling storage

Table (5): Reduction percent of the treated samples in the 18th day of the experiment

Microorganism	6 mM ZnO NPs	12 mM ZnO NPs
Aerobic plate count	98.9	99.7
Anaerobic bacteria count	100.0	100.0
E. coli	99.6	99.9
S. Typhimurium	99.6	99.9

DISCUSSION

Minced meat is an excellent medium for microorganism growth. The microorganisms normally encountered on meat surface are distributed thoroughly into the meat product and start reproducing when the conditions are favorable during grounding, mixing, storing and packaging, causing loss of product quality and creating potential health hazards **(Saad et al. 2018).**

Food borne diseases caused mainly by *E. coli*, *Salmonella* species and *S. aureus* are the major cause of mortality and infections especially in the developing countries. These pathogens are transmitted mainly through consumption of contaminated food and the presence of these organisms in meat and raw meat products has relevant public health implications **(Bintsis 2017).**

According to the recorded results of some food poisoning bacteria in the examined minced meat samples, **Fig. (1)** revealed that *E. coli, Salmonella* species and *S. aureus* were detected in the prevalence of 18.4%, 1.6% and 23.3% of the total examined samples, respectively; while, *Shigella* species was not detected in any of the examined samples. Superiority of *S. aureus* as the most detected foodborne bacteria in the examined minced meat samples can be referred to the more handling practices, improper hygienic practices during mincing and packaging processes, and other added spices **(Kabwang et al. 2019).**

The recovery of *E. coli* from meat samples indicates fecal contamination and implies that other pathogens of fecal origin may be present. The increased incidence of *E. coli* in the examined samples may be due to mishandling during production, processing and distribution or to the use of contaminated water during evisceration and slaughtering **(Gwida et al. 2014).** In addition, presence of *S. aureus* in meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils **(Edris et al. 2018).**

In relation to the locality of collection, Toukh city showed the highest prevalence of bacterial contamination, followed by Kaha and Kafr-shokr, Qalub and Benha cities, respectively which may be attributed to variation in the hygienic knowledge and government censorship during slaughtering,

transportation, processing and storage in civilized and rural cities **(Cook et al. 2017).**

Regarding to the previous records, the current obtained results came lower than those obtained by **Erdem et al. (2014)** who recorded detection of *E. coli* and *S. aureus* in the incidence of 63.3% and 96.6%, respectively, and **Saad et al. (2019)** (40% for *S. aureus*). While, it came in line with those recorded by **Heweidy (2016)** (22.9% and 25.7% for *E. coli* and *S. aureus*, respectively) and **El-Bagory et al. (2020)** (20% for *E. coli* detection); meanwhile, it was higher than those recorded by **Ibrahim et al. (2015)** (16% for *E. coli* detection). Variation between different authors can be referred to variation in the area of collection and the level of hygienic practices application during processing and storage between different sources **(Bloomfield et al. 2006)**.

The emergence of antimicrobial resistance among some foodborne bacteria including *E. coli*, *Salmonella* and *S. aureus* strains of meat origins have important public health implications. Several studies showed that multidrug resistant foodborne bacterial infections have been occurred occasionally in humans **(Ventola 2015)**. Besides that, the pathogenicity of them could be attributed to their adhesion factors that enhance the microbial attachment and initiate infections. For enteropathogenic *E. coli*, these factors including *eaeA,* that produces intimin which has been necessary to produce the attaching-and-effacing lesion **(Louie et al. 1993)**. Meanwhile, for *S. aureus,* clumping factor A (*Clf*A), a cell-wall–anchored protein from *S. aureus*, is a virulence factor in various infections and facilitates the colonization and bacterial adhesion to the blood plasma protein fibrinogen **(Herman-Bausier et al. 2018).**

The results of antibiotic sensitivity tests for the isolated *E. coli* **(Table, 2)** showed the highest resistance to oxytetracycline, streptomycin, ampicillin and amoxicillin, respectively, which came in line with those obtained by **Olukemi et al. (2015), Rasmussen et al. (2015) and Abdel Alim (2017).** Moreover, the in-vitro sensitivity tests for the isolated *S. aureus* **(Table, 3)** showed that, the isolated *S. aureus* were highly resistant for oxacillin (83.9%) and methicillin (80.7%) followed by various sensitivity reactions to the other used antibacterial agents, which came in agree with the previously recorded results by **Momtaz et al. (2013), Abd El-Tawab et al. (2015), Olukemi et al. (2015), Zogg et al. (2016), Edris et al. (2018)** and **Amer et al. (2021).**

The PCR results for *E. coli* showed that, intimin or *E. coli* attaching and effacing gene (*eaeA*) virulence gene in *E. coli* isolates was amplified in both examined *E. coli* strains as was shown in **Fig. (2)**. These results came in accordance with those recorded by **Abdel Alim (2017)**, while disagreed with those recorded by **El-Bagory et al. (2020)** who did not detect *eaeA* gene in 80% of their examined *E. coli* isolates. Moreover, detection of *blaSHV* gene as was shown in **Fig. (3)** came in line with those recorded by **Zogg et al. (2016)** and **Zhang et al. (2021).**

The PCR results for *S. aureus* showed that, clumping factor A(*clf*A) and *mecA* genes were detected in both the examined isolates as was shown in **Figs. (5 and 6)**, which came in agree with the recorded results of **Younis et al. (2019) and Amer et al. (2021)**.

Zinc oxide (ZnO) is listed as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). Recently, ZnO-nanoparticles have demonstrated a broad-spectrum antibacterial effect on foodborne pathogens and even some resistant microorganisms **(Li et al. 2009)**.

Regarding with the recorded sensory and bacteriological quality of the treated minced meat with zinc oxide nanoparticles as were shown in **Figs. (6 to 10),** significant improvement in the sensory and bacteriological quality was observed which was mainly attributed to the antimicrobial effect of ZnO, especially in the nano-size, which maximizes its interaction with the bacterial surface and/or with the bacterial core where it act on the cell membrane and deeply in its DNA **(Zanet et al. 2019)**; therefore, it may be considered as a multi-target compound and affect several structures of bacteria cells, but their main mechanism of action is in the cytoplasmic membrane, being other structure effects a consequence/secondary effect after the membrane rupture **(Mendes et al. 2022)**.

The significant enhanced sensory and bacteriological quality of the treated minced meat samples came in agree with those previously recorded results by **Ahmed et al. (2011)** who recorded that *S. Typhimurium* and *S. aureus* exposure to their relevant minimal inhibitory concentrations from ZnO NP reduced the cell number to zero within 8 and 4h, respectively; **Mirhosseini and Arjmand (2014) and Abd El-Aziz et al. (2020)** who recorded a significant antibacterial effect of zinc oxide nanoparticles on different foodborne bacteria with enhanced sensory quality and extending shelf-life of the treated meat samples

CONCLUSION

As a conclusion, multidrug resistant *E. coli* and *S. aureus* were of high prevalence in the examined minced meat samples, that making them of high-level risk for the consumer's health. In addition, using of ZnO Np enhance the sensory and bacteriological quality of minced meat with significant extension in the shelf-life in chilling condition. Further investigations on the safety margin and technical application in the food industry is highly recommended.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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