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ZINC PEROXIDE NANOPARTICLES: PROMISING SOURCE COMBATING CHICKEN MEAT-CONTAINING PATHOGENS

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ABSTRACT

Due to the increase of the chicken meat consumption, the ensuring of microbial safety is essential. Therefore, our study aims to assess the microbiological quality of retailed chickens in local markets at El-Sharkia Governorate, Egypt. Besides, investigating zinc peroxide nanoparticles (ZnO_2-NPs) antibacterial activity against pan drug-resistant bacteria (PDR). For this purpose, an Antibiotic susceptibility test was performed using 10 different antibiotics to detect MDR and PDR bacteria in retail chicken meat cuts-up. All PDR bacteria were selected and identified using 16S rRNA. Also, the activity of ZnO_2-NPs was investigated against PDR isolates. Our results showed that the average total viable count (TVC) of fresh, chilled and frozen meat estimated by 6.84, 3.75 and 2.1 log_{10} CFU/g, respectively. The antibacterial susceptibility test revealed that 70% (84/124) of all bacterial isolates were MDR bacteria. Out of these isolates 5 PDR isolates were detected and identified as *Pseudomonas aeruginosa* IMD85, *Escherichia coli* O157:H7 and *Salmonella enterica* ATCC 8387, *Staphylococcus aureus* 191 and *Staphylococcus epidermidis* BGHMC11. The antibacterial susceptibility test of ZnO_2.NPs against these PDR isolates showed an outstanding activity at different concentrations (50, 100, 150 and 200 µg/ml). The MIC value for the PDR bacterial isolates ranged from 25-150 µg/ml and MBC value ranged from 50-300 µg/ml. ZnO_2-NPs have exhibited efficiency as an active antibacterial agent against PDR pathogens suggesting that the ZnO_2-NPs are potentially valued for food safety applications.

KEYWORDS: Foodborne diseases, Zoonotic pathogens, Antibiotics, Multidrug-resistant, Pan drug resistant, Metal oxides nanoparticles.

1. INTRODUCTION

Poultry is an important, inexpensive source of animal protein, rich in nutrients, minerals, and vitamins. Chicken meat comprises about 66% of the total production in the world (**Ruban et al., 2010**). Chicken carcasses can be contaminated through the processing operation during scalding, de-feathering, evisceration, and contamination from other birds. Chicken carcasses are considerably contaminated with pathogens from either intestinal tract or fecal material on feed and feathers (**Dincer and Baysa, 2004**). Zoonotic pathogens that cause foodborne illness are of the major concerns all over the world. Recently, more than 250 various foodborne diseases were described and more than 65% of foodborne outbreaks are caused by zoonotic pathogens. The use of antibiotics acts to remove the sensitive pathogenic bacteria and leave the resistant bacteria and thus these resistant bacteria be dominant and can transfer-resistant genes to other bacteria (**Laxminarayan et al., 2013**). These resistance genes can transfer to the human via consuming or handling this contaminated meat (**Van Looveren et al., 2001**; **Ali et al., 2019**) which are difficult to be treated, thus resulting in increased morbidity and mortality (**Smith and Coast, 2013**). Hence, there is a mass need to solve this serious problem by innovating new antimicrobial agents. Zinc peroxide nanoparticles (ZnO_2-NPs) was represented in this study where metal oxide nanoparticles (MO-NPs) have attract the scientist's attention as it was reported as safe for both human and animals (**Jacob et al., 2014**). Also, these particles have unique properties like antimicrobial, good chemical stability and low toxicity (**Samanta, 2017**; **Ali et al., 2017**). The antibacterial activity of MO-NPs has been associated with their morphology including, surface area and particle size (**Sirelkhatim et al., 2015**). Metal oxide nanoparticles of smaller size (<100 nm) and larger surface area have reflected effectiveness as antibacterial agents due to its ability to penetrate, interact, and damage the bacterial membranes (**Gordon et al., 2011**). Therefore, this study aimed to assess the microbiological quality of retail chicken meat in Sharkia Governorate, Egypt and to evaluate the effectiveness of zinc peroxide nanoparticles against PDR bacteria isolated from chicken meat.

2. MATERIALS AND METHODS

2.1. Samples collection and preparation

Chicken meat cuts-up samples were purchased from different local markets during the period from August 2018 to February 2019 from Sharkia Governorate, Egypt. A number of 120 random samples of chicken meat cuts-up were divided into four groups including breast, thigh, liver and gizzard, 30 of each. According to the status of the samples, each group consisted of fresh, chilled and frozen samples, 10 of each. Every single sample was transported to the laboratory in a sterilized bag to avoid cross-contamination. The samples were prepared according to the International Commission on Microbiological Specifications for Foods technique (**ICMSF, 1986**). In brief, a weight of 25 grams from different parts of each sample was separately grounded in a sterile mechanical blender, and mixed with 225 ml of sterile buffered peptone water 0.1% in a stomacher bag and homogenized for 2 min in a Stomacher (Seward, BA6021, UK). According to **ICMSF** (**1986**), a volume of 1 ml of the homogenate was used to prepare tenfold serial dilution (10^{-4}-10^{-8}).

2.2. Microbiological quality evaluation and isolation

A volume of 1 ml from each sample of each dilution was used for enumeration of aerobic bacteria. Serial dilution agar plating method was used to determine the total viable count (TVC) on Plate Count Agar (Oxoid, Basingstoke, Hampshire, UK) in triplicate and the plates were incubated for 48 h at 37ºC (**Marzan et al., 2017**). Total *Enterobacteriaceae* count (TEC) and the total count of coliform bacteria (TCC) were carried out on violet red bile glucose agar (VRBG) (Oxoid, England) and MacConkey agar (Oxoid, England) with crystal violet, respectively (**Kornacki and Johnson, 2001**).

2.3. Antimicrobial susceptibility test

The susceptibility of 120 bacterial isolates against 10 different antibiotic discs (Oxoid, England) was determined by the disc diffusion technique according to the Clinical and Laboratory Standards Institute guidelines and breakpoint (M100, **CLSI, 2017**). The selected antibiotics were Ampicillin (30 µg/disc), Aztreonam (30 µg/disc), Cefotaxime (30 µg/disc), Chloramphenicol (30 µg/disc), Ciprofloxacin (5 µg/disc), Colistin sulphate (25 µg/disc), Co-trimoxazole (25 µg/disc), Gentamicin (10 µg/disc), Imipenem (10 µg/disc) and Tetracycline (30 µg/disc). From each isolate, a standard suspension was adjusted to 0.5 McFarland standard (**Bauer et al., 1966)**. A volume of 100 µl from bacterial isolates suspension was inoculated onto Müller Hinton agar medium (Oxoid, UK). Antibiotic discs were placed on the surface of the inoculated plates and incubated at 37°C overnight. The produced inhibition zone (mm) for each disc was measured and values were interpreted. Bacterial isolates which yielded intermediate results were considered resistant. The multiple antibiotic resistance (MAR) index of an isolate was calculated as *a/b*, where *a* represent the antibiotics number to which the isolate was resistant and *b* represents all tested antibiotics number (**Krumperman, 1983**).

2.4. Characterization and identification of bacterial isolates

Characterization of the selected bacterial isolates was performed using colonies morphological examination and Gram's staining. Afterwards, subjected to the characterization using VITEK®2 automated systems (BioMérieux, Marcy-L'E'toile, France) at Mabaret El-Asafra hospital.

The molecular identification was carried out to by extracting the genomic DNA using Qiagen DNA extraction kit (Qiagen, USA) according to the manufacturer's instructions. Oligonucleotide primers (27F and 1492R) targeting 16S rRNA gene (**Senthilraj et al., 2016**) were used. The amplified products were purified according to the instructions protocol by the QIAquick PCR purification kit (Qiagen, USA) and visualized by 1.5% agarose gel electrophoresis. The PCR products sequencing were performed using ABI 3730xl DNA Sequencer (ABI, USA). The obtained sequences were compared using BLAST searches **(**http://blast.ncbi.nlm.nih.gov/Blast.cgi**)**. Neighbor-joining tree based on 16S rRNA which was conducted using MEGA (version 6.0).

2.5. Synthesis of ZnO_2-NPs

A pure phase of ZnO_2-NPs with a transition temperature of 211°C and size ranged from 15 to 25 nm was synthesized using the co-precipitation method (**Ali et al., 2017**). A volume of 10 ml of ammonium hydroxide (NH_4OH) was mixed with 20 ml of 0.1 M of zinc acetate dihydrate using magnetic stirring. Acetone (70 ml) and of glycerol (3 g) were homogenized and added to the prepared solution. A volume of hydrogen peroxide (40 ml; 40%) was added to the prepared solution with stirring at 25°C for 30 min. The precipitate was centrifuged and washed with distilled water (**Ahmadi and Vossoughi**, **2013; Siddiqui et al., 2016**).

2.6. Antimicrobial activity of ZnO_2-NPs

A stock solution of ZnO_2-NPs (2 g/ml) was used to prepare various concentrations (50, 100, 150 and 200 µg/ml) to carry out the antibacterial activity of ZnO_2-NPs was performed by preparation of various concentrations (50, 100, 150 and 200 µg/ml) of a stock solution (2 g/ml). Filter paper discs (6 mm) were dipped in the previously prepared solutions and then placed on Muller Hinton agar plates inoculated with the bacterial isolates and incubated at 37°C for 4 days. Distilled water was considered as a control for the antibacterial activity. Triplicates were maintained and the mean values and standard error were calculated for all inoculated plates (**Ali et al., 2017**). The Minimum inhibitory concentration (MIC) of ZnO_2-NPs was determined by disc diffusion methods (**CLSI, 2017**). A different concentrations were prepared (10, 15, 20, 25, 50, 100, 150, 200, 250 and 300 µg/ml), and 2 µl of the bacterial concentration which was

adjusted to 7 log_{10} colony-forming units/ml (CFU/g) were added to every well and incubated at 37°C for 24 h. Minimum inhibitory concentration is the lowest concentration of ZnO_2-NPs at which no growth was observed. For minimum bactericidal concentration (MBC), a volume of 10 µl of the aliquots from the wells, which were used in MIC assays and showed no visible growth was sub-cultured on nutrient agar. The plates were incubated overnight at 37°C. The least concentration that did not show any growth of the tested organisms was considered as the MBC value of ZnO_2-NPs against the selected bacterial isolates. MBC was defined as the lowest concentration of ZnO_2-NPs at which there was no formation of colony after 18 h of incubation (**Ali et al., 2017**).

2.7. Statistical analysis

To compare the TVC, TEC and TCC data; all count techniques were transformed using Logarithm to the base 10 (Log_{10}) before the statistical analysis. Our herein obtained data were analyzed statistically using NCSS 2019 data analysis to detect the significance degree using ANOVA (one-way analysis of variance) and t-test at probability level ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Microbiological quality assessment

Retail marketed chickens which were purchased from different markets in Sharkia Governorate were subjected to microbiological quality evaluation by determination of TVC, TEC and TCC in fresh, chilled and frozen chicken meat cuts-up. As shown in Table 1, TVC, TEC and TCC of fresh chicken meat were significantly higher ($P \leq 0.05$) than chilled and frozen meat. Our obtained data showed that all TVC of fresh meat is higher than 5 log_{10} CFU/g. These data prove that all fresh meat is unacceptable according to the recommended limit of bacterial contamination for foods by international microbiological standards which estimated the maximum level of contamination by 5 log_{10} CFU/g for total bacterial plate count (**Odetunde et al., 2011**; **Refai, 1979**). Also, Centre Nationale d'Etudeset de Recommendations sur la Nutrition et l'Alimentation (**CNERNA, 1996**) reported a guideline of 5.7 log_{10} CFU/g for the maximum level of the bacterial count. Additionally, according to the Egyptian organisation for standardization (**EOS**, **2005**), the microbial contamination limit is 5 log_{10} CFU/g. Hence, chilled and frozen meat bacterial quality is considered acceptable where it was lower than 4.3 log_{10}CFU/g. Similar results were reported by **Chaiba *et al*.** (**2007**) who studied the quality of marketed chicken meat in Morocco and reported that the TVC was 6.18 log_{10} CFU/g. Also, **Barbuddhe *et al*.** (**2003**) analyzed the microbial load of mesophiles and psychrophiles in 37 poultry meat samples and reported that the mean total mesophilic count was 7.24 log_{10} CFU/g. However, lower contamination levels were observed by different authors (**Daoud, 2012**). The obtained data revealed that the average value of total *Enterobacteriaceae* count of fresh meat (5.54 log_{10} CFU/g) was higher than chilled (3.03 log_{10} CFU/g) and frozen meat (1.72 log_{10} CFU/g) by 54.78 and 30.98%, respectively. Total coliform count of fresh meat (3.81 log_{10} CFU/g) also was significantly higher ($P \leq 0.05$) than chilled meat total coliform count (2.12 log_{10} CFU/g) by 57.7% and significantly higher ($P \leq 0.05$) than frozen meat (1.23 log_{10} CFU/g) by 23.3% as shown in Table 1. This variation between values reflects the high contamination rate of fresh chicken meat. A similar value was reported by **Kilonzo-Nthenge et al**. (**2013**) who stated that 95.2% of poultry meat samples were positive for the presence of *Enterobacteriaceae*. The contamination level with *Enterobacteriaceae* in raw meats ranged from 3.26-4.94 log_{10} CFU/g. These findings are supported by previous studies (**Meldrum et al., 2005**; **Wong et al., 2007**) because the TEC is an indicator of hygiene and post-processing contamination of retail meats. **Zhang et al**. (**2016**) reported that the maximum limit of *Enterobacteriaceae* count is 3.27 log_{10} CFU/g. Therefore, *Enterobacteriaceae* contamination of fresh chicken meat observed in our study clearly highlights a possible breakdown of hygienic handling practices at different stages of the manufacturing processing (deboning, cutting, mincing, and mixing). Also, air and equipment surfaces are considered sources of contamination (**Dincer and Baysa, 2004**).

Table 1: Analytical results of microbiological quality evaluation of chicken carcasses.

Chicken meat cuts-up	TVC (n=10)			TEC (n=10)			TCC (n=10)		
	Fresh	Chilled	Frozen	Fresh	Chilled	Frozen	Fresh	Chilled	Frozen
Breast	7.73±0.44^a	3.87±0.25^a	2.17±0.21^a	5.20±0.52^a	3.27±0.20^a	2.11±0.11^a	3.8±0.45^a	2.8±0.51^a	1.13±0.12^a
Thigh	6.22±0.51^b	3.51±0.41^{ab}	1.49±0.37^b	5.51±0.23^a	3.12±0.35^{ab}	1.17±0.35^b	2.95±0.48^b	1.05±0.30^b	1.06±0.15^a
Liver	5.46±0.35^b	3.25±0.28^b	1.95±0.15^{ab}	4.92±0.29^a	2.99±0.25^b	1.51±0.26^b	3.85±0.11^a	2.15±0.43^a	1.13±0.17^a
Gizzard	7.95±0.54^a	4.35±0.45^c	2.77±0.27^c	6.51±0.33^b	2.75±0.28^c	2.07±0.15^a	4.62±0.23^c	2.47±0.44^a	1.85±0.50^b
Average	6.84	3.75	2.10	5.54	3.03	1.72	3.81	2.12	1.29
P-value	0.879	0.652	0.615	0.884	0.268	0.721	0.834	0.862	0.783
F value	0.131	0.449	0.514	0.125	1.531	0.34	0.185	0.152	0.252

Values were measured by log_{10} CFU/g and represented as the mean of three replicates (mean±SD).

Values with the same letter in the same column are insignificant ($p \leq 0.05$).

3.2. Antimicrobial susceptibility test

One hundred and twenty bacterial isolates were encoded (TSX1 to TSX120) and tested against 10 antibiotics agents belonging to 10 antibacterial classes. The drug resistance profiles of each tested isolate are listed in Table 2 which concluded that among 120 bacterial isolates, 84 (70%) isolates were MDR to at least 3 antibiotics different classes. However, only 5 isolates (4%) were pan drug-resistance (PDR) which was resistant to all antimicrobial agents. Similar findings were reported by Kilonzo-Nthenge *et al*. (2013) who found that 84.9% of the bacterial isolates from poultry meat were MDR bacteria. Also, Saud et al. (2018) who studies the antimicrobial susceptibility test of the zoonotic pathogen and reported that the MDR bacteria were higher in chicken meat in comparison to buffalo meat. Besides, the identification of these isolates revealed the presence of *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Citrobacter* spp., *Proteus* spp. and *S. aureus.* Our obtained results exhibited that 67% (80/120) of the bacterial isolates were resistant to ampicillin. However, 53% (63/120) were resistant to tetracycline. The resistance to ciprofloxacin and cefotaxime estimated by 47% (56/120) Also, 46% (55/120), 43% (51/120), 41% (49/120) and 40% (48/120) of bacterial isolates were resistant to imipenem, colistin sulphate, aztreonam and chloramphenicol, respectively. Also, 38% (45/120) and 33% (39/120) were resistant to co-trimoxazole and gentamicin, respectively. Hence, most bacterial isolates were resistant against penicillins and were susceptible to aminoglycosides. Similar findings were reported by Davis et al. (2018) tested the resistance prevalence in chicken and turkey meat and reported that resistance prevalence was high for 50% of tested antibiotics also reported that the resistance to penicillin and tetracycline was significantly high. Table 3 showed that twelve distinct resistance patterns were detected with a variation in the susceptibility to antibiotics. The multiple antibiotic resistance (MAR) showed a variation with an average estimated by 0.55. All the bacterial isolates with MAR index higher than 0.2 consider MDR bacteria where it was resistant to more than 3 different classes of antibiotics (Rolain et al., 2016) and estimated by 84 isolates (70%) out of these isolates 5 isolates (TSX11, TSX18, TSX23, TSX57 and TSX109) were PDR where it was resistant to all classes of tested antibiotic (Magiorakos et al., 2012). The high multiple antibiotic resistance reflected the high-risk of the sample origin and the variation in the MAR index could be due to the variation of samples sources (Morshdy et al., 2018). The overuse and misuse of drugs have led to the development of MDR and PDR bacteria and the acquisition of resistance genes that made the treatment is difficult and in some cases lead to death (Van Looveren et al., 2001; Gieraltowski et al., 2016).

Table 2: Antibiotic susceptibility of the isolated bacteria from chicken meat cuts-up.

Antibiotic class	Antibiotic disc	Code	Sensetive		Intermediate		Resistant	
			No.	%	No.	%	No.	%
Penicillins	Ampicillin	AMP	40	33.3	7	5.8	73	60.8
Monobactams	Aztreonam	ATM	71	59.2	4	3.3	45	37.5
Aminoglycosides	Gentamicin	CN	81	67.5	6	5.0	33	27.5
Phenicols	Chloramphenicol	C	72	60.0	5	4.2	43	35.8
Fluoroquinolones	Ciprofloxacin	CIP	64	53.3	5	4.2	51	42.5
Lipopeptides	Colistin sulphate	CT	69	57.5	4	3.3	47	39.2
Third-generation cephalosporin	Cefotaxime	FEB	64	53.3	6	5.0	50	41.7
Carbapenems	Imipenem	IPM	65	54.2	4	3.3	51	42.5
Folate pathway inhibitors	Co-trimoxazole	SXT	75	62.5	7	5.8	38	31.7
Tetracyclines	Tetracycline	TE	57	47.5	5	4.2	58	48.3

Table 3: Antibiotic resistance pattern and MAR index of all bacterial isolates.

Resistance pattern	Resistance profile	Number of bacterial isolates	MAR
I.	AMP, ATM, CN, C, CIP, CT, FEB, IPM, SXT, TE	5	1
II.	AMP, ATM, CN, CIP, CT, FEB, IPM, SXT, TE	4	0.9
III.	ATM, CN, C, CIP, CT, FEB, IPM, SXT	8	0.8
IV.	ATM, CN, C, CT, FEB, IPM, SXT	12	0.7
V.	ATM, CIP, CT, IPM, SXT, TE	10	0.6
VI.	AMP, C, CIP, CT, FEB, TE	6	0.6
VII.	AMP, C, CIP, FEB, TE	11	0.5
VIII.	AMP, C, CT, IPM, SXT	6	0.5
IX.	ATM, CN, FEB, IPM	10	0.4
X.	AMP, CIP, TE	12	0.3
XI.	AMP, TE	15	0.2
XII.	AMP	21	0.1
Average			0.55

3.3. Isolation and molecular identification

The biochemical and molecular identification of the selected PDR isolates revealed that TSX11, TSX23 and TSX109 were closely related to the phylum *Proteobacteria* and all these isolates belonging to the same class (γ-*Proteobacteria*). However, TSX18 and TSX57 were closely related to the phylum *Firmicutes* as shown in Table 4. The phylogenetic tree (Figure 1) showed that two isolates (TSX11 and TSX57) were closely related (>99% similarity) to *Pseudomonas aeruginosa* IMD85 and *Staphylococcus aureus* 191, respectively. While TSX18, TSX23 and TSX109 were closely related to genus *Staphylococcus epidermidis* BGHMC11, *Escherichia coli* O157:H7 and *Salmonella enterica* ATCC 8387, respectively.

Our obtained data were nearly similar to the results reported by Ahmed and Sarangi (2013) who reported the presence of *E. coli*, *Staphylococcus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Streptococcus* sp., *Serratia* sp., *Shigella* sp., and *Salmonella* sp. in raw meat samples. Also, Olukemi et al. (2015) who studied the bacteriological status of chicken carcass in Nigeria and the results indicated the presence of *S. aureus*, *Arcobacter* spp. and *E. coli*. Furthermore, Nagarajan et al. (2018) recorded the bacterial contamination of chicken meat in Indian market and noted the occurrence of *Enterococcus* sp., *Klebsiella* sp., *E. coli*, and *Proteus* sp.. This variation in results from different studies proves that bacterial contamination and bacterial prevalence varies according to area and meat type. Hence, it is urgent to find a suitable and wide spectrum antibacterial agent.

Table 4: Molecular identification and resistance profiles of PDR bacterial isolates.

PDR Isolates code	Group	Closest relative according to molecular identification	Identity (%)
TSX11	*Proteobacteria*	*Pseudomonas aeruginosa* IMD85	99.10
TSX18	*Firmicutes*	*Staphylococcus epidermidis* BGHMC11	98.93
TSX23	*Proteobacteria*	*Escherichia coli* O157:H7	98.20
TSX57	*Firmicutes*	*Staphylococcus aureus* 191	99.74
TSX109	*Proteobacteria*	*Salmonella enterica* ATCC 8387	93.68

Abbreviations: AK: Amikacin; **AMP**: Ampicillin; **AX**: Amoxicillin; **ATM**: Aztreonam; **FEP**: Cefotaxime; **C**: Chloramphenicol; **CIP**: Ciprofloxacin; **CN**: Gentamicin; **CT**: Colistin sulphate; **IPM**: Imipenem; **SXT**: Co-trimoxazole; **TE**: Tetracycline; **MAR**: Multiple antibiotic resistance.

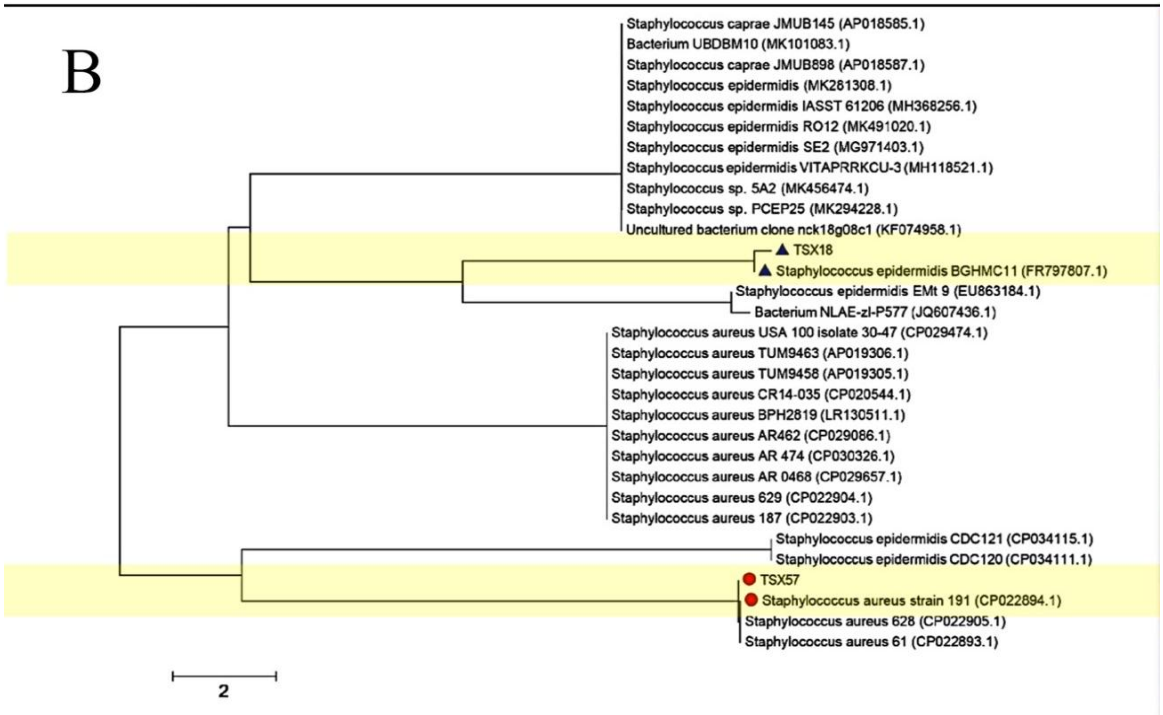

Fig. 1: 16S rRNA gene sequence analysis dendogram, showing the PDR bacteria isolated from chicken meat. A: PDR bacterial isolates belonging to *Proteobacteria*; B: PDR bacterial isolates belonging to *Firmicutes*.

3.4. Antimicrobial activity of ZnO_2-NPs

Several reports investigated the activity of metal oxide nanoparticles (MO-NPs) as antimicrobial agents due to their unique properties (Dakal et al., 2016; Hemeg, 2017; Slavin et al., 2017). This study attempted to evaluate the efficiency of ZnO_2-NPs against zoonotic pathogens isolated from retail chicken meat. Antimicrobial activities of synthesized ZnO_2-NPs are presented in Table 5. The ZnO_2-NPs show outstanding inhibitory activity against PDR bacterial isolates with a variation in the susceptibility to ZnO_2-NPs. The proportional effect proves the susceptibility of bacterial isolates to a high dose of tested antibacterial agents. At a concentration of 50 µg/ml of ZnO_2-NPs, the most susceptible bacterial isolate was the Gram-positive bacteria (*S. aureu* and *S. epidermidis*) with an inhibition zone 15.0 and 13.0 mm, respectively. However, Gram-negative bacteria (*E. coli*, *S. enterica*, and *P. aeruginosa*) showed lower inhibition zone ranged from (7.0-10.0 mm) as shown in Table 5. The statistical analysis indicating the significant ($p \leq 0.05$) inhibition zone of all Gram-negative bacteria. At 100 and 150 µg/ml, all bacterial isolates showed susceptibility to ZnO_2-NPs with insignificant ($p \leq 0.05$) inhibition zone ranged from. However, *P. aeruginosa* showed significant inhibition zone. This behavior was changed at the high dose of ZnO_2-NPs (200 µg/ml) where all bacterial isolates showed outstanding susceptibility to ZnO_2-NPs against zoonotic PDR pathogens. The MIC and MBC values for *S. epidermidis*, *E. coli* and *S. enterica* were estimated as 50 and 100 µg/ml, respectively. However, MIC value for *P. aeruginosa* and *S. aureus* was 150 and 25 µg/ml and MBC value was 300 and 50 µg/ml, respectively as shown in Table 5.

Table 5: Antimicrobial activity of ZnO_2-NPs against PDR bacteria.

Organism	Concentrations of ZnO_2-NPs (µg/ml)				MIC (µg/ml)	MBC (µg/ml)
	50	100	150	200		
	Diameter of inhibition zone (mm)					
S. aureus	15.0±3.0^{a}	18.0±3.0^{a}	24.0±1.0^{a}	33.0±4.0^{a}	25	50
S. epidermidis	13.0±1.0^{a}	18.0±3.0^{a}	22.0±3.0^{ab}	25.0±3.0^{b}	50	100
E. coli	10.0±1.0^{b}	16.0±1.0^{a}	24.0±2.0^{a}	30.0±2.0^{ac}	50	100
S. enterica	10.0±1.5^{b}	18.0±2.0^{a}	20.0±1.0^{b}	35.0±4.0^{a}	50	100
P. aeruginosa	7.0±1.0^{c}	11.0±0.5^{b}	15.0±1.0^{c}	28.0±2.0^{bc}	150	300
***P*-value**	0.876	0.887	0.953	0.808	ND	ND
F value	0.133	0.121	0.048	0.217	ND	ND

Values are the mean of three replicates (mean±SD).
Values with the same letter in the same column are insignificant ($p \leq 0.05$).

Ali et al. (**2017**) reported the activity of ZnO_2-NPs against MDR bacteria isolated from burn wound infections. Also, **Duffy et al**. (**2018**) estimated the activity of different MO-NPs against *Campylobacter* sp. and *Salmonella* sp. isolated from chickens and reported that MO-NPs exhibit antibacterial activity that will lead to many potentially useful applications in the industrial field. In this concern, **Nagarajan and Kuppusamy** (**2013**) explained the mechanism of antibacterial activities of MO-NPs and reported that the small particle size of MO-NPs is associated with a larger bandgap causing undesirable conditions that prevent the recombination of excitons. So that more available excitons will cause the formation of a higher concentration of reactive oxygen species (ROS) which cause significant damage to bacterial cell structure and enhance the antibacterial activities of MO-NPs.

Liu et al. (**2009**) reported similar results indicating the antibacterial activity of zinc oxide nanoparticles (ZnO-NPs) against *E. coli* (Gram negative bacteria) and mentioned the gradual activity of inhibitory effect along with the concentration of the nanoparticles. Also, the activity of ZnO-NPs against *S. aureus* (Gram positive bacteria) was investigated by **Narasimha et al**. (**2014**) who found that the prepared ZnO-NPs by a chemical method has an excellent antibacterial effect. Additionally, the nanoparticles acted by destroying the bacterial cell wall membrane by altering the the membrane permeability and inducing oxidative stress leading to bacterial cell death (**Xie et al., 2011**; **Narasimha et al., 2014**). **Reddy et al.** (**2011**) also reported that the nanoparticles also inhibited invasion internalization by non-phagocytic cells. Also, **Hsueh et al. (2015)** reported that the accumulation of nanoparticles in the cytoplasm or on the outer membranes of the bacteria resulted in cell death.

Furthermore, **Rago et al**. (**2014**) suggesting that the morphology of the bacterial cell influenced the antibacterial activity of the formulation and this conclusion was in agreement with our results where all Gram negative PDR bacteria were less susceptible to ZnO_2-NPs than Gram-positive bacteria. Another studies indicated that the antibacterial activity of ZnO-NPs depending on the size and concentration of the nanoparticles (**Raghupathi et al., 2011; Hsueh et al., 2015**). Hence, in the future, nanotechnology will play a vital role against food-borne diseases causing agents.

4. CONCLUSION

The antibacterial techniques based on nanotechnology may contribute as alternative approaches for mitigation of multiple and pan drug resistant. Our results showed that among all meat samples, fresh meat were unacceptable because of the high TVC of bacterial contaminants were higher than $\log_{10}$ 5 CFU/g. The antibacterial susceptibility test showed that 70% were MDR and out of these isolates 5 PDR isolates were detected and identified as *Pseudomonas aeruginosa* IMD85, *Escherichia coli* O157:H7 *and Salmonella enterica* ATCC 8387, *Staphylococcus aureus* 191 and *Staphylococcus epidermidis* BGHMC11. Zinc peroxide nanoparticles showed a gradual activity against these PDR pathogens at a concentration from 50-200 µg/ml. the MIC value of bacterial isolates ranged from 25-100 µg/ml and MBC values ranged from 50-300 µg/ml. ZnO_2-NPs have exhibited efficiency as antibacterial activity against zoonotic PDR pathogens reflecting that the ZnO_2-NPs are potentially valued MO-NPs for food safety applications.

Conflict of interest

The author declares no conflict of interest statement.

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