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USING ZINC OXIDE NANOPARTICLES AS AN ANTIBACTERIAL AGAINST MULTIDRUG-RESISTANT BACTERIA FROM BROILER FARMS

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ABSTRACT

The global healthcare system is at risk due to antibiotic resistance. The study evaluates the antibacterial susceptibility of green synthesis of zinc oxide nanoparticles (ZnO NPs) against *Escherichia coli* and *Salmonella* species from chicken farm waste, environment, and farmworkers. A total of 100 samples from chicken farms (15 feces, 75 poultry environments, and 10 workers' hands) were isolated to identify bacterial strains. Disk diffusion was employed to evaluate the antibiotic susceptibility of 28 bacterial isolates. Minimum inhibitory concentration (MIC) was conducted on ZnO NPs against *Salmonella* species and *E. coli* and confirmed by conventional polymerase chain reaction (PCR). The obtained results revealed that 20% were positive for *E. coli*, with serotypes O55:K59 (4), O44:K79 (3), O86:K61 (5), O126:K71 (4), and O111:K58 (4). Another 8% were positive for *Salmonella* species, with serotypes *S. typhimurium* (2), *S. anatum*(1), *S. belegdam*(2), *S. montevideo* (2), and *S. lumberhurst* (1). The majority of *Salmonella* and *E. coli* isolates were multidrug-resistant to colistin (100%), amoxyclav (62.5%–80%), cefotaxime (75%–60%), sulpha and trimethoprim (50%–80%), gentamicin (75%–100%), amikacin (55%–57%), and ciprofloxacin (30%–70%), neomycin (87.5%–100%), levofloxacin (30–60%). The cytotoxicity IC_{50} of ZnO NPs was 130.3 µg/ml, with a zeta potential of +83.5 mV and a size of 6.35 nm, and the MIC was 5 mg/ml. The isolated *Salmonella* species and *E. coli* were validated using conventional PCR. The results showed that the ZnO NPs were more effective against *Salmonella* species than *E. coli* isolates. Eco-friendly ZnO NPs, when utilized as a treatment for multidrug-resistant *E. coli* and *Salmonella* species, can replace conventional antibiotic treatments.

Keywords: Zinc propolis nanoparticle, *Salmonella* spp., *E.coli*, MDR, broiler

INTRODUCTION

Bacterial diseases pose a significant threat to the poultry industry in Egypt and worldwide, leading to substantial economic losses and health challenges. Among the most critical pathogens affecting the poultry industry are *Salmonella* and *Escherichia coli*. Gram-negative bacteria are commonly found in the gastrointestinal tract of chickens. These organisms, particularly prevalent in newly hatched chicks, can cause severe disease outbreaks, hinder growth, and increase mortality rates (Shao *et al.,* 2014).

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Avian pathogenic *Escherichia coli* (APEC) is responsible for a wide range of clinical conditions collectively referred to as avian colibacillosis. These include colisepticemia, airsacculitis, enteritis, and salpingitis (Dziva *et al.,* 2008). The disease is often associated with co-infections and results in high morbidity and mortality, as well as reduced market value due to stunted growth in surviving birds (Matin *et al.,* 2017; Stacy *et al.,* 2014). Similarly, *Salmonella* infections, particularly those caused by *S. typhimurium* and *S. enteritidis*, constitute a significant concern due to their zoonotic potential and their role in both poultry and human diseases (Abd ElGhany *et al.,* 2012). These infections are frequently vertically transmitted and cause severe clinical signs such as diarrhea, low body weight, and increased chick mortality, accompanied by lesions like hepatitis, splenitis, and omphalitis (Bhatti *et al.,* 2013; Sedeik *et al.,* 2019; Soufy *et al.,* 2016). Beyond animal health, *Salmonella* remains a leading cause of foodborne illnesses in humans, underlining its public health significance (Fatima *et al.,* 2022).

The rise in antimicrobial resistance (AMR), primarily driven by the misuse of antibiotics in poultry production, has compounded the problem, making conventional treatments less effective and raising public health concerns, especially in the context of zoonotic pathogens (Boyle *et al.,* 2007). This has prompted an urgent need for alternative therapeutic strategies. One promising avenue involves nanotechnology-based antimicrobials, or nanobiotics, which leverage the unique physicochemical properties of nanoparticles to combat multidrug-resistant bacterial strains (Ye *et al.,* 2020).

Recently developed in agricultural and biological fields, nanotechnology is the most often used strategy. It is employed in molecular and atomic-scale material synthesis and management. Nanomaterials are normal materials reduced to 1–100 nm. Thus, nanoparticles (NPs) exhibit chemical and physical properties. In particular, these additional NP properties have facilitated their application in emerging areas such as drug delivery systems, antibacterial agents, and anticancer agents (Abdelsattar *et al*., 2024).

Several NPs were synthesized using chemical, physical, biological, and biosynthetic methods. Though these methods have been used for years, the biosynthetic method has a more substantial impact and less toxicity. Biosynthesized NPs are biodegradable, biocompatible, and easy to make. NPs can be made from plants, algae, fungi, bacteria, and propolis (Chokkareddy *et al*., 2018). Applying NPs in a resinous mixture improves their efficiency. Waxes, essential oils, polyphenolic acids, aromatic acids, phenolic acid esters, flavonoids, and terpenoids are all part of propolis, which is used as a coating agent for nanoparticles made of metals and metal oxides. Thus, it is helpful in antimicrobial, anti-inflammatory, antioxidant, and anticancer therapies (Salem and Fouda, 2021; Saadawi *et al*., 2022). Propolis extract has been employed as a reducing agent in the manufacture of metal nanoparticles, including ZnO–propolis composites, demonstrating inhibitory effects on various cell lines (Salama *et al*., 2023).

ZnO NPs are relatively simple to produce using basic chemicals and standard equipment, making them accessible for general laboratory applications. Also, they are commercially available, allowing for their use in microbial detection and PCR applications, even in laboratories without advanced characterization instruments (Upadhyay *et al.,* 2020; Mohamed *et al.,* 2024).

The polymerase chain reaction (PCR) is a widely used technique for detecting *Salmonella*, known for its speed, accuracy, and ability to work with complex samples such as food. Due to these advantages, PCR serves as an effective tool for

identifying both *Salmonella* and *Escherichia coli*, contributing to safer food handling practices (Prayoga & Wardani, 2015). A common PCR target for *Salmonella* detection is the *invA* gene, which contains unique DNA sequences specific to this pathogen (Abdel-Aziz, 2016). In the case of *E. coli* isolated from poultry farms, the 8F and 1492R primers are frequently used to amplify and sequence the 16S rRNA gene for identification and analysis (Leis *et al.,* 2019).

MATERIALS AND METHODS

Collection of samples

From the poultry environment in Giza province, 100 samples were taken: 15 from fecal matter, 15 from tap water, 15 from feeds, 15 from drinkers, 15 from feeders, 15 from litter, and 10 from workers' hands. After processing, the samples were properly stored in sterile screw-top bottles and plastic bags. Before sampling, each swab was put into 0.1% buffer peptone water (BPW; Oxoid, Ltd., Basingstoke, UK). Then, 10 ml of BPW was placed on the swabs, following the procedure described by Adzitey *et al.* (2012).

Tenfold serial dilutions were prepared from each sample using BPW, which was pre-enriched. Sterile swabs collected from feeders, drinkers, and workers' hands were added to 0.1% BPW (Oxoid, Ltd, Basingstoke, UK).

Isolation and identification of bacterial pathogens

Salmonella isolation and identification (ISO 6579: 2017)

The sample combinations were kept in an incubator at 37 ± 1°C for 18 ± 2 hours. Subsequently, 0.1 ml of the broth culture was introduced into various selective enrichment broths, including Muller–Kauffmann Tetrathionate Novobiocin broth (MKTTn) (Oxoid), Rappaport-Vassiliadis soya broth (RVS broth) (MERCK). These broths were then incubated at 41.5 ± 1.0°C for 24.0 ± 3.0 hours and 37.0 ± 1.0°C for 24.0 ± 3.0 hours, respectively. A portion of each broth culture was added to Xylose Brilliant Green agar media (HiMedia) and Lysine deoxycholate agar (XLD) (Oxoid) as a selective plating medium. Then, the mixture was incubated at 37.0 ± 1.0°C for 24 ± 3 hours. *Salmonella* suspected growing colonies (pink, with or without a black center) were streaked onto the nutrient agar and incubated at 37°C for 24 hours. Afterward, they were maintained at 4°C for further biochemical identification (Hammack *et al.*, 2001).

E. coli isolation and identification (Nolan *et al.,* 2013)

Usually, samples are enriched for 24 hours in buffered peptone water broth under aerobic conditions at 37°C. *E. coli* was selectively colonized using two differential media: Eosin methylene blue (EMB) agar and MacConkey's agar. From every sample, a loopful of broth was inoculated onto the plates and then incubated at 37°C for 24 hours. Measuring 1-2 mm in diameter, the probable colonies exhibited a hot-pink color on MacConkey agar and a metallic shine on EMB agar. Single, separate *E. coli* colonies were kept for later examination. Indole reaction, methyl red, Voges–Proskauer, citrate usage, catalase, sugar fermentation, oxidase, and urea agar tests were among the biochemical procedures performed.

Serological identification of *Salmonella*

Biochemically validated *Salmonella* isolates underwent serological identification using monovalent antisera via the slide agglutination test following ISO (2014), part III. The study utilized diagnostic antisera from Denka Seiken Co., Ltd. for *Salmonella* O and H, alongside both polyvalent and monovalent formulations of the vaccine, specifically F-67, A-67, and A-E.

E. coli isolates serological characterization (Schouler *et al.*, 2012)

Slide agglutination testing, in conjunction with conventional polyvalent and monovalent antisera, was used for *E. coli* isolate classification at the Animal Health Research Institute. Serotyping was performed on the isolates using an assay developed and supplied by Safin Antisera Co. of Germany. The assay used antisera that targets the somatic (O) antigens of *E. coli*.

Antimicrobial susceptibility testing

Escherichia coli and *Salmonella* isolates were subjected to disc diffusion antimicrobial susceptibility testing under Clinical and Laboratory Standards Institute guidelines (CLSI, 2020).

The test assessed the susceptibility of the isolated strains to nine antimicrobial discs from six categories: penicillins (AMC, 30 µg), cephalosporines (CTX, 30 µg), aminoglycosides (amikacin, 30 µg; neomycin, 30 µg; gentamycin, 10 µg), and sulphonamides (trimethoprim/sulfametho xazole, 25 µg). The Clinical and Laboratory Standards Institute assessed the inhibition zone and classified them as sensitive or resistant.

Multiple Antimicrobial Resistance (MAR) Index

The MAR was determined as MAR index = number of antimicrobials that show resistance/number of total antibiotics that were used (Christopher *et al.,* 2013). The interpretation of results was conducted using the MAR index ≤ 0.2, which indicated a low risk, while the value ≥0.2 represents a high risk of antimicrobial resistance (Akande *et al.,* 2019)

Green preparation and characterization of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnO NPs) were synthesized using a green coprecipitation method, adapted from the technique outlined by Singh *et al.* (2011). In this approach, a natural reducing agent was prepared by combining 20 g of propolis with 200 mL of Milli-Q water, then heating the mixture at 100°C on a magnetic stirrer-equipped hot plate for 10 minutes. After allowing the solution to cool, it was centrifuged twice at 4500 rpm for 10 minutes each. The clarified supernatant was filtered through Whatman filter paper and kept at 4°C until use in nanoparticle synthesis.

A 0.10 M solution of zinc nitrate was prepared to initiate nanoparticle formation. Then, the stored propolis extract was added slowly to the zinc nitrate solution under constant stirring at room temperature (25°C), maintaining equal volumes of both components. The resulting suspension was subjected to two centrifugation cycles at 8000 rpm for 20 minutes each. The recovered solids were thoroughly rinsed with bi-distilled water and then dried at 100°C, yielding ZnO NPs in powder form (Salama *et al.,* 2023).

Nanoparticle Characterization

Multiple analytical tools were used to characterize the physical and chemical properties of the ZnO NPs. The particle size distribution, polydispersity index (PDI), zeta potential, and electrical conductivity were assessed using the Microtrac Wave system (version 12.0.1.0). High-resolution imaging was performed via transmission electron microscopy (HRTEM) using a JEM 1400F model operating at an accelerating voltage of 300 kV. Additionally, Fourier-transform infrared spectroscopy (FTIR) was employed to identify the functional groups on the nanoparticle surface, conducted at the Research Park of Cairo University's Faculty of Agriculture.

Cytotoxicity of zinc oxide nanoparticles

The cytotoxic potential of the synthesized nanoparticles was assessed through the MTT assay, a colorimetric method based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial enzymes such as

succinate dehydrogenase. Once internalized by viable cells, MTT is metabolized into insoluble formazan crystals, which are later solubilized in isopropanol. The resulting solution's optical density is measured at 570 nm, using 630 nm as the background wavelength, with absorbance values corresponding to cell viability (Mosmann, 1983).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Zinc oxide nanoparticles against *E. coli* and *Salmonella* isolates, according to **Parvekar *et al.* (2020)**

- The bacterium was viable and preserved as stock. Subsequently, the concentration was calibrated using a spectrophotometer to achieve an optical density of 0.10 at 600 nm (10^8 CFU/ml, 0.5 McFarland standard).
- The susceptibility of isolates to ZnO NPs was assessed using the microbroth dilution method, which was established according to protocols authorized by the Clinical and Laboratory Standards Institute (CLSI). A total of 50 µl of Mueller–Hinton broth (MHB) was introduced into wells 1 to 12. Subsequently, 100 µl of nanoparticle stock solution (5 mg/ml for ZnO NPs) was added to well 1, and a serial dilution was conducted in well 1 to well 10. Then, 50 µl of bacteria with a concentration of 10^8 CFU/mL were introduced into all wells, except the negative control well (well 12). The microplate was thereafter incubated at 37°C for 24 hours. The initial dilution at which no growth was observed was determined by using a spectrophotometer to achieve an optical density of 0.10 at 600 nm.
- For the measurement of the minimum bactericidal concentration (MBC) NP, 50 µL from all wells exhibiting no detectable bacterial growth were seeded onto Mueller–Hinton (MH) agar plates and incubated for 24 hours at 37°C. MBC endpoint refers to the point at which 99.9% of the bacterial population is killed at the minimal concentration of a nanoparticle agent.

Detection of *Escherichia coli* and *Salmonella* species by conventional polymerase chain reaction (PCR):

DNA extraction:

The samples were subjected to DNA extraction using the QIAgen Cat. no. 69504 and 69506 DNA Easy Blood and Tissue Kits. Then, 200 µl of the isolated sample was put into sterile 1.5 mL Eppendorf tubes. In summary, the process was carried out exactly as the manufacturer had instructed: 180 µl of ATL buffer and 20 µl of proteinase K were added to the samples and then incubated for two hours at 56ºC. After that, samples were mixed with 200 µl of AL buffer and heated to 70°C for five minutes. Then, 200 µl of ethanol was added. The lysate was then transferred onto the spin columns included with the kit, and the tubes were centrifuged. AW1 and AW2 buffers were used twice to wash the resin-bound DNA. Finally, 50 µL was used to elute DNA. The cleaned DNA was kept in storage.

PCR amplification:

Cosmo PCR Red Master Mix (Catalog no. W1020300x, Willow Fort, UK) was used. The mix includes all ingredients for a successful PCR and the Cosmo DNA polymerase enzyme. $MgCl_2$, dNTPs, storage buffer, and Cosmo Taq DNA polymerase are the ingredients of the Cosmo PCR Master Mix was used according to White *et al.* (1990). The primers are summarized in Table 1.

Each sample requires the following reagents: 10 µI of Cosmo master mix, 2 µI of specific primers (*invA*) or (8F/1492R) with 3 µI from nuclease-free water. Then, 5 µI of DNA extracted from the sample was added lastly (20 µI total volume). The reaction consisted of one cycle of initial denaturation at 95°C for two minutes. This

was followed by 27 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 58°C for 20 seconds, and extension at 72°C for one minute. Finally, there was one cycle at 72°C for 10 minutes.

Table (1): The primers used in this study of both bacteria

Gene	Forward and Reserve	Amplified region	References
(*invA*) (*Salmonella* pecies)	GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C	284 bp	**Upadhyay *et al*, (2010)**
8F/1492R (*Escherichia coli*)	AGAGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	1000bp	**Turner *et al.* (1999)**

Electrophoresis of PCR products:
Agarose gel electrophoresis was used to examine the PCR amplification products in the gel documentation system by ethidium bromide staining to visualize the results. The combination of amplicon and gel loading buffer (50% glycerol/0.1M EDTA, pH 8.0/1% SDS/0.1% bromophenol blue/0.0% xylene cyanole) was loaded into 1.5% agarose in 1x TBE (89 mM Tris/89 mM boric acid/2 mM EDTA, pH 8.0). A 100 bp ladder was the size standard (Gibco, BRL).

Gel band intensity measurement:
PCR products were loaded onto an agarose gel in the same volume as the DNA ladder. Following migration, the Image Lab software (BioRad) was updated with the DNA ladder band concentrations for molecular weight analysis. Rectangles of the same dimension covered the PCR product-specific band, and the program used the supplied ladder concentrations to determine the band's concentration. A bar chart was then created to visualize the computation results.

RESULTS

1. Prevalence of *E. coli* and *Salmonella* isolates in the examined samples

The results have demonstrated a notable incidence of *E. coli* among samples, with a total rate of 20% (20/100) and *Salmonella* with a rate of 8% (8/100) (Tables 2, 3).

Table (2): Prevalence of *E. coli* and *Salmonella* isolates

Source of sample	***E. coli* isolates**		***Salmonella* isolates**	
	No.	**%**	**No.**	**%**
Fecal droppings (n = 15)	6	40 %	2	13.33%
Tap water (n = 15)	1	6.67%	0	0
Feeds (n = 15)	2	1.33%	0	0
Drinkers (n = 15)	3	20%	1	13.33%
Feeders (n = 15)	2	1.33%	2	26.67%
Litter (n = 15)	4	26.67%	2	26.67%
Worker's hand swabs (n = 10)	2	20%	1	10%

*Percent was calculated according to the total number of samples

2. Antimicrobial susceptibility of *Salmonella* species and *E. coli*

The results of antibiotic susceptibility testing *Salmonella* isolates showed resistance against colistin (100%), amoxyclav (64%), cefotaxime (41%), sulpha and trimethoprim (50%), gentamicin (12.5%), amikacin (25%), ciprofloxacin (30%), levofloxacin (30%), and neomycin (75%), (Table 4).

The results of antibiotic susceptibility testing *E. coli* isolates showed resistance against colistin (100%), Amoxyclav (85%), Cefotaxime (70%), Sulpha & Trimethoprim (80%), Gentamicin (60%), Amikacin (65 %), Ciprofloxacin (40%) Levofloxacin (40%), Neomycin, (55%) (Table 4).

Table (3): Serotyping *of E. coli* and *Salmonella* isolates.

Serovars of *E. coli* isolates	No. (%)*	Serovars of *Salmonella* isolates	No.(%)*1
O55:K59	4(20%)	*S.Typhimurium*	2(25%)
O44:K79	3(15%)	*S.Anatum*	1(12.5%)
O86:K61	5(25%)	*S.Belegdam*	2(25%)
O126:K71	4(20%)	*S. Montevideo*	2(25%)
O111:K58	4(20%)	*S.Lumberhurst*	1(12.5%)
Total	20(100%)	Total	8(100%)

*The percent was calculated according to total no. of *E. coli* isolates (20)
%*1 was calculated according to total no of *Salmonella* isolates (8)

Table (4): Antimicrobial susceptibility pattern of *Salmonella* and *E. coli* isolates

Antibiotic agents	*Salmonella* isolates (n=8)				*E. coli* isolates (n= 20)			
	Resistant		Sensitive		Resistant		Sensitive	
	No.	%	No.	%	No.	%	No.	%
Colistin sulfate (CT) 10µg	8	100	0	0	20	100	0	0
Amoxicillin/clavulanic acid (AMC) 30µg	5	64	3	36	17	85	3	15
Cefotaxim (CTX) 30 µg	3	41	5	59	14	70	6	30
Trimethoprim + Sulphamethoxazole (SXT) 25 µg	4	50	4	50	16	80	4	20
Gentamicin (CN) 10µg	1	12.5	7	87.5	12	60	8	40
Amikacin (AK), 30µg	2	25	6	75	13	65	7	35
Ciprofloxacin (CIP) 5 µg	3	30	5	70	8	40	12	60
Levofloxacin (LEV) 5 µg	3	30	5	70	8	40	12	60
Neomycin (N), 30µg	6	75	2	25	11	55	9	45

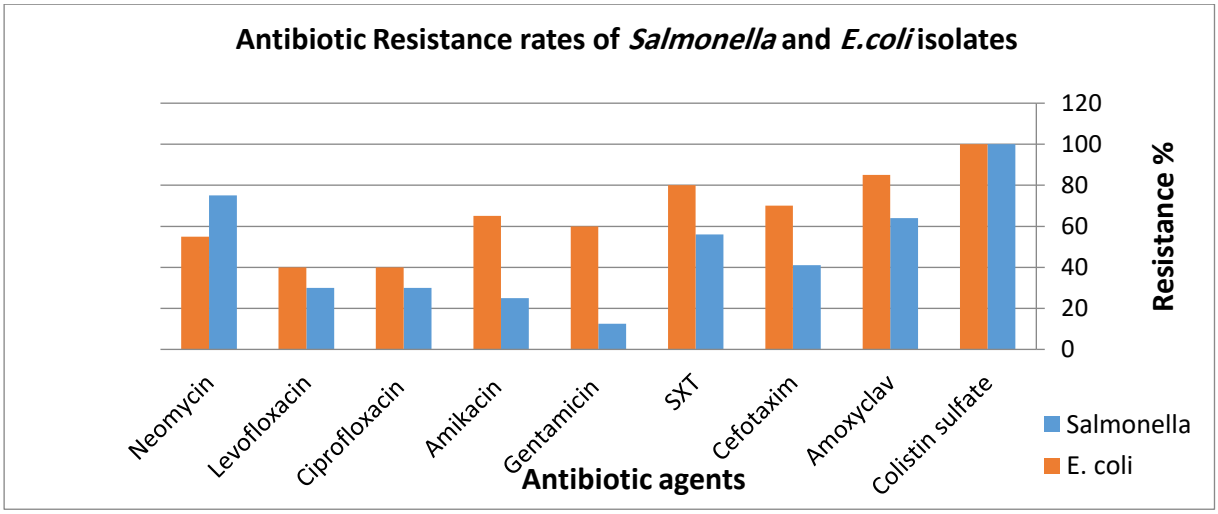

Figure (1): Antibiotics resistance rates of *Samonella* Species and *E.coli* isolates

Table 5: Antibiotic resistance profile of *Salmonella* isolates with their MAR index.

Salmonella Strains (No. of isolates)	**Antimicrobial Resistance Profile**	MAR Index (No .of isolates)
S.Typhimurium (2)	CT10, SXT25, LEV5, CN10, AK30	0.55
S.Anatum (1)	CT10, AMC30, N30, CTX30,	0.44
S.Belegdam (2)	LEV5, CT10, AK30, N30, AMC30, CTX30	0.66
S. Montevideo (2)	N30, SXT25, CT10, AMC30, CIP5	0.55
S.Lumberhurst (1)	CT10, AK30, CIP5, N30	0.44

amoxicillin/clavulanic (AMC), Cefotaxime (CTX), Amikacin (AK), Neomycin (N), Gentamycin (CN), Trimethoprim sulfamethoxazole (SXT), Levofloxacin (LEV), ciprofloxacin (CIP), colistin (CT), 10µg

Table 6: Antibiotic resistance profile of *E. coli* isolates with their MAR index.

E.coli Strains (No.of isolates)	**Antimicrobial Resistance Profile**	MAR Index (No.of isolates)
O55:K59 (4)	SXT25, CT10, N30, CN10, AK30, AMC30	0.66
O44:K79 (3)	SXT25, CT10, N30, CTX30, CN10, CIP5	0.66
O86:K61 (5)	CN10, CT10, CTX30, AMC30, CIP5, SXT25, AK30	0.77
O126:K71 (4)	CT10, N30, AK30, AMC30, LEV5, CTX30,	0.66
O111:K58 (4)	CT10 , AMC30, CN10, , LEV5, SXT25	0.55

amoxicillin/clavulanic (AMC), Cefotaxime (CTX), Amikacin (AK), Neomycin (N), Gentamycin (CN), Trimethoprim sulfamethoxazole (SXT), Levofloxacin (LEV), ciprofloxacin (CIP), colistin (CT), 10µg.

3. Characterization of zinc nanoparticle:

The zeta potential results indicated that zinc and propolis extract, when combined, could interact to give the surface of the nanoparticle a positive charge. The synthesized nanoparticle displayed zeta potentials of +83.5 mV with PDI 0.0979.

The MTT assay demonstrated that propolis-mediated ZnO NPs exhibited low cytotoxicity at concentrations below 100 µg/mL, suggesting their potential for biomedical applications with $IC_{50\%}$ = 130.3 µg/mL. FTIR spectra demonstrated the presence of phenolic and flavonoid compounds from propolis on the ZnO NP surface, hence proving effective bio-functionalization. Peaks at 3450.55 cm^{-1} (O-H stretching) and 1637 cm^{-1} (C=O stretching) and Zn–O bonding peaks 501.77 cm^{-1} were detected as illustrated in Figure 2.

4. The findings revealed that ZnO NPs exhibited notable antibacterial activity against various *E. coli* and *Salmonella* isolates, with a MIC of 5 mg/mL for all tested strains. The MBC tests demonstrated complete inhibition of bacterial growth in all *Salmonella* isolates. On the other hand, for *E. coli*, four out of five isolates showed no bacterial growth at the MBC level, while one isolate, serotype O_{126}, exhibited limited growth.

5. The results of PCR helped assess *Salmonella* and *Escherichia coli* isolates, which were treated with MIC 5 mg/ml ZnO NPs, where five samples from each species isolate were compared to qPCR without ZnO NPs. Both *Salmonella* and *Escherichia coli* were amplified at 284 bp and 900 bp, respectively. The result of the untreated *Salmonella* isolates with ZnO in four

samples have a strong band; however, one isolate (Table 5) had a faint band. Conversely, the treated *Salmonella* isolates with MIC 5 mg/ml ZnO NPs in four samples have a faint band, and one isolate (Table 6) is at zero concentration. The results of the untreated *Escherichia coli* isolates with zinc oxide in five samples show a strong band concentration. On the other hand, the results of the treated *Escherichia coli* isolates with MIC 5 mg/ml ZnO NPs in five samples have a very faint band concentration, as shown in Figures 3-4.

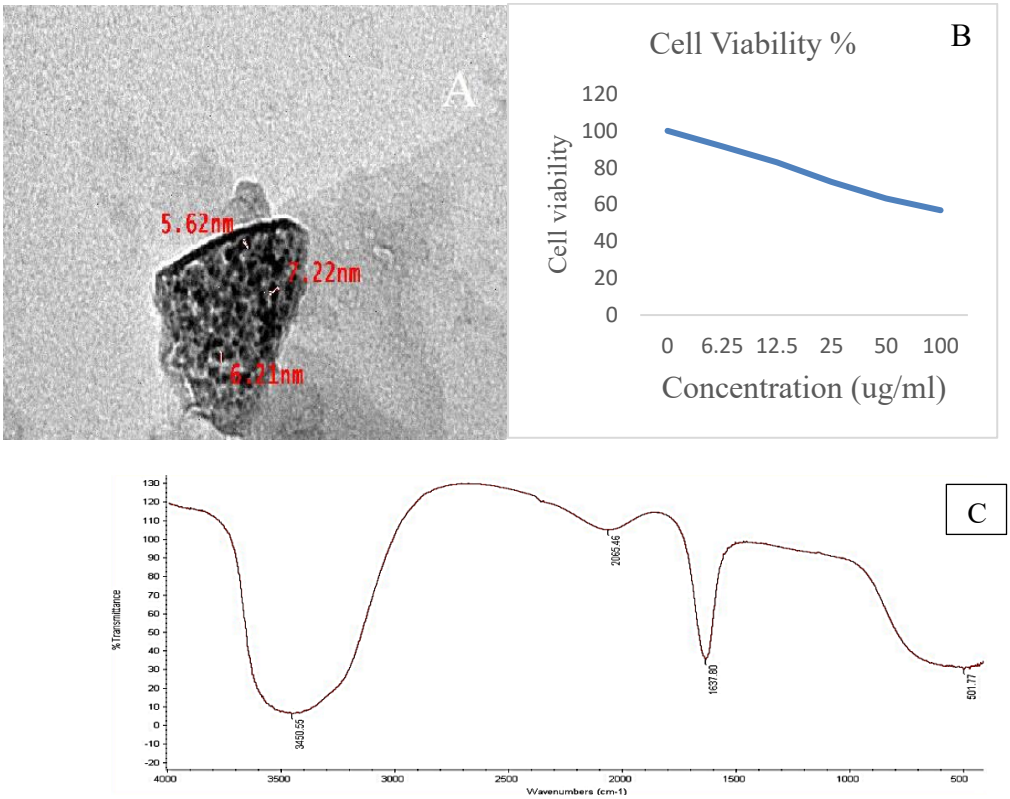

Figure (2) A: HRTEM revealed that droplets size was 6.35 nm, there no aggregation, size homogeneity and spherical nature. (**B**) Cell viability % of ZnO NP effect on Vero cells IC_{50} was determined to be 130.3 ug/ml. (**C**) FT-IR Analysis of ZnO NP for functional groups

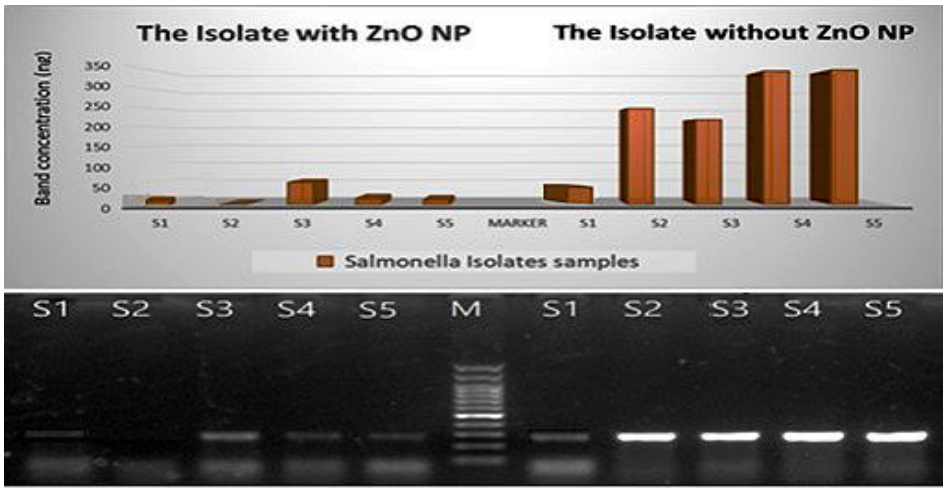

Figure (3): Conventional PCR amplification of *Salmonella* species at (284 bp), there specific fragment with and without mic 5 mg /ml ZnO NPs.

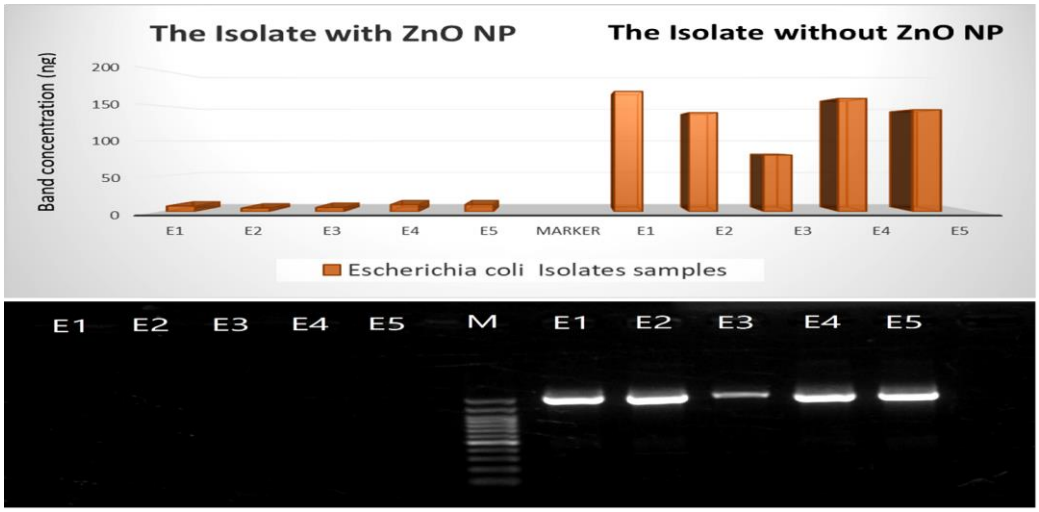

Figure (4): Conventional PCR amplification of *Escherichia coli* at (1000 bp), the specific fragment with and without mic 5 mg /ml ZnO NPs

DISCUSSION

The isolation of pathogenic micro-organisms, particularly *Salmonella* and *Escherichia coli*, from poultry farm environments is essential for assessing both animal health and food safety. These pathogens are commonly implicated in various poultry infections and present significant zoonotic risks. In the current study, the incidence of *E. coli* was 20%, which is lower than the 34.3% reported by Sri Poernomo *et al.* (1992). The highest proportion of avian pathogenic *E. coli* (APEC) was found in bird droppings, accounting for 40%, aligning closely with findings by Saha *et al.* (2020), who reported a rate of 33.33%.

The incidence of *Salmonella* in this study was 8%, which is comparable to the 15.4% reported by Yang *et al.* (2019) in broilers. In contrast, a lower prevalence was observed by Bayu *et al.* (2013), who reported a 2.5% isolation rate from local chickens in Tanzania. These differences in prevalence may be attributed to variations in sample sources, geographic locations, biosecurity measures, and sampling periods. In this study, different *E. coli* serotypes were detected, including Four isolates of *E. coli* serotypes O126, O55, and O111 were detected, each representing 20% of the total, while three isolates of O44 accounted for 15% and five isolates of O86 accounted for 25%. These findings differ from those of Ibrahim et al. (2024) but are relatively similar to those reported by Elnagar *et al.* (2021), who recorded *E. coli* O44 and O55 at 16.7% in Egypt.

In this study, *Salmonella* isolates were serotyped as *S. typhimurium* (2/8; 25%), *S. blegdam* (2/8; 25%), *S. montevideo* (2/8; 25%), *S. anatum* (1/8; 12.5%), and *S. lamberhurst* (1/8; 12.5%). These findings are consistent with those of Hee *et al.* (2007), who found that *S. typhimurium* is the most common serotype (23.4%) among broiler chicken isolates. However, the current results are higher than those of Narapati (2007), who reported 15.38% for *S. typhimurium*, and lower than those reported by Chaiba *et al.* (2008), who indicated a prevalence of 40.35%.

The antibiotic susceptibility patterns of *Salmonella* and *E. coli* isolates revealed high resistance levels to multiple antibiotics. Notably, both pathogens exhibited 100% resistance to colistin. *Salmonella* isolates had the highest sensitivity to gentamicin (87.5%), while resistance was variable across the other antibiotics: amoxyclav (64% vs. 85% for *E. coli*), cefotaxime (41% vs. 70%), sulphamethoxazole/trimethoprim (50% vs. 80%), gentamicin (12.5% vs. 60%),

amikacin (25% vs. 65%), ciprofloxacin (30% vs. 40%), levofloxacin (30% vs. 40%), and neomycin (75% vs. 55%).

When compared with previous studies, our findings differ from those recorded by Al-Baqir *et al.* (2019), where *Salmonella* showed 100% resistance to gentamicin and 65% to cefotaxime. Additionally, ciprofloxacin resistance (65%) in their study was notably higher than in ours (30%). Our results were more than those reported by Ibrahim et al. (2021) regarding sulphamethoxazole / trimethoprim resistance, though they reported lower ciprofloxacin resistance (4.8-23.8%). These differences may be attributed to geographic variations and differing practices in antibiotic usage and management.
For *E. coli*, our resistance rates were comparable to those reported by Enany *et al.* (2019), who observed 80.92% resistance to amoxyclav, 75% to sulphamethoxazole/trimethoprim, and 50% to gentamicin. These consistently high resistance rates highlight the continued overuse and misuse of antibiotics in poultry production systems.

A health risk assessment using the Multiple Antibiotic Resistance (MAR) index showed that 100% of both *Salmonella* and *E. coli* isolates had MAR indices ≥0.2, indicating exposure to high-risk sources of contamination. This finding is supported by the results of Ibrahim et al. (2021), who reported that most *Salmonella* and *E. coli* isolates exhibited MAR indices above 0.2. Furthermore, the high MAR values emphasize the need for stricter antimicrobial stewardship and monitoring practices in poultry farming to curb the spread of resistant pathogens.

Various environmentally friendly methods have been explored for synthesizing ZnO NPs using biological materials. For example, extracts from *Cayratia pedata* have been used to produce NPs with an average size of 52 nm and characteristic Zn–O bond signals observed between 400 and 600 cm^{-1}. Similarly, green algae like *Spirogyra hyalina* have generated particles ranging from 25 to 60 nm, showing Zn–O stretching vibrations around 553 cm^{-1}. Also, citrus peel extracts, including those from grapefruit, orange, and lemon, were employed, producing spherical NPs between 30 and 70 nm, with infrared signatures indicating Zn–O bonds near 553 cm^{-1}.

PCR remains a fundamental tool for identifying microbial DNA, valued for its precision in amplifying targeted gene regions (Mohamed *et al.,* 2024). In the current study, samples that were not treated with ZnO NPs displayed successful DNA amplification, indicating the presence of intact nucleic acids. Conversely, isolates exposed to 5 mg/mL of ZnO NPs displayed a marked decrease in nucleic acid levels. This decline is likely due to the unique characteristics of ZnO NPs—particularly their high surface area and efficient heat conduction—which may compromise DNA structure. However, studies suggest that, at lower concentrations, these NPs exhibit minimal cytotoxicity (Xu & Yao, 2013; Upadhyay *et al.,* 2020).

CONCLUSION

This study successfully demonstrated a green synthesis approach for producing ZnO NPs using propolis extract characterization techniques, such as TEM and FTIR confirmed the formation of stable, well-dispersed nanoparticles coated with bioactive compounds. The observed low cytotoxicity suggests that these ZnO NPs hold promise for use in drug delivery systems and as antibacterial agents.

PCR was utilized to assess the impact of ZnO NP treatment, revealing a reduction in nucleic acid content in treated samples, which indicates a potential decrease in microbial viability.

The presence of *E. coli* and *Salmonella* in poultry environments, likely stemming from contaminated feed, water, bedding,

personnel, or slaughter processes, highlights the ongoing risk of bacterial transmission. Furthermore, these findings emphasize the importance of implementing stricter biosecurity protocols, enhanced sanitation practices, and responsible antibiotic use to mitigate the spread of resistant pathogens in poultry production systems.

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استخدام جزيئات أكسيد الزنك النانوية كمضاد بكتيري ضد البكتيريا المقاومة للأدوية من مزارع التسمين

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تُواجه الأنظمة الصحية العالمية تحديًا متصاعدًا نتيجة انتشار البكتيريا المقاومة للمضادات الحيوية. تهدف هذه الدراسة إلى تقييم فعالية جزيئات أكسيد الزنك النانوية المُحضّرة بطريقة صديقة للبيئة في مقاومة وعلاج بكتيريا *Escherichia coli*و*Salmonella* المعزولة من مخلفات مزارع الدواجن، والبيئة المحيطة، وأيدي العاملين بتلك المزارع. تم جمع 100 عينة من إحدى مزارع الدواجن شملت: 15 عينة من الزرق، 75 من البيئة، و10 من أيدي العاملين. أُجري العزل والتشخيص الميكروبيولوجي القياسي، وتم تحديد 28 عزلة بكتيرية. كما تم استخدام اختبار الانتشار بالأقراص (Disk Diffusion) لتقييم الحساسية تجاه مجموعة من المضادات الحيوية، بالتوازي مع اختبار فعالية جزيئات أكسيد الزنك النانوية ضد العزلات. أظهرت النتائج أن 20% من العينات كانت موجبة لبكتيريا *E. coli*، وتوزعت الأنماط المصلية بين O55:K59، O44:K79، O86:K61، O126:K71 ، و O111:K58. أما *Salmonella* فقد تم عزلها من 8% من العينات، وشملت الأنماط: S. *Typhimurium*، *S. Anatum*، *S. Belegdam* ، *S. Montevideo* و *S.* Lumberhurst. اتضح أن معظم العزلات أظهرت مقاومة متعددة للمضادات الحيوية، بما في ذلك الكوليستين، الأموكسيسيللين/كلافولانيك، السيفوتاكسيم، السلفا/تريميثوبريم، الجنتاميسين، الأميكاسين، النيوميسين، والسيبروفلوكساسين، بدرجات متفاوتة. تم تحضير جسيمات أكسيد الزنك النانوية من مستخلصات البروبوليس الطبيعية، وتميزت بخواص فيزيائية فعالة مثل حجم الجسيمات (20 نانومتر)، وجهد السطح (+83.5 mV)، ومؤشر التشتت (0.0979)، مع تركيز تثبيطي أدنى بلغ 5 ملغ/مل. كما أظهرت الفعالية البيولوجية لتلك الجزيئات من خلال قدرتها على تثبيط تضخيم الحمض النووي في اختبار PCR، خاصة لعزلات *E. coli* .تشير هذه النتائج إلى إمكانية استخدام جزيئات أكسيد الزنك النانوية كبديل فعال وآمن للعلاجات الكيميائية التقليدية في مواجهة البكتيريا متعددة المقاومة، مما يسهم في خفض الاعتماد على المضادات الحيوية وتقليل مخاطر المقاومة في قطاع الدواجن.

الكلمات الدالة: جزيئات الزنك النانو مصنعة من بروبوليس – السالمونيلا - الإشريكية القولونية – مقاومة متعددة للأدوية - دجاج التسمين