Special Issue:

Veterinary Medicine between Sustainable Development and Public Health to Confront Global Changes

Antibacterial Effect of Zinc Oxide Nanoparticles on Drug Resistant *E. coli* Isolated from Chicken with a Zoonotic Perspective

Mohamed Ahmed Abaza[1], Amany O. Selim[2], Mona Abdallah[3], Shimaa A.E. Atwa[4], Hala El Daous[5], Mona Abd-Allah Abd-Elrehim[6], Mohamed M.S. Gaballa[7], Reda R. Fathy[1]*

[1]Avian and Rabbit Diseases Department, Faculty of Veterinary Medicine, Benha University, 13736, Mushtuhur, Toukh, Qalioubia, Egypt: [2]Bacteriological department, Animal Health Research Institute (AHRI), Banha branch, Agricultural Research Center (ARC), Doki, Giza, Egypt; [3]Zoonoses department, Faculty of Veterinary Medicine, Benha University, 13736, Mushtuhur, Toukh, Qalioubia, Egypt; [4]Biochemistry and Molecular Biology department, Faculty of Veterinary Medicine, Benha University, 13736, Mushtuhur, Toukh, Qalioubia, Egypt; [5]Department of Hygiene and Veterinary Management, Faculty of Veterinary Medicine, Benha University, 13736, Mushtuhur, Toukh, Qalioubia, Egypt; [6]Biochemistry department, Animal Health Research Institute (AHRI), Banha branch, Agricultural Research Center (ARC), Doki, Giza, Egypt; [7]Pathology department, Faculty of Veterinary Medicine, Benha University, 13736, Mushtuhur, Toukh, Qalioubia, Egypt.

Abstract | Escherichia coli (*E. coli*) infection has significant public health impact on both chickens and human. Antibiotic resistance as well as antibiotic residues in chicken meat are some of the negative outcomes of the traditional antibiotic-based approach to prevent and control bacterial infections. Therefore, the main goal of current investigation was to control the drug-resistant E. coli O6 infection using nano-production of zinc oxide (ZnO-NPs) in both in vitro and vivo studies. ZnO-NPs was applied in one day old specific pathogen free chicks to evaluate the antibacterial effectiveness of 50mg/kg ration dosage compared with colistin as commercial antibiotic at 5 days old. *E. coli serotype O6* was the highest prevalent and pathogenic multi drug resistant bacterial strain. The assessment parameters were clinical signs, post-mortem lesions and histopathological picture which showed effective role of ZnO-NPs as bacterial inhibitor in the treated groups compared to control one. Quantitative analysis showed that ZnO-NPs significantly lowered gross lesion scores in the liver, cecum, colon, spleen, heart, and lungs compared to the *E. coli*-infected group. These findings solidified our central hypothesis which was to evaluate the antimicrobial and antioxidant efficacy of ZnO-NPs against pathogenic bacterial strain of *E. coli* in broiler chicken as a powerful, safe alternative to antibiotics.

Keywords: Nano-production of zinc oxide (ZnO-NPs), Specific pathogen free chicks, *Escherichia coli (E. coli)*, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)

Received | June 02, 2024; **Accepted** | July 21 2024; **Published** | August 31, 2024
***Correspondence** | Reda Refat Fathy, Avian and Rabbit Diseases Department, Faculty of Veterinary Medicine, Benha University, 13736, Mushtuhur, Toukh, Qalioubia, Egypt; **Email:** redarefat2015@gmail.com
Citation | Abaza MA, Selim AO, Abdallah M, Atwa SAE, El Daous H, Abd-Elrehim MAA, Gaballa MMS, Fathy RR (2024). Antibacterial Effect of zinc oxide nanoparticles on drug resistant *E. coli* isolated from chicken with a zoonotic perspective. Adv. Anim. Vet. Sci. 12(s1): 75-89.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.s1.75.89
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

Recently, poultry producers has faced a great challenges in preserving the health and safety of their flocks due to the prevalence of various bacterial infections, particularly those caused by *Escherichia coli (E. coli)* (Swelum *et al*., 2021). Gram-negative pathogenic bacteria called *E. coli* are frequently found in the gastrointestinal tracts of chickens. These bacteria cause substantial economic losses, severely impair the growth performance of chickens, and can even be fatal, especially in young chicks who have just hatched (Jiddu Joseph *et al*., 2023). Because certain strains of *E. coli* are known to be zoonotic, these infections not only threaten the health of poultry but also have consequences for food safety and public health (Zachary *et al*., 2017). Several poultry species, specially, chickens, are particularly susceptible to *E. coli spp.* infection owing to several factors such as high population density, stress factors, and bad breeding conditions (Mughini-Gras *et al*., 2018).

Because *E. coli* infections in chickens can result in a range of clinical symptoms, such as respiratory, gastrointestinal, and reproductive problems, the poultry industry faces severe financial losses because of the infected birds' higher mortality rates, decreased growth rates, and decreased egg production (Kromann and Jensen, 2022) . Furthermore, *E. coli*-contaminated the poultry products as meat and eggs and pose a significant transmission of pathogenic *E. coli* strains from poultry to human (Raheel *et al*., 2022). Zoonotic transmission of *E. coli* raises critical concerns, as it causes a wide range of symptoms among people, from minor gastrointestinal distress to severe and perhaps fatal infections (Mellata *et al*., 2018).

The extensive proliferation of antibiotic resistance has become more alarming in recent years and presents a significant worldwide threat to public health, resulting in millions of fatalities worldwide (Abdus Salam *et al*., 2023). This problem increased when bacteria form biofilms, which can greatly enhance bacterial resistance up to 1000 times and contribute to multidrug resistance (MDR) infections. The control measures implementation can lead to emergence of antibiotic- *E. coli* resistant strains, which increase the problem and reduces the available treatment choices for both poultry and human (Christodoulou, 2023). The lack of new powerful antimicrobial chemicals is connected to the growth in this resistance (Helal *et al*., 2023). Therefore, it is important to create new innovative alternative to prevent and control *E. coli* infections in poultry, in order to protect the poultry industry and ensure public health care (Serwecińska, 2020). To tackle this issue, this study was searching about novel techniques that have antibacterial properties and a high level of safety.

Nanotechnology has become a popular option because of its tiny particle size and enhances the absorption of materials. The use of nanoparticles show many optimistic results due to their unique physical and chemical properties, as well as their possible applications in disease prevention (Mubeen *et al*., 2021). Zinc is an essential micronutrient for health and biological procedures in the body. Inorganic forms of zinc, such as zinc oxide and zinc sulfate, have traditionally been used in poultry feed because it is inexpensive. However, its bioavailability is limited, which necessitates adding high levels to the feed which can lead to various problems (Hidayat *et al*., 2023). Zinc oxide nanoparticles (ZnO-NPs) have become an alternative for wider kinds of uses including veterinary field, however there are still concerns regarding their safety. ZnO-NPs have obtained considerable attention due to their antimicrobial properties and wide-ranging uses in industries such as agriculture and medicine (Xian-Qing *et al*., 2023). Contrasting to these findings, several additional research trials examining the positive impacts of zinc supplementation and didn't record performance improvements. However, they generally noticed an improved bird's immunological state (Yogesh *et al*., 2013). The central hypothesis for the this research was to assess the antimicrobial effectiveness of ZnO-NPs in vitro and in vivo against specific pathogenic bacterial strain of *E. coli* in broiler chicken as a potential alternative to antibiotics.

Therefore, this study aimed to investigate the zoonotic potential of *E. coli* infection, different serotypes prevalence, the development ability of biofilms, antibiotic resistance of *E. coli* among chicken and human populations. The results of this study could be utilized to advocate for the utilization of ZnO-NPs as a potent alternative antimicrobial supplement in chicken feed, given its safety, strong capacity to be absorbed by the body, and ability to kill bacteria.

MATERIALS AND METHODS

Ethical Considerations

All procedures described in this study were ethically approved from the Ethical Approval Committee of the Faculty of Veterinary Medicine, Benha University, Egypt for the use of cell line, chicken, and human samples (No: BUFVTM 16-1-23). In this study we followed the ethical guidelines indicated by previously mentioned committee as the chicks were reared on caged system within a high level of sanitary conditions in segregated, cleaned, and disinfected rooms at the center of animal research at the faculty of Veterinary Medicine, Benha University, Egypt.

Samples Collection

Chicken samples: two hundred polled surveyed random samples (lung and liver 100 pooled samples each) were

randomly collected from 100 diverse poultry farms in Qalioubia governorate, Egypt. Qalioubia governorate is recognized as one of the most prominent governorates for poultry production in Egypt. Liver and lung samples were hygienically collected under complete aseptic conditions. Each individual organ was carefully collected into a sterile labelled packet in an ice bag at 4°C without any unnecessary delay for bacteriological analysis.

Human samples: twenty samples (10 human urine samples and 10 nasal swab) were collected from persons who admitted to Benha Teaching Hospital at Qalioubia governorate, Egypt. All samples were transferred to the lab into sterile labelled packets in an ice box at 4°C, for bacteriological examination.

Bacteriological Isolation

For enrichment the collected samples were incubated in nutrient broth at 37°C for 24 h. The enriched samples were streaked on Eosin Methylene Blue agar (EMB) plates and incubated aerobically at 37°C overnight according to (Markey *et al*., 2013).

Biofilm Formation

Assessment of biofilm production was based on the distinctive appearance of colonies grown on Congo Red Agar (CRA) medium. Notably, colonies exhibiting a deep black hue, coupled with a dry and crystalline consistency, were indicative of robust biofilm production according to (Freeman *et al*., 1989).

Serological Identification of *E. coli spp.*

The serotyping process, pivotal for characterizing *E. coli* isolates that exhibited high biofilm production, was carried out using the slide agglutination technique. For serotyping, the "SEIKEN" antisera, a well-recognized reagent supplied by MAST ASSURE™, was employed. The serological reaction was executed by combining a 24 h-old colony of the tested *E. coli* strain, previously cultured on nutrient agar, with a drop of physiological saline on a slide. The resultant mixture was emulsified using a loop and thoroughly mixed with a drop of the designated "SEIKEN" antiserum according to (Markey et al., 2013)..

Antibiotic Sensitivity Assay

A panel of antibiotics commonly used for *E. coli* infections in both chickens and human was selected such as Norfloxacin, Gentamicin, DE Oxytetracycline, Chloramphenicol, Colistin, Trimethoprim + Sulfamethoxazole, Azithromycin, Amikacin, Neomycin and Ampicillin Clavulanic Acid. The Kirby-Bauer disk diffusion method was employed to determine the sensitivity profiles of the four serotypes of isolated *E. coli* strains O1, O119, O6, and O44 to antibiotics as described by Markey *et al*. (2013).

Zinc Oxide Nanoparticles (ZnO-NPs)

ZnO-NPs provided by the Nanoparticles Unit at the Animal Health Institute in Cairo, Egypt. The structure of the produced ZnO-NPs was analyzed using High-Resolution Transmission Electron Microscopy (HR-TEM). The ZnO-NPs' physicochemical characteristics were examined via a UV-visible spectrophotometer (SHIMADZU-2600i, USA). The dimensions and electric charge of the produced ZnO-NPs were measured using a NANOTRAC-WAVE II Zeta sizer (MICROTRAC, USA). The surfaces plasmon resonance was measured by means of UV-Visual spectroscopy, whereas the ZnO-NPs average particle size was calculated via more than 300 particles with ImageJ software (National Institute of Health, Bethesda, MD, USA).

Cytotoxicity Assay

The cytotoxicity effect of ZnO-NPs was assessed applying the sulforhodamine B (SRB) assay, using Vero cell line obtained from Nawah Scientific Inc. Mokatam, Cairo, Egypt. Vero cells were cultivated in DMEM media with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum. The cells were maintained at 37°C with 5% CO2. The cultured cells, consisting of 5 x 10^3 cells, were placed in 96-well plates and allowed to grow in a nutrient solution for a period of 24 h. After that, cells were exposed to different quantities of ZnO-NPs ranging from 0.01 µg/mL to 100 µg/mL that were suspended in the solution. After exposed for 72 h, the cells were treated with 150 µL of 10% trichloroacetic acid for fixation. Then, they were rinsed five times with distilled water. Then, 70 microliters of 0.4% solution of SRB dye were added to each well and kept in the dark condition at room temperature for 10 min. Following application of a 1% acetic acid wash and overnight air-drying, the protein-bound SRB stain was dissolved by the addition of 150µL of 10mM TRIS solution. The measurement of absorbance was conducted at a wavelength of 540 nm using BMG LABTECH®- FLUOstar Omega microplate reader (Allam *et al*., 2018).

In vitro Study

Preparation of bacterial cultures and biofilm formation: The isolated *E. coli O6* strain was cultured overnight in nutrient media. The overnight cultures of bacterial isolates were properly diluted to 1:100 in Tryptic Soy Broth (TSB) which enriched by 1% glucose, conducive to biofilm development. Subsequently, aliquots of approximately 100µL from these diluted cultures were carefully dispensed into wells of a sterile microtiter plate. The plate incubated for 48 hours to promote the development of a strong biofilm. (Basumatari *et al*., 2021).

Evaluation of antimicrobial and antibiofilm efficacy of ZnO-NPs: The evaluation of biofilm elimination

and antibacterial efficacy of ZnO-NPs performed using different concentrations of ZnO-NPs 100, 50, 25, 12.5, 6.25µg/mL into microtiter plate. To create acceptable benchmarks, negative control wells without bacterial growth and positive control wells without ZnO-NPs were maintained for each isolate. The microtiter plate was then incubated in a controlled environment at a temperature of 37°C for a duration of 24 h. The efficacy of ZnO-NPs in eliminating bacterial biofilms was assessed by inoculating samples onto nutritional agar and Congo Red Agar (CRA) media, using the method outlined by (Basumatari *et al*., 2021).

In vivo Study

Animal model: One hundred specified pathogen free (SPF) one-day-old chicks were acquired from Al-Fayemi hatchery in Egypt. These chicks were reared in a battery system under strict hygienic circumstances in sanitized rooms at the Animal Research Center at Benha University's Faculty of Veterinary Medicine. They were used for field assessment of ZnO-NPs in starter feed ration against *E coli* infection challenge.

Experimental design: The chicks were divided into five distinct groups of twenty chicks per group. Group I served as the negative control which received no treatment, while group II represented the positive control for *E. coli* O6 strain infection without any intervention. Chicks in group III were receiving ZnO-NPs at one day old till the end of experiment with a dose of 50 mg/kg in feed. Group IV was given colistin as commercial antibiotic in DW which coincide with the time of challenge at 5 days of age, each challenged chick in group (II), (III) and (IV) received 0.1 ml of *E. coli* strain ($5x10^{10}$) CFU for 3 successive days. Group V was designated as the ZnO-NPs toxicity group receiving ZnO-NPs in feed at concentration 50mg per kg. Daily activities included clinical observations, post-mortem examinations, and monitoring of both living and diseased chicks. At the 5th day post-infection, all birds from each group were euthanized in accordance with ethical standards.

Lesions in internal organs such as heart, lung, intestine, and spleen were visually scored as per established protocols (Peighambari *et al*., 1995). The identification and culture of bacteria were conducted utilizing tissue swabs that were obtained post-mortem from poultry in each group on the 5th day following infection. Bacterial re-isolation was executed employing the previously established isolation method. Histopathological examinations were conducted on samples from cecum, liver, lung, spleen, and heart. Additionally, comprehensive hematology and biochemical analysis were conducted, collected blood samples were determined by spectrophotometer in which sera were separated by centrifugation at 2500 RPM. for 15 min and kept in a deep freeze at -20°C till used for determination of the biochemical parameters: Serum L-MDA (Mesbah *et al*., 2004), liver function tests as serum AST, ALT (Murray, 1984) ALP (John 1982) and serum urea according to (Patton and Crouch, 1977) Serum uric acid according to (Young, 2001). Serum Creatinine (Henry, 1974), serum total protein (Tietz, 1994), Albumin (Doumas *et al*., 1971), Enzymatic Antioxidants as CAT (Luck, 1974), Non-Enzymatic Antioxidants as GSH (Moron *et al*., 1979).

Statistical Analysis

SPSS software (version 25.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The significance between the mean values was set at $P < 0.05$. We analyzed the statistical differences among treated and untreated groups by using Analysis of variance (ANOVA) according to (Booth *et al*., 1981).

RESULTS AND DISCUSSION

Bacterial Isolation and Characterization

Twenty isolates tested positive for *E. coli* out of the 100-chicken lung pooled samples that were examined. On the other hand, the prevalence rate of *E. coli* in the chicken liver samples (44%) was significantly higher than other samples. Regarding the human urine samples had prevalence rate of *E. coli* 30% higher than that of urine samples (20%) (Table 1).

Table 1: Prevalence of E. coli among different tested chicken samples.

Number of tested samples/tested organ	Total samples No.	Positive No.	Prevalence %.
Lung	100	20	20%
Liver	100	44	44%
Urine	10	3	30%
Nasal swabs	10	2	20%

Biofilm Formation

Among twenty *E. coli* lung isolates, 5 were high-level biofilm producers, as indicated by extremely dark colonies; 9 were intermediate; and 6 were low-level biofilm producers. Eight of the liver isolates were very powerful, 25 were moderately strong, and 11 were weak. Two of the urine isolates were very productive, and the third was a partial producer. Finally, the two isolates from nasal swabs were both potent biofilm formers (Figure 1).

Serotyping Using *E. coli* Polyvalent and Monovalent O Antisera

The results revealed a predominance of strains belonging to serotypes O1, O6, O44, and O119. The isolates from lung samples, all 5 were typed as O6 and O119 serotypes. The 8 isolates from liver samples were typed as O6, O119, and

O44 serotypes. The urine and nasal swab samples each had 2 isolates belonging to the O1 and O6 serotypes (Table 2).

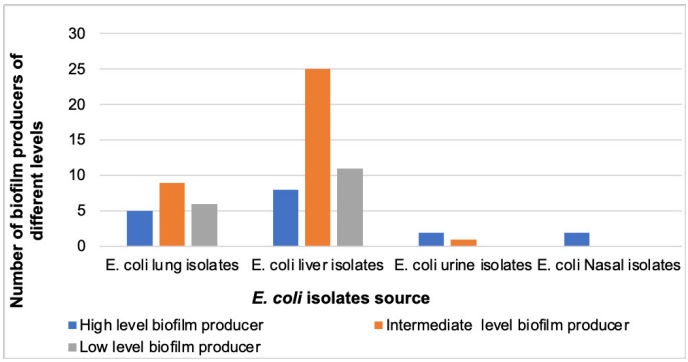

Figure 1: Prevalence of biofilm producers among different tested E. coli spp. positive samples.

Table 2: Serotyping of E. coli strains isolates that exhibited high biofilm production.

Sample	Number of high biofilm producers	Serotyping
Lung	5	2 (O6), 3(O119)
Liver	8	2 (O6), 3 (O119), 3 (O44)
Urine	2	1(O1), 1(O6)
Nasal swab	2	1(O1), 1(O6)

Table 3: Antibiotic sensitivity testing results for 4 different E. coli serotypes - O1, O6, O119, and O44.

Antibiotic disc/ *E. coli*	O1	O6	O119	O44
Norfloxacin	R	R	R	R
Gentamicin	S	R	S	S
De oxytetracycline	S	R	R	R
Chloramphenicol	R	R	S	S
Colistin	S	R	S	S
Trimethoprim + sulfamethoxazole	R	R	S	S
Azithromycin	S	S	S	S
Amikacin	R	R	R	R
Neomycin	R	R	R	R
Ampicillin clavulanic acid	R	R	R	R

R: Antibiotic-resistant, **S:** Antibiotic-susceptible

The Antibiotic Sensitivity Assay

The antibiotic sensitivity assay findings for four distinct serotypes of *E. coli*, namely O1, O6, O119, and O44, are presented in Table 3 and Figure 2. Across the serotypes, there was a high level of antibiotic resistance. Norfloxacin, amikacin, neomycin, and the ampicillin-clavulanic acid combination were all resistant to all four serotypes. O6 exhibited the most widespread multi-drug resistance, being resistant to 9 of the 10 antibiotics examined. O1 has intermediate levels of resistance, with 6 antibiotics resistant. O44 and O119 exhibited the least antibiotic resistance, being susceptible to 5 of the 10 antibiotics tested, including Gentamicin, Chloramphenicol, Colistin, Trimethoprim-sulfamethoxazole, and Azithromycin.

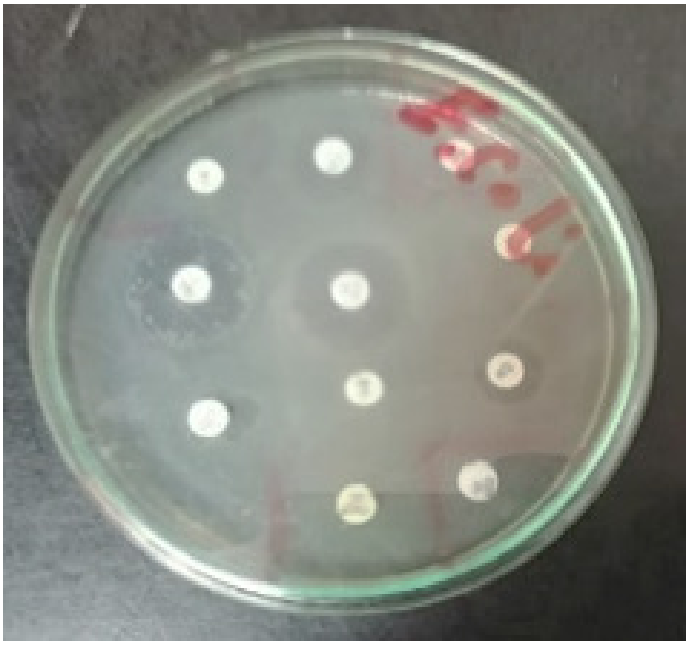

Figure 2: Antibiotic sensitivity assay of different antibiotic disks effect against *E. coli* serotype *O6*.

Characterization of ZnO-NPs

The results of Zetasizer showed that the average particle size of synthesized ZnO-NPs is 163.6 nm with a narrow size distribution (poly dispersity index (PDI) is 0.04). Surface charge of synthesized NPs is -25.0 mV (Figure 3A) confirmed by TEM results showed spherical shape nanoparticles well dispersed without agglomeration ranged in size from 19.5 to 23.8 nm (Figure 3B). ZnO-NPs showed strong absorption beak at 371 nm with optical density of 1.137 (Figure 3C).

Cytotoxicity Assay of ZnO-NPs

The cytotoxicity of synthesized ZnO-NPs was evaluated using Vero cell line through SRB assay at concentration ranged from 0.01- 100 µg/ml. In SRB assay, ZnO-NPs showed no observed toxicity till concentration of 100 µg/ml that cells have the same characteristic features compared to control ones and the cell viability rate ranged from 103.177 to 93.0227 % at concentration ranged from 0.01 to 100 µg/ml, IC50 concentration is >100 µg/ml (Figure 4).

In vitro Antibacterial Activity and Antibiofilm Effects of ZnO-NPs

At a concentration of 50 µg/ml, ZnO-NPs exhibited significant antibacterial effects against *E. coli* serotype O6 which was identified as a multi drug resistant isolate (Figure 5A). Additionally, ZnO-NPs demonstrated antibiofilm activity against *E. coli* serotype O6 at a concentration of 6.25 µg/ml (Figure 5B).

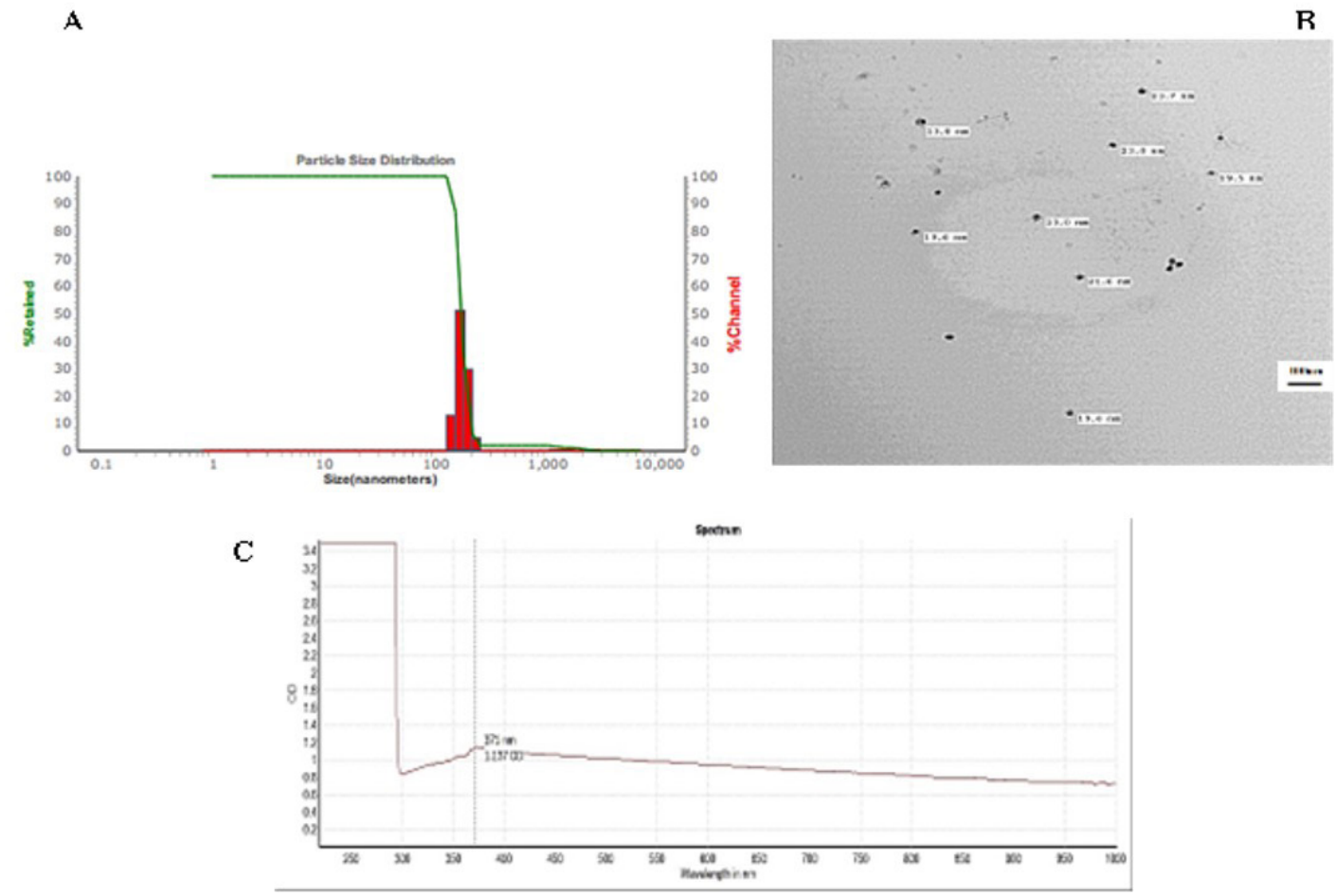

Figure 3: Characterization of synthesized ZnO-NPs. (A) particle size pattern of synthesized NPs showing size distribution of ZnO-NPs of 163.6 nm, (B) TEM imaging of ZnO-NPs showed well dispersed spherical nanoparticles without agglomeration and size ranged from 19.5 to 23.8 nm, Scale bar = 100 nm.(C) UV analyses of synthesized ZnO-NPs at wavelength range of 200-1000 nm showed absorption peak at 371 nm.

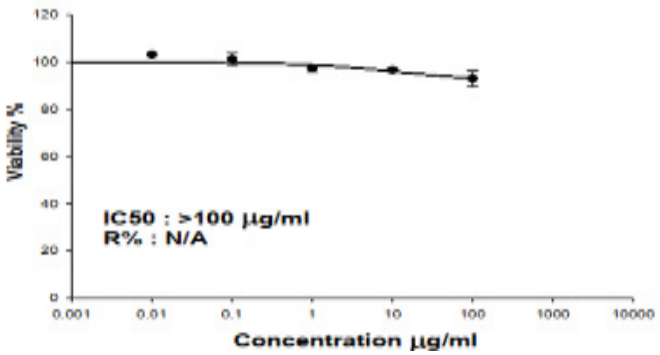

Figure 4: SRB cytotoxicity assay of ZnO-NPs at different concentrations ranged from 0.01 to 100 µg/ml

In vivo Study

Clinicopathological picture: In control *E. coli* serotype O6-positive group, chicks showed depression, pasty vent with severe profuse whitish and greenish diarrhea, respiratory manifestation as swelling of infraorbital sinus, conjunctivitis, and nasal discharge in some birds. While post-mortem lesions revealed petechial hemorrhage in the liver with impacted cecum along intestinal tract mainly at

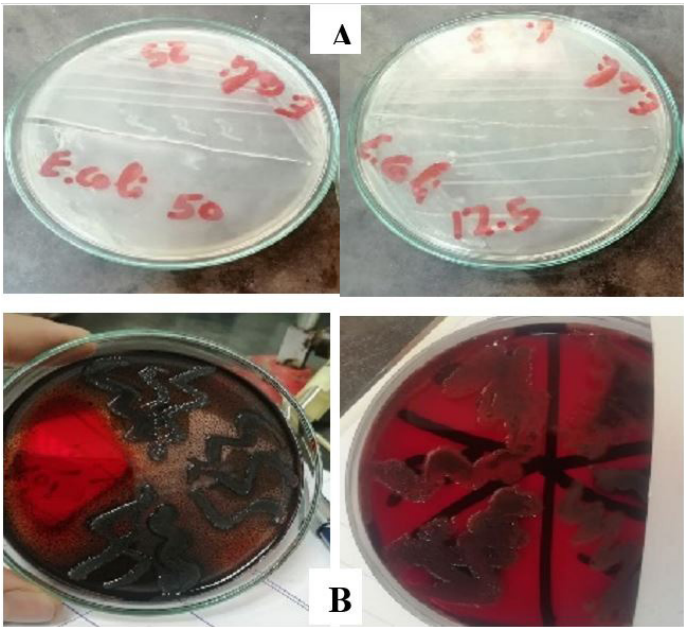

Figure 5: (A) Different concentration of ZnO-NPs showed antibacterial activity against *E. coli serotype O6*, (B): Antibiofilm activity of ZnO-NPs against *E. coli serotype O6.*

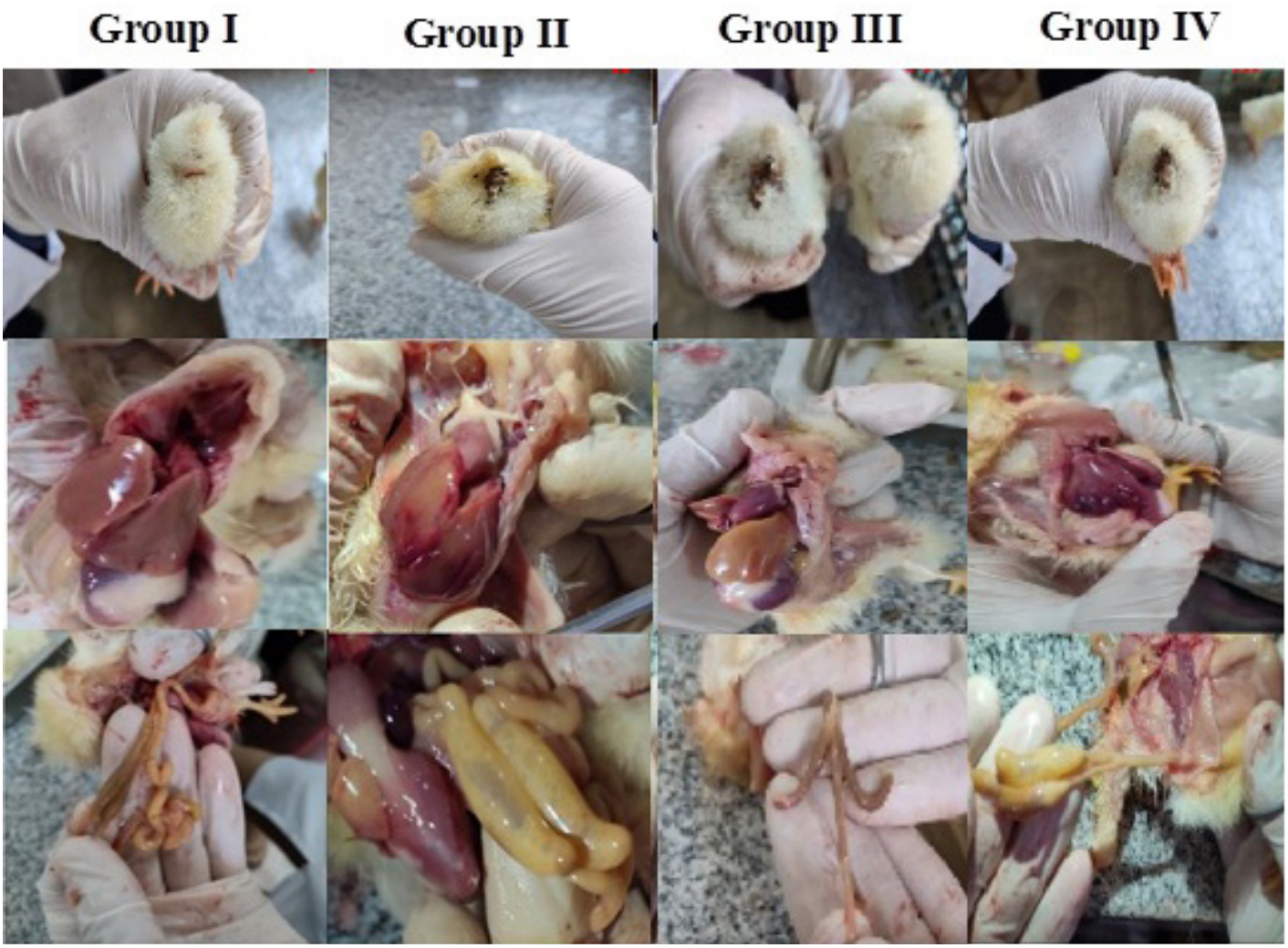

Figure 6: Effect of ZnO-NPs on clinicopathological findings of SPF chicks infected with *E. coli* serotype *O6* at 5 dpi. control negative (I), *E. coli* infection control positive (II), ZnO-NPs treatment (III), Colistin treatment (IV). Comparison in pathological picture showed pasty vent with severe profuse whitish and greenish diarrhoea in group II, control group (I) normal anatomical structure, control positive group (II) showed marked petechial hemorrhage congested liver as similar picture of colistin treated group (IV), while in ZnO-NPs there is no marked changes in liver. In cecum, showed enteritis with distended cecum with gas in colistin treated group and in control positive group, whereas Zno-NPs showed similar picture as control negative group (I).

duodenum and jejunum. Treatment with Colistin sulphate effectively mitigated the severity of clinical symptoms, although liver congestion persisted. In contrast, ZnO-NPs treated group resulted in no clinical signs and a near-normal appearance of internal organs, show casing its protective effects (Figure 6).

Quantitative analysis revealed that ZnO-NPs significantly reduced gross lesion scores in various organs compared to the *E. coli*-infected group, including the liver, cecum, intestine, spleen, heart, and lungs, suggesting their potential in mitigating *E. coli* serotype O6 infections in chicks. In the colistin treated group, revealed congested liver in comparison with ZnO-NPs group, there is no marked changes in the liver. In case of cecum, control positive group (*E. coli* serotype O6 infected) showed enteritis with distended cecum with gas in comparison the zinc oxide nanoparticle normal content of cecum as demonstrated in Table 4.

Histopathological picture: Histopathological examination across different organs revealed diverse outcomes, all examined organs from the negative control group (I) have typical histological alterations. While in *E. coli*-infected group (II), liver showed focal to diffuse areas of hepatocytic necrosis, eosinophilic fibrillar masses, infiltration of inflammatory cells and fragmented nuclei or complete nuclear absence indicative of severe tissue damage. ZnO-NPs treated group revealed preserved hepatic structure with minimal changes while, colistin treatment mitigated necrosis and inflammation. However, a toxic dose of ZnO-NPs induced mild to moderate hydropic vacuolization and portal area thickening. Lung tissue examination showed that chickens infected with *E. coli* displayed significant lung tissue alterations, including serofibrinous exudates,

Table 4: Gross lesion score in internal organs at 5th day post infection scorings.

Organs	Postmortem findings	Groups				
		Group I	Group II	Group III	Group IV	Group V
Liver	Congestion Petechial haemorrhage	0^{d}	3.6 ± 0.33^{a}	1.2 ± 0.5^{c}	1.67 ± 0.3^{b}	3.2 ± 0.2^{ab}
Lung	Pneumonia	0^{d}	3.4 ± 0.33^{a}	1.1 ± 0.33^{c}	2.33 ± 1.15^{bc}	2.6 ± 0.33^{ab}
Spleen	Congestion Mottled appearance	0^{d}	2.9 ± 0.5^{a}	1.33 ± 0.44^{c}	1.4 ± 0.4^{c}	2.67 ± 1.31^{b}
Cecum	Impacted cecum Caecal core	0^{d}	2.67 ± 1.32^{a}	1 ± 0.37^{c}	1.2 ± 0.16^{c}	2.1 ± 0.3^{bc}
Heart	Inflammation Haemorrhage	0^{d}	3 ± 0.49^{a}	0.67 ± 0.44^{c}	1.4 ± 0.8^{c}	1.33 ± 0.33^{b}

Post-mortem lesion scores mean the following: (0) no, (1) mild, (2) moderate, and (3) severe lesion, was expressed as mean ± standard error. control negative (I), *E. coli* infection control positive (II), ZnO-NPs treatment (III), Colistin treatment (IV) and ZnO-NPs toxic dose (V).

cellular debris, neutrophil accumulation, and alveolar interval was widened with dilated and congested capillaries. ZnO-NPs maintained pulmonary structure while, colistin treatment alleviated symptoms, limiting infiltration. In the spleen, *E. coli*-infected groups displayed notable changes, included a reduction in certain regions, accompanied by lymphocyte apoptosis and the presence of nuclear debris ZnO-NPs caused mild expansion of blood sinusoids whereas, colistin treatment resulted in lesser lymphoid depletion. A toxic dose of ZnO-NPs significantly dilated blood sinusoids in the red pulp. Cecum tissue examination revealed that chickens infected with *E. coli* showed severe desquamation of the lining epithelium and necrotic debris mixed with inflammatory cells. ZnO-NPs treatment maintained a healthy cecum structure while, colistin treatment reduced changes and showing mild leukocytic infiltration, in toxic dose of ZnO-NPs group resulted in epithelial desquamation. In heart tissue examination, control group (II) showed that focal areas of disrupted and fragmentated cardiac muscle fibers with mononuclear inflammatory cells indicating an inflammatory response. On other hand, both ZnO-NPs and colistin treatments preserved overall heart muscle structure. Although a toxic dose of ZnO-NPs caused mild myocardial fibre changes as shown in Figure 7 and Table 5.

Biochemical Analysis

Table 6 showed the significant differences ($P < 0.05$) among the treatment groups in serum AST, ALT, ALP, TP, Albumin, Globulin, Urea, Creatinin and Uric acid levels. There was significant decrease in AST, ALP, ALT, Creatinin and Uric acid serum levels and significant increase in TP, Globulin, albumin and Urea levels in *E. coli* infected group and Zno-NPs treated group. On the other hand, there was no significant difference between *E. coli* infected group and colistin treated group except for ALT, AST, urea serum levels. Additionally, uninfected Zno-NPs toxic dose group showed significant decrease in ALT, TP, Albumin, globulin, Urea and uric acid levels and showed significant increase in AST, ALP and Creatinin levels when compared with control negative group. The results revealed that the lowest level of AST and A/G ratio showed in infected group treated with ZnO-NPs when compared to the other groups.

Table 5: Grading and scoring of histopathological lesions of examined sample.

Treatments	Degenerative and necrotic changes of hepatocytes	Inflammatory cells infiltration	Hepatic vasculatures/ sinusoidal dilatation
Group (I)	-	-	-
Group (II)	+++	+	-
Group (III)	-	-	-
Group (IV)	+	-	
Group (V)	++	-	

Table 6: Effect of different treatment on liver and kidney function tests in SPF chicks infected by *E. coli.*

Group/Parameter	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Urea (mg/dL)	Creatinin (mg/dL)	Uric acid (mg/dL)
Control negative group	19.33 $\pm1.45^{b}$	84.33 $\pm1.45^{ab}$	1298.66 $\pm13.56^{c}$	4.93 $\pm0.04^{a}$	2.31 $\pm0.05^{bc}$	2.58 $\pm0.01^{a}$	0.89 $\pm0.02^{d}$	21.06 $\pm0.37^{b}$	0.45 $\pm0.01^{cd}$	8.16 $\pm0.31^{a}$
Control positive group	24.66 $\pm2.60^{a}$	93.00 $\pm6.35^{a}$	1391.00 $\pm8.08^{a}$	4.12 $\pm0.02^{b}$	2.40 $\pm0.02^{abc}$	2.04 $\pm0.03^{b}$	1.02 $\pm0.02^{abc}$	20.30 $\pm0.40^{b}$	0.47 $\pm0.01^{bcd}$	7.36 $\pm0.49^{ab}$
E. coli and ZnO-NPs group	16.66 $\pm3.75^{b}$	65.66 $\pm13.56^{b}$	1312.00 $\pm6.92^{b}$	4.61 $\pm0.16^{ab}$	2.08 $\pm0.01^{d}$	2.37 $\pm0.23^{ab}$	0.93 $\pm0.03^{cd}$	24.40 $\pm0.46^{a}$	0.45 $\pm0.02^{cd}$	6.03 $\pm0.81^{cd}$
Clostin treated group	19.00 $\pm1.15^{b}$	89.66 $\pm3.75^{ab}$	1376.66 $\pm20.49^{ab}$	4.84 $\pm0.35^{a}$	2.47 $\pm0.11^{ab}$	2.59 $\pm0.11^{a}$	1.05 ±0.05ab	20.00 $\pm0.46^{b}$	0.43 $\pm0.01^{d}$	5.76 $\pm0.08^{cd}$
ZnO-NPs toxicity	36.66 $\pm2.60^{a}$	90.66 $\pm2.02^{a}$	1371.00 $\pm7.23^{ab}$	4.99 $\pm0.13^{a}$	2.29 $\pm0.08^{bc}$	2.32 $\pm0.08^{ab}$	0.98 $\pm0.003^{bcd}$	20.16 $\pm0.49^{b}$	0.51 $\pm0.01^{ab}$	5.50 $\pm0.05^{cd}$

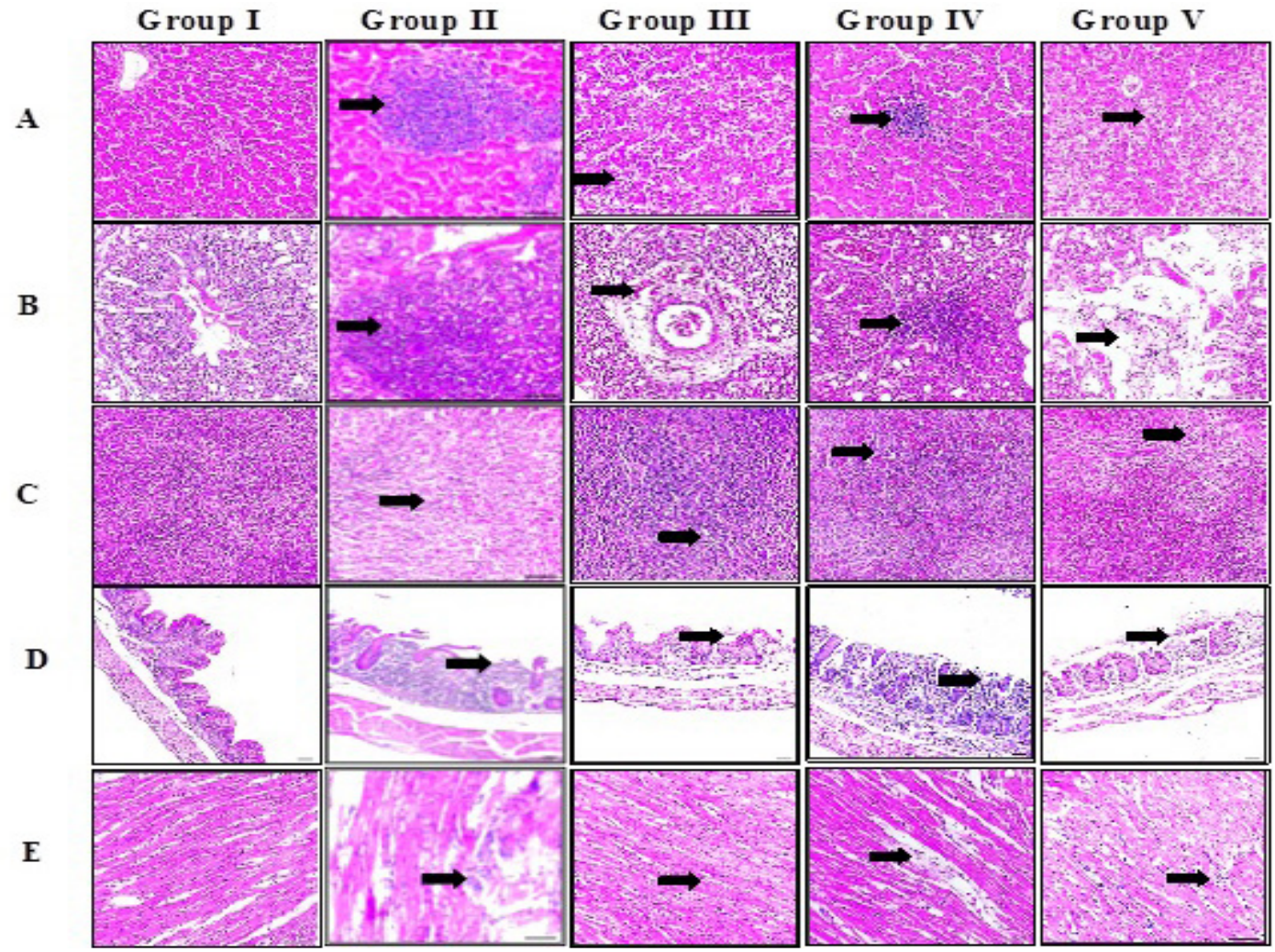

Figure 7: Illustrates the histopathological differences in liver (A), lung (B), spleen (C), cecum (D) and heart (E) across five groups: control negative (I), *E. coli* infection control positive (II), ZnO-NPs treatment (III), Colistin treatment (IV) and ZnO-NPs toxic dose (V). The control group exhibited normal tissue structures in all organs. In the *E. coli* infection group, significant hepatic necrosis and inflammation, lung serofibrinous exudates with neutrophil accumulation, spleen lymphocyte apoptosis with reduced mass, severe cecum epithelial desquamation and necrosis, and disrupted cardiac muscle fibers were observed. ZnO-NPs treatment maintained structural integrity in most tissues but resulted in minimal pathological changes in the liver and cecum. Colistin treatment ameliorated these effects, showing reduced liver necrosis and inflammation, mild lung heterophil infiltration, lesser lymphoid depletion in the spleen, and mild heart disruptions while preserving cecum structure. However, a toxic dose of ZnO-NPs induced moderate liver hydropic vacuolization, lung epithelial cell desquamation, significant dilation of spleen blood sinusoids, and myocardial fiber degeneration in the heart.

Regarding antioxidants, *E. coli* infection induced oxidative stress, as evidenced by elevated levels of MDA and catalase and decreased GSH. ZnO-NPs further increased these antioxidant markers, indicating enhanced antioxidant defences in response to infection. Colistin drug increased MDA and catalase levels but decreased GSH, suggesting a different antioxidant response. When ZnO-NPs were administered without infection, they increased MDA, catalase, and GSH levels compared to controls, indicating their influence on antioxidant parameters independently. These comprehensive results highlight the effectiveness of Colistin and ZnO-NPs in mitigating the impact of *E. coli* infection in chickens, offering valuable insights into potential therapeutic strategies and the associated biochemical responses (Table 7).

Infectious microorganisms provide a significant challenge to the poultry business since they negatively impact growth rate, efficiency, and result in substantial economic losses estimated by several millions $ due to enteric infections such as *E. coli* infection (Kromann and Jensen, 2022). The poultry industry frequently uses antibiotics as growth promoters and to control pathogenic microbes. Nevertheless, the extended utilization of antibiotics resulted in the development of antimicrobial resistance (Raquel *et al*., 2023), and the possibility for transmission to humans and the sharing

of genes for resistance between multiple kinds of bacteria resulted in the emergence of MDR (multidrug-resistant) bacteria (Helal *et al*., 2023). Multidrug resistant bacteria are a major problem because they are resistant to multiple antibiotics which affect public health, veterinary medicine and agriculture. MDR bacteria affects a wide and diverse area as it is not limited to a specific region or group of people (Catalano *et al*., 2022). As a result, there have been global efforts to develop new and improved antimicrobial drugs, as well as creative and efficient methods for administering antibiotics. Combating MDR bacteria is possible only with the help of the complex use of traditional and non-traditional approaches. Thus, probiotics, bacteriophages, nanoparticles, and AMPs are different strategies with their benefits and limitations. Further studies and innovations in these areas and other novel approaches are believed to offer better and lasting strategies to tackle antibiotic resistance on an international level. (Zeinab and Rafik, 2023).

Table 7: Effect of different treatments on antioxidants parameters in SPF chicks infected by *E. coli*

	MDA (n.mol\ul)	CAT(u\l)	GSH(u\l)
Control negative group	1.27±.08^{d}	15.66±.18de	3.79±.02^{b}
Control positive group	1.55±.08^{bcd}	15.89±.65^{cd}	3.78±.16^{b}
E. coli and ZnO-NPs group	2.67±.03^{a}	21.47±.37^{a}	4.25±.06^{a}
Clostin treated group	2.32±.10^{ab}	16.95±.45^{c}	2.11±.03^{d}
ZnO-NPs toxicity	1.46±.03^{cd}	18.33±.44^{b}	4.14±.06^{a}

Mean values with different superscript letters in the same row are significantly different at (*P<0.05*). Data are presented as (Mean ± Standard error). Antioxidants: Serum L-Malondialdehyde (L-MDA) (MDA (n.mol\ul), Catalase CAT(u\l) and Reduced Glutathione GSH(u\l)

A possible use in the poultry business is using trace minerals, particularly Zinc oxide in the form of nanoparticles ZnO-NPs, as a suitable alternative to bigger particles. The inclusion of ZnO-NPs in the diet of broiler chickens led to improved zinc uptake and bioavailability of Zn and shown potential antibacterial properties against the many pathogens (Hidayat *et al*., 2023). This study compared the efficiency of ZnO-NPs as antibacterial and antibiofilm against *E. coli O6* serotype isolated from chickens and humans, in vitro with colistin. It also reviewed the side effects on the liver and kidney functions, antioxidants and toxicity when used as a dietary supplement.

The isolation results in this study showed that 44% of the samples were positive for *E. coli* by which the previous studies also reported that *E. coli* was highly prevalent in poultry, for example 66. 3% prevalence which was observed by Abdelkarim et al. (2020). This underlines the importance of poultry as the most common source of pathogenic and antibiotic-resistant *E. coli* strains that can be transmitted to humans through the food chain or physical contact (De Mesquita *et al*., 2022). The higher isolation rates from poultry samples imply that perhaps, eradicative hygienic measures and public health interventions are more effectively controlling the spread of *E. coli* in humans than in birds (Reed *et al*., 2023).

The prevalence of the highly capable biofilm producers *E. coli*. strains in different sample sources highlight the need of minimizing contamination, as biofilms have been linked to increased antibiotic resistance and virulence (Murugesan *et al*., 2022). The results revealed that the predominance of *E. coli* strains was present in serotypes *O1, O6, O44*, and *O119*. Lung samples which were positive for of 5 isolates, all of which were of serotypes O6 and O119. All the 8 liver isolates were further identified into the serotypes *O6*, *O119*, and *O44*. Urine samples and nasal swab had two isolates of serotype *O1* and *O6*. The junction of these data suggests the potential for zoonotic transmission among diverse hosts. There is several evidence that support the fact that retail chicken meat can transmit pathogenic *E. coli* to humans (Dipak *et al*., 2021).

Antibiotic resistance exhibited a remarkable prevalence within different serotypes; however diverse resistance patterns were detected. Serotype *O6* showed multidrug resistance against all tested antibiotics except Azithromycin. The occurrence of multidrug resistance in *O6* is quite troublesome and suggests the probable idea for antibiotic selection for local poultry industry (Veloo *et al*., 2022). These findings highlight the need for tailored strategies to tackle antibiotic resistance in chickens recorded in various serotypes of *E. coli*.

The physicochemical profiles of synthesized ZnO-NPs were carefully investigated that would help to understand their possible antibacterial and antibiofilm activities. Further characterization as well as analysis using XRD test proved that the ZnO-NPs are crystalline and the atomic structure matches the previous literature works (Król-Górniak *et al*., 2023). The shape, nanostructure, size, and dispersion of the NPs were investigated using TEM where it was confirmed that the ZnO-NPs were successfully synthesized (El-Ghwas *et al*., 2022). Due to the smaller size of ZnO-NPs they have a large surface area to volume ratio which makes them more reactive and more capable to absorb more. This enhanced reactivity is essential for their antibacterial property since the destruction of bacterial cell membrane and interference of biofilm formation is directly proportional to the reactivity between the NPs and bacterial cells. Also, the large surface area provides a better opportunity to interact with microbial cells; this attribute increases the ZnO-NPs to penetrate and erode biofilms, making them suitable for use as antibiofilm agents (Hi-

dayat *et al*., 2023). These property understandings can well account for the antibacterial and antibiofilm effects evidenced in ZnO-NPs.

Determining the biocompatibility of Vero cells requires first evaluating their cytotoxicity. In vitro tests on ZnO-NPs cytotoxicity against cell lines proved its cytotoxicity. From cytotoxic perspective, the ZnO-NPs are suitable for therapeutic applications due to their high cytocompatibility, as showed by their high cell viability IC` 54W32 value across the concentration range and lack of noticeable toxicity (De Berardis *et al*., 2010; Martinez *et al*., 2011).

The antibacterial and antibiofilm characteristics of ZnO-NPs were determined in vitro by testing them against *E. coli* serotype *O6* isolates. The results indicated that 50 µg/ml had strong antibacterial effects and 6.25 µg/ml was effective in breaking up biofilms. Helal *et al*. (2023) investigated how quickly nano formulations can pass through biological barriers, such as biofilms, due to their small size, large surface area, and intense reactivity. These nano-formulations show promise for treating multidrug-resistant (MDR) pathogens and decreasing biofilm development. They also show a high preference against bacterial cell walls. Antibacterial mechanism of ZnO-NPs as shown by Krishnamoorthy *et al*. (2022), who involves making more malondialdehyde and reactive oxygen species (ROS) effects in bacterial cells. Which leads to membrane peroxidation, decreased permeability, denaturation of intracellular proteins, DNA damage, and membrane leakage. The information we've gathered supports the conclusions of (Mohd Yusof *et al*., 2020; Ashengroph *et al*., 2020), who also documented that ZnO-NPs had notable antibacterial effects against foodborne pathogens such *S.aureus* , *E.coli*, and *Salmonella spp*. that are important to poultry. According to these findings, ZnO-NPs may eventually take the place of traditional antibiotics in the raising of chickens. We investigate the impact of giving one-day-old SPF chicks 50 mg ZnO-NPs /kg of feed based on the earlier findings. Evaluating this supplementation to the commercial antibiotic colistin, we will examine whether it functions as an antibacterial agent against multidrug-resistant *E. coli* serotype O6.

At the time of the bacterial challenge, the results showed that ZnO-NPs successfully decreased clinical symptoms and post-mortem lesions, suggesting its potential for therapeutic use. These results are agreed with earlier research that demonstrated ZnO-NPs antibacterial properties in living organisms (Hameed *et al*., 2016). Our study's results are consistent with those of Hidayat *et al*. (2023), who showed that ZnO-NPs had antibacterial properties against gastrointestinal infections in broiler chickens, including *Enterococcus spp*. and *E. coli*. ZnO-NPs is safe and does not upset the balance of commensal bacteria, hence they advised using it at a dosage of 100 mg/kg. Li *et al*. (2021) and Long *et al*. (2022) have also revealed equivalent outcomes, demonstrating that high bioavailability zinc supplements improve antioxidative activity and less liver damage in animals. Furthermore, our results are consistent with theirs because none of the treatment groups' liver samples showed any symptoms of hepatotoxicity, such as hepatic bleeding or swelling.

The biochemical changes seen were a significant increase in the liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT); also seen was a significant decrease in the total serum protein, albumin and globulin. These changes suggest hepatocellular injury, and this could have a negative impact on the overall health and productivity of the poultry because the liver plays an important role in the metabolisms of protein. In comparison with the group III treated with ZnO-NPs, the level of which significantly decreased in globulin, albumin, AST, ALT, ALP and TP, the A/G ratio of the control positive group II sharply increased. This implies that there is a differential effect to ZnO-NPs, which calls for evaluation of dosage to prevent hepatotoxic effects. In the ZnO-NPs hazardous dosage group V, all the investigated biochemical parameters such as ALT, AST, ALP, TP, albumin, and globulin were found to be increased showing signs of liver stress not even when but by the toxic effect of ZnO-NPs alone. But there was relatively close similarity with the infected group IV treated with colistin, which indicates that both treatments are equally likely to have toll on the biochemical stress in the liver.

These results are in support with Sonwane *et al*. (2017) where they presented that hepatocellular damage could have caused hepatocyte membrane damage associated with hepatocyte content leakage during the detoxification of *E. coli* and bacterial toxins. This might partly explain why the liver enzymes are highly raised, which are very key indicators of liver dysfunction. AST and ALT enzymes as biomarkers in toxicological investigations are very sensitive to reveal that an enlarged liver is a sign of an underlying disease that suggests inflammation of the organ. In addition, the effects of the observed decrease in serum total protein, albumin and globulin levels would be to suggest that liver synthetic function is impaired in the face of the growing broiler industry and their overall health and productivity could be adversely affected. Kaneko *et al*. (1997) noted that renal affection and hepatocyte damage can cause failure in the plasma protein synthesis and therefore, protein losses due to low serum urea, uric acid, and creatinine.

Antioxidant activity and stress response are the two important factors that affect the health and production of

poultry. In this study, the *E. coli* infection group treated with ZnO-NPs had the highest MDA, GSH in serum and significantly higher CAT level (P < 0. 05). The CAT, MDA, and GSH of Group V were significantly higher than those of the control group after the exposure to a more toxic number of ZnO-NPs. These enzymes include glutathione (GSH) that help in removal of free radicals and catalase (CAT) that help in the removal of hydrogen peroxide which are forms of oxidative stress. Similarly, Hafez *et al*. (2020) and Zhao *et al*. (2014) reported higher serum GSH and CAT activity in broiler chicks fed with ZnO-NPs. Equally, Abd-El Rhman et al. (2018) observed higher serum MDA concentrations in the *E. coli* infected chickens because of endotoxins resulting from E. coli infection. These endotoxins cause an increase in the production of ROS and RNS which in turn result in the damage of proteins and nucleic acids and increased lipid peroxidation. The applicability of these results in poultry farming is quite profound. Thus, improved antioxidant activity, which is supported by the increase in GSH and CAT, may help to decrease the level of oxidative stress resulting from bacterial infections and environmental stressors. This can result in better health status of the birds by lowering cellular damage, increasing immune function, and increasing general growth and production in poultry. In addition, the knowledge of the processes that occur within the framework of oxidative stress and its effect on health also paves the way for effective interventions to improve the birds' resistance to infections and stress, for example using antioxidants or nanoparticles such as ZnO-NPs.

The following should be considered as future investigation and prospects: the chronic influence of ZnO-NPs on the health and performance of chickens, mechanism pathways of antibacterial activity of ZnO-NPs for efficient prevention and control of some poultry economic effective diseases, and large-scale field trials to evaluate the ZnO-NPs in the commercial environment.

CONCLUSIONS AND RECOMMENDATIONS

The results of our study confirmed our main hypothesis, which aimed to assess the effectiveness of ZnO-NPs in combating pathogenic multi-drug-resistant *E. coli* bacteria in broiler chickens. We specifically focused on the antibacterial and antioxidant properties of ZnO-NPs, with the goal of finding a safe and potent alternative to antibiotics for the treatment and prevention of drug-resistant *E. coli* O6 infections. The in vivo study demonstrated that ZnO-NPs are capable of alleviating *E. coli* induced- signs, macroscopic and microscopic lesions, and possess a potent antioxidant agent. These findings suggest that ZnO-NPs could serve as an alternative to the antibiotic Colistin in the field to combat antibiotic resistance in both humans and chickens. This is one of the unintended consequences of the conventional wisdom that antibiotics are the most effective method of preventing and controlling bacterial infections.

ACKNOWLEDGEMENTS

The authors grateful thank Dr.Dalia EL-Masery at nanotechnology unit of Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Doki, Giza, Egypt for her valuable assistance during the practical part of research.

NOVELTY STATEMENT

Availability of ZnO-NPs as antibacterial agent for efficient prevention and control of some poultry economic diseases.

AUTHOR'S CONTRIBUTION

All authors participated in the experimental work design, analysis, observation, data collection, results, correction, and overlooking. Writing of the original outline and statistically analysis of results was done by Reda, Fathy, and all authors have revised and agreed on the manuscript

Conflicts of Interest

The authors declare no conflict of interest

REFERENCES

Abdallah A.G., Mamduh O., Abd-Elsamee, K., Elkloub, M.E. Moustafa, M. A., Khattab, A. and Rehan, A.A. Effect of Nanosilica and Bentonite as Mycotoxins Adsorbent Agent in Broiler Chickens' Diet on Growth Performance and Hepatic Histopathology Animals, 2021.11: 21-29. https://doi.org/10.3390/ani11072129

Abdelkarim, E.A., Hafez, A.-E.E., Hussein, M.A. & Elsamahy, T.S. (2020). Prevalence of Escherichia coli in Marked Poultry Carcasses in Egypt. Advances in Animal and Veterinary Sciences,8.)s1):55- 61. http://dx.doi.org/10.17582/journal.aavs/2020/8.s1.55.61

Abd-El Rhman S.L., Mohammed F. K, Samir F.A. Effect of probiotics and chelated zinc on *E. coli* infected broilers. Benha Vet Med J, 2018; 35:510-25. https://doi.org/10.21608/bvmj.2018.96446

Abdus Salam, M., Yusuf A., Moushumi T.S., Jogendra, S., N.A., Ali A.R. and M.A.A. Antimicrobial Resistance: A Growing Serious Threat for Global Public Health Healthcare, 2023, 11:1946. https://doi.org/10.3390/healthcare11131946

Allam, R.M; Al-Abd, A.M.; Khedr, A., Sharaf, O.A.; Nofal, S.M.; Khalifa, A.E.; Mosli, H.A. and Abdel-Naim, A.B. Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. Toxicology Letters, 2018, 291: 77- 85 https://

doi.org/10.1016/j.toxlet.2018.04.008

Arjmand F., Tean Z., Kaushik P., Rao Z.A., Tayyaba A. and Mahumoud M.S. Zinc Oxide Nanoparticles Significant Role in Poultry and Novel Toxicological Mechanisms .Biol Trace Elem Res, 2024, 202, 1:268-290. https://doi.org/10.1007/s12011-023-03651-x

Ashengroph, M.; Khaledi, A.; Bolbanabad, E.M. Extracellular biosynthesis of cadmium sulphide quantum dot using cell-free extract of Pseudomonas chlororaphis CHR05 and its antibacterial activity. Process Biochem, 2020, 89, 63-70. https://doi.org/10.1016/j.procbio.2019.10.028

Basumatari, M., Devi, R.R., Gupta, M.K., Gupta, S.K., Raul, P.K., Chatterjee, S. and Dwivedi, S.K. Musa balbisiana Colla pseudostem biowaste mediated zinc oxide nanoparticles: Their antibiofilm and antibacterial potentiality. Current Research in Green and Sustainable Chemistry, 2021, 4, 100048. Elsevier. https://doi.org/10.1016/j.crgsc.2020.100048

Booth, G.D., Steel, R.G.D., Torrie, J.H. Principles and Procedures of Statistics: A Biometrical Approach. Journal of the American Statistical Association, 1981, 76: 753. https://doi.org/10.2307/2287561

Catalano, A., Domenico, I., Jessica, C., Domenico, S., Federica, G., Carmela, S., Stefano, A., Camillo, R., Maria, S.S. Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies Molecules,2022,27,616. http://doi.org/10.3390/molecules27030616

Christodoulou, M.K. 2023. Comparison of Antimicrobial Resistance in *Escherichia coli* Strains Isolated from Swine, Poultry, and Farm Workers in the Respective Livestock Farming Units in Greece. Cureus, 15, 12: e51073. https://doi.org/10.7759/cureus.51073

De Berardis, B., Civitelli, G., Condello, M., Lista, P., Pozzi, R., Arancia, G. , Meschini, S. Exposure to ZnO nanoparticles induces oxidative stress and cytotoxicity in human colon carcinoma cells. Toxicology and Applied Pharmacology, 2010, *246*:116–127. Toxicol Appl Pharmacol. https://doi.org/10.1016/j.taap.2010.04.012

De Mesquita, M.S.S.; Kelvin, L.; Daniel, F.M.M.; Patrícia, E.N.G.; Lucas, B.R.A.; Oliveiro, C. de Neto; Samuel, K.A.B; Júnior, E.J.B.O.; Wondwossen,A.G.Antimicrobial resistance in the globalized food chain: A One Health perspective applied to the poultry industry. Brazilian Journal of Microbiology., 2022, 53:465–486 https://doi.org/10.1007/s42770-021-00635-8

Dipak, K., Dhanashree, L., Sochina, R., Gireesh, R. Avian Pathogenic *Escherichia coli* (APEC): An Overview of Virulence and Pathogenesis Factors, Zoonotic Potential, and Control Strategies. Pathogens, 2021, 10: 467. https://doi.org/10.3390/pathogens10040467

Doumas, B.T.; Watson, W., Biggs, H.G. Albumin standards and the measurement of serum albumin with bromcresol green. Gun. Chim. Acta, 1971, 31: 87. https://doi.org/10.1016/0009-8981(71)90365-2

EL-Ghwas, D.E., Al-Nasser, A.S., A. Zamil, G. Zinc Oxide Nanoparticles Bacterial Synthesis and Application. Research Journal of Pharmacy and Technology, 2022, 15: 471–480. https://doi.org/10.52711/0974-360X.2022.00077

Esraa A. Abdelkarim, Abd-Elsalam E. Hafez, Mohamed A. Hussein, Tamer S. Elsamahy. Prevalence of *Escherichia coli* in Marked Poultry Carcasses in Egypt Advances in Animal and Veterinary Sciences, 2020, 8:1, 55. https://doi.org/10.17582/journal.aavs/2020/8.s1.55.61

Freeman, D.J., Falkiner, F.R, Keane, C.T. New method for detecting slime production by coagulase negative staphylococci. Journal of Clinical Pathology, 1989, 42:872–874. BMJ Publishing Group. https://doi.org/10.1136/jcp.42.8.872

Hafez, A.; Nassef, E.; Fahmy, M.; Elsabagh, M.; Bakr, A.; Hegazi, E. Impact of dietary nano-zinc oxide on immune response and antioxidant defense of broiler chickens. Environ. Sci. Pollut. Res, 2020, 27, 19108–19114. https://doi.org/10.1007/s11356-019-04344-6

Hameed, A.S.H., Karthikeyan, C., Ahamed, A.P., Thajuddin, N., Alharbi, N.S., Alharbi, S.A. Ravi, G. . In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae. Scientific Reports. 2016, *6*, 24312. Nature Publishing Group. https://doi.org/10.1038/srep24312

Helal F.H, Yasmin R, Alhanouf I.AH, Esraa AA, Basem B, Noura HE, Stefania Z, Matthew GD. Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. Biomedicines, 2023;11, 413. https://doi.org/10.3390/biomedicines11020413

Henry, R.J., Clinical chemistry, principles and technics. Second Edition, Harper and row, 525, 1974. Colorimetric determination of creatinine.

Hidayat, M.Y., Nor'Aini, A.R., Rosfarizan, M., Uswatun, H.Z., Muhammad, A.A., Anjas, A.S. Effects of dietary zinc oxide nanoparticles supplementation on broiler growth performance, zinc retention, liver health status, and gastrointestinal microbial load. Journal of Trace Elements and Minerals., 2023, 4. https://doi.org/10.1016/j.jtemin.2023.100072

Jiddu Joseph, Li, Z., Pratima, A., Jeffrey D. Evans and Reshma Ramachandran. Avian Pathogenic Escherichia coli (APEC) in Broiler Breeders: An Overview. Pathogens., 2023, 12: 1280. https://doi.org/10.3390/pathogens12111280

John, D. 1982. Laboratory method for estimation of alkaline phosphatase. 9th Ed 81.

Kaneko, J.J., Harvey, J.W., Bruss, M.L. 1997. Serum proteins and the dysproteinemias. Clinical Biochemistry of Domestic Animals, Acad. Press, New York, NY,; 117-138. https://doi.org/10.1016/B978-012396305-5/50006-3

Krishnamoorthy, R.; Athinarayanan, J.; Periyasamy, V.S.; Alshuniaber, M.A.; Alshammari, G.; Hakeem, M.J.; Ahmed, M.A.; Alshatwi, A.A. Antibacterial Mechanisms of Zinc Oxide Nanoparticle against Bacterial Food Pathogens Resistant to Beta-Lactam Antibiotics. Molecules., 2022, 27:2489. 22. https://doi.org/10.3390/molecules27082489

Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V. Buszewski, B.. Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. Advances in Colloid and Interface Science., 2017, 249, 37–52. Elsevier. Retrieved from. https://doi.org/10.1016/j.cis.2017.07.033

Kromann, S. Jensen, H.E. In vivo models of Escherichia coli infection in poultry. Acta Veterinaria Scandinavica., 2022, 64, 33. BioMed Central Ltd. https://doi.org/10.1186/s13028-022-00652-z

Li, T., He, W.X. Liao, X. Lin, L. Zhang, L. Lu, Y. Guo, Z. Liu, Luo, X. Zinc alleviates the heat stress of primary cultured hepatocytes of broiler embryos via enhancing the antioxidant ability and attenuating the heat shock responses, Anim. Nutr., 20217: 621–630, https://doi.org/10.1016/j.aninu.2021.01.003

Long, L., Zhao, X., Li, H., Yan, X., Zhang, H. Effects of zinc lactate supplementation on growth performance, intestinal

morphology, serum parameters, and hepatic metallothionein of Chinese yellow-feathered broilers. Biol. Trace Elem. Res ,2022;200 1835–1843. https://doi.org/10.1007/s12011-021-02785-0

Luck, H. 1974. Estimation of catalase. In: methods in enzymatic analysis. 2nd edition, Bergmeyer, Academic Press, New York. pp. 885-890.

Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A., Maguire, D. 2013. Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto

Martinez, C.R., Joshi, P., Vera, J.L., Ramirez-Vick, J.E., Perales, O., Singh, S.P. Cytotoxic studies of PEG functionalized ZnO nanoparticles on MCF-7 cancer cells, in 2011: Proceedings of the NSTI Nanotechnology Conference Expo, NSTI Nanotech.

Mellata, M., Johnson, J.R. and Curtiss, R. *Escherichia coli* isolates from commercial chicken meat and eggs cause sepsis, meningitis and urinary tract infection in rodent models of human infections. Zoonoses and Public Health.,2018; *65*: 103–113. John Wiley & Sons. https://doi.org/10.1111/zph.12376

Mesbah,L.; Soraya, B. ; Narimane, S.; Jean, P. F. Protective effect of flavonoides against the toxicity of vinblastine, cyclophosphamide and paracetamol by inhibition of lipid-peroxydation and increase of liver glutathion Haema., 2004;7(1): 59-67.

Mohd-Yusof, H.; Mohamad, R.; Zaidan, U.H.; Rahman, N.A. Sustainable microbial cell nanofactory for zinc oxide nanoparticles production by zinc-tolerant probiotic Lactobacillus plantarum strain TA4. Microb. Cell Fact., 2020, 19, 10. https://doi.org/10.3390/app10196973

Moron, M.S.; Depierre, J.W. and Mannervik, B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica et Biophysica ACTA, 1979; 582, 67-78. https://doi.org/10.1016/0304-4165(79)90289-7

Mubeen, B., Ansar, A.N., Rasool, R., Ullah, I., Imam, S.S., Alshehri, S., Ghoneim, M.M., Alzarea, S.I., Nadeem, M.S. Kazmi, I. Nanotechnology as a Novel Approach in Combating Microbes Providing an Alternative to Antibiotics. Antibiotics.,2021;10, 1473. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/antibiotics10121473

Mughini-Gras, L., van Pelt, W., van der Voort, M., Heck, M., Friesema, I. Franz, E.. Attribution of human infections with Shiga toxin-producing Escherichia coli (STEC) to livestock sources and identification of source-specific risk factors, The Netherlands (2010-2014). Zoonoses and Public Health., 2018;65,e8–e22. John Wiley & Sons, Ltd. https://doi.org/10.1111/zph.12403

Murray 1984. Report on the symposium "drug effect in clinical chemistry methods for Estimation of AST. Eur J Clin Chem clin Biochem . 34:1112-1116.

Murugesan, S., Madeline, C. McCarthy, Michelle, K. S., Linzhi, W., Jo-Anne, R.D., Joseph, E.R. and Aaron, P. White. Biofilm Formation and Antimicrobial Susceptibility of *E. coli* Associated With Colibacillosis Outbreaks in Broiler Chickens From Saskatchewan.Frontiers in Microbiology., 2022, 13 :847516. https://doi.org/10.3389/fmicb.2022.841516

Patton, C.L. and Crouch, S.R., 1977. Colorimetric determination of urea Analytical chemistry, 49: 464-469. https://doi.org/10.1021/ac50011a034

Peighambari, S.M., Julian, R.J., Vaillancourt, J.P. Gyles, C.L. *Escherichia coli* cellulitis: Experimental infections in broiler chickens. Avian Diseases., 1995, 39, 125-134. American Association of Avian Pathologists. https://doi.org/10.2307/1591991

Raheel, S., Sabahat, Z., Alma, A.R. *Escherichia Coli* O157:H7 in meat and poultry: Transmission, Consequences on Human Health and Impact of non-Thermal Decontamination technologies .Agricultural Sciences Journal., 2022; 4(1):52-62. https://doi.org/10.56520/asj.004.01.0134

Raquel, A., Teresa, S.L., Eva, C., L.T., Manuela, O. Antimicrobial Drug Resistance in Poultry Production. Current Status and Innovative Strategies for Bacterial Control. Microorganisms. 2023;11, 953. https://doi.org/10.3390/microorganisms11040953

Reed, W., Adelumola, O., Zaid, A. Chicken Production and Human Clinical Escherichia coli Isolates Differ in Their Carriage of Antimicrobial Resistance and Virulence Factors. Applied and Enviromental Microbiology. 2023; 89(2):1-18 https://doi.org/10.1128/aem.01167-22

Serwecińska, L. 2020. Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health. Water., 12:3313. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/w12123313

Sofie Kromann and Henrik Elvang Jensen. In vivo models of Escherichia coli infection in poultry. Acta Veterinaria Scandinavica, 2022, 64:33. https://doi.org/10.1186/s13028-022-00652-z

Sonwane S, Ingole R, Hedau M, Rathod P, Hajare S, Ingawale M. Ameliorative effect of Andrographis paniculata on hematobiochemical parametersin Escherichia coli induced broilers. J Pharmacogn Phytochem, 2017, 6:1284–8.

Swelum, A.A., Elbestawy, A.R., El-Saadony, M.T., Hussein, E.O.S., Alhotan, R., Suliman, G.M., Taha, A.E., Ba-Awadh, H., El-Tarabily, K.A. Abd El-Hack, M.E..Ways to minimize bacterial infections, with special reference to Escherichia coli, to cope with the first-week mortality in chicks: an updated overview.Poultry Science., 2021100: 101039. Elsevier. https://doi.org/10.1016/j.psj.2021.101039

Tietz NW 1994. Determination of Total Proteins. Fundamentals of Clinic. Chemis. Saunders Co., London, Philadelphia, 692-772.

Xian-Qing, Z., Zakir, H., Dong-Dong, Z., Meng-Yao, L., Si, H., Qiong, W., Yu-Fei, C., Ying, Y. Zinc Oxide Nanoparticles: Synthesis, Characterization, Modification, and Applications in Food and Agriculture. Processes., 2023;11: 1193. https://doi.org/10.3390/pr11041193

Veloo, Y.; Syahidiah, S.A.T.; Sakshaleni, R.; Lim, K.H.; Norazah, A., Vickneshwaran M.; Rafiza, S. Multidrug-Resistant Gram-Negative Bacteria and Extended Spectrum b-Lactamase-Producing Klebsiella pneumoniae from the Poultry Farm Environment Microbiology Spectrum, 2022;10: 3. https://doi.org/10.1128/spectrum.02694-21

Yogesh, K.; Chandra, D.; Shrivastava, H.P.; Mandal, A.B. Growth Performance, Carcass Yield, and Immune Competence of Broiler Chickens as Influenced by Dietary Supplemental Zinc Sources and Levels. Journal of Agricultural Research., 2013; 2(3). https://doi.org/10.1007/s40003-013-0067-5

Young, D.S., 2001. Effects of disease on clinical lab. Tests, 4th ed AACC,Colorimetric determination of serum uric acid.

Zachary, R.S., James, R.J., John, M.F., Jacquelyn, K., Angelica, V.G., Roy, C., Melha, M. Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. PLoS ONE., 2017; 12(7):

e0180599. https://doi.org/10.1371/journal.pone.0180599

Zeinab, B. and Rafik, K. Design and Synthesis of Novel Antimicrobial Agents.Antibiotics., 2023,12: 628. https://doi.org/10.3390/antibiotics12030628

Zhao, C.Y., Tan, S.X., Xiao, X.Y., Qiu, X.S., Pan, J.Q., Tang, Z.X. Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. Biol. Trace Elem. Res., 2014;160:361–367. https://doi.org/10.1007/s12011-014-0052-2