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# Highlighting on the antimicrobial effect of silver nanoparticles on *E. Coli* Isolated from Newly Weaned Rabbits

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## ABSTRACT

Rabbits reared for meat in industrial farms exhibit the highest rates of antimicrobial usage compared to other food-producing animals. E. coli is principally responsible for neonatal and post-weaning colibacillosis in rabbits, which is frequently accompanied by enteritis and diarrhea. A total of 300 rectal swabs were obtained from diarrheic live newly weaned (30 - 40 days old) Newzeland white rabbits from 3 farms from different localities in Shakira province, which suffered from diarrhea, high morbidity and mortality rates. Bacteriological isolation of *E. coli from* diarrheic weaned rabbits, Biochemical and Serological identification of isolated strains as well as detection of some virulence genes in isolated strains, antimicrobial Susceptibility Testing of isolated strains, assessment of the efficacy of silver nanoparticles on *E. coli* isolates, scanning electron microscopy (SEM) To determine the influence of the silver nanoparticles on the morphology of *E. coli*. Experimental modelling in E. coli free rabbits. The allover incidence of *E. coli* isolation from rabbits with diarrhea was 28.33% (85/300). Serotypes of the isolated *E. coli* strain were O stereotype in order of frequency O158, O128, O125, O18, O119, O148 and untypable (25%, 15%, 20,10%, 10%, 5% and 15 % isolates respectively). *E. coli* isolates were highly resistance to Ampicillin (AM) (83.3%; 70/85), Cefoxitine (CF) (60%; 51/85), Sulfamethoxazole (SXT) (56.5%; 3156/85), chloramphincol (CHI) (57.7%; 56/85), Kanamycin (KAN) and Streptomycin (S) (41/85; 48.8%).Hundred percent ( 6|6) of tested *E. coli* isolates carry eaeA gene, while 66.7 %( 4/6) of the tested *E. coli* isolates were positive to Tsh gene. The MIC50 of AgNPs-H2O2 was 0.625 µg/mL against E. coli. Electron microscopy scanning (SEM) was utilized to determine the differences in bacterial morphology after application of the of AgNPs-H2O2at 1 MIC and 2 MIC concentrations. Significant alterations (exhibited varying degrees of distortion) in the morphology of treated cells. Experimental infection results revealed that the nanotechnology-treated group exhibited a reduction in bacterial load and clinical signs, indicating potential treatment efficacy.

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## INTRODUCTION:

Rabbits reared for meat in industrial farms exhibit the highest rates of antimicrobial usage compared to other food-producing animals, leading to alarming rates of antimicrobial resistance within the industry **(Crovato et al. 2023).**

Rabbits are raised for a variety of purposes, including their use in laboratory, and fur, which constitute a valuable by-product, meat, besides being efficient converters of vegetable protein into high quality animal protein. The production of rabbit meat on an industrial scale has been very slow to develop due to excessive mortality among growing rabbit which hinder mass production (Okerman, 1999) *E coli* bacterium is a member of the family *Enterobacteriacae* facultative anaerobic, gram-negative short rods and considered a common inhabitant of the gut of the worm- blooded animals, including man ***(*WHO, 1996*)***.

*E.coli* infection is the primary causative agent in most outbreaks of diarrhea in newly weaned rabbits. Several strains of varying virulence cause diarrhea in rabbits belong to different serotypes **(*Percy* et al. *1993*).** *E. coli* is principally responsible for neonatal and post-weaning colibacillosis in rabbits, which is frequently accompanied by enteritis and diarrhea **(Adriana Silva, 2024)**

The enterotoxigenic E. coli (ETEC) is leading to infectious diarrhea worldwide (**Wolf, 1997*)***. The adherence of bacteria to the enterocytes is mediated by intimin, an outer membrane protein encoded by eaeA that mediates close attachment of enteropathogenic bacteria to apical surfaces of epithelial cells, is required for formation of the attaching-effacing lesions and for full pathogenesis of the bacteria **(Nataro and Kaper, 1998)**.

Tsh gene, is another adhesion-related factor. The tsh gene encoding a temperature-sensitive hemagglutinin, was isolated and characterized by and may act as an adhesion, particularly in the initial stages of bacterial colonization. The Tsh autotransporter seems to be one of the factors associated with induce fluid accumulation in the rabbit gut **(Maluta et al. 2014).**

Antibiotic resistance in commensal bacteria from food animals is a global concern, with research focusing on the effects of antibiotic use on animals and the potential transmission of resistant bacteria to humans. The use of antibiotics in food-producing animals has led to the development of MDR food bacteria like E. coli. While studies have primarily focused on E. coli's prevalence in other livestock animals, there is a significant lack of research on rabbits **(Ramos et al. 2020)**.

Silver nanoparticles (AgNPs) exhibit biocidal effects against various bacteria. They can interact with the cell surface of Gram-negative bacteria, causing damage and structural changes that enhance bacterial permeability **(Jones et al. 2008).**

In Egypt there is a little literature on newly weaned rabbit diarrhea causes by *E. coli*. Therefore, this study aims to determine the occurrence and antimicrobial resistance patterns of *E. coli* in newly weaned rabbits. Evaluation of the antibactericidal efficacy of silver nanoparticles (AgNPs -H2O2) against *E. coli* this can be achieved by: Isolation of *E. coli* from diarrheic weaned rabbits, Biochemical and Serological identification of isolated strains as well as detection of some virulence genes in isolated strains, antimicrobial Susceptibility Testing of isolated strains, assessment of the Efficacy of silver nanoparticles on E. coli isolates, Scanning electron microscopy (SEM) To determine the influence of the silver nanoparticles on the morphology of E. coli. Experimental modelling in E. coli free rabbits.

## MATERIAL AND METHODS

### Sample:

A total of 300 rectal swabs were obtained from live newly weaned (30 - 40 days old) Newzeland white rabbits from 3 farms from different localities (100 each) in Shakira province, which suffered from diarrhea, high morbidity and mortality rates during extended periods of seasons (summer, winter, autumn and spring). Samples were directly transferred to

the bacteriological laboratory examination without delay.

**Bacteriological examination:**

**Isolation and biochemical identification of *E. coli:***

Collected samples were enriched first on buffered peptone broth incubated aerobically at 37ºC for 24 hours, then a loopful from each sample was inoculated separately onto Mac-Conkey agar and Eosin Methylene blue agar (EMB). The inoculated plates were aerobically incubated at 37ºC for 24h. Suspected colonies were subjected to morphological and biochemical identification according to **Cruickshank et al. (1982).**

**Serological identification:**

Suspected E*. Coli* isolates were subjected to serological identification according to **Quinn et al. (2002)** for determination of (O) antigen using slide agglutination test.

**Antimicrobial Susceptibility Testing:**

Agar disc diffusion assay was used to test antimicrobial sensitivity according to the **Clinical and Laboratory Standards Institute standards (CLSI, 2017).** The antimicrobials used were ampicillin (AM; 10 µg), tetracycline (TE; 30 µg), gentamicin (GE; 10 µg), sulfamethoxazole (SXT;25 µg), amikacin (AK;30 µg), ceftriaxone (Cef;30 µg), Streptomycin (S 30 µg), Chloramphenicol (CHI 30 µg), Kanamycin (KAN 30 µg), Cefoxitin (Cf 30 µg), Nalidixic acid (Na 30 µg), Ofloxacine (Ofx 5 µg) and Ciprofloxacin (Cip 5 µg). These antimicrobials are commmon utilized in veterinary field.

**Molecular detection of virulence genes in *E. coli* isolates using PCR:**

It was carried out on 6 isolates (two isolates from each farm).

- Extraction of DNA according to QIAamp DNA mini kit instructions.
-Preparation of PCR master mix according to Emerald Amp GT PCR master mix (Takara).
- Cycling conditions of the primers during cPCR:

Table 1. Cycling conditions of the different primers during cPCR .

| Target Gene | Primary denaturation | Secondary denaturation | Annealing | Extension | No. of cycles | Final extension | references |
|---|---|---|---|---|---|---|---|
| *eaeA* | 94˚C 5 min. | 94˚C 30 sec. | 51˚C 30 sec. | 72˚C 30 sec. | 35 | 72˚C 7 min. | Bisi-Johnson *et al.* 2011 |
| *Tsh* | 94˚C 10 min. | 94˚C 45 sec. | 54˚C 45 sec. | 72˚C 45 sec. | 35 | 72˚C 10 min. | Delicato *et al.* 2003 |

**Evaluation of the Efficacy of silver nanoparticleson *E. coli* isolates:**

Silver nanoparticles combined with Hydrogen peroxide (AgNPs-H2O2) were used to control some virulent organisms. This study will make atrial to investigate the Efficacy of Silver nanoparticles combined with Hydrogen peroxide on E. coli isolates.

**Antimicrobial effect of silver nanoparticles on *E. coli* isolates:**

Silver nanoparticles (AgNPs-H2O2) was obtained as a commercial product from El-Delta Center for Nano silver Technology Company, Mansoura, Egypt. The stock solution of the product contained 45-nm silver nanoparticles (0.00004467 mL/liter), hydrogen peroxide (50% per liter) and natural herb mint (1 mL/liter) at a concentration

of 5 mL/liter of water. The particles size was previously determined to be 30.17–67.92 nm with a zeta potential estimation of −0.192 mV **(El-Gohary et al. 2020)**. The AgNPs-H2O2 mixture was prepared by diluting the stock solution in sterile dis- tilled water to achieve the desired commercial concentration. The minimum inhibitory concentrations (MIC50 and MIC90) of AgNPs-H2O2 were determined against E. coli isolates by the broth micro dilution method (CLSI; 2012). Briefly, microliter plate wells were supplemented with various concentrations of AgNPs-H2O2 ranging from 100, 50, 25, 10, 5, 2.5 1.25, 0.625, 0.312, 0.156 and 0.078 µg/ml. E. coli isolates colonies were added in Muller Hinton broth and adjusted to the density of a 0.5 McFarland standard (1 × 108 cfu/ml). Each well received a final inoculum of 5 × 105 cfu/mL, and the plates were incubated for 24 h at 37 °C. A well E. coli isolates 1 alone and another well included E. coli isolates with AgNPs-H2O2 were used as reference control. The lowest agent con- centration that entirely prevents an organism's observable growth is known as the MIC endpoint. The MIC50 and MIC90 were calculated using an orderly array method **(Hamilton-Miller 1991)** where the middle value was selected as MIC50. The MIC90 was determined in the same way by selecting the appropriate value from the orderly array.The inhibitory effect of nanoparticles product on E. coli isolates was performed using disc diffusion method according to **(CLSI 2017).**

**Scanning electron microscopy (SEM):**

To determine the influence of the silver nanoparticles combined with Hydrogen peroxide on the morphology of *E. coli*, SEM was used in conjunction with a few modifications to the technique reported by **Bajpai et al. (2013**).

The Silver nanoparticles combined with Hydrogen peroxide were added at (MIC and 2 MIC) concentrations to working cultures. After 1 hour at 37 °C, 1 ml sections of each tube were collected and centrifuged for 10 minutes at 4000 rpm. Fix with 2.5% buffered gluteraldehyde in 0.1 M PBS pH 7.4 at 4˚C for 2 hours. Following fixation in 1% osmic acid for 10 minutes, wash three times with PBS (10 minutes between each wash) (30min). Three PBS washes (10 minutes each), followed by 30 minutes of dehydration with an ascending sequence of ethyl alcohol concentrations (30, 50, 70, 90, and absolute alcohol) infiltrated with acetone. SEM samples were dried with liquid CO2, supplied by SPI supplies®, using a critical point drying device. In an SPI- Module TM Vac/Sputter, mounted on aluminum stubs and gold-coated. Scanning electron microscope JEOL JSM-5200LV (SEM, Hitachi, and Tokyo, Japan). Electron Microscope Unit at Tanta University.

**Experimental model:**

**Animals**

Thirty days old 120 rabbits were used in this study, divided into four groups. Rectal swabs were examined to assure pathogenic E. coli free rabbits. Good housing and feeding were supplied.

They were infected orally with an infective dose of1 0 9mo. /ml. **(Stas, 1999).**

**Strains:**

*E. coli*, serotype O158, isolated from rectal swab of diarrheic rabbit (Farm 1) in the current study.

**Experimental design:**

Animals were divided into 4groups (30 rabbits each)
G1: Control negative (non- infected)
G2: Control positive (infected and non-treated).
G3: infected and treated with Silver nanoparticles combined with Hydrogen peroxide.
G4: infected and treated with Norfloxacine.

Treatments were added to drinking water from the first day of infection at a dose and duration according to the instruction of the producing companies.

Clinical signs and mortality rates were recorded daily from 0 day of infection till slaughter day

**Reisolation of experimental *E.coli* strain:**

Reisolation rates of E. coli experimental strain were assessed from day 0 to slaughter day. Two rectal swabs were taken from each rabbit from the day of infection, up to the day of slaughter. One swab was then streaked directly Onto EMB agar (Oxoid). The other was broken into a separate bijou bottle containing 2 ml of Mackonky broth (Oxoid) to determine the count of E. coli on each swab. For this, the broth was mixed on a vortex mixer (Stuart Scientific GB) for 60 sec, 0.1 ml was taken from the bijou bottle and 10-fold dilutions were prepared in sterile Mackonky broth at pH 7.2. Viable counts were then determined.

## RESULTS:

**Results of isolation and biochemical identification of *E. coli*:-**

*E. coli* isolates were Gram negative, medium sized bacilli to coccobacilli, non sporulated and arranged single, in pairs or in short chains. Appeared as smooth, shinny, strong lactose fermenting colon and on MacConkey's agar. Characteristic greenish metallic sheen on EMB.

Suspected *E. coli* isolates were lactose fermenting colonies and positive Indole, methyl red and catalase. Meanwhile, all isolates were negative oxidase, urea hydrolysis, citrate utilization, and voges-proskauer and not produced H2S.

The allover incidence of *E .coli* isolation from rabbits with diarrhea as shown in table (2) was 28.33% (85/300). *E. coli* Was recovered with high rate (46%) from farm (3) , followed by Farm (1): (22%) and Farm (2) : (17%). Seasonal prevalence of *E. coli* isolates from weaned rabbits revealed that the highest incidence occurred in summer season (44%). Followed by spring (31%), then in autumn (22%), and finally in winter (13.33%).

Table 2. The prevalence of *E. coli* isolated from diarrheic Rabbits from different farm:

| Farm | Number samples | Number of E. coli isolates | Percentage |
|---|---|---|---|
| **1** | 100 | 22 | 22% |
| **2** | 100 | 17 | 17% |
| **3** | 100 | 46 | 46% |
| **Total** | 300 | 85 | 28.33% |

Table 3. Seasonal prevalence of E. coli recovered from weaned rabbits.

| Season | No. of examined samples | Incidence | |
|---|---|---|---|
| | | No. | % |
| Autumn | 50 | 11 | 22 |
| Winter | 75 | 10 | 13.33 |
| Spring | 100 | 31 | 31 |
| Summer | 75 | 33 | 41 |
| Total | 300 | 85 | 28.33 |

**Results of serological identification of isolated *E. coli*:**

Serotyping was applied to isolated *E. coli* (table 4), the isolated *E. coli* strain, from diarrheic rabbit were O stereotype in order of frequency O158, O128, O125, O18, O119, O148 and untypable (25%, 15%, 20%, 10%, 10%, 5%, and 15% isolates respectively).

Table 4. *E. coli* serotypes recovered from diarrheic rabbits:

| Serotype | Number | Percentage |
|---|---|---|
| O158 | 5 | 25 |
| O128 | 3 | 15 |
| O125 | 4 | 20 |
| O18 | 2 | 10 |
| O20 | 2 | 10 |
| O148 | 1 | 5 |
| Untypable | 3 | 15 |
| Total | 20 | 100 |

**Results of antimicrobial Susceptibility Testing:**

*E. coli* isolates were highly resistance to Ampicillin (AM) (83.3%; 71/85), Cefoxitine (CF) (60%; 51/85), Sulfamethoxazole (SXT) (56.5%; 3156/85), chloramphincol (CHI) (57.7%; 56/85), Kanamycin (KAN) and Streptomycin (S) (41/85; 48.8%) (Table 5). Resistance to other antimicrobial agents was always less than 30%.

Table 5. Antibacterial resistance of tested *E. coli* isolates

| Resistanceantibiotics | Total n = 85 (%) |
|---|---|
| Ampicillin(AMP) | 71(83.3) |
| Gentamycin(GM) | 10(11.9) |
| Sulfamethoxazole(SXT) | 55 (56.5) |
| Cloramphenicol(CHI) | 56 (57.7) |
| Kanamycin (KAN) | 41 (48.8) |
| Streptomycin (S) | 41(48.8) |
| Cefoxitin (Cf) | 51 (60) |
| Nalidixicacid(Na) | 22 (26,2) |
| Amikacin (AK) | 5 (5.9) |
| Ofloxacine(Ofx) | 10 (11.9) |
| Ceftriaxone(Cef) | 21 (26.9) |
| Norfloxacin (Nx) | 5 (5.9) |
| Ciprofloxacin(Cip) | 1 (1.1) |

**Results of cPCR FOR detection of *E. coli* virulence genes:**

PCR assay was carried out on 6 *E. coli* isolates (two isolates from each farm) serotypes (O158, O128, O125, O119, O18 and O148) to detect two virulence genes (eaeA and tsh). It was found that 100 %( 6/6) of tested *E. coli* isolates carry eaeA gene, while 66.7 %( 4/6) of the tested *E. coli isolates* were positive to tsh gene

Table 6. Prevalence of virulence genes (eaeA, tsh,) using cPCR among Isolated *E. coli* isolates:

| Gene | *E. coli* isolates | Percentage |
|---|---|---|
| eae A | 6/6 | 100% |
| Tsh | 4/6 | 66.7% |

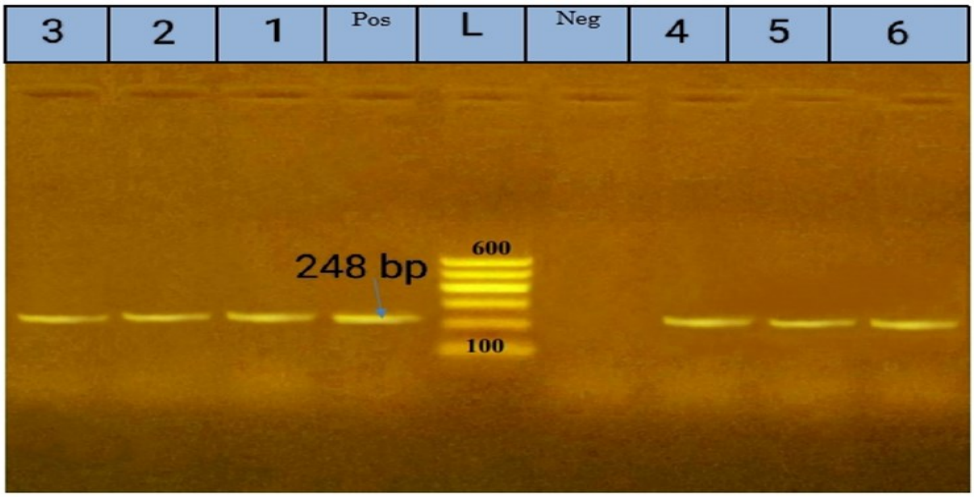



Fig (1): Agarose gel electrophoresis of conventional PCR for detection of *eaeA* showing amplification of 620 bp. fragment.
L (ladder): 100-600 bp.
(Pos): positive control.
(Neg): negative control.
Lanes: (1, 2, 3.4, 5, 6) positive samples.

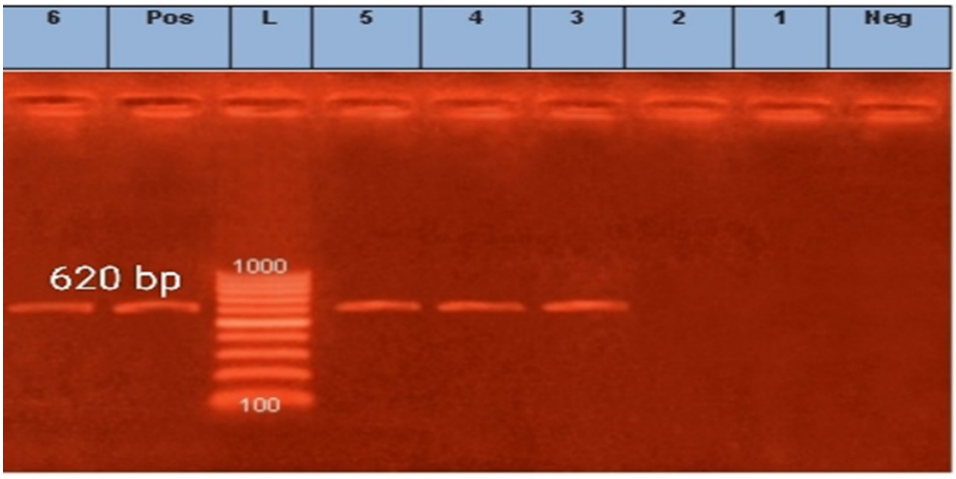



Fig (2): Agarose gel electrophoresis of conventional PCR for detection of *Tsh* showing amplification of 620 bp. fragment.
L (Ladder): 100-600 bp.
(Pos): positive control.
(Neg): negative control.
Lanes (3, 4, 5and 6): positive samples.
Lane (1and 2): negative sample

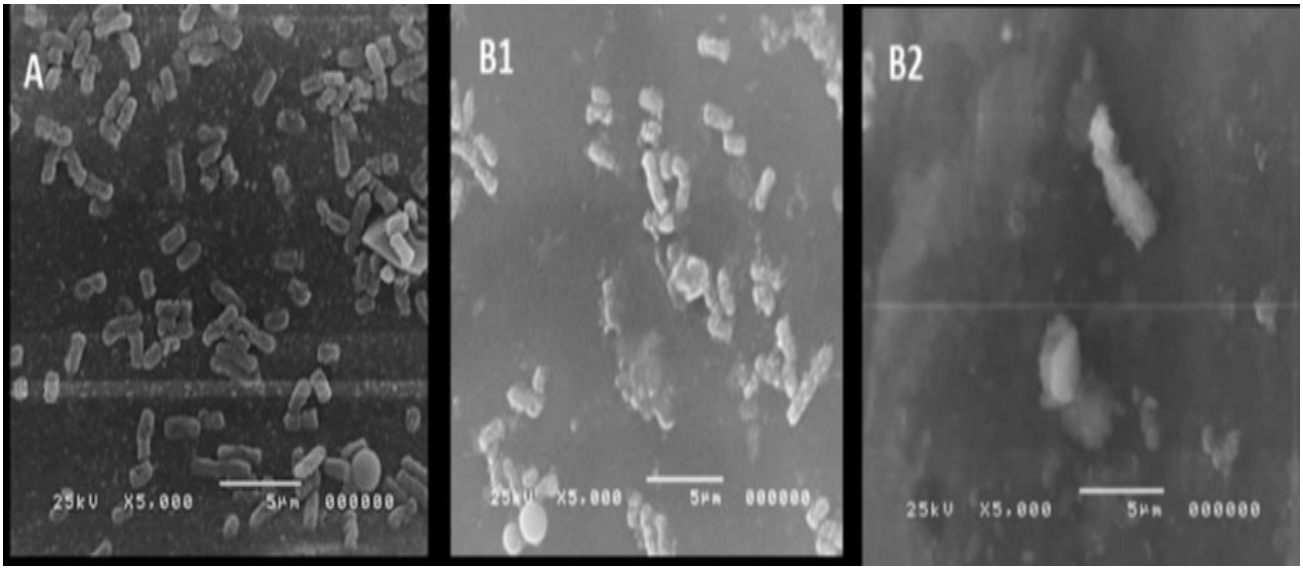
Table 7. The distribution of minimum inhibition concentration (MIC) values of AgNPs concentrations against *E. coli* isolates:

| Source | No. of sensitive *E. coli* isolate at different AgNPs concentrations (µg/mL) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 5 | 2.5 | 1.25 | 0.625 | 0.312 | 0.156 | 0.078 |
| Diahrroeic rabbits | 2 | *2* | 3 | 5 | 0 | 0 | 0 |

**Results of electron microscopy scanning:**

SEM was utilized to determine the differences in bacterial morphology after application of the of AgNPs-H2O2 For 1 hour treatment, a sample suspension of *E. coli* was mixed with AgNPs-H2O2 at 1 MIC and 2 MIC concentrations, and the microstructure was examined (Fig. 3). Bacteria that had not been treated were used as a control. SEM was used to examine changes in bacterial morphology that had been treated and had not been treated. Significant alterations (exhibited varying degrees of distortion) in the morphology of microbial cells treated with AgNPs-H2O2 (Fig 3B1, B2) were detected when compared to untreated cell which exhibited rod-like form with smooth undamaged surfaces (Fig 3A).

Figure (3): Scanning electron micrographs. (A): untreated *E. coli,* (B1): E. coli treated with AgNPs-H2O2 at 1 MIC, (B2):E. coli treated with AgNPs-H2O2 at 2 MIC.




Untreated E. coli Treated E. coli with 1 MIC AgNPs-H2O2 Treated E. coli with 2 MIC AgNPs- H2O2

Table 8. Results of experimental infection with *E. coli* in 30 days old rabbits

| | Clinical signs and mortality rates \days of infection | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | 0 | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th |
| G1 No. (30) | - | - | - | - | - | - | - | - | - | - | |
| G2 No. (30) | - | - | 1 diarrhea & weakness | 4 diarrhea weakness ruffed fur. | 5 bloody diarrhea +1 mortal | 7 diarrhea 3 mortal | 13 diarrhea 2 mortal | 15 Diarrhea 5 mortal | 17 Diarrhea 2 mortal | 20 Diarrhea 2 mortal | 20 Diarrhea 2 mort |
| G3 No. (30) | - | - | - | - | - | 1 Slight diarrhea | - | - | 1 Slight diarrhea | - | |
| G4 No. (30) | - | - | - | 1 diarrhea | 2 Diarrhea +weakness | 1 mortal +5 diarrhea | 1 mort +1 diarrhea | - | 1 mort 4 diarrhea | - | 1 Mort 7 diarrhea |

Table 9. Reisolation rates of *E. coli* experimental isolate from experimentally infected rabbits:

| | 0 | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | 10th |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G1 | - | - | - | - | - | - | - | - | - | - | - |
| G2 | - | $10^5$ | $10^9$ | $2*10^9$ | $3*10^9$ | $4*10^9$ | $5*10^9$ | $6*10^9$ | $6*10^9$ | $5*10^9$ | $3*10^9$ |
| G3 | - | - | - | - | - | - | - | - | - | - | - |
| G4 | - | - | - | $10^3$ | $10^3$ | $10^5$ | $10^6$ | $10^6$ | $10^7$ | $10^8$ | $10^9$ |

## DISCUSSION

Diarrhea is one of the major problems facing rabbitries in Egypt, causing high mortalities **(Stakenborg et al. 2006).**

Three hundred rectal swabs from diarrheic rabbits were subjected for isolation and identification of *E. coli* The result indicated that 85 isolates were biochemically identified as *E. coli*, the allover incidence of *E. coli* isolation from a rabbit with diarrhea was 28.33% (85/300). This result is less than that isolated by **Morsy et al. (2002)** as they recovered *E. coli* from newly weaned rabbit by 80% from examined samples (60/90). While **Shahin et al. (2011*)*** isolated *E. coli* by 65.7% from diarrheic rabbit.

Concerning, seasonal prevalence of *E. coli infection* in weaned rabbits. The result showed that, summer season was found to be the most important season that influenced the post weaning diarrhea in rabbits. (44%) during summer compared to 22%, 13.33% and 31% during autumn, winter and spring seasons respectively. This result higher than **Habeeb et al. (1997)** who showed that, the mortality rate was found to be 18% in summer season, while no mortality was recorded during winter season. While, **Shehata et al. (1998)** recorded

18.52% mortality rate during summer season compared to (3.70, 7.41and 7.41%) during spring, autumn and winter seasons, respectively.

Concerning, serological serotyping in the current study, 20 *E coli* isolates recovered from weaned rabbits were distributed among 6 different O serotype groups besides untypable ones. The most prevalent serotypes were O158 (25%), O125(20), O128 (15%) followed by O18, O119 (10%), O148 (5%) and untypable strains were (15%). Such results were similar to **Percy et al. (1993)** as they isolated 0128, and untyped from newly weaned diarrheic rabbits. **Leroy et al. (1994)** recovered 0128. **Saad (1994)** isolated E. coli O125 from weaned rabbits. **Aisha and Youseif (1999)** isolated 0128, O125, O158 and untyped strains. **Alshimaa (2007)** isolated *E. coli* serogroup O125 from rabbits with enteritis. **Morsy et al. (2002)** found that, Serotypes associated with diarrhoea in newly weaned rabbits in Ismailia were (O119, O103, O55, O153, O128) and untypable ones with variable percentages. **Blanco et al. (1997)** and **Marches et al. (2000) found** that, most common serotypes among E. coli strains associated with diarrhea in rabbit in order of frequency were (0103, 049, 026, 0128, 092) on the other hand **(Leroy et al 1994)** recorded the isolation of 6 nonpathogenic diarrheic *E. coli* belonged to (0128) and (0132).

The majority of isolates had high resistance to Ampicillin (AM) (83.3%), Cefoxitin (CF) (60%), Sulfamethoxazole (SXT) (56.5%), chloramphenicol (CHI) (57.7%), Kanamycin (KAN), and Streptomycin (S) (48.8%). Antimicrobial resistance develops as a consequence of antibiotic consumption, along with transmission of resistant genes and bacteria across animals, animal products, humans and the surrounding area. Antibiotic-resistant bacteria may be spread directly between animals and people, as well as to soil, food, and underground water. **(Wichmann et al. 2014).**

The highest drug resistance found could be due to the widespread use of antimicrobials as a therapeutic and preventative treatment in animal production farms **(Mohammed et al. 2014)** and **(Taye et al. 2013).** These findings show that rabbits may act as a reservoir for antibiotic-resistant E. coli, as well as a vector for their dissemination and a health danger. Many studies have indicated that isolated E. coli was resistant to erythromycin (100%), ampicillin (50%), tetracycline (75%), streptomycin (50%), but was highly susceptible to penicillin (100%), chloramphenicol (75%), gentamicin (75%) and amoxicillin (50%) recorded by **(Kindu et al. 2019).**

Results of PCR analysis (table: 6) showed that (6/6) 100% of tested E. coli strains isolated from weaned rabbits with diarrhea carried (eaeA) virulence gene. This result agreed with **Blanco et al. (2005)** who reported that, fecal culture examination of 20 rabbits yielded 48 *E. coli* isolates, 83% of which were eaeA positive. **Alexis and James (2003)** found that, (25%) of 28 rabbits were positive for eaeA gene.

Based on the obtained molecular results, it explained the severity of clinical signs and morbidity and mortality of weaned rabbits where, The intimin (eaeA gene) considered as indicator of attaching and effacing pathogenicity factor. It was present in100% of tested isolates. So, this gene is clearly associated with diarrhoeagenic E. coli which increased the severity and duration of diarrhea as well as mortality and rabbit inflammatory response **(Mashood et al. 2009).**

Concerning, tsh gene, is another adhesion factor. The result of this study proved that *E. coli* isolates for the presence of temperature sensitive hemagglutinin gene (tsh) revealed that (4/6) 66.7% of the examined E.coli strains from weaned rabbits with diarrhea bearing the virulence gene (tsh). Which go in parallel with **Hanchun et al. (2004)** who detected tsh gene in 93% of E. coli isolates from diseased animals with diarrhea While, this result not matched with **Abhirrosh and Asit (2013)** who did not detect tsh gene in the tested E. coli strains isolated from diarrheic rabbits. Meanwhile, **Maluta et al. (2014)** suggested that, EPEC might induce fluid accumulation in the rabbit gut. **Hagedorn et al. (2011) reported** that, although tsh gene associated with the bird, it was also found in 46% of *E. coli* isolates.

Nanoparticles, such as silver nanoparticles, are considered alternatives to antibiotics for treating various infections. Silver nanoparticles have large surface area to volume ratio, allowing for increased contact with bacteria and resulting in direct interaction with the bacterial cell wall to produce antibacterial activity **(Castillo RR, et al. 2019).** Results showed that the MIC50 of AgNPs-H2O2 was 0.625 µg/mL against E. coli. **Ahmed et al. (2023)** reported that AgNPs-H2O2 at concentrations of 0.625, 1.25, 2.5 and 5 µg/mL showed complete bacterial growth inhibition. Another study reported that the average MIC value of AgNPs against ESBL-producing E. coli was 27 µg/ml. Krishna et al (2018) reported that the in vitro antibacterial tests against C. jejuni showed a minimal inhibitory concentration of AgNP at the level of 50 ppm. **Shafreen, et al. (2017)** argued that silver nanoparticles suspensions prepared by biological methods and with concentrations higher than 100 µg/mL may lose their antibacterial effect on microorganisms.

SEM was used to examine the morphological alterations of both treated and untreated E.coli. Untreated *E. coli* cells (control) which exhibited usual rod-shaped characteristics, with normal and smooth cell surfaces (Fig. 3A). After 60 min of treatment with the AgNPs-H2O2 at 1 MIC, the majority of bacteria treated grew irregular with varying degrees and the membrane has burst in some cells (as shown in Fig.3B1). Greater effects to the *E. coli* cell membrane were seen after 60 min of treatment with AgNPs-H2O2 at 2 × MIC, nearly every cell exhibits membrane breakdown and rupture, (Fig.3B2).

According to **Zhang et al. (2016),** the unfavorable morphological changes of *E. coli* cells might be caused by the rupture of the *E. coli* membrane and the loss of intracellular components. Bacterial cells were likely destroyed as a result of the cytoplasmic membrane being broken or penetrated by an interfacial contacting inhibitory action on the surface (Kong *et al.,* 2008). The targeted cells deformity induced by AgNPs-$H_2O_2$ depends on specific dose that was corroborated by other investigations **(Diao et al, 2014).** The recorded SEM pictures in this work are similar to prior studies on the effects of various antimicrobial drugs on *E. coli* cells, such as cinnamon oil **(Ma et al. 2016 and Zhang et al. 2016).** Other antibacterial agents include mustard EO **(Turgis et al., 2009),** cold nitrogen plasma, and clove oil **(Cui et al. 2016),** a thymus nanoemulsion **(Moghimi et al. 2016).** These SEM findings corroborate our antibacterial activity findings, which revealed that AgNPs-$H_2O_2$ was capable of killing *E. coli* at 1 MIC and were significantly more effective at 2 MIC.

Concerning experimental infection, the negative control group showed no signs of infection, validating the health status of the non-infected rabbits. In contrast, the positive control group exhibited clinical symptoms and significantly elevated bacterial load, indicating successful induction of E. coli infection and expressed high virulence as clinical manifestations and modalities occurred specially in control positive group which reached its peak at the $10^{th}$ day. The Norfloxacin-treated group demonstrated a notable reduction in bacterial load and clinical improvement compared to the positive control group. Similarly, the nanotechnology-treated group exhibited a reduction in bacterial load and clinical signs, indicating potential treatment efficacy. The findings highlight the potential of both Norfloxacin and nanotechnology in reducing E. coli infection. Norfloxacin, a widely used antibiotic, demonstrated its effectiveness in mitigating bacterial load and clinical symptoms. However, concerns about antibiotic resistance necessitate exploring alternative treatments. The use of nanotechnology in managing E. coli infection presents a promising avenue as it minimized bacterial shedding. Nanoparticles have shown antimicrobial properties and the ability to target specific pathogens, making them a potential alternative or adjunct to traditional antibiotics

## CONCLUSION:

Silver nanoparticles products are considered a promising alternative medication for treatment E. coli infection and could reduce the risk of antimicrobial re-

sistance.

Further intensive studies are required for implementation of the effective alternative medication to reduce the risk of antimicrobial resistance.

**Ethics approval**:

The study received approval from the Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC) under approval number
ZU-IACUC/2/F/117/2023.

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