Synthesis & Antibacterial Effect of Metallic Oxide Nanoparticle against of Escherichia coli Isolated of Tabriz Aviculture

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ABSTRACT
Escherichia coli is one of the common microbial flora of gastrointestinal and respiratory tracts of poultry but may become pathogenic to both. Antibiotic treatment is considered the most important issue that promotes the emergence, selection and deploying of antibiotic-resistant microorganisms in veterinary medicine. With the increase of resistant to antibiotics, many researchers have tried to develop new. So, antibacterial nanoparticles have attracted great interest. The aim of this research is comparison antibacterial activity of Ag/ZnO with Zinc Oxide and Silver nanoparticles, against E.coli Isolated of Tabriz Aviculture. Nanoparticles are synthesis via wet method and avouch with oxalate decomposition in high temperature (500°C). FT-IR, XRD, SEM and TEM were used for determination of characterization of samples, respectively. Also the nanoparticles were digested and break down by ICP-AES for defining the presence of residual chemical element in the nanoparticles. Bacterial sensitivity to nanoparticles, after sonication, was commonly tested using by disc diffusion test and agar dilution test, also with determination of minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). The particles size were less of 12 nm, approximately. This study shows that the Ag/ZnO nanoparticle has great antimicrobial agent against E.coli and just mixture of ZnO and Ag nanoparticles, give increase their bactericidal effect.

1. Introduction

At the recent time, multi drug resistant strains of E. coli are presentsin both human and animal isolates (Amara, Ziani, & Bouzoubaa, 1995) and multiple drug resistant, nonpathogenic E. coli found in the intestine is probably an important reservoir of resistance genes (Osterblad et al., 2000) and momentarily drug-resistant E. coli of animal origin may colonize the human intestine (Marshall, Petrowski, & Levy, 1990). Acquired multi drug resistance to antimicrobial agents make an extensive trouble in case of the management of intra and extra intestinal infections caused by E. coli, which are a major source of illness, death, and increased healthcare costs (Gupta, Hooton, & Stamm, 2001) and cause a variety of lesions in immune-compromised hosts moreover in poultry. Among the diseases some are often severe and sometimes lethal infections such as meningitis infection, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (Daini, Ogbolu, & Ogunledun, 2004) and Yolk Sac Infection, omphalitis, cellulitis, swollen head...
syndrome, Coli granuloma, and Colibacillus’s (Gross, 1994). Antibacterial properties of nano metal oxide have been discovered as new generation of antimicrobial agent and researchers have introduced the use of silver and zinc ions as superior disinfectants for from hospitals infectious microorganisms (Lin, Vidic, Stout, & Victor, 1996; Sondi & Salopek-Sondi, 2004; Yu-sen, Vidic, Stout, McCartney, & Victor, 1998). Although, they have believed that residual these metal ions may adversely affect human health (Blanc, Carrara, Zanetti, & Francioli, 2005), but scientists’ experiments demonstrated selectivity in the toxic nature of ZnO nanoparticle to different bacterial systems and human T lymphocytes. They suggested that ZnO nanoparticle may potentially useful as nanomedicine based antimicrobial agents (Sondi & Salopek-Sondi, 2004). We know that the bactericidal effect of metal nanoparticles have been ascribed to their size, photo-catalytic activity, which allows them to interact with microbial membranes and is due to the release of metal ions in solution (Morones et al., 2005). While various hypotheses have been proposed to explain the mechanism of antimicrobial activity of silver nanoparticle, it is widely believed that silver nanoparticle are incorporated in the cell membrane, which causes leakage of intracellular substances and eventually causes cell death (Cho, Park, Osaka, & Park, 2005; Thirumurugan, Shaheedha, & Dhanaraju, 2009). Some of the silver nanoparticle also inter penetrate into the cells. It is also reported that bactericidal efficiency is affected by the type of microorganism. In investigate with gram negative, E. coli, and gram positive, S. aureus, Kim reported greater biocidal efficiency of silver nanoparticle for E. coli and attributed it to difference in cell wall structure between gram negative and gram positive microorganisms (Kim et al., 2007). Also, Jayesh suggested that combination of metal oxide nanoparticles may give rise to more complete bactericidal effect against bacterial population (Jafari, Ghane, & Arastoo, 2011; Jafari, Ghane, Sarabi, & Siyawashifar, 2011). This study has been undertaken to isolate and characterize E. coli strains from aviculture of Tabriz for assessing their susceptibility patterns to some nanoparticle antimicrobials.

2. Materials and Methods

2.1. Sampling from excrement swab

Sampling for 4 months, from 15 April to 15 July 2015 was conducted. After the autopsy, samples were prepared from liver of chicken with sterileswabs from different aviculture of Tabriz and were transferred via BHI broth to the microbiology laboratory.

2.2. Bacteriological analysis

A loop sample from Lung swab were spread on the Eosine-Methylene Blue (EMB) agar medium (MERCK, Germany). All samples were incubated for 24 hours at 37°C into EMB for successful isolation of typical colonies. Identification was done base of biochemical tests included gram staining, tests for oxidase, Methyl red, Voges-Proskauer reactions, Indole, Catalase, Urea hydrolysis, Citrate, Gelatin hydrolysis tests, also Lactose fermentation, Nitrate reduction, casein hydrolysis and sugar fermentation.

2.3. Synthesis of ZnO nanoparticle

Zinc acetate (MERCK, Germany) was added to ethanol (MERCK, Germany) in a two-neck flask giving a 0.3 M white solution. The temperature was elevated to 50°C and after 30 min of continuous stirring oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Zn:OA was 1. The system was kept at 50°C under reflux for 2 h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and the arising viscous gel was dried at 80°C overnight. The dried Zinc oxalate was ground and calcined at 550°C for 2 h.

2.4. Synthesis of Ag monometallic nanoparticle

Silver nitrate (MERCK, Germany) was added to ethanol (MERCK, Germany) in a two-neck flask giving a 0.3 M gray solution. The temperature was elevated to 50°C and after 30 min of continuous stirring oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Ag:OA was 1. The system was kept at 50°C under reflux for 2 h and a white precipitate was obtained; then the acetic acid and some of the ethanol were released moisture and
the arising viscous gel was dried at 80 °C overnight. The dried silver oxalate was ground and calcined at 550 °C for 2 h.

2.5. Synthesis of Ag/ZnONanoparticle

Zinc chloride (MERCK, Germany) and silver nitrate (MERCK, Germany) were added to ethanol (MERCK, Germany) in a two neck flask giving a 0.3 M gray solution. The temperature was raised to 50 °C and after 30 min of continuous stirring, oxalic acid (MERCK, Germany) was rapidly added to the solution. The molar ratio Zn/Ag:OA was 1. The system was kept at 50 °C under reflux for 2 h and a gray precipitate was obtained; then the resulting viscous gel was dried at 80 °C overnight. The dried Ag/ZnO oxalate was ground and calcined at 550 °C for 2 h.

2.6. Characterization

Experiences of dependent on the crystallinity of the nanoparticles were carried out using a X-ray diffractometer set (XRD, Bruker D8-Advance Diffractometer using Cu Kα radiation). Also the nanoparticles were digested and analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, LIBERTY-RL, Varian Australia Co.) for determining the presence of residual chemical element in the nanoparticles. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker spectrophotometer in KBr pellets. Surface morphology of product was characterized by using a scanning electronic microscopy (SEM, Cam Scan MV2300) with an accelerating voltage of 30 KV and TEM.

2.7. Disk diffusion test

Bacterial sensitivity to antibiotics is commonly inspected by a disk diffusion test, employing antibiotic impregnated disk (Case & Johnson, 1984). A 10 ml suspension of each nanoparticle (approximately, 16384 μgml⁻¹) was prepared into the Muller Hinton Broth medium (MERCK, Germany) and then each nanoparticle was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute. The bacterial inoculum (1.5×10⁸ CFUml⁻¹) was cultured completely on the surface of a Muller Hinton agar plate before sonication, and then 100 μg.ml⁻¹ from suspension of nanoparticles were filled into the cavities. The plate was incubated at 35°C for 24h, after which the average diameter of the inhibition zone enclosing the cavities was measured with a ruler with up to 1 mm resolution. The examination was also replicated, and so the results were compared together. Subsequently, the tests were reported for each nanoparticle and with E. coli strain on three replicates.

2.8. Agar Dilution test

A 16384 μgml⁻¹ suspension of each nanoparticle was prepared into the Muller Hinton Broth medium, approximately, and then each nanoparticle was sonicated at room temperature and frequency of 28 KHz, during at the 10 minute. The bacterial inoculum (1.5×10⁸ CFUml⁻¹) was cultured completely on the surface of a Muller Hinton agar plate before sonication, and then 100 μg.ml⁻¹ from suspension of nanoparticles were filled into the cavities. The plate was incubated at 35°C for 24h, after which the average diameter of the inhibition zone enclosing the cavities was measured with a ruler with up to 1 mm resolution. The examination was also replicated, and so the results were compared together. Subsequently, the tests were reported for each nanoparticle and with E. coli strain on three replicates.

2.9. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The lowest concentration of material that inhibits the growth of an organism was defined as the minimum inhibitory concentration (Sondi & Salopek-Sondi, 2004)(MIC). From the serial dilution method, were employed for determining the MIC of the nanoparticles (Daini et al., 2004). The test tubes were filled with 1ml of the liquid Muller Hinton broth medium. Into each of the test tubes number 1 and 2, one ml solution containing 16384 μg.ml⁻¹ of nanoparticles that had been sonicated at room temperature and frequency of 28 KHz, during at the 10 minute,
already was added and mixed thoroughly with the culture medium. The concentration of nanoparticles in each test tube become 8192 μg.ml⁻¹. Then, 1 ml of the content of test tube number 2 was added to test tube number 3 and mixed completely. This process was performed serially to test tube number 16. Consequence, 1 ml content of test tube number 16 was discarded. In order to have equal amounts of material in all the test tubes, 0.9 ml of test tube number 1 was discarded. Finally, 0.1 ml of microbial suspensions (E.coli) containing 1.5x10⁸ CFU ml⁻¹ microorganism, were added to test tubes number 2 to 17, and the test tubes were incubated at 35 °C for 24 h. Then, the microbial growth was studied by turbidimetric measurement, using a spectrophotometer (Nanovolume-spectrophotometer, Scandrop 250, Analytikjena Co.). The experiments also included a positive control (test tube containing nanoparticle and Muller Hinton broth medium, devoid of inoculum) and a negative control (test tube containing inoculum and Muller Hinton broth medium, devoid of nanoparticle). The negative controls indicated the microbial growth profile in the absence of nanoparticle(Jafari, Ghane, Sarabi, et al., 2011; Kawashita et al., 2000; Yang et al., 2006). All the experiments were carried out in triplicate. The minimum bactericidal concentration (MBC), i.e., The lowest concentration of nanoparticles that kills 99.9% of the bacteria was also determined from the batch culture studies. For growth inhibitory concentration (MIC) the presence of viable microorganisms was tested and the lowest concentration causing bactericidal effect was reported as MBC as suggested by Avadi(Avadi et al., 2004). Two experiments for bactericidal effect, a loopful from each test tube (Especially, negative & positive test tubes) was inoculated on Muller Hinton agar and incubated at 35 °C for 24 h. The nanoparticle concentration illustrating the bactericidal effect was picked out based on absence of colonies on the agar plate. The release of Ag⁺ and Zn²⁺ ions from the nanoparticles into DI water and Muller Hinton broth medium were deliberated by suspending 10 mg of nanoparticles in 100 ml DI water/medium and sonicating with ultrasonic set (PARSONIC 7500s, Pars Nahand ENGG. Co. IRAN) for 10 min. The suspension was kept in a rotary shaker (Gyrotwister 3-Dshaker, labnet Co. USA) under the same conditions as in the above studies and residual Ag⁺ and Zn²⁺ concentration in the aqueous phase was defined by ICP-AES after 24 h.

2.10. Antibiotic Sensitivity Test

To determine the sensitivity of standard strain of E.coli to common antibiotics, Disc Diffusion AgarTest with Ceftriaxone (30μg), Gentamicin (10μg), Ciprofloxacine (5μg) and Cefazolin (30μg) antibiotic disks on Mueller Hinton agar medium was done. After 24 hours of incubation, the diameter of growth inhibition around each disc was measured and compared. The disc, which has the maximum diameter of the inhibitory zone; it is more effective to destroy bacteria.

3. Results

3.1. Bacteriological analysis

Escherichia coli isolated from poultry and poultry environment, was subjected to various morphological and biochemical tests used for identification. The distribution pattern and the biochemical tests for identification of E. coli isolated from poultry sources is summarized in Table 1.

3.2. The FT-IR spectra analysis

Fig. 1 shows FT-IR spectra of (a) Ag, (b) ZnO and (c) Ag/ZnO. The supplement of oxalic acid to the ethanol solution of Ag caution was caused to the precipitation of a gray solid of silver oxalate as shown by FT-IR spectrum in Fig. 1a. The broadband at 3427.33 cm⁻¹ was allocated to both the ν(O-H) and ν₆(H₂O) of hydration water. The extreme band at 1634.68 cm⁻¹ was allocated to asymmetric and water tensional tremble δ(H-O-H). The shoulder at 1428.89 cm⁻¹ is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 875.31 cm⁻¹ and 577.35 cm⁻¹ are present in the spectrum evidence of (O-C-O) tensional tremble and (M-O) tremble, respectively. Fig. 1b depended to ZnO FT-IR spectrum. The broadband at 3445.05 cm⁻¹ was allocated to both the ν₆(O-H) and ν₆(H₂O) of hydration water. The extreme band at 1629.57 cm⁻¹ was allocated to asymmetric and water tensional tremble δ (H-O-H). The shoulder at 1428.89 cm⁻¹ is present in the spectrum evidence of (N-O) tremble and the
closely spaced bands at 876.14 cm\(^{-1}\) and 551.12 cm\(^{-1}\) are present in the spectrum evidence of (O-C-O) tensional tremble and (Zn-O) tensional tremble respectively. Also, Fig. 1c conclude Ag/ZnO FT-IR spectrum. The broadband at 3426.47 cm\(^{-1}\) was allocated to both the \(v_3(O-H)\) and \(v_\text{as}(O-H)\) of hydration water. The extreme band at 1628.14 cm\(^{-1}\) was allocated to asymmetric and water tensional tremble \(\delta (H-O-H)\). The shoulder at 1458.22 cm\(^{-1}\) is present in the spectrum evidence of (N-O) tremble and the closely spaced bands 625.36 cm\(^{-1}\) are present in the spectrum evidence of (Ag/ZnO) tensional tremble respectively.

3.2. The XRD spectra analysis

The XRD pattern of Ag, ZnO and Ag/ZnO nanoparticles (Fig. 2 a, b and c) were compared and interpreted with standard data of International Centre for Diffraction Data (ICDD). The average crystallite sizes (C.S) of the nanocrystals were calculated using the Debye-Scherer Equation from the major diffraction peaks (C.S= \(K\lambda / \beta \cos \theta\)). Where At half maximum (FWHM) of the diffraction peak in radiant and \(\theta\) is the Bragg angles of the main planes. The average crystallite size of the Ag, ZnO and Ag/ZnO were 8.66 nm, 24.75 nm and 12.15 nm, respectively.

3.3. The ICP-AES spectra analysis

By ICP-AES analysis, we succeed to estimation of residual ions, after digestion of nanoparticles by sonication. They indicated ions levels of 120 ppm of silver and \(\leq 1\) ppm of zinc oxide, in the silver/Zinc Oxide nanoparticles, respectively.

3.4. The TEM and SEM images analysis

TEM images of silver nanoparticles were taken (Fig. 3) and approved that the metal particles were in the nano range, approximately. However, SEM images (Fig. 4) of nanoparticles were shown that silver; zinc oxide and silver/zinc oxide metal particles were exactly in the shape of spherical and clustered.

3.5. Antibiotic Sensitivity Test

After 24 hours of incubation, the diameter of growth inhibition around each disc was measured. In fact, the disc, which has the maximum diameter of the inhibitory zone; it is more effective to destroy bacteria. The results in depended of the antibacterial effects of currency antibiotic against \(E. coli\) via the disc diffusion test show that the \(E. coli\) had most sensitivity against of Ciprofloxacin (5\(\mu\)g) with \(\geq 30\) mm inhibition zone.

3.6. The antibacterial activity of Ag/ZnO analysis

The antibacterial activity of silver, zinc oxide and silver/zinc oxide nanoparticles were compared for \(E. coli\) using the diameter of inhibition zones in disk diffusion test and Agar dilution test. In fact, the diameter of inhibition zone (DIZ) reflects the dimension of impressionability of the bacteria. We knew, the strain susceptible to disinfectants demonstrates larger DIZ, while resistant strain exhibit smaller DIZ. The disks with silver and Zinc oxide nanoparticles were compared to the silver/zinc oxide nanoparticles for \(E. coli\) strain. The DIZ for zinc oxide and silver/zinc oxide nanoparticles impregnated disks were almost greater than that studied with the silver nanoparticles impregnated disks for \(E. coli\) strain. Correspondingly, for \(E. coli\), the silver and silver/zinc oxide nanoparticles impregnated disks were found to be more effective compared to zinc oxide nanoparticles impregnated disks, however the difference in the DIZ was merely 10–15% percent. Since DIZ was measured on agar plates using a ruler with 1 mm resolution, the possibility of measurement errors exists. The results in depended of the antibacterial effects of nanoparticles against different of bacterial via the disc diffusion test, the agar dilution test the MIC and the MBC are summarized in Table 2. A greater lag phase and lower maximum absorbance (at 600 nm) were observed as the concentration of nanoparticles increased.

A Similar observation was reported by Sondi (Sondi & Salopek-Sondi, 2004). We investigate silver, Zinc oxide and silver/zinc oxide nanoparticles against \(E. coli\). The bactericidal effect of nanoparticles is dependent on the concentration of nanoparticles and the initial bacterial concentration. In this study, the initial bacterial concentration was constant at \(1.5 \times 10^8\) CFU ml\(^{-1}\) regardless of nanoparticles concentration and microbial strain. The MIC observed for zinc oxide nanoparticle was 64\(\mu\)gm l\(^{-1}\) for \(E. coli\). Surprised, antibacterial
effect of the silver nanoparticle was weak for *E. coli*. The MIC observed for silver nanoparticle was 2048 µg ml⁻¹. In contrast with the nanoparticles that picked out for this study, the most antibacterial effect was seen to silver/zinc oxide nanoparticle. Interestingly, the *E. coli* was most sensitivity against of silver/zinc oxide nanoparticle. In fact, our research shows that silver/zinc oxide nanoparticle has got antibacterial effects against *E. coli*. The MBC observed in this study for silver/zinc oxide nanoparticle was 128 µg.ml⁻¹.

4. Discussion

Studies of Ruparelia indicated that in aqua medium, no systematic change in the size of nanoparticles observed after 24 h (Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008). In the current study, after synthesis of nanoparticles of metal oxides Ag, ZnO and combined nanoparticles of Ag/ZnO, their antibacterial effects compared. Though, studies of several authors in recent years, confirmed the antibacterial effects of Ag nanoparticle (Marshall et al., 1990; Williams, Ehrman, & Holoman, 2006). In the current study, disc diffusion and agar dilution methods used for determining the antibacterial effects of nanoparticles. Rupareliaand Jafari, et al. performed extensive experiments in the determination of microbial sensitivity of various bacteria to silver and copper nanoparticles, using Disc Diffusion Method (Jafari, Ghane, & Arastoo, 2011; Ruparelia et al., 2008). Regarding that the diameter of inhibition zone (DIZ), reflects the sensitivity of the organism, strains of sensitive, show larger DIZ and the resistant strains show smaller DIZ. The Results of the disc diffusion with Ag nanoparticle, by Thirumurugan against strains of pathogens *E.coli, salmonella typhi, Bacillus subtilis, Staphylococcus aureus*, indicated higher sensitivity to silver nanoparticle which is in contrast with the results of our study (Thirumurugan et al., 2009). Cho studies the MIC of Ag nanoparticle against *Pseudomonas aeruginosa* in which its growth in a concentration of 7.5 µg ml⁻¹ completely inhibited (Cho et al., 2005). Following their research project, Cho reported the MIC rate of silver nanoparticle for *Staphylococcus aureus* as 12.6 µg.ml⁻¹, but the MIC for these bacteria affected by silver nanoparticle reported 2048 µgml⁻¹. The *E. coli*, used in the current study, showed the least and the most sensitivity to silver and Ag/ZnO nanoparticle, respectively. Actually, the least degree of MIC in *E. coli*were related to combined nanoparticles of silver and zinc with concentration of 128 µg.ml⁻¹. i.e, notwithstanding those nanoparticles had the most growth inhibitory effect in *E. coli*. Lock concluded that the most the dimension of silver nanoparticle, the better would be the MIC and their antibacterial effect would decrease (Lok et al., 2006). The aim was to indicate that the sensitivity of different strains of one bacteria to silver nanoparticle shows meaningful difference. Kim studied the gram negative bacteria *E. coli* and gram positive *Staphylococcus aureus*, also reported that the antibacterial silver nanoparticle mostly affects the *E. coli*, which is due to the difference between cell wall of gram negative & positive microorganisms (Kim et al., 2007). Rate of MIC obtained by Ping Li (Li, Li, Wu, Wu, & Li, 2005) for silver nanoparticle and against *Staphylococcus aureus* were 0.625 µg ml⁻¹. Limited studies performed on the antibacterial properties of ZnO. Reddy were amongst few authors worked on the toxicity of the ZnO nanoparticle in gram negative & positive bacteria (Reddy et al., 2007). They found nanoparticle is able to completely inhibit the growth of *E. coli*. Up to now, no complete and comprehensive study reported in the field of combining antibacterial nanoparticles and the comparison of their antibacterial properties for *E. coli*. Amongst few studies, Yang combined silver nanoparticle with Zn to improve the antibacterial activity of Zn nanoparticle and investigate the antibacterial effect of Zinc oxide and silver nanoparticles and also comparing them with Ag/ZnO nanoparticle (Yang et al., 2006). They obtained interesting results. According to the findings of Kawashita and Park & Jang, 2003) found that silver nanoparticles significantly increases antimicrobial activity. Actually, Yang believed that the photocatalytic ability of ZnO nanoparticle plus silver nanoparticle improves and also increases its oxidation and reduction abilities, while suppressing bacteria growth (Yang et al., 2006). However, silver ions, eventually, release during sterilization and kill bacteria due to their high antibacterial activation. They theorized that silver ion release following bacteria death and
Figure 1. FT-IR pattern of (a) Ag, (b) ZnO and (c) Ag/ZnO nanoparticles
Figure 2. XRD pattern of (a) Ag (b) ZnO and (c) Ag/ZnO nanoparticles
colloid with other bacteria and repeat their sterilization behavior. It was also mentioned that silver covered on the surface of Zn nanoparticle has the ability to involve the electrons produced through photo-catalytic reactions of Zn nanoparticle which increases electron isolation and makes gaps in the cell membrane, so increase its antimicrobial activity. Regarding studies of these authors, antibacterial property of silver and zinc oxide nanoparticles improves with their combination. In fact, our study confirmed that the gram negative strains of bacteria had most sensitive to silver/zinc oxide nanoparticle. Further, our study approved that the Ag/ZnO nanoparticle has a great antimicrobial agent against E.coli and just combination of zinc oxide and silver nanoparticles, give increase their bactericidal effect.
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References


Sondi, I., & Salopek-Sondi, B. 2004. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science. 275(1); 177-182.


