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# COMPARATIVE STUDIES BETWEEN USING ANTIMICROBIAL EFFECT WITH SILVER NANO PARTICLES EFFECTS FOR *E. COLI*

**Lina Shaheed[2], Noor Abady[1*], Shaimaa obaid Hasson[1], Ilham Bunyan[3] and Ban Mahmood Shaker Aljoda[3]**

[1]Department of microbiology, University of Al-Qasim Green, Iraq
[2]Department of veterinary medicine, University of Al-Qasim Green, Iraq
[3]Department Microbiology, College of medicine, University of Babylon, Iraq
[3]Department of chemistry and biochemistry, College of medicine, University of Babylon, Iraq
Corresponding Author: Noor Abady
Email : noormohammedheath@yahoo.com
Phone: +9647822268229

## Abstract

Several key factors, including different extracellular appendages, are implicated in E. coli surface colonization and their expression and activity are finely regulated, both in space and time, to ensure productive events leading to mature biofilm formation. In the current study, *E.coli* were used is a multidrug resistance and biofilm formation which cause serious infections are difficult to eradicate by ordinary antibiotics, so it need novel and effective antibacterial materials to deal with it. Silver nanoparticle AgNPs have vastly antibacterial application today were used in this study. The antibacterial activity of AgNPs was evaluated by agar well diffusion method. *E. coli* showed highly resistance rate to all tested antibiotics in Vitek2 AST in contrast the result of sliver nano particles effects showed antibacterial activity with inhibition zone revealing sensitivity of *E coli*. This reseach point to the some differences in the antimicrobial effect to get better understanding of resistant isolate.

## Introduction

Milk is a good medium for bacterial growing, which can cause infections in consumers, and spoils the milk and associated products (Oliver *et al*., 2005). Many types of microorganisms can get access to milk and its products including *E. coli*, which is an indicator of milk contamination and establishing a public health problems (Virpari *et al*., 2013) especially when people drinking raw milk without pasteurization(Nalband, 2015) This organism is one of the greatest significant opportunistic gram-negative, rod-shaped, facultative anaerobic bacteria found in the environment, foods and the intestines of animals and people which can be transmitted by the contact with infected people and animals or the contamination of food CDC 2018 (Kuhar *et al*., 2018) . It can cause diverse virulence factors and it is resistant to many antimicrobial agents (Baker *et al*., 2017). In human and veterinary medicine the antimicrobial resistance had rising threat worldwide and that emerge from random used of antimicrobial agents among pathogenic and commensal bacteria predominant in environment and food cause complicated morbidity, mortality and increase cost of treatment of diseases (Brown and Wright, 2016) In last few years *E.coli* resistance to antimicrobial agents has emerged and is a major health problem (Sahm *et al*., 2001). Thousands of food borne illnesses, hundreds of hospitalizations and deaths had been estimated by this microorganism worldwide each year (Mesa *et al*., 2006). For that, in this article the silver nanoparticles (AgNPs) are evaluated for their part in increasing the antimicrobial activities of various antibiotics against this bacteria. That is one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in biomedical applications. AgNPs play an important role in Nano-science and nanotechnology, particularly in nanomedicine. Although several noble metals have been used for various purposes, AgNPs have been focused on potential applications in cancer diagnosis and therapy (Zhang *et al*., 2016).

## Materials and Methods

### Collection of samples

One hundred milk and seventy five cheese samples were collected from Al–Qasim village in Iraq, all the samples were cultured in routinely culture media nutrient, blood, and MacConkey agar) were incubated at $37^0$C for 24hrs.

### Biofilm Detection

To detect biofilm-forming bacteria by Congo red agar method according to (Freeman *et al*., 1989) by prepared a Congo red stain as stock solution, autoclaved at 121°C for 20 min. then added to autoclaved brain heart infusion broth with agar and 5% sucrose at 55°C (Hassan *et al*., 2011). The bacterial strains were inoculated and incubated at 37 °C for 24 to 48 hrs. then read the result as following: if the bacteria formed black colonies with a dry crystalline consistency that was mean it biofilm producer isolates while if it formed red colonies that was mean the non-biofilm producer isolates (Kaiser *et al*., 2013).

### Antibiotics susceptibility tests

VITEK 2 AST system to determine the Minimum inhibitory concentration (MIC) to many tested antibiotics. All the following steps were done according to the manufacturer's instructions as VITEK 2 AST system supplemented with antimicrobial susceptibility testing cards Enterobacteriaceae contains more than 15 antibiotics (Table 1). The results read also digitally on monitor connected to VITEK system apparatus.

**Table 1 :** Antibiotics provide by VITEK AST card for Enterobacteriaceae with MIC breakpoints according to M100 (10):

| Antibiotic | MIC Breakpoints (µg/mL) | | | Antibiotic | MIC Breakpoints (µg/mL) | | |
|---|---|---|---|---|---|---|---|
| | S | I | R | | S | I | R |
| Ticarcillin | ≥8 | 16 | ≤32 | Cefepime | ≥2 | - | ≤16 |
| Ticarcillin/ clavulanic acid | ≥16/2 | 32/2-64/2 | ≤128/2 | Trimethoprim /Sulfamethoxazole | ≥2/38 | - | ≤4/76 |
| piperacillin | ≥16 | 32-64 | ≤128 | Gentamicin | ≥4 | 8 | ≤16 |
| Piperacillin-tazobactam | ≥16/4 | 32/4-64/4 | ≤128/4 | Tobramycin | ≥4 | 8 | ≤16 |
| Aztroenam | ≥4 | 8 | ≤16 | Ciprofloxacin | ≥1 | 2 | ≤4 |
| Meropenem | ≥1 | 2 | ≤4 | Amikacin | ≥16 | 32 | ≤64 |
| Ceftazidime | ≥4 | 8 | ≤16 | Minocycline | ≥4 | 8 | **≤16** |
| Imipenem | ≥1 | 2 | ≤4 | | | | |

**Silver nanoparticles synthesis:** it was synthesis by chemical synthesis method briefly:

Twenty drops of 0.1M $AgNO_3$ was added dropwise (1 drop per sec.) to 50ml of 0.001M $NaBH_4$ in beaker (250ml) on magnetic stirrer at (400  for 30 min in dark condition and in room temp.) then the change in color was noted. The reaction mixture was stirred vigorously on a magnetic stirrer (11, 12) according to equation below:

$$AgNO_3 + NaBH_4 \rightarrow Ag + H_2 + B_2H_6 + NaNO_3$$

**Optimize silver nanoparticles characterization**

The silver nanoparticles were characterized by UV. Spectrophotometer and Size analyzer(Gomaa, 2017). All these analyses were carried out at pharmacy and Science College, Kufa and veterinary college of Al-Qasim green university.

**UV–visible spectrophotometer analysis**

The Surface Plasmon Resonance (Teeling *et al.*) of silver nanoparticles was measured by UV–visible spectrophotometer at wave length ranging from 300-500 nm. By sampling 1ml of AgNPs solution to different wave length were measured every ten degree at resolution of 1nm (14).

**Size analyzer**

Laser diffraction particle size analyzers, which measure light scattering and assume an index of refraction to calculate the particle size distribution  (15). Silver nanoparticles sample was examination in size analyzer after incubated in sonicator water bath at 35C for 30 min. Emulsion diluted sample with deionized water were put in grove of apparatus and the size were measured during 5 min. by using laser beam scattering in beta sizer apparatus. The results were monitoring on computer's screen.

**Antimicrobial activity assay of AgNPs**

The antimicrobial activity of AgNPs were evaluated by agar well diffusion method by using Muller Hinton plate inoculated with tested biofilm-forming bacteria at inoculum 1.5 x $10^8$ CFU/ml by streaking method and waited 10 min. to dry then made well by cork borer in the center of inoculated plate and fill the well with 100 µl of filtered AgNPs, incubated at 37C to 24 hrs. at dark condition. After that, the inhibition zone diameter were measured by ruler and compared to the nearest whole millimeter.

## Results and Discussion

After the culturing and diagnosis the milk and chesses samples, the results revealed as table 2.

| Source | | | |
|---|---|---|---|
| Milk | 100 | 64 | 64.00% |
| chesses | 75 | 43 | 57.33% |

*E. coli* is gram negative bacteria belong to enterobactericeae family have green metallic sheen appearance on Eosin Methylene Blue (Beloin *et al.*, 2008) Fig. 1.

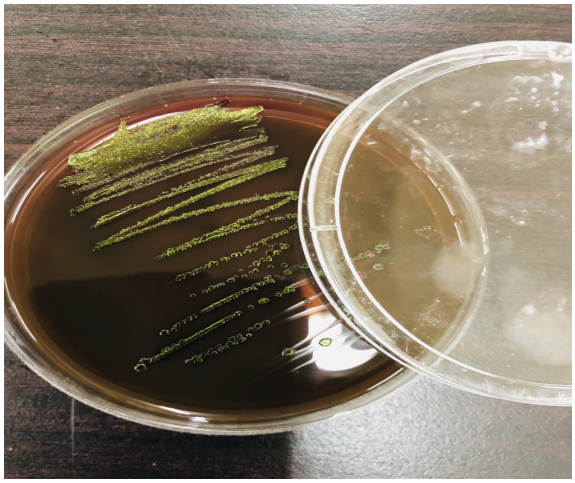

**Fig. 1:** Green metallic sheen of E. coli isolates isolated from milk and cheese samples.

All *E. coli* isolates isolated from both sources revealed biofilm formation which detected by congo red method with control non –biofilm *E. coli* (Fig. 2).

In milk and milk product as liquid environment, the biofilm bacteria are submitted to forces of hydrodynamic. At the same time, bacteria developed active motility to overcome the hydrodynamic and electrostatic forces by repulsive power and that lead to increase the interaction chances of bacteria to container surface (Beloin *et al.*, 2008). Pratt and Kolter found that flagellar motility were the main aid factor to form biofilm, they supposed that,  in addition to aid the bacteria to overcome repulsive forces, flagella may also assist bacteria spreading over the surface (Pratt and Kolter, 1998). Recently, it thought that conjugate plasmid may *E. coli* bearing have main role in biofilm formation

which express highly adhesion force to the surface (Ghigo, 2001)

Some of adherent bacterial cell remain attach to surface and arrested to be irreversible attachment. Bacterial appendages (flagella, pilli, fimbriae) and even EPS stimulate chemical reaction between bacterial cell and surface to unit bonds, which act as a bridge between bacteria and surface and that depend on degree of hydrophobicity and hydrophillicity of interaction surface. (Liu *et al*., 2004, Kokare *et al*., 2009, Joo and Otto, 2012).

The black color colony in figure above indicate biofilm formation of bacteria due to stain exopolysaccharide matrix producing during biofilm process by Congo red stain (Bose *et al*., 2009).

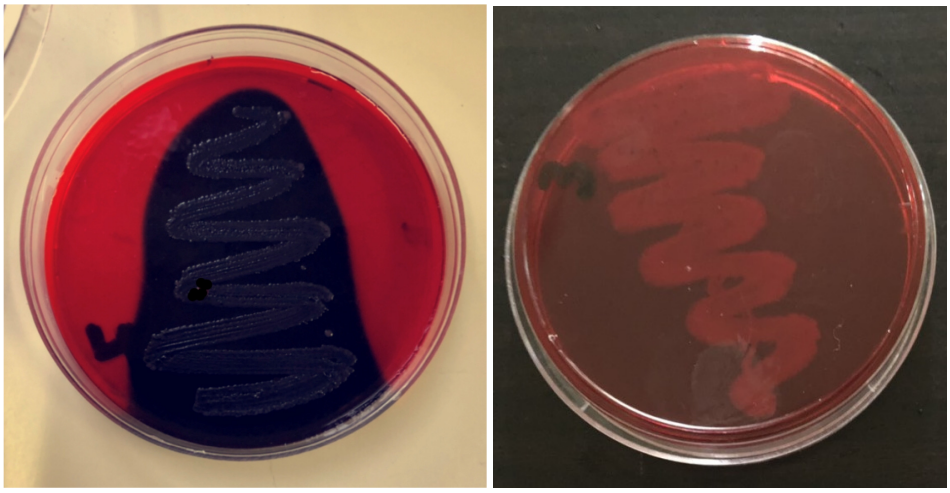

**Fig. 2:** Congo red agar indicating A= *E.coli* biofilm formation B= non –biofilm *E.coli* as control.

**Antibiotic susceptibility testing:**

**Table 3:** Vitek AST results of biofilm forming *E coli.*

| Antibiotic | MIC | Interpretation | Antibiotic | MIC | Interpretation |
|---|---|---|---|---|---|
| Ticarcillin | 64 | I | Cefepime | ≤1 | S |
| Ticarcillin / clavulanic acid | ≤8 | R | Trimethoprim /Sulfamethoxazole | ≤20 | R |
| piperacillin | ≤4 | R | Gentamicin | ≤1 | R |
| Piperacillin-tazobactam | ≤4 | R | Tobramycin | ≤1 | R |
| Aztroenam | ≤1 | R | Ciprofloxacin | ≤0.25 | S |
| Meropenem | ≤0.25 | S | Amikacin | ≤2 | R |
| Ceftazidime | ≤4 | R | Minocycline | ≤1 | S |
| Imipenem | ≤0,25 | S | | | |

Antibiotic susceptibility profile by Vitek AST to *E.coli*

*E. coli* showed highly resistance rate to all tested antibiotics in Vitek2 AST in contrast the result of sliver nano particles effects showed antibacterial activity with inhibition zone revealing sensitivity of *E. coli*. This reseach point to the some differences in the antimicrobial effect to get better understanding of resistant isolate.

**Silver Nanoparticles Synthesis**

The AgNPs were used in current study synthesis as previous study (Abady, 2019). chemical synthesis method were used in AgNPs production according to equation below:

$$AgNO_3 + NaBH_4 \rightarrow Ag + H_2 + B_2H_6 + NaNO_3$$

The characterization of AgNPs product briefly: the UV. Spectrophotometer analysis showed that high rate of absorbance at 390 nm. while the Size analyzer test showed the size of synthetic nano at 5 nm.

**The antibacterial activity of synthetic AgNPs**

In the current study, *E. coli* were used is a multidrug resistance and biofilm formation which cause serious infections are difficult to eradicate by ordinary antibiotics, so it need novel and effective antibacterial materials to deal with it. AgNPs have vastly antibacterial application today. The antibacterial activity of AgNPs was evaluated by agar well diffusion method.

The results revealed that antibacterial effect of synthetic AgNPs against biofilm forming *E. coli* inhibition zone at average 33 nm (Fig. 1) and that average is highly relatively in compared to previous studies (Hasson, 2019, Abady, 2019).

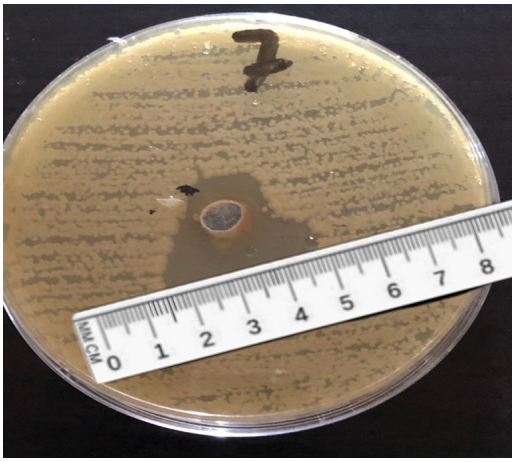


**Fig. 1:** Zone of inhibition of biofilm formation *E. coli* growth as a result antibacterial activity of synthetic AgNPs.

The antibacterial activity of AgNPs against biofilm forming bacteria studies is limited except littles studies (Mathur *et al*., 2006, Guzmán *et al*., 2009, Hasson, 2019) especially in biofilm forming *E. coli* (Yu *et al*., 2018).

The antibacterial activity of AgNPs suppose related to many mechanisms but it still unknown, AgNPs may interact with the bacterial cell membrane lead to disturb the permeability and functions of respiration (Kvitek *et al*., 2008) and may penetrate the bacteria cell (Morones *et al*., 2005). Many researchers also proposed that $Ag^{+}$ ions interact with the thiol groups in bacteria proteins, affecting the replication of DNA (Marini *et al*., 2007).

## Conclusion

*E Coli* showed highly resistance rate to all tested antibiotics in Vitek2 AST in contrast the result of sliver nano particles effects showed antibacterial activity with inhibition zone revealing sensitivity of *E coli*. This reseach point to the some differences in the antimicrobial effect to get better understanding of resistant isolate.

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