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The Effect of Some Nanoparticles on The Growth of Multi- Resistant Escherichia Coli Bacteria Isolated from Different Sources in Diyala University

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Abstract. Background: Escherichia coli bacteria possess a number of virulence factors, in addition to their ability to acquire enterotoxin genes through plasmids or bacteriophages, or through genes acquired from other pathogenic bacterial genera through bacterial conjugation. The resistance to antibiotics that bacteria possess is considered one of the most important economic and health problems around the world. Nanoparticles are mainly used in antibacterial applications due to their long-term stability and biocompatibility. The mechanisms behind the antimicrobial effect of these nanoparticles are metal ion release, oxidative stress, and non-oxidative stress that occur simultaneously. Mineral nanoparticles are characterized by their broad-spectrum properties against both Gram-negative and Gram positive bacteria. Objectives: The study aimed to determine the effect of nanomaterials on the growth of E. coli bacteria Methods: Been collected of 100 samples collected from urinary tract infections and stool in Sterile bottles in different hospitals in Baquba city (Baquba Teaching Hospital and Al- Batool Hospital) It was subjected to detecting the resistance of the isolates to six antibiotics and the extent of the effect of nanomaterials on bacterial growth Results: Thirty-three isolates of E. coli bacteria were obtained. From urine and stool, the isolates were tested for resistance to six antibiotics: Amoxicillin-Clavulanic acid, Cefotaxime, Cefepime, Ciprofloxacin, Ampicillin, and Tetracycline. The effect of nanomaterials, silver and zinc nanoparticles, on bacterial growth was determined, as the results showed a decrease in bacterial growth as a result of the use of nanomaterials. Conclusion: Escherichia coli isolates are characterized by their multiple resistance to antibiotics, which increases their pathogenicity. The use of nanoparticle inhibitors of silver and zinc led to a decrease in bacterial growth, which makes them used as alternatives in treatment.

Highlights:

1. E. coli shows virulence, gene transfer, and antibiotic resistance.
2. Evaluate nanomaterials' effect on E. coli bacterial growth.
3. Silver, zinc nanoparticles reduce E. coli growth; potential antibiotic alternatives.

Keywords: Escherichia coli. Nanoparticles; urinary tract infection; Antibiotic resistance

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Introduction

Escherichia .coli bacteria are the main part of the Enterobacteriaceae family that invade the intestines after birth, where they coexist naturally, while some of their strains have the opportunistic ability to infect the host with diseases when appropriate conditions for growth are available (1)

The incidence of urinary tract infections (UTIs) varies according to age groups and gender, as approximately 50% of all women will suffer from a urinary tract infection. The reason for this is the anatomical differences between women and men as the urethra is much shorter in women, the shorter distance from the opening of the urethra to the bladder. Pyelonephritis is less common than (2) ،. . lower urinary tract infection

Escherichia coli bacteria possess a number of virulence factors, in addition to their ability to acquire enterotoxin genes through plasmids or bacteriophages, or through genes acquired from other pathogenic bacterial genera through bacterial conjugation. The resistance to antibiotics that bacteria possess is considered one of the most important economic and health problems around the world. This has prompted scientists and researchers to search for alternative ways to eliminate resistant bacteria, as infection with resistant bacteria leads to a longer treatment period. (3) . There are four mechanisms for bacterial resistance to antibiotics, which include changing the site targeted by the antibiotic, reducing the absorption of the antibiotic, efflux pumps, and disabling the action of the antibiotic. These mechanisms may be carried on the bacterial chromosome, as they occur naturally in all strains, or they are acquired through a plasmid (4) (5) . Mentioned that metal nanoparticles play a major role in many biomedical applications, including antibacterials, due to their tunable properties that depend on shape and size. Nanoparticles such as copper, titanium, silver, zinc, and iron can be used against multidrug resistant (MDR) microorganisms due to their antibacterial nature, noted (6).

Nanoparticles are mainly used in antibacterial applications due to their long-term stability and biocompatibility. The mechanisms behind the antimicrobial effect of these nanoparticles are metal ion release, oxidative stress, and non-oxidative stress that occur simultaneously. Mineral nanoparticles are characterized by their broad-spectrum properties against both Gram-negative and Gram-positive bacteria. Whereas copper nanoparticles and silver nanoparticles showed antimicrobial activity against S.aureus,

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E.coli, and AgNPs showed antimicrobial activity against microbes P.aeruginosa, E.coli, and Bacillus anthracis. (7), and nano-zinc oxide showed inhibitory activity against S.aureus, P.aeruginosa, and E.coli bacteria.(8) .Nanoparticles affect several main goals, including inhibiting the synthesis or destruction of the cell wall, and they affect the process of translation and reproduction during the process of protein and DNA synthesis, as well as their effect on the bacterial respiratory system, which leads to stimulating the production of reactive oxygen species (ROS) by influencing the bacterial antioxidant system. Thus, nanoparticles can be used to overcome the problem of antibiotic resistance (9) . Nanoparticles contribute to enhancing the antimicrobial effectiveness of traditional antibiotics, such as Penicillin G, Rivamicin, and Novobiocin, when applied together. A comparative study between biological and chemical nanoparticles showed that biological nanoparticles have a higher antimicrobial effect than chemically synthesized nanoparticles. (10,9).

The study aims to detect the resistance of E.coli bacteria to multiple antibiotics and the extent of the effect of nanomaterials on their growth .

Methods

The study was approved by the Ethics Board of the university of Diyala and informed written consent was taken from each participant in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Isolation and Identification of Escherichia col

A total of 100 samples were collected from different clinical sources (urinary tract infections , stool) in sterile containers in different hospitals in Baquba city (Baquba Teaching Hospital and Al-Batool Hospital). During sample collection from 13/9/2024 to 12/11/2024.The samples were cultured on MacConkey`s agar and blood agar plates, and then incubated for 24 hours at 37◦C. The isolates were identified depending on the morphological and biochemical tests and compared to the scheme described by (11). Morphological and Microscopic Identification: it was possible to see the form, size, texture, and colony organization of the bacteria. Colonies were isolated and stained using Gram stain. In order to determine their form and length, they were ultimately viewed under a microscope. Testing biochemical reactions using indole and oxidase (12)

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Antimicrobial susceptibility

Sex antimicrobial discs to determine their sensitivity, and the antimicrobial susceptibility test (13). Bacterial isolates were grown in MacConkey broth over night at 37°C. Muller Hinton agar is made and put in petri dishes. In 5 ml of ordinary salineIsolated colonies were resuspended using a vortex. Checked for recognizability and comparison with standard McFarland solutions. sex antimicrobial antibodies were used to show the desalination of an isolate of the bacterium Proteus mirabilis, after an absence of the method of distributing cotton in three directions by means of the protein for the protein plate at 60°C for each direction. Within room temperature, the vertical plate was turned upside down at room temperature . She was treated with various antibiotics and then admitted at 37°C overnight. With the help of an area distillation ruler, the fluorescence areas were measured in millimeters and the results were compared with those of the National Laboratory Standards Laboratory.

Table (1) showing the antibodies used in the study

Manufacturer (origin)	micrograms/tablet	The Name of antibiotic
Bioanalyse (Turkey)	30	Tetracycline
Bioanalyse (Turkey)	30	Cefepime
Bioanalyse (Turkey)	5	Ciprofloxacin
Bioanalyse (Turkey)	30	Cefotaxime
Bioanalyse (Turkey)	10	Ampicillin
Bioanalyse (Turkey)	30	Amoxicillin-Clavulanic acid

The inhibitory effect of nanoparticles on the growth of Escherichia coli bacteria

The liquid nutrient dilution method was used to estimate and determine the inhibitory effect of nanoparticles prepared by Sky Spring Nano Materials, according to what was reported by (14).

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Results and Discussion

Isolation of E.coli

This study included 100 samples of different clinical sources (urinary tract infections, , stool), the rate of male patients was 37% and female patients 63% their age ranged between 15 to 35 years. Thirty-three isolates belong to E. coli bacteria and sixty-seven isolates belong to other species.

Antimicrobial Susceptibility

The sensitivity of P. mirabilis strains isolated from (urinary tract infections, wounds, stool) was tested against antibiotics, including, Amoxicillin-Clavulanic acid , Cefotaxime, Cefepime, Ciprofloxacin, Ampicillin ,Tetracycline , in Table(2) .

A table showing the resistance of E. coli bacteria to antibiotics

Antibiotic	Resistant
Amoxicillin-Clavulanic acid	100%
Cefotaxime	**94%**
Cefepime	%90
Ciprofloxacin	%50
Ampicillin	100%
Tetracycline	**90%**

The effect of silver nanoparticles and zinc nanoparticles on the growth of E. coli bacteria .

The effect of nanoparticles on the growth of E. coli bacteria was studied at different concentrations, where the results showed a clear effect on the growth of bacterial isolates.

Discussion

The rate of bacterial resistance to the antibiotic Ampicillin reached 100%, and this result was consistent with each of the results reached by the researcher (15) in

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Diyala Governorate, as his study included 100 isolates of E.coli bacteria to investigate their ability to resist antibiotics, as the results showed that the rate of resistance to the antibiotic Ampicillin was 100%. With the results reached by (16), the rate of resistance to the antibiotic Ampicillin was (97.5%) The result agreed with(17) , with a resistance rate of 100%. which is considered an antibiotic that targets cell wall manufacturing. Many β-lactamase enzymes controlled by genes carried on the plasmid in Gram-negative bacteria can degrade each of the antibiotics Ampicillin.

Which included the antibiotics Cefotaxime, Cefepime, it was in agreement with (16), as it was found that the E.coli bacteria had a high resistance to the antibiotic Cefepime, which reached (80%), The results of a study that were inconsistent with (18) regarding the antibiotics Cefotaxime and are (70%) The reason for the high resistance of E.coli bacteria to antibiotics is that they possess Extended Beta-Lactamase (ESβL) enzymes. These enzymes are able to degrade cephalosporins and penicillins by breaking the beta-lactam ring, after which the antibiotic becomes ineffective, as the resistance genes are carried either on plasmids or on chromosomes, and this leads to multiple resistance to various antibiotics (19).

As for the tetracycline antibiotic, the results of the study agreed with (16,17), as the percentage of resistance to the tetracycline antibiotic in E. coli bacteria was (80%) and (96%), respectively.

There are many reasons that make E.coli bacteria resistant to the Tetracycline group of antibiotics, namely their containment of efflux pumps, especially those belonging to the RND-type efflux pumps family, and the change in the location of the target in addition to the change in the permeability of the outer membrane (20). As for the antibiotic Ciprofloxacin, the rate of bacterial resistance was 50%, and this result was consistent with the results reached by (21), where the rate of resistance to the antibiotic Ciprofloxacin was (52%), and with (22) in Iran, which showed that the rate of resistance of E.coli bacteria to the antibiotic Ciprofloxacin was (55%).

The antibiotic Ciprofloxacin is one of the most widely used quinolone antibiotics in the treatment of urinary tract infections. Excessive use of this antibiotic has led to a clear increase in the rate of resistance in recent years.

The current results showed the effect of silver and copper nanoparticles on the growth of E.coli bacteria, and this result agreed with (23,24,25) about the effectiveness

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of biosynthetic silver nanoparticles (AgNPs) for a group of bacteria pathogenic to humans, including E. coli bacteria, and their effect on bacterial growth. It was shown that silver nanoparticles have an effective effect on bacterial growth with increasing concentration, and this Agree with what the current study .found The lethal effect of nanoparticles is due to the small size of the particles and the large surface area, which gives them a lethal effect(26), in addition to their effect on the cellular membrane and disrupting its basic functions such as permeability and respiratory processes. Nanoparticles are also able to easily enter the cellular membrane through their interaction with compounds containing sulfur and phosphorus, such as DNA (27).

Conclusion

1- Escherichia coli isolates are characterized by their multiple resistance to antibiotics, which increases their pathogenicity.

2- The use of nanoparticle inhibitors of silver and zinc led to a decrease in bacterial growth, which makes them used as alternatives in treatment.

References

[1] S. N. Gharajalar and V. H. Sofiani, "Patterns of Efflux Pump Genes Among Tetracycline Resistance Uropathogenic Escherichia coli Isolates Obtained from Human Urinary Infections," Jundishapur J. Microbiol., pp. 1–5, 2017.

[2] H. Kornfält Isberg, K. Hedin, E. Melander, S. Mölstad, and A. Beckman, "Uncomplicated urinary tract infection in primary health care: presentation and clinical outcome," Infect. Dis., vol. 53, no. 2, pp. 94–101, 2021. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/33073654/

[3] K. Krause, A. Serio, T. Kane, and L. Connolly, "Aminoglycosides: An Overview," Cold Spring Harb. Perspect. Med., vol. 6, no. 6, 2016.

[4] P. Pachori, R. Gothalwal, and P. Gandhi, "Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit: a critical review," Genes Dis., vol. 6, no. 2, p. 109, 2019. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/31194018/

ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.141

[5] A. J. Huh and Y. J. Kwon, "A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era," J. Control. Release, vol. 156, pp. 128–145, 2011.

[6] S. Zaidi, L. Misba, and A. U. Khan, "Nano–therapeutics: A revolution in infection control in post-antibiotic era," Nanomed. Nanotechnol. Biol. Med., vol. 13, pp. 2281–2301, 2017.

[7] B. Saud, P. Pandey, G. Paudel, G. Dhungana, and V. Shrestha, "In-vitro Antibacterial Activity of Probiotic against Human Multidrug Resistant Pathogens," Arch. Vet. Sci. Med., vol. 3, pp. 31–39, 2020.

[8] N. S. Rezouqi, A. Ramadhan, B. Nasir, and W. A. A. Hasin, "A Study of the Effect of Some Growth Conditions on Inhibitory Activity of Lactobacillus delbrueckii and Lactobacillus fermentum to reduce growth of E. coli," Iraqi J. Sci., pp. 1357–1364, 2016.

[9] P. She et al., "Effects of exogenous glucose on Pseudomonas aeruginosa biofilm formation and antibiotic resistance," Microbiol. Open, vol. 8, no. 12, p. 933, 2019.

[10] P. Singh, N. Verma, P. Kumar, and P. Nagu, "Review on a potential of antibiotics," J. Drug Deliv. Ther., vol. 8, no. 5, pp. 35–40, 2018.

[11] N. Jones, B. Ray, K. Ranjit, et al., "Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms," FEMS Microbiol. Lett., vol. 279, no. 1, pp. 71–76, 2008.

[12] N. Lee, C. Lim, T. Kim, et al., "Which hazard category should specific nanomaterials or groups of nanomaterials be assigned to and how," World Health Organization, Geneva, 2017.

[13] CLSI, Performance Standard for Antimicrobial Susceptibility Testing, 27th ed., CLSI Supplement M100, Wayne, PA: Clinical and Laboratory Standards Institute, pp. 32–41, 2021.

[14]] W. S. Dawood, "Molecular and susceptibility study of antibiotic resistance genes in E. coli isolated from selected Iraqi patients," Syst. Rev. Pharm., vol. 11, no. 9, pp. 214–223, 2020.

[15] X. Shi et al., "In situ structure and assembly of the multidrug efflux pump AcrAB-TolC," Nat. Commun., vol. 10, no. 1, pp. 1–6, 2019.

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ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.141

[16] H. M. Radif and R. M. Al-Bahrani, "Green synthesized of silver nanoparticles and study their properties and applications," J. Biotechnol. Res. Cent., vol. 13, no. 2, pp. 5–9, 2019.

[17] G. Procop, D. Church, G. Hall, et al., Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 7th ed., Lippincott Williams & Wilkins, Philadelphia, 2016.

[18] A. Ahmed, "Molecular detection of some virulence genes in E. coli isolated from women with urinary tract infections," M.Sc. thesis, College of Science, University of Diyala, 2021. [Online]. Available: https://sciences.uodiyala.edu.iq/uploads/biology%20Master%20Thesises/Master%20Thesis%202019-2020/E.%20coli%20final%20paper_edit17.pdf

[19] R. M. Abdullah and A. F. Mahdi, "Identification of Pseudomonas aeruginosa from clinical specimen by using 16S rDNA," Iraq Acad. Sci. J., vol. 10, no. 1, pp. 45–49, 2016.

[20] G. Kapoor, S. Saigal, and A. Elongavan, "Action and resistance mechanisms of antibiotics: A guide for clinicians," J. Anaesthesiol. Clin. Pharmacol., vol. 33, no. 3, pp. 300–305, 2017. [Online]. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC5672523/

[21] A. Gupta, S. Mumtaz, C. Li, et al., "Combatting antibiotic-resistant bacteria using nanomaterials," Chem. Soc. Rev., vol. 48, no. 2, pp. 415–427, 2019.

[22] Y. Malekzadegan, R. Khashei, H. Sedigh Ebrahim-Saraie, and Z. Jahanabadi, "Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic Escherichia coli isolates from Iranian patients," BMC Infect. Dis., vol. 18, no. 1, pp. 1–9, 2018. DOI: 10.1186/s12879-018-3467-0.

[23] I. M. AL-Dulaimy, "Molecular investigation of some virulence gene of multidrug resistant Proteus mirabilis isolated and study of the nano effect on gene expression for some swarming gene," M.Sc. thesis, College of Science, Diyala University, 2023. [Online]. Available: https://cajmns.centralasianstudies.org/index.php/CAJMNS/article/view/1480

[24] A. Al–Atbi, "Molecular study for one gene responsible for biofilm formation and the effect of some biofilm inhibitors in Proteus mirabilis isolated from burns and wounds," M.Sc. thesis, College of Science, Diyala University, 2020.

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ISSN 3063-8186. Published by Universitas Muhamamdiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.141

[25] F. Y. Ramírez-Castillo, A. C. Moreno Flores, F. J. Avelar González, F. Márquez-Díaz, J. Harel, and A. L. Guerrero Barrera, "An evaluation of multidrug-resistant Escherichia coli isolates in urinary tract infections from Aguascalientes, Mexico: Cross-sectional study," Ann. Clin. Microbiol. Antimicrob., vol. 17, no. 34, pp. 1–13, 2018. [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/30041652/

[26] S. K. Sarkar, A. Bhattacharyya, and S. M. Mandal, "YnfA, a SMR family efflux pump is abundant in Escherichia coli isolates from urinary infection," Indian J. Med. Microbiol., vol. 33, no. 1, pp. 139–142, 2015.

[27] M. Rojas-Lopez, R. Monterio, M. Pizza, M. Desvaux, and R. Rosini, "Intestinal pathogenic Escherichia coli: Insights for vaccine development," Front. Microbiol., vol. 9, p. 440, 2018.