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The Impact of Gold Nanoparticle Susceptibility on Drug Resistance Phenotypes in Uropathogenic *Escherichia coli*

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ABSTRACT

Introduction: The widespread use of antibiotics has contributed to the dissemination of multidrug-resistant pathogens. This study aimed to assess the prevalence of *Escherichia coli* strains associated with urinary tract infections, characterized by diverse drug-resistance phenotypes. Additionally, the antibacterial properties of gold nanoparticles were examined against each phenotype to determine their effectiveness. **Methods:** This cross-sectional study was conducted on 170 *E. coli* strains isolated from 250 urine samples collected from symptomatic and asymptomatic patients with urinary tract infections (UTIs) between August 2022 and July 2023. The antibiotic susceptibility profiles across various classes of antibiotics were determined using the Kirby-Bauer method. At the same time, the minimum inhibitory concentrations of gold nanoparticles were assessed through the microdilution broth test. **Results:** Among *E. coli* isolates, 91 (53.53%), 45 (26.47%), and 17 (10%) isolates were identified as multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR), respectively. The MIC of gold nanoparticles that inhibited the growth of 90% of PDR isolates (MIC90=200 ppm) was two times higher than the MIC90 against XDR isolates (MIC90=100 ppm) and four times higher than the MIC90 against MDR isolates (MIC90=50 ppm). There was also a significant difference between the MIC90 and the MIC of gold nanoparticles that inhibited the growth of 50% of PDR and XDR bacteria (P<0.05). **Conclusion:** The emergence of MDR, XDR, and PDR uropathogenic *E. coli* isolates represents a significant societal health concern. Considering the favorable *in vitro* antimicrobial potential of gold nanoparticles against uropathogenic *E. coli* isolates, it is recommended to further analyze their applicability as antibiotic alternatives by conducting *in vivo* studies.

INTRODUCTION

Nosocomial infections, also known as hospital-acquired infections, are acquired during hospitalization. These infections can manifest either during the hospital stay or after the patient's discharge. *Escherichia coli*, a prominent pathogen, is responsible for causing nosocomial infections affecting various anatomical sites, such as the genitourinary system, appendix, peritoneum, and gall bladder. Urinary tract infection (UTI) is the prevailing nosocomial infection, frequently instigated by diverse pathogens, including *E. coli*, which can be transmitted via perineal or digestive routes or ascend through the urethral canal. A positive infection is indicated when a mid-stream urine sample, collected in a standard manner, reveals the presence of over 10^5 microorganisms per milliliter [1-3]. Recent reports have highlighted a significant and rapid increase in the resistance rates of *E. coli* strains to a wide range of antibiotics, encompassing beta-lactams, aminoglycosides, fluoroquinolones, and cephalosporins. The formidable challenge posed by treating these infections has elevated this issue to a grave concern within both developed and developing nations, warranting urgent attention in the realm of public health. The escalating prevalence of antimicrobial resistance worldwide can be attributed, in part, to the escalating and inappropriate utilization of antibiotics [4, 5]. Antibiotic resistance in bacteria can develop through various mechanisms, including the acquisition of drug-resistant genes through chromosomal

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alterations, plasmid transfer, or genetic mutations. This phenomenon has given rise to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) clinical strains, particularly prevalent in low- and middle-income countries [6]. Strains of *E. coli* are classified as MDR when they exhibit non-susceptibility to one or more antibiotics belonging to a minimum of three distinct antibiotic classes. In contrast, XDR strains are non-susceptible to all antibiotic classes except for two. On the other hand, PDR strains display non-susceptibility to all antibiotic classes [7]. Antibiotic resistance can potentially prolong illnesses, escalate morbidity and mortality rates, and impose a substantial financial burden on patients and healthcare systems. Despite efforts to raise awareness and address this issue, antibiotic resistance continues to escalate. Therefore, the identification and characterization of antibiotic-resistant strains, as well as the development of alternative compounds, are of paramount importance, particularly in mitigating treatment failures.

Applying nanoparticles (NPs), specifically metal NPs, offers a highly effective strategy for combating drug-resistant strains. In recent years, the exceptional optical, thermal, chemical, and antimicrobial properties of gold nanoparticles (AuNPs) have garnered significant attention from the research community. The main characteristic of these NPs lies in their minute size relative to their expansive surface area, thereby augmenting their biochemical and biological potential, encompassing solubility, membrane permeability, and antibacterial efficacy [8].

Infrared spectroscopic analysis of unbound antibiotics and antibiotics coated with AuNPs has revealed a pronounced and robust binding affinity between these nanoparticles and the structural rings of certain antibiotics. Furthermore, AuNPs can be synthesized via the chemical reduction method, exhibiting minimal toxicity compared to other nanomaterials. This characteristic renders them highly suitable for diverse therapeutic, diagnostic, and biological applications [9, 10].

Given that the examination and management of drug-resistant infections have the potential to yield novel medical guidelines, the objective of this study was to explore the antibiotic resistance patterns in *E. coli* strains responsible for UTIs. Additionally, this investigation sought to determine the frequency of drug-resistant isolates and evaluate the minimum inhibitory concentration (MIC) of AuNPs against these isolates.

MATERIAL AND METHODS

Sample collection and bacteriological assessment. This descriptive-analytical research study was conducted between 2022 and 2023, encompassing 250 symptomatic or asymptomatic individuals with UTIs. The participants were both in-patients and out-patients, and the study took place across six hospitals in Gorgan City and its suburbs in Northern Iran. The inclusion criterion for this study required participants to have refrained from antibiotic usage within the preceding 20-day period. The sample size was determined using a confidence level of 95%. UTI was diagnosed through a positive urine culture, specifically, 10^5 microbial colonies in asymptomatic patients and 10^4 microbial colonies in symptomatic patients [11]. In adherence to the ethical guidelines for human subject research outlined by the World Medical Association Declaration of Helsinki, written informed consent was obtained from all patients. Demographic data, encompassing gender, age, infection frequency, and underlying diseases such as diabetes, high blood pressure, and kidney diseases, were meticulously recorded. Patients with autoimmune diseases and under 12, regardless of gender, were excluded from the study. Early morning urine samples were obtained and subsequently subjected to culturing procedures. The samples were incubated at 37 ^{o}C for 18-24 h. Ultimately, E. coli strains were identified and confirmed by utilizing microbiological and biochemical methods, specifically employing the API20E system (bioMérieux, France) [12].

Antibiotic susceptibility assay. For the Kirby-Bauer disk diffusion method, a half McFarland turbidity bacterial suspension was prepared from overnight cultures of *E. coli* and subsequently inoculated onto Mueller Hinton agar plates. The antibiotic susceptibility of the *E. coli* isolates was determined using a panel of specific antibiotic disks, including gentamicin (GM10), imipenem (IPM10), tetracycline (TE30), colistin (CS10), ciprofloxacin (CIP5), aztreonam (ATM30), cefoxitin (FOX30), cefazolin (CZ30), ticarcillin-clavulanic acid (TCC 75+10), fosfomycin (FOS50), chloramphenicol (C30), ampicillin-sulbactam (SAM10+10), cefotaxime (CTX30), cefepime (FEP30), sulfamethoxazole-trimethoprim (SXT 1.25 + 23.75), and piperacillin-tazobactam (TZP110). The antibiotic disks utilized in this study were procured from Padtan Teb Co., Iran. Following their placement on the bacterial cultures, incubation was carried out for 16-18 h at 37 °C. The resulting inhibition zones around each disk were measured and interpreted per the guidelines outlined in CLSI-M100 [13].

MIC and MBC of AuNPs. For the preparation of the initial stock solution of AuNPs, a 20 ml volume of the colloidal suspension of spherical-shaped AuNPs, with a diameter of 30 nm, obtained from Nanopishgaman Co., Iran, was subjected to agitation in a shaking incubator for a duration of 4 h. Following centrifugation at 3,800 rpm, the resultant pellet was resuspended in 10 ml of distilled water. To validate the binding of NPs to bacterial surfaces, the absorption spectrum was acquired within the wavelength range of 600-700 nm using spectroscopy. To ascertain the Minimum Inhibitory Concentration (MIC) through the broth microdilution test, 100 µl of the initial stock solution of AuNPs was inoculated into the wells of a 96-well microplate, each containing Mueller Hinton

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broth (Merck, Germany). Concurrently, with the inoculation of a drug-resistant bacterial suspension at a concentration of 3×10^5 CFU/ml, the initial stock solution of AuNPs was subjected to serial dilutions to achieve a concentration range of 0.3-200 ppm. Subsequently, the microplate was incubated at 37 °C for 20-24 h. A well containing AuNPs within the culture medium was the negative control, while *E. coli* ATCC25922 was utilized as the standard strain.

The minimum bactericidal concentration (MBC) was determined by inoculating 50 µl of the contents from wells exhibiting no turbidity onto Mueller Hinton agar plates, followed by incubation at 37 °C for 24 h. Subsequently, colony counting was performed to identify the lowest concentration of gold nanoparticles (AuNPs) that inhibited the growth of 99.99% of *E. coli* strains, defined as the MBC. Subsequently, the drug-resistant isolates were divided into control and AuNP-treated groups. In the case of AuNP treatment, a sterile test tube was utilized to combine 10 µl of the colloidal solution of AuNPs with 200 µl of Mueller Hinton broth. Following the inoculation of the bacterial suspension at a concentration of 10^5 CFU/ml and 24 h of incubation, 10 µl of the tube contents were inoculated onto McConkey agar. Subsequently, the number of colonies (cfu) was enumerated, and the absorbance at 570 nm was measured.

Data analysis. Following data collection, the findings were presented as frequency tables, graphs, and numerical indices. All data were subjected to statistical analysis using the Chi-square test in the GraphPad Prism software. The significance level for the analysis was set at 0.05.

RESULTS

Frequency and demographics of *E. coli* isolates. Of the 250 samples assessed, 170 bacterial isolates (68%) were identified as *E. coli*. The mean age of the included patients was 50.03 ± 12.08 years. Most of the isolates were derived from female patients (n=93; 57.70%) and individuals below 40 (n=106; 62.35%). Furthermore, a significant proportion of the bacterial isolates, specifically 101 isolates (59.41%), were obtained from patients with recurrent UTIs, defined as experiencing the infection more than three times. Additionally, 87 isolates (51.18%) were derived from patients with underlying medical conditions, among which 49 isolates (56.32%) were specifically isolated from individuals with a history of kidney diseases.

Table 1. Antibacterial susceptibility of *E. coli* isolates from patients diagnosed with UTI

Antibiotic	Susceptibility result	Frequency of *E. coli* isolates		
		Number	%	Chi-Squared (χ2)
Imipenem	R	29	17.06	51.77*
	S	116	68.24	
Gentamicin	R	29	17.06	60.05*
	S	119	70	
Aztreonam	R	42	24.70	48.32*
	S	110	64.70	
Cefoxitin	R	35	20.59	59.87*
	S	115	67.65	
Cefazolin	R	33	19.41	67.68*
	S	112	65.88	
Tetracycline	R	91	53.52	21.90
	S	75	44.12	
Ticarcillin-clavulanic acid	R	48	28.23	29.01
	S	77	45.29	
Ciprofloxacin	R	29	17.06	54.88*
	S	112	70.59	
Colistin	R	19	11.17	76.76*
	S	142	83.53	
Gemifloxacin	R	17	10	74.02*
	S	150	88.23	
Fosfomycin	R	18	10.50	60.11*
	S	148	87.06	
Chloramphenicol	R	82	48.23	19.89
	S	41	24.12	
Ampicillin-Sulbactam	R	60	35.29	20.69
	S	77	45.29	
Cefotaxime	R	28	16.47	49.81*
	S	122	71.76	
Cefepime	R	21	12.35	55.23*
	S	125	73.53	
Sulfamethoxazole -trimethoprim	R	38	22.35	21.72
	S	89	52.35	
Piperacillin-tazobactam	R	30	17.64	29.03
	S	92	54.11	

* Statistically significant difference (P<0.05). R: resistant; S: sensitive.

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Antibiotic susceptibility patterns. Among the *E. coli* isolates examined, tetracycline exhibited the highest resistance rate at 53.52%, while gemifloxacin demonstrated the highest sensitivity at 88.23% (Table 1). The prevalence of MDR, XDR, and PDR isolates was found to be 91 (53.53%), 45 (26.47%), and 17 (10%), respectively. The majority of MDR strains were found to be isolated from female patients aged above 40, individuals with recurrent UTIs, and those with underlying diseases (Table 1). A statistically significant association was observed between UTI incidence and the abundance of *E. coli* isolates (P<0.05). Conversely, no significant relationship was identified between UTI occurrence and the presence of an underlying disease (Table 2).

Broth microdilution test. The investigation into the impact of various concentrations of AuNPs on the growth of resistant *E. coli* isolates revealed their inhibitory effect on 90% of the bacterial population in a dose-dependent manner, with a minimum inhibitory concentration (MIC) of 100 µg/ml or higher. The minimum concentration of AuNPs required to inhibit the growth of 90% of PDR isolates, as determined by the MIC90 value, was 200 ppm. This concentration was twice as high as the MIC90 against XDR isolates (MIC90=100 ppm) and four times higher than the MIC90 against MDR isolates (MIC90=50 ppm). No significant difference was observed between the MIC of AuNPs required to inhibit the growth of 50% of MDR isolates (MIC50=12.5 ppm) and the MIC90 value (25 ppm) (P=0.1). However, a noteworthy distinction was found between the MIC50 and MIC90 of AuNPs against PDR and XDR isolates (P<0.05) (Table 3).

Table 2. Characteristics of the study population and frequency of MDR, XDR, and PDR *E. coli* isolates

Variables		MDR (n=91)	XDR (n=45)	PDR (n=17)	*P*-value
Gender	Female	52 (57.14)	29 (64.44)	10 (58.82)	0.17
	Male	39 (42.86)	16 (35.56)	7 (41.18)	
Frequency of infection	First	21 (23.07)	8 (17.78)	2 (11.76)	0.03*
	Second	30 (32.97)	17 (37.78)	7 (41.18)	
	≥Third	40 (43.96)	20 (44.44)	8 (47.06)	
Age (years)	<40	31 (34.07)	18 (40)	4 (23.52)	0.02*
	>40	60 (65.93)	27 (60)	13 (76.48)	
Underlying disease	Yes	49 (53.85)	35 (77.78)	9 (52.94)	0.23
	No	42 (46.15)	10 (22.22)	8 (47.06)	

* Statistically significant difference

Table 3. Comparison of MIC and MBC of AuNPs against drug-resistance *E. coli* isolates

AuNPs Concentration range (ppm)		MIC50*	MIC90**	MBC***	χ2	*P*-value
E. coli	MDR	6.25-12.50	25-50	50	5.17	**0.1**
E. coli	XDR	12.50-25	50-100	200	9.33	**0.02***
E. coli	PDR	25-50	100-200	200	11.08	**0.01***
P-value			0.01*	0.01*		

* Minimum inhibitory concentration that inhibited growth of 50% of *E. coli* isolates compared to the negative control group.

** Minimum inhibitory concentration that inhibited the growth of 90% of *E. coli* isolates compared to the negative control group.

*** The minimum bactericidal concentration compared to the negative control group.

Following the treatment of drug-resistant *E. coli* isolates with AuNPs, a significant reduction in absorbance at a wavelength of 570 nm was observed (P=0.01, Figure 1).

DISCUSSION

Identifying patients with nosocomial infections, particularly those caused by MDR pathogens, holds paramount importance in controlling and preventing such infections. Among bacterial infections, UTIs are widely recognized as one of the most prevalent types. *E. coli*, being one of the primary causative agents, accounts for 80-90% of all community-acquired UTIs and 30-50% of

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nosocomial UTIs. Nosocomial infections, the leading cause of hospitalization, can give rise to notable clinical complications, heightened mortality rates, and escalated healthcare expenditures, mainly when treatment failure occurs due to drug resistance. Consequently, it is imperative to consistently and diligently investigate and identify pathogenic bacteria exhibiting multidrug resistance. Such efforts can potentially enhance treatment and improve patient outcomes [14, 15].

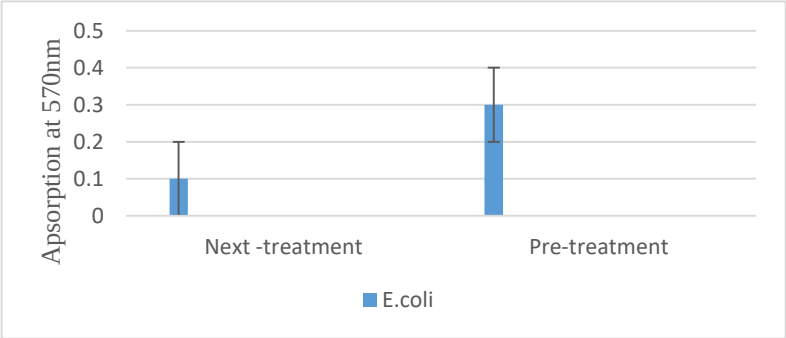


Fig .1. Comparison of mean absorbance at 570 nm in drug-resistant *E. coli* isolates before and after treatment with AUNPs

In the current investigation, the uropathogenic *E. coli* isolates demonstrated the highest susceptibility to gemifloxacin (88%), whereas tetracycline exhibited the highest resistance rate (53.5%). These findings align with a recent study conducted in Iran, which reported a 70% resistance rate of *E. coli* isolates from UTIs to tetracycline [16]. Additionally, another study indicated that 87% of the isolates were susceptible to gemifloxacin [17]. The substantial resistance of uropathogenic *E. coli* isolates to tetracycline has been reported in various regions worldwide, including Asia (93%) and Europe (67%) [18, 19]. Within the scope of this study, out of the 170 *E. coli* isolates examined, 91 (53.53%) were classified as MDR. Furthermore, 45 (26.47%) and 17 (10%) isolates were identified as XDR and PDR, respectively. The prevalence of MDR *E. coli* isolates implicated in UTIs exhibits variations across different countries. For instance, studies conducted in different regions have reported varying frequencies of MDR strains. In India, the frequency of MDR strains was reported as 75% [20], while in South Korea, it was 21.9% [21]. A study in Bangladesh in 2015 reported a prevalence of 70.67% MDR *E. coli* isolates and 14% XDR isolates, although no PDR isolates were detected [22]. In Iraq, a recent study conducted in 2023 reported a prevalence of XDR isolates at 21.24% and PDR isolates at 1.77% [23]. Notably, the PDR rate in our study was higher than in the report mentioned above. Among the identified isolates, MDR was most prevalent in females, patients aged 40 years and older, individuals with recurrent UTIs occurring three times or more, and those with underlying medical conditions. This finding aligns with a previous study conducted in Iran [16]. The relatively shorter length of the urethra in females may contribute to a higher incidence of UTIs, a trend that tends to increase with advancing age. Considering the clinical importance of a notable prevalence of uropathogenic *E. coli* in urinary and gastrointestinal samples [24-26], recent research endeavors have prioritized the exploration of alternative antimicrobial agents, particularly NPs. This pursuit aims to mitigate the overreliance on antibiotics and curb the proliferation of antibiotic-resistant strains. The intracellular penetration of NPs has been recognized as a promising avenue for achieving notable therapeutic effects. Specifically, gold AuNPs have been acknowledged for their potential to provide groundbreaking and highly efficacious solutions in tackling critical biomedical obstacles, such as drug delivery and cancer treatment [27]. Nanoparticles are widely recognized as viable biosensors due to their small size range (3 to 100 nm) and ability to be detected using optical absorption, magnetic force measurement, and electric current analysis. Their inherent characteristics make them well-suited for sensing applications. Multiple studies have consistently demonstrated that the antibacterial efficacy of AuNPs is contingent upon their size and morphology, with smaller sizes yielding more pronounced antibacterial effects [28]. Our findings corroborate this observation, potentially attributable to diminished bacterial adhesion to solid surfaces. Previous investigations have similarly established nanoparticles of smaller dimensions (> 100 nm) exhibit superior antimicrobial properties. In the current investigation, the utilization of AuNPs with a size of 30 nm exhibited noteworthy antibacterial activity, surpassing that observed in prior studies [29-31]. Grace *et al*. (2007) demonstrated the efficacy of AuNPs against Gram-positive and Gram-negative bacteria [32]. Furthermore, a previous study conducted in Iran demonstrated the antibacterial properties of AuNPs against a spectrum of clinical and environmental isolates [33]. Our findings indicate that higher concentrations of AuNPs (up to 200 mg/ml) enhanced the inhibitory effects observed. In line with these findings, a study conducted in Pakistan similarly revealed that an elevated concentration of AuNPs is required to eradicate Gram-positive bacterial strains [34]. The outcomes of our study underscore the

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notable prevalence of MDR *E. coli* isolates and the emergence of XDR and PDR strains within the clinical samples obtained from patients diagnosed with UTIs. Such trends could potentially result in substantial financial burdens and adverse health consequences. Given the promising potential of high-dose AuNPs in addressing drug-resistant uropathogenic *E. coli*, it is highly recommended to pursue *in vivo* studies to comprehensively evaluate the prospective application of these nanoparticles in controlling and treating drug-resistant *E. coli* infections. Additionally, it is prudent to consider assessing the potential ecological toxicity of these nanoparticles in both aquatic and terrestrial ecosystems as part of future investigations. Such research endeavors will provide valuable insights into the efficacy and environmental impact of AuNPs, thereby advancing our understanding of their broader implications.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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