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PLASMID CURING AND ANTIBIOTIC RESISTANCE REVERSAL IN MULTIDRUG-RESISTANT ESCHERICHIA COLI USING SILVER NANOPARTICLES AND SDS

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ПЛАЗМИДНАЯ ЭЛИМИНАЦИЯ И УСТРАНЕНИЕ УСТОЙЧИВОСТИ К АНТИБИОТИКАМ У ПОЛИРЕЗИСТЕНТНЫХ ESCHERICHIA COLI С ИСПОЛЬЗОВАНИЕМ НАНОЧАСТИЦ СЕРЕБРА И ДСН

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Abstract

Background: The gram-negative bacterium known as *Escherichia coli* is one of the most prevalent types of bacteria that are responsible for opportunistic infections, especially in clinical settings. As a result of the proliferation of multidrug-resistant (MDR) *E. coli* bacteria, which represent a substantial risk to public health, many traditional antibiotics are no longer effective against them. On account of this growing resistance, there is an immediate and pressing need to investigate alternate treatment approaches.

Objectives: This study aimed to characterize plasmid profiles associated with antibiotic resistance in *E. coli* isolates from clinical specimens and evaluate the effectiveness of silver nanoparticles (AgNPs) and sodium dodecyl sulfate (SDS) in plasmid curing and reversing antibiotic resistance.

Materials and Methods: Multidrug resistance was assessed using the agar disk diffusion method by measuring the inhibition zones around antibiotic-impregnated disks. Plasmids were extracted and analyzed via agarose gel electrophoresis to identify resistance-associated genetic elements. Plasmid curing was performed using 10% SDS, AgNPs, and a combination of both agents. Antibiotic susceptibility was retested post-curing to assess any changes in resistance.

Results: Among the tested *E. coli* isolates, 75% showed resistance to tetracycline, erythromycin, and chloramphenicol. Plasmid profiling confirmed the presence of resistance-carrying plasmids in these strains. Following plasmid curing, previously resistant isolates demonstrated restored sensitivity to the antibiotics, confirming the plasmid-mediated nature of resistance. Isolates lacking plasmid bands remained sensitive throughout. Notably, AgNPs alone showed significant antibacterial activity, especially against gram-negative bacteria such as *E. coli*,

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which may be attributed to the structural differences in the bacterial cell wall and the presence of fimbriae that enhance nanoparticle uptake.

Conclusion: This study highlights the potential of silver nanoparticles, alone or in combination with SDS, as promising agents for plasmid curing and reversing antibiotic resistance in multidrug-resistant *E. coli*. The findings provide insight into the mechanism by which nanoparticles interact with bacterial cells and offer a foundation for future development of novel antibacterial therapies.

Keywords: plasmids, nanoparticles, Escherichia coli, antibiotic resistance, Silver Nanoparticles, sodium dodecyl sulfate.

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Резюме

Введение: Грам-отрицательная бактерия *Escherichia coli* является одним из наиболее распространенных видов бактерий, вызывающих оппортунистические инфекции, особенно в медицинских учреждениях. В результате пролиферации полирезистентных (МЛУ) E. coli, представляющей значительный риск для общественного здравоохранения, многие традиционные антибиотики теряют свою эффективность против таких бактерий. Возрастающая распространенность такой резистентности существует определяет необходимость к проведению безотлагательных исследований по поиску альтернативных подходов к лечению.

Цели: Целью данного исследования было изучение плазмидных профилей, связанных с устойчивостью к антибиотикам, у изолятов E. coli, выделенных из клинических образцов, и оценка эффективности наночастиц серебра (AgNP) и додецилсульфата натрия (ДСН) в плазмидной элиминации и устранении устойчивости к антибиотикам.

Материалы и методы: Множественную лекарственную резистентность оценивали с помощью метода диффузии в агаровых дисках путем измерения зон подавления роста вокруг дисков, пропитанных антибиотиком. Плазмиды были выделены и проанализированы методом электрофореза в агарозном геле для выявления генетических элементов, связанных с резистентностью. Плазмидная элиминация проводилось с использованием 10% ДСН, AgNPs и комбинации обоих агентов. Чувствительность к антибиотикам была дополнительно оценена после плазмидной элиминации для оценки любых изменений резистентности.

Результаты: Среди исследованных изолятов E. coli 75% проявили резистентность к тетрациклину, эритромицину и хлорамфениколу. Плазмидное профилирование подтвердило наличие плазмид, несущих

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резистентность, в указанных штаммах. После плазмидной элиминации ранее резистентные изоляты демонстрировали восстановление чувствительности к антибиотикам, что подтверждает плазмидно-опосредованную природу резистентности. Изоляты, не содержащие полос плазмид, сохраняли чувствительность на протяжении всего исследования. Примечательно, что AgNPs сами по себе продемонстрировали значительную антибактериальную активность, особенно в отношении грамотрицательных бактерий, таких как E. coli, что может быть связано со структурными различиями в клеточной стенке бактерий и наличием фимбрий, усиливающих захват наночастиц. Выводы: настоящее исследование подчеркивает наличие потенциала для применения наночастиц серебра, как изолированно, так и в сочетании с ДСН, в качестве перспективных агентов для плазмидной элиминации и снижения устойчивости к антибиотикам у полирезистентных E. coli. Результаты исследования проливают свет на механизм взаимодействия наночастиц с бактериальными клетками и служат основой для дальнейшей разработки новых антибактериальных препаратов.

Ключевые слова: плазмиды, наночастицы, Escherichia coli, устойчивость к антибиотикам, наночастицы серебра, додецилсульфат натрия.

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1 Inroduction

Plasmids are additional pieces of genetic material discovered in bacteria and a lot of cells that commonly bestow a unique attribute to the cell [22]. Plasmids transfer several traits, including virulence factors and genes involved in symbiosis [10]. global health challenges over the years due to multidrug-resistant (MDR) bacteria [21]. plasmid curing, the process of eliminating plasmids from bacterial cells, is a valuable tool for studying the genetic basis of these traits [9].

Traditional curing agents, such as ethidium bromide, acridine orange, and SDS have been widely used, but their efficacy can vary [16]. Recently, nanoparticles have emerged as promising alternatives due to their unique physicochemical properties and antimicrobial activity [25]. Nanoparticles are distinct from bulk particles due to their elevated surface-to-volume ratio. Because of their better catalytic ability, optical characteristics, and compatibility with biological matters, nanoparticles can be applied to medicine, environment, and biotechnology [6]. Nanoparticles' properties (Physical and chemical) are affected by size, shape, and crystal structure. Dimensions and morphology affect the optical properties of metal nanoparticles [12].

Several industrial sectors adopt silver nanoparticles (AgNPs) because these nanomaterials exhibit outstanding physical properties together with chemical behavior and biological effects [13]. These nanoparticles show strong antimicrobial functions which help them function in medicine and antibacterial coatings besides wound healing applications [1,18]. The scientific community deploys AgNPs in biosensing as well as water purification along with drug delivery systems and catalytic operations [17]. The diagnostic and imaging possibilities benefit from the large surface area together with plasmonic properties. The ability of silver nanoparticles to excel in medicinal research together with their versatility ensures an essential role in nanotechnology-based medical developments [1,18].

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Plasmid curing is required for antibiotic resistance and pathogenicity research. Traditional curing agents like acridine dyes and SDS are toxic and less effective. Nanoparticles are a potential plasmid curative agent due to their unique physicochemical characteristics [24,27]. This study aimed to evaluate the effectiveness of silver nanoparticles (AgNPs) as plasmid-curing agents in *Escherichia coli* and to compare their efficacy with SDS.

2 Materials and Methods

Bacterial Isolates and Plasmid Isolation

Escherichia coli was chosen for their multidrug resistance, making them ideal candidates for plasmid-curing experiments. As the antibiotic sensitivity test has shown [14].

Curing Agents and Experimental Design

The isolate was grown in 5ml of brain heart infusion broth to mid log phase. Then 0.05 ml of inoculums of the culture were inoculated in a series of 5 ml fresh BHI broth tubes containing various concentrations of SDS and AgNPs and mixed with SDS (1%-10%), then were incubated at 37 °C for 24 – 48hrs. The growth density was observed to determine the effect of SDS, AgNPs and the combination of AgNPs with SDS on bacterial growth. The highest concentration that allows the growth of bacteria was observed at 6% and 8% respectively. The AgNPs size range 50-150nm, a mix of rutile and anatase con.33.37 w%, dissolved in water with Mwt 79.90 (Sigma/ USA) [15]. Appropriate dilutions of treated cultures were spread on brain heart infusion agar, and 100 colonies were selected for further analysis. Cured colonies were identified by their inability to grow on selective media containing specific antibiotics.

DNA Extraction and Analysis

Extraction of DNA was done using the salting-out procedure [27]. Cultures of *Escherichia coli* were grown on brain heart infusion (BHI) broth, the pellets were obtained from 20 mL by centrifuge for 15 minutes at 6,000 rpm. The pellets were

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washed with 3 mL of SET buffer and suspended in 1.6 mL of the same buffer. Subsequently, 1 mL of 10% SDS and AgNPs were added and gently mixed by inversion before incubation at room temperature for 15 minutes. Chloroform of equal volume was added then the mixture was mixed by inversion of for 15 minutes, and centrifugation at 6,000 rpm for 20 minutes at 4°C. The aqueous phase was pipetted into a sterile tube, and DNA was precipitated by adding 0.6 volumes isopropanol, inverting the mixture, and standing at room temperature for 5 minutes. Finally, the sample was centrifuged at 13,000 rpm for 15 minutes at 4°C, and the supernatant was removed. The DNA pellet was dissolved in 100 μL of TE buffer and kept at -20°C for storage.

Plasmid Isolation

Plasmid was isolated from according to Plasmid Miniprep Kit (Promega, USA). 600 μL of bacterial culture was cultured to obtain the plasmid DNA, and it was stored at -20°C for future use.

Agarose Gel Electrophoresis

Plasmid DNA was analyzed on 0.7% agarose gel electrophoresis following the method [21], Agarose gels in 1X Tris-borate-EDTA (TBE) buffer were made. DNA samples were mixed with 1/10 volume of loading buffer and loaded into wells. Electrophoresis was done at 5 V/cm for 2–3 hours maintaining the buffer level at the gel surface. Following electrophoresis, the gels were stained with ethidium bromide (0.5 μg/mL) for 30–45 minutes and destained in distilled water for 30–60 minutes. The stained gels were then examined under UV light at 302 nm to assess the plasmid DNA bands

Plasmid Curing Experiment

Plasmid curing experiments were conducted to determine the correlation between plasmid content and antibiotic resistance in *Escherichia coli* (E3). curing experiment was performed on *Escherichia coli* (E3) by using SDS as a curing agent

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[16], and to test the AgNPs and the combination of AgNPs with SDS as a curing agent other than SDS.

The bacterial isolates were first grown in 5 mL of BHI broth to mid-log phase. A sample of 0.05 mL of the culture was then inoculated into fresh BHI broth tubes containing various concentrations (1–10%) of SDS, AgNPs, or a mixture of SDS and AgNPs. The tubes were incubated at 37°C for 24–48 hours. The minimum inhibitory concentration (MIC) of every curing agent was determined by observing the highest concentration at which the growth of bacteria was permitted. For the isolation of survivor colonies, samples from tubes containing the lowest concentration of curing agents that permitted growth were diluted and spread onto BHI agar plates. The plates were incubated overnight at 37°C to obtain isolated survivor colonies.

Selection of Cured Colonies

To identify cured colonies, survivor colonies were replica-plated on to BHI agar master plates and BHI agar supplemented with antibiotics to which the original isolates were resistant. Colonies that could grow on the master plate but not on plates carrying antibiotics were considered cured. The selected cured colonies were picked and their sensitivity to several other antibiotics were tested and compared to that of the original isolate to determine which antibiotic marker had been lost in addition to the original one because of treatment with the curing agents SDS, AgNPs and mixed with SDS.

3 Results

Isolation and identification of *Escherichia coli*

Ten clinical samples were obtained from Al-Kadymia Hospital in Baghdad. The specimens consisted of stool samples from patients with urinary tract infections and diarrhea. Isolates were identified using morphological, biochemical, and physiological characteristics, following conventional cultural procedures.

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The standard disk diffusion method was used to determine the susceptibility of *E. coli* to different antibiotics. It was found that 75% of isolates were resistant to tetracycline, erythromycin, and chloramphenicol as shown in Table 1. The clinical isolates showed multiple resistances to various antibiotics, with a complete sensitivity of 100% to Cefixime, which may serve as an effective treatment for inhibiting growth. Notably, five isolates (83%) demonstrated resistance to Tetracycline and Erythromycin, while four isolates (67%) showed resistance to Chloramphenicol. Additionally, three isolates (50%) were resistant to Novobiocin, and two isolates (33%) exhibited resistance to both Neomycin and Kanamycin.

Plasmid isolation

The plasmid was isolated to establish a plasmid profile prior to curing by using Plasmid Miniprep kit (Promega/ USA). The results show that the isolate had tiny plasmid DNA bands. The bacterial isolates examined in this study contain small plasmid DNA bands and additional plasmids that were not discovered, maybe due to their huge size.

The relationship between plasmid curing by SDS and Nanoparticles

Plasmid curing was used to decide whether the genes responsible for antibacterial resistance and virulence factors are located on the plasmid or not. As the results in table 2 show that the highest concentration of SDS allows the growth of *E. coli* was 8%. While by using the nano AgNPs (v/v) the highest concentration allows the growth of *E. coli* was also 6%. The highest concentration of mixer of SDS and nano AgNPs that allows the growth of *E. coli* was 4%. The *Escherichia coli* colonies were assessed on a selective medium containing a specific antibiotic to identify the cured colonies. These cured colonies were unable to proliferate on the antibiotic-containing medium due to the loss of the plasmid. It is important to note that the absence of a detectable band alone does not confirm curing without corroborative evidence from selective media.

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DNA Purity and Concentration

After the treatment with different curing agents the purity and quantity of the extracted DNA was recorded as shown in Table 3, which explained the improvement in the quality of DNA. The recorded range of DNA concentration was 98-205 ng/µl and the purity of extracted DNA from *E. coli* was 1.6 and became 1.7 to 1.8 when DNA extracted with nanoparticles, which show increase in the concentration of the DNA extracted by the nanoparticles.

The present breakthrough with AgNPs in DNA extraction indicates new possibilities for plasmid curing and antimicrobial techniques in biological studies. (Figure 1).

4 Discussion

According to these results the isolate E3 was chosen because of their multi-resistance to antibiotics. Other studies show that *E. coli* is MDR to different antibiotics globally [11]. Meanwhile, this study results show that all isolates 100% are resistant to Chloramphenicol, and 43% were Cefixime resistant in contrast to other research which is greatest at 95%, due to different of environmental condition. Resistance between bacteria occurs due to plasmids that carry resistance genes [8].

Plasmid curing of bacterial isolate was used to determine whether the genes responsible for antibacterial resistance and virulence factors are located on the plasmid or not. The results pattern indicates that SDS at high doses damages bacterial cell membranes, resulting in reduced viability. While the highest concentration of AgNPs that allows the growth of *E. coli* was 6% the antimicrobial action of silver nanoparticles becomes effective due to ROS production while it breaks membranes and interacts with bacterial intracellular elements to cause bacterial cell death [20].

In contrast, a mixture of SDS and AgNPs at a volume-ratio of 0.4:1 allowed E. coli to grow even at the highest concentration tested.

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The research findings confirm AgNPs have strong antimicrobial properties that become even more effective when SDS is added. Clinical applications of antibacterial medicines would benefit from the SDS-AgNPs combination due to its improved bactericidal properties when used against multidrug-resistant bacterial pathogens. Future research needs to study the molecular bond formations between SDS molecules and AgNPs to enhance their bactericidal capabilities. While other studies used crude oil as curing agent where the biodegradation efficiency of wild and plasmid cured bacteria was 85% and 81.63% [5].

Cell lysis or DNA release occurs better when AgNPs are present because they break bacterial membranes and interact with intracellular substances [24]. The combined application of AgNPs with SDS yielded a DNA concentration of 198 ng/µl, surpassing the results obtained from SDS treatment alone. This mixture demonstrated an interaction effect that optimized DNA extraction processes and minimized degradation. The integration of AgNP treatment improved the efficiency of DNA extraction, thereby enhancing DNA purity to a level of 1.7. The AgNP-SDS combination treatment achieved a maximum purity of 1.8 for extracted *E. coli* DNA by effectively eliminating contaminants, resulting in higher quality DNA. All DNA extraction procedures were conducted in triplicate to ensure reproducibility. The reported concentrations and purity values represent mean results from independent technical replicates. Treatment with AgNPs shows a dual benefit because it leads to increased DNA concentration alongside improved DNA purity while efficiently breaking bacterial cells for DNA release. AgNPs demonstrate antimicrobial and membrane-disrupting capabilities that assist better DNA extraction efficiency. This combination SDS and AgNPs approach creates a synergistic action which produces both pure DNA and high DNA yield outcomes. Molecular biology researchers must pay close attention to this discovery because high-quality DNA forms the essential precursor for PCR and sequencing methods along with genetic engineering applications.

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The potential biomedical and microbiological applications of AgNPs as a novel tool become more viable because the nanoparticles both break target bacterial cells and amplify the acquisition of DNA molecules. Scientific research should expand its investigation of molecular AgNPs-bacterial DNA interactions for optimizing their performance in DNA extraction and plasmid removal applications. The higher sensitivity of Gram-negative bacteria to AgNPs may be attributed to the presence of fimbriae, which enhances nanoparticle adhesion [2,7].

5 Conclusion

This study illustrated the considerable potential of AgNPs in improving DNA extraction efficiency and eliminating bacterial plasmids, thus advancing molecular biology and combating antibiotic resistance. The observed improvement in DNA extraction is due to the capacity of AgNPs nanoparticles to break cell membranes and effectively engage with extraction proteins. The Bacterial isolate plasmid removal proves to be an effective strategy against antibiotic resistance.

More investigations have become necessary to understand how different types of nanoparticles affect plasmid repair in various bacterial species and strains despite this research demonstrating AgNPs' potential applications. Further research combining with the findings from this project will produce better insights into nanoparticle therapies in molecular biology and the mechanisms behind antibiotic resistance.

The research showed the value of studying new techniques including AgNPs to address extraction problems and antimicrobial resistance developments. Researchers can achieve improved bacterial treatment and prevention methods through continuous tool evaluation and enhancement thus limiting the development of antibiotic resistance. The evaluation has significant restrictions based on inconsistent bacterial responses to AgNPs treatment that varies by strain and environmental effects on nanoparticle behavior that can yield different results. Such findings typically apply to restricted bacterial species and environmental conditions.

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Researchers must explore the efficiency of AgNPs to combat bacterial strains from a broad perspective and under optimal laboratory conditions. The study investigated only the positive effects AgNPs produce for DNA extraction and plasmid curing without mentioning nanoparticle toxicities. Additional studies must analyze how AgNPs affect both bacterial and human cells together with studying their lifetime stability. The complete understanding of AgNPs impact on DNA extraction and plasmid curing needs more research because researchers do not understand how AgNPs cause cell membrane lysis while interacting with proteins. The future research should focus on three components: worldwide risk analysis of nanomaterials and their characteristics and molecular mechanism clarifications. Scientists need to study nanoparticles under clinically appropriate condition. Reduction of AgNP limitations will strengthen their molecular biological applications while advancing antibiotic resistance combat programs to create new microbiological and biotechnological tools.

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Competing financial interests

The authors have no conflict of interest.

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ТАБЛИЦЫ

Table 1. Antibiotic susceptibility of E. coli isolate.

Antibiotics	Code	E1	E2	E3	E4	E5	E6
Tetracyclin	TE	R	S	R	R	R	R
Cefixme	CFM	S	S	S	S	S	S
Erythromycin	E	R	S	R	R	R	R
Neomycin	N	S	S	R	S	R	S
Kanamycin	K	R	S	R	S	S	S
Chloramphenicol	C	R	S	S	R	R	R
Novobiocin	K	S	S	R	R	R	S

Notes: R: Resistance, S: Sensitive

Table 2. Effect of SDS and AgNPs and mixer of both AgNPs and SDS on the growth of *E. coli* (E3).

Concentration %	1 %	2%	3 %	4 %	5 %	6 %	7 %	8 %	9 %	10 %
Bacterial growth (E3) (SDS)	+++	+++	++	++	++	+	+	±	-	-
Bacterial growth (E3) (AgNPs)	+++	+++	++	++	+	±	-	-	-	-
Bacterial growth (E3) (SDS and AgNPs)	+++	+++	++	++	+	±	-	-	-	-

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Notes: (+++): very good growth, (++): Good growth, (+): Moderate growth, (±): Slightly growth, (-): No growth.

Table 3. The concentration and purity means DNA extracted from the independent technical replicates indicate of E. coli.

Extracted DNA From	**DNA concentration ng\µl**	**Purity of DNA (260 /280)**
E. coli (E3) DNA with SDS	104.8	1.6
E. coli with AgNPs	205	1.7
E. coli with AgNPs and SDS	198	1.8

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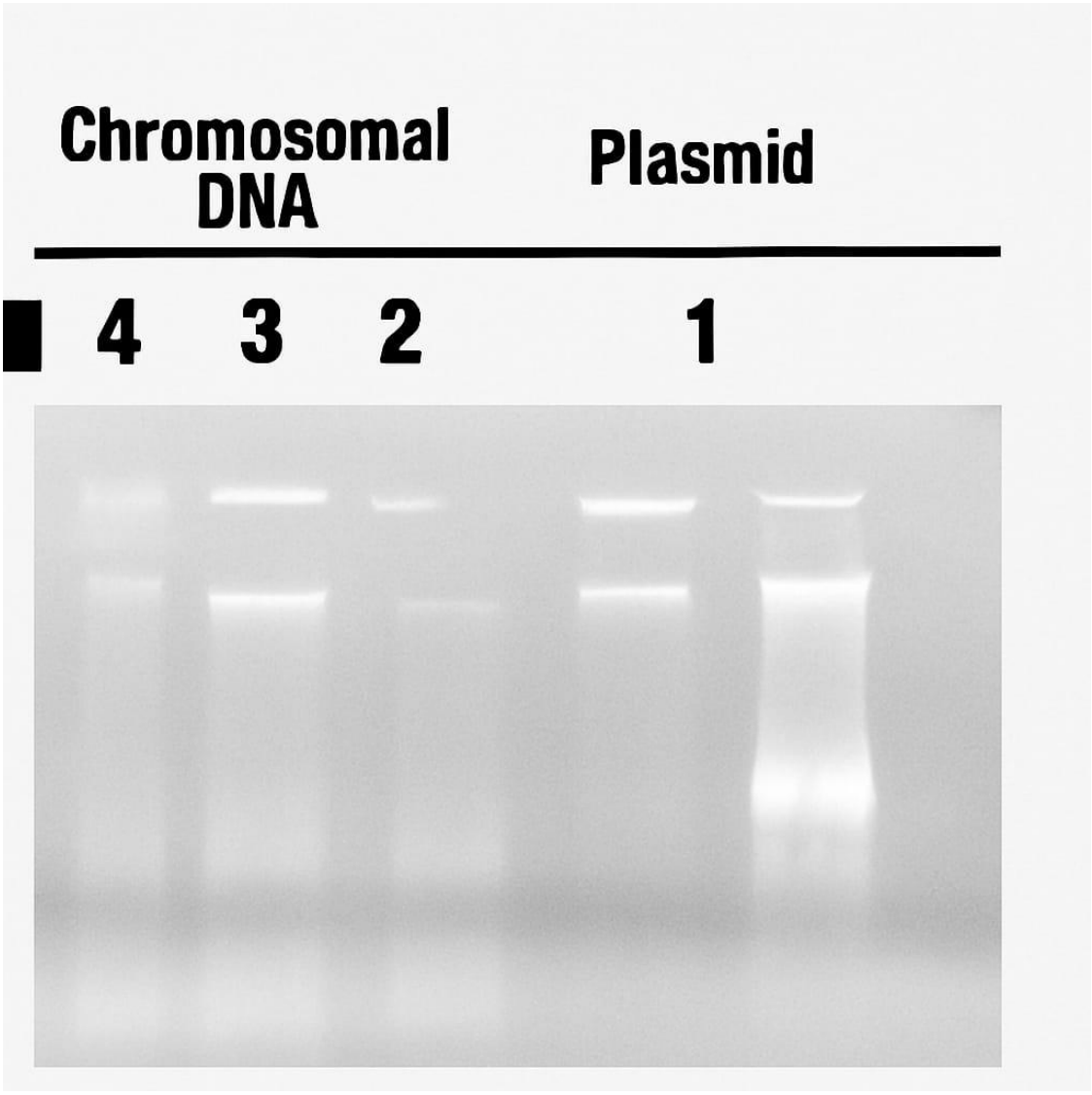
РИСУНКИ

Figure 1. Gel electrophoresis of plasmids content of E. Coli before and after treatment with SDS and AgNPs and the mixer on agarose gel (0.7%) in TBE buffer at 5V/cm. lane 1-3 are cured strains while lane 4 is non cured strain.



Notes:

1: Plasmid content of cured strain with TiO2 and SDS (E3) *

2: Plasmid content of cured strain with TiO2 (E3) *

3: Plasmid content of cured strain with SDS (E3) *

4: Plasmid content of *E. Coli* (E3). *

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Блок 3. Метаданные статьи

PLASMID CURING AND ANTIBIOTIC RESISTANCE REVERSAL IN MULTIDRUG-RESISTANT ESCHERICHIA COLI USING SILVER NANOPARTICLES AND SDS

ПЛАЗМИДНАЯ ЭЛИМИНАЦИЯ И УСТРАНЕНИЕ УСТОЙЧИВОСТИ К АНТИБИОТИКАМ У ПОЛИРЕЗИСТЕНТНЫХ ESCHERICHIA COLI С ИСПОЛЬЗОВАНИЕМ НАНОЧАСТИЦ СЕРЕБРА И ДСН

Сокращенное название статьи для верхнего колонтитула:

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Keywords: plasmids, nanoparticles, Escherichia coli, antibiotic resistance, Silver Nanoparticles, sodium dodecyl sulfate.

Ключевые слова: плазмиды, наночастицы, Escherichia coli, устойчивость к антибиотикам, наночастицы серебра, додецилсульфат натрия.

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