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Comparison of the Effect of Nano Curcumin on the Expression of some Drug Resistance Genes in Gram-negative and Gram-positive Bacteria

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ABSTRACT

This research aims to investigate the molecular aspects of aminoglycoside resistance genes in *S. dysenteriae* obtained from children and evaluate their expression when influenced by curcumin nanoparticles. Also this study seeks to assess the impact of nano curcumin on the expression of multidrug-resistant efflux pump genes in *Staphylococcus aureus* using quantitative PCR (qPCR). The identification of the *aad-E*, *aac-A*, *aph-D*, and *aph* genes in *Shigella dysenteriae* was successfully confirmed using multiplex PCR techniques. Furthermore, the expression of the aad-E gene was examined in the presence of curcumin nanoparticles using real-time PCR. The study also monitored the expression of efflux pump genes (*mexA*, *mexB*, *norA*, *norB*) in *S. aureus* when subjected to curcumin nanoparticles compatibility. In the analysis of *S. dysenteriae* isolates, it was found that 83.3% of the samples were positive for the *aad-E* gene. Additionally, six isolates were identified to carry the *aac-A* and *aph-D* genes, while one isolate possessed the *aph* gene. The expression of the *aad-E* gene exhibited a 1.03-fold change when treated with curcumin nanoparticle at a sub-minimal inhibitory concentration (SubMIC) of 128 µg/ml. The results indicate that Curcumin effectively reduced NorA gene expression in the efflux pump for over 82% of the *S. aureus* samples examined. Furthermore, a decrease in *NorA* gene expression was observed in more than half of the *S. aureus* strains tested. The prevalence of the *aad-E* gene in most Shigella dysentery isolates shows the necessity of effective treatment. The prevalence of the *aad-E* gene in the majority of *S. dysenteriae* isolates underscores the necessity for effective treatment strategies. Curcumin nanoparticles exhibit potential in reducing the expression of aminoglycoside resistance genes and significantly influence *norA* gene expression, indicating its potential as an alternative treatment for drug-resistant strains.

Keywords:Drug Resistance, Nano Curcumin, Gene expression, Gram-negative, Gram-positive

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INTRODUCTION

Shigellosis, also known as bloody diarrhea, is a significant cause of death in children under 5 years old, particularly in developed and developing countries. It is caused by a bacterium called *Shigella*, which is non-motile and lacks spores [1, 2]. *Shigella* has four subgroups: *S. dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*. The disease poses global health challenges due to its low infectious dose, antibiotic resistance, complications, and high mortality rates [3, 4]. Each year, Shigella is responsible for approximately 165 million cases of bloody diarrhea. Fecal-oral transmission is the primary mode of spread, particularly in impoverished communities [5, 6].

The bacterium invades mucosal epithelial cells (M cells), causing damage to the coating tissue and ulcers in the intestinal mucosa. This leads to bloody and mucous diarrhea due to the loss of blood, release of inflammatory elements and mucus, and prevention of water absorption. *Shigella* isolated from humans has shown an increasing rate of antimicrobial resistance in the past two decades. Recent studies reveal that *Shigella* can acquire antibiotic-resistance genes, such as aminoglycosides, through plasmid and integrin [7-10]. Aminoglycosides play a critical role in the inhibition of bacterial protein synthesis by binding to bacterial ribosomes, which subsequently diminishes pathogenicity. These antibiotics demonstrate efficacy against a diverse range of bacteria, including gram-negative organisms, as well as staphylococci and streptococci. *Shigella*, for example, possesses aminoglycoside-modifying enzymes (AMEs) that cause resistance through adenylation, phosphorylation, and acetylation reactions. Enterobacteriaceae, particularly Shigella, carry *aadA1* and *aadA2* genes, conferring resistance to streptomycin and spectinomycin [11, 12]. *Campylobacter jejuni* strains with *aph3-1* and *aadE* genes exhibit resistance to aminoglycosides, with *aadE* gene specifically inducing resistance to streptomycin [13]. The *aph* gene confers resistance to neomycin and kanamycin in gram-negative gut bacteria. The *aac* gene is a key resistance gene causing gentamicin resistance in gram-negative bacteria like *Shigella*. Unfortunately, gentamicin resistance is now widespread in these strains [14]. Curcumin, extracted from turmeric, is a widely used polyphenolic compound in the food industry. It has been

used for centuries in traditional Asian medicine as an antibacterial, anti-inflammatory, and anti-cancer agent. Curcumin interacts with various biological molecules to exert therapeutic effects. Scientists have found that it can form ion channels, leading to the death of bacteria by causing pores in the cell wall [15, 16]. It also inhibits bacteria DNA repair, acting as a bacteriostatic agent. Curcumin has shown the ability to inhibit the growth of different Shigella species. In order to investigate the difference in the effectiveness of nano curcumin and amoxicillin on *S. dysenteriae*, *Streptococcus pneumoniae*, *S. aureus*, and *Escherichia coli*, a study was conducted and its high effectiveness was observed [17, 18].

This study aims to examine the molecular resistance genes associated with aminoglycosides in *S. dysenteriae* isolates obtained from children. Additionally, it seeks to explore the effects of curcumin nanoparticles on the expression of the *aadE* gene [19].

In various researches and studies, anti-infective properties anti-inflammatory, and anti-inflammatory and antioxidant Turmeric have been considered for centuries Scientists update the proven facts and call it a cure. Supplement for Alzheimer's, diabetes, asthma, and wound Stomach [20-27]. The potential antibacterial properties of curcumin are well-documented; however, its low bioavailability and solubility significantly limit its effectiveness in this regard. According to a study, nanotechnology improves the antibacterial effect of curcumin [28]. *S. aureus* is a round gram-positive bacterium that causes a wide range of diseases, such as skin, endocarditis, pneumonia, meningitis, and septicemia. Among the virulence factors of this bacteria, we can mention enzymes, toxins, and sticky surfaces [29, 30]. *S. aureus* is known for its high antibiotic resistance and can cause severe diseases. The global spread of multidrug-resistant *S. aureus* is a significant concern [31, 32]. Appropriate antibiotic treatment is crucial for managing *S. aureus* infections, but resistance to common drugs has been increasing in recent years, making treatment challenging. Efflux pump systems are one of the most influential bacterial defense systems. In efflux pump systems, removing toxic substances and bacterial metabolites prevents the creation of poisonous concentrations of these substances inside the cell and thus prevents the death of bacteria. The efflux system is one of the principal defenses of *S. aureus* against antibiotics [29, 33, 34]. As of now, over 10 efflux pumps have been identified and characterized in *S. aureus*. Most of them belong to the major superfamily (MFS), including NorA, NorB, NorC, MdeA, and SdrM (chromosomally encoded) and QacA/B pumps (plasmid-encoded) [34].

NorA is responsible for exporting many types of drugs and chemical substances, such as ethidium bromide, fluoroquinolones, benzalkonium chloride, cetrimide, acriflavine, and tetraphenylphosphonium bromide multi-drug efflux pumps actively remove antimicrobial substances from bacterial cells, increasing resistance. These strains, equipped with specific plasmids, pose a complex challenge in treating diseases and infections as they are resistant to most antibiotics [33]. Multiplex PCR amplifies multiple target genes simultaneously, using specific primer sets for each gene. This saves time and effort by detecting multiple DNA sequences in one reaction. Through the analysis of the sizes of DNA fragments generated, it is possible to ascertain the identity of the organism, characterize its genotype, or detect the presence of specific genes. This allows for the simultaneous detection of multiple pathogens in a sample. PCR is now widely used in pathogen identification, enabling species, serotypes, antibiotic resistance, and pathogenicity determination [35]. This research presents a novel approach aimed at decreasing the expression of resistance genes in two distinct bacterial strains using nanotechnology.

MATERIALS AND METHODS

Sampling and Isolation

S. dysenteriae

In this study, 60 stool samples were randomly collected from children under 10 years old with diarrhea who were referred to Tehran hospitals in 2022. The samplings were dispatched to Pasargad Laboratory. Initially, the samples were cultured on MacConkey agar and Salmonella-Shigella agar (Merck Co., Germany), and subsequently incubated at 37 °C for 24 hours. Upon confirming the presence of *Shigella* bacteria, further tests were performed, including including TSI (Merck Co., Germany), IMViC, urease, and decarboxylation of amino acids ornithine and mannitol were conducted to diagnose the *S. dysenteriae* species. The disk diffusion method was used to conduct an antibiotic sensitivity test according to the instructions of the Laboratory and Clinical Standards Institute (1977). The test was performed on Mueller Hinton Agar medium (Merck, Germany) using gentamicin (10μg), kanamycin (30 μg), vancomycin (30 μg), Tobramycin (30 μg), and amikacin (30 μg) antibiotics from Himedia, India [1, 36].

S. aureus

One hundred *S. aureus* isolates were isolated from nose, sputum, trachea, and bladder samples from infants and adults at Milad Hospital (Tehran, Iran) and transferred to the Microbiology Laboratory of Mofid University (Tehran, Iran). The identification of the bacteria was conducted using established methodologies [47]. The isolates were stored in 20% glycerol at −80 °C. Then, it was cultured in a nutrient agar medium and incubated at 37 °C. *S. aureus* ATCC 25923 was used as a control strain [52]. In order to investigate the microbial susceptibility test of ciprofloxacin, the disk diffusion method was used in Mueller Hinton Agar medium (Merck, Germany) according to the Clinical Laboratory Standards Institute

(CLSI, 2015) and the incubation time was 24 hours at 37 °C [22, 39].

DNA Extraction and Multiplex PCR Reaction

S. dysenteriae

The bacteria DNA extraction was performed using the Cinnagen DNA extraction kit. The purity of the extracted DNA was confirmed using a nanodrop from Bio-Rad. Multiplex PCR was conducted to amplify the *aadE*, *aac-A*, *aph-D*, and *aph* genes. The amplification was performed in a final volume of 20 µl, utilizing specific primers as detailed in Table 1. The PCR reaction mixture contained 10 µl of 2× PCR master mix concluding Taq DNA polymerase, $MgCl_2$, and dNTPs. Additionally, 1 µl of each primer was incorporated, along with 4 µl of template DNA (40 ng), and 4 µl of double-distilled water. The amplification process was conducted over 33 cycles, consisting of an initial denaturation at 95 °C for 30 seconds, followed by primer annealing at 30 °C for 55 seconds, and an elongation step at 72 °C for 60 seconds. To conclude, the PCR products were analyzed using a 1% agarose gel [19,40, 41].

S. aureus

In the study, 100 strains of *S. aureus* were collected from patients. The studied gene, sequence and primer used are shown in Table 2. DNA was extracted from *S. aureus* using PCR. Specific primers were used to determine the presence of NorA efflux pump genes. The sample was prepared and transferred to a thermocycler (Gradient Mastercycler Eppendorf, Germany) in a final volume of 20 µl.

Preparing Solution

The reason for preparing 0.5 McFarland is that in the sense of standard inoculation for microbial tests, standard barium sulfate equal to half-McFarland standard should be used. To prepare this standard, 0.05 ml of barium chloride 1% was mixed with 9.95 ml of sulfuric acid 1%. Bacterial culture is required for 24 hours to prepare microbial suspension. Therefore, 24 hours before the experiment, the culture is inoculated with nutrient agar in a sloping medium and incubated for 24 hours at 37 °C. A 24-hour bacterial culture was used and dissolved in normal saline. The suspension was returned at 530 nm with the absorption of 0.5 McFarland solution. In other words, the produced suspension should contain 1.5×10^8 [43].

Preparation of Curcumin Nanoparticle Suspension

Nanoparticle formulations were examined using electron microscopy and found to be between 2 and 40 nm in size. To create the nanoparticle stock solution, 10 grams of nanoparticles were mixed with one liter of 50% dimethyl sulfoxide (DMSO) as a secondary solvent. Ultrasonic RK31H was employed for 30 minutes to ensure proper dispersion [28,44,45].

MIC Test

Determination of MIC Nano Curcumin in *S. dysenteriae*

A 96-well microplate with 12 rows containing sterile Mueller Hinton Broth medium was utilized. Curcumin nanoparticle solution with a concentration of 1024 µg/ml was added to the first row, and a serial dilution was prepared ranging from 128-1024 µg/ml. Half-McFarland microbial suspension (100 µl) was added to all wells. Two wells served as positive controls (culture media and microbial suspension), while another two wells served as negative controls (culture media with curcumin nanoparticles). The microplates were then incubated in a shaker incubator at 37 °C and 200 rpm for 24 hours [46].

Determination of *S. aureus* MIC by Microdilution Method

In this study, the microdilution method was utilized to measure the minimum inhibitory concentration (MIC) of an antimicrobial substance. According to CLSI 2015, an antimicrobial agent was employed. The MIC represents the lowest concentration of the substance that prevents bacterial growth 16 mg of ciprofloxacin powder was dissolved in 3 ml of water. The concentrations for these antibiotics were 5120 µg/ml, during work, this solution was diluted 1/10. The MIC was determined using a 96-well microplate filled with Muller-Hinton broth. The process involved adding 100 microliters of the medium to each well. Then, we added 100 µl of the antibiotic to the first row of the microplate and carried out serial dilution in the form of a column. The final concentration was nearly 5×10^5 CFU/mL. *S. aureus* ATCC 29213 was used as a control strain [37]. The microplates were then incubated at 37 °C for 48 hours. Behind the incubation period, a visual inspection was conducted to determine bacterial growth.

Table 1 Primers used in Multiplex-PCR for detection of targeted genes (19)

GEN	Primer sequence	Size
aadE	GCCCTTGGAAGAGTTAGATAATT CGGCACAATCCTTTAATAACA	198 bp
aacA-aphD	CCAAGAGCAATAAGGGCATA CACTATCATAACCACTACCC	220 bp
aph	GAGGGCTTTAGGAATTACGC ACACACCGACCAACAGAGG	125 bp

Table 2 Primers used in this study (42)

Gene	Primer	Primer sequence	Product size (bp)	Reference
norA	norA-F	GACATTTCACCAAGCCATCAA	102	42
	norA-R	TGCCATAAATCCACCAATCC		
gmK (internal control)	gmK-F	TCAGGACCATCTGGAGTAGGTAAAG	108	42
	gmK-R	TTCACGCATTTGACGTATTG		

A scoring system ranging from zero to four was used, with zero indicating no growth and four indicating 100% growth compared to the control well. The MIC was determined as the dilution that completely inhibited bacterial growth according to the established scoring approach.

In summary, the study utilized the microdilution method to measure the MIC of an antimicrobial substance. The method involved serial dilution of nanoparticles, the addition of bacterial suspension, and visual observation of bacterial growth to determine the MIC and Sub MIC.

RNA Extraction

S. dysenteriae

RNA was isolated from *aadE* gene-carrying strains in both treated and untreated groups during the logarithmic growth phase (OD_{600} = 0.4-0.6) using the Qiagen RNeasy microkit. DNA removal was performed using the Qiagen DNase kit. The extracted RNA quality was evaluated with a nanodrop. Roche's Reverse AMV enzyme was used to synthesize cDNA. In preparation for the real-time polymerase chain reaction, a 20 µl reaction volume was established, utilizing the Genet bio-CAT kit. The amplification reaction was carried out in a Corbet machine with the following program: initial denaturation at 95 °C for 1 minute, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 59 °C for 40 seconds, and extension at 72 °C for 60 seconds. The 16s rRNA home gene served as an internal control for the test [46].

S. aureus

Genomic RNA extraction of ciprofloxacin-resistant strains was performed using the RNX-PLUS kit (Cat. No. RN7713C/EX6101). The purified RNA was used for creating cDNA.

cDNA Synthesis

S. dysenteriae

The cDNA synthesis process involved using Reverse AMV enzyme (concentration: 25 lµ/unit, Roche). The extracted RNA was incubated at 65 °C for 3 minutes. Reverse transcription (RT) was carried out at 42 °C for 60 minutes using 2 µl of Random Primer, 0.8 µl of AMV Reverse Transcriptase enzyme, 2 µl of 10 mM dNTP, 1 µl of Rnase inhibitor, and 2 µl of 10× buffer. Following, the AMV enzyme was deactivated by incubating it at 99 °C for 5 minutes [19].

S. aureus

cDNA was synthesized using a reverse transcriptase reaction, DNasel, RNase-free Kit (cat. No: PR891627), according to the manufacturer. cDNA Synthesis was performed for both groups of RNA (extracted in the presence and absence of curcumin). cDNA was used for Real-time PCR. The concentration of cDNA was measured by nanodrop.

They mixed 10 µl RNA, 10 µl 10× reaction buffer with $MgCl_2$, and 5µl DNasel, RNase-free. Then, incubate at 37 °C for 30 minutes. After, we added 10 µl 50 mM EDTA and incubated at 65 °C for 10 min. RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent. Then, the prepared RNA is employed as a template for reverse transcriptase. We added 10 µl of this RNA to 2 µl random hexamer and 8µl distilled water. Then, PCR is accomplished. The program of PCR was 45 °C for 59 minutes, and after that 95 °C for 5 minutes [34].

Quantitative PCR Test

S. dysenteriae

A 20 µl PCR reaction was conducted using Genet bio sybr green. The reaction mixture included 5 µl DEPC water, 1 µl forward primer, 1 µl reverse primer, 1 µl Rox dye, and 2 µl cDNA. The amplification was performed in a Corbet qPCR machine with a denaturation step at 95 °C for 3 min, followed by 35 cycles of amplification at 95 °C for 30 s, 59 °C for 40 s, and 72 °C for 60 s. The 16s rRNA gene was used as an internal control. The presence of the *qepA* gene was determined to calculate its frequency and generate graphs using specialized software. The expression level was analyzed by comparing mRNA expression to a standard. The extracted genomes of all isolates were confirmed using 1% agarose gel electrophoresis, and the resulting bands were documented.

S. aureus

Method Real-time PCR was used to determine the effect of curcumin on norA gene expression. gmk is a *S. aureus* housekeeping gene and it was used as an internal control 2. The total volume of materials used in the reactions was 20 µl including 1 µl cDNA as a template 10 µl Power SYBR® Green PCR Master Mix (Applied Biosystems) and 0.5 µl of each F and R primers and 8 µl distilled water. The qPCR cycling for the *norA* was performed at 94 °C for 10 min, followed by 40 cycles at 94 °C for 12s and 37s at 57 °C and finally a melting stage (72 °C for the 20s) to determine the unspecific PCR product or possible primer dimers. Couple of a negative control were contained in all qPCR runs, and the gmk gene was used as an internal

control. The relative expression of the norA efflux pump gene was analyzed using the ΔΔCT method [47].

RESULTS

Cultivation, Isolation, and Identitying of Bacteria

A total of 60 stool samples suspected of *S. dysenteriae* were obtained from 4- to 10-year-old children with diarrhea at Tehran Hospitals in Pasargad Research Laboratory, Iran. Positive samples were identified by colorless colonies on McConkey agar and Salmonella-Shigella agar. Out of the samples analyzed, 12 isolates were identified as *S. dysenteriae*. These isolates exhibited specific biochemical characteristics, including being lactose, gas, indole, and urease negative. They also showed non-motion, ornithine, and mannitol decarboxylation reaction negative, and citrate negative results. Furthermore, the isolates tested positive for methyl red and negative for Voges Proskauer [19].

Sensitivity Test

S. dysenteriae

Out of the 8 isolates tested, they were resistant to gentamicin and tobramycin, while 1 isolate showed resistance to amikacin and kanamycin. None of the isolates exhibited resistance to vancomycin. Additionally, only 2 isolates showed no signs of resistance [19].

S. aureus

Out of 100 samples of *S. aureus* isolated, 31 cases were resistant to ciprofloxacin. Out of 31 isolates resistant to ciprofloxacin, 13 had an active efflux pump [29].

Multiplex PCR Reaction

S. dysenteriae

From 12 isolates of *S. dysenteriae* identified, 10 isolates (83.3%) carried the *aadE* gene, 6 isolates carried the *aacA, aphD* gene, and 1 isolate carried the *aph* gene [19].

MIC Test

S. dysenteriae

The results of the MIC of curcumin nanoparticles in S. dysenteriae showed that the minimum concentration of growth inhibition in the presence of curcumin nanoparticles was 225 μg/mL and the Sub MIC of curcumin nanoparticles to reduce *aadE* gene expression was 128 μg/ml.

S. aureus

To assess the antimicrobial properties of curcumin solution, different concentrations of a primary solution (5120 μg/ml) were tested on the expression of the *norA* gene, which is highly abundant. The microdilution method was used to determine the minimum inhibitory concentration (MIC) of the collected *S. aureus* strains. MIC and Sub MIC are indicated [29].

DISCUSSION

The real-time PCR method was used to investigate the expression level of the Ciprofloxacin-resistant *S. aureus* gene in the presence of curcumin. Based on the obtained results, curcumin extract significantly reduces the expression of the NorA gene ($P<0.05$). In more than 82% of the samples, curcumin decreased the rate of *norA* gene expression and also decreased the expression of NorA gene in more than half of the strains in 18% of the samples, the reduction was more than 100 times and this is an excellent result. In only 2 samples we have an increase in *norA* expression, while in one sample the increase is very slight and insignificant [29]. The *aadE* gene expression was assessed using the qPCR technique to determine the impact of curcumin nanoparticles. The isolates treated with curcumin nanoparticles exhibited a significant alteration in the relative expression of the *aadE* gene compared to the untreated isolates. Statistical analysis revealed a *P*-value below 0.05, indicating a significant difference in gene expression between the treated and untreated groups. The Fold Change parameter for the *aadE* gene was −1.03, indicating a 1.03-fold reduction in gene expression in the treated group relative to the untreated group [19] (Figs. 1 and 2). Shigellosis, also known as bloody diarrhea, is a concerning condition due to its high mortality rate in children under 5 years old and the prevalence of antibiotic-resistant strains.

Early diagnosis and antibiotic treatment are crucial to reduce complications. *Shigella* strains have been found to possess aminoglycoside-modifying enzymes (AMEs) that confer resistance to aminoglycosides through various reactions. Similar resistance genes have been identified in other bacteria such as *Pseudomonas aeruginosa* and *Campylobacter jejuni*. However, the distribution of these genes differs among bacterial isolates. Our study focused on *S. dysenteriae* and found that the *aadE* gene, associated with streptomycin resistance, was the most common gene. This contradicts findings in Campylobacter jejuni. Another study demonstrated that the *aac-A, aph-D* gene, responsible for gentamicin resistance, was present in both *S. aureus* and *S. dysenteriae* strains [13, 48, 49]. These findings highlight the presence of aminoglycoside resistance genes in both gram-positive and gram-negative bacteria. Turmeric, known for its antibacterial and therapeutic properties, contains curcumin, a phenolic compound responsible for its pharmacological effects. However, curcumin's bioavailability is limited, and nanotechnology has been employed to enhance its efficacy. Our study investigates the impact of curcumin nanoparticles on the expression of the *aadE* gene, prevalent in 83.3% of *S. dysenteriae* isolates. Previous studies have shown the inhibitory effects of curcumin on bacteria such as *Helicobacter pylori* and *Pseudomonas aeruginosa*, albeit with varying results.

Table 3 MIC and Sub MIC concentrations of curcumin that inhibited the growth of bacteria and altered gene expression, respectively

Isolate No.	Curcumin MIC	Curcumin Sub MIC (for extraction)
160	32	16
113	32	16
133	32	16
164	32	16
22	32	16
128	R	R
68	R	R
85	16	8
161	32	16
54	16	8
104	32	16
43	2	1
90	32	16

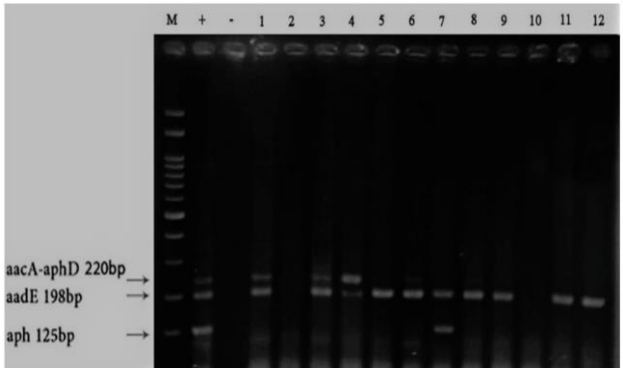

Fig. 1 Results of Multiplex PCR and electrophoresis, 100 bp ladder, +: positive control, and -: negative control.

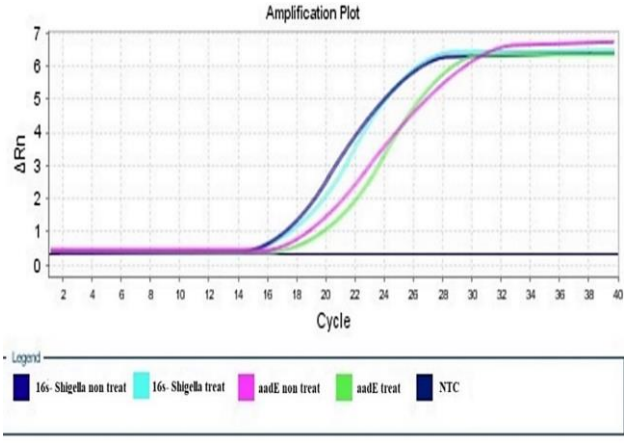

Fig. 2 Results of Real Time PCR reproduction curve of *aadE* gene related to aminoglycoside resistance.

In our study, we utilized dimethyl sulfoxide to dissolve curcumin nanoparticles, yielding a similar minimum inhibitory concentration (MIC) as reported in previous research [17, 35, 45, 50]. Another study demonstrated MIC values for curcumin against *S. dysenteriae* and *Campylobacter jejuni*. By employing nanotechnology, we achieved a lower MIC of 225 for curcumin against *S. dysenteriae* compared to the previous study. Hence, nanonization of curcumin particles proves more effective in combating *S. dysenteriae* than curcumin alone.

S. aureus is a gram-positive, eukaryotic cluster. It is a human and animal pathogen. This bacterium can cause skin infections such as jaundice, gout burns, boils, and obesity. It may also cause meningitis, bacteremia, endocarditis and septicemia. *S. aureus* through the formation of Biofilm, can stick to surfaces and create resistance Antibiotics play a role in their colonies However, the treatment of *S. aureus* infections faces challenges due to antibiotic resistance [51]. A study examined the effects of curcumin treatment on the *norA* gene in *S. aureus*, showing promising therapeutic effects, reduced drug consumption, and cost savings. Further research is needed to measure key variables and investigate curcumin's potential in epidemiological studies and control strategies. The inhibitory concentration of curcumin in the current study was found to be 16 µg/ml [29].

In a study conducted by Jabir *et al* in 2022 to investigate the antibacterial effect of curcumin nanoparticles in the production of alpha-hemolysin in *S. aureus* bacteria, the results of the data indicated that Cur-Au@ZnO nanoparticles showed the highest antibacterial effect in S. aureus., in such a way that the diameter of the aura of lack of growth was estimated to be more than 30 mm (Fig. 3) [52].

In Tyagi's research conducted in 2015, the effective dose of curcumin nanoparticles to investigate the toxicity of curcumin on *S. aureus* was reported to be 100 µM, and in this study, 100 percent of the bacteria were destroyed due to the pores created in the membrane [53]. In the study conducted to investigate the antibacterial effect of samarium oxide nanoparticles synthesized by curcumin on Gram-negative *P. aeruginosa* and Gram-positive *S. aureus*, the lowest MIC value was 3.12 µg/ml for *S. aureus* and the highest MIC for *P. aeruginosa* was obtained at the rate of 25 µg/ml. The results obtained indicate that the

combination of samarium oxide nanoparticles and curcumin may effectively diminish the resistance of *S. aureus* and *P. aeruginos*a.

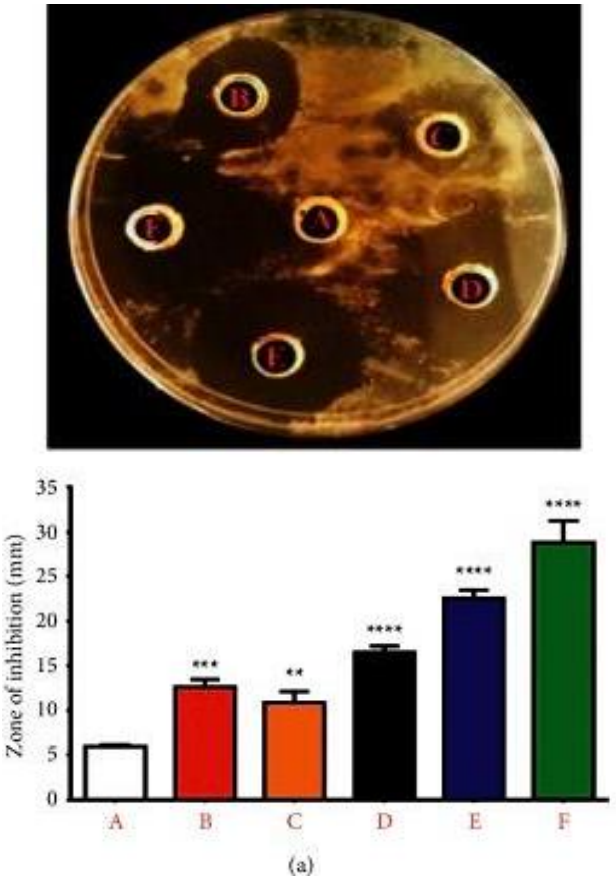

Fig. 3 Antibacterial activity of prepared NPs against *S. aureus*. (a) Zone of inhibition: (A) control untreated bacterial strain. The bacterial strains were treated with NPs as follows: (B) ZnO NPs, (C) Cur NPs, (D) Au NPs, (E) Au@ZnO NPs, and (F) Cur-Au@ZnO NPs.

This finding highlights the potential for using this combination in developing strategies to combat these bacterial strains [54]. According to the investigation carried out in *E. coli* bacteria, the minimum growth inhibitory concentration of curcumin nanoparticles was reported to be 400-500 µM, and it can be concluded that it is an active approach for water-soluble compounds with low bioavailability [55]. Also, the study conducted on the antibacterial effect of curcumin nanoparticles on biofilm gene (*rsmZ*) and efflux pump genes (*mexD*, *mexB*, and *mexT*) In *P. aeruginosa*, the minimum effective dose was 128 µg/ml [50].

On the other hand, nano curcumin technology with other compounds has also shown good antimicrobial effects, for example, the MIC of curcumin and zinc nanoparticles in *S. epidermidis*, *S. hemolyticus*, and *S. saprophyticus* resistant to methicillin was reported as 1000, 750 and 500 µg/ml, respectively [56]. According to Adeyem *et al*.'s research, curcumin nanoparticles of MIC of 600 µg/ml for *S. aureus* caused an antibacterial effect on the L-tryptophan mechanism, and in MIC of 400 µg/ml in *E. coli*, it caused lipid peroxidation and also increased the DNA destruction of both bacteria [57].

Using the colony count technique, a study was conducted to investigate the resistance of *S. aureus* resistant bacteria in 2019. In this study, the appropriate MIC value in the growth of resistant bacteria was recorded as 100 µg/ml [58].

The virulence factors of Helicobacter pylori, including urease enzyme, haemagglutination activity, and the biofilm created, decreased significantly in the dose of 50 µg/ml curcumin, so it can be concluded that Helicobacter pylori is one of the effective factors in stomach infection and Also, the antibacterial effects of curcumin on this bacteria, curcumin was used to treat stomach infection [59].

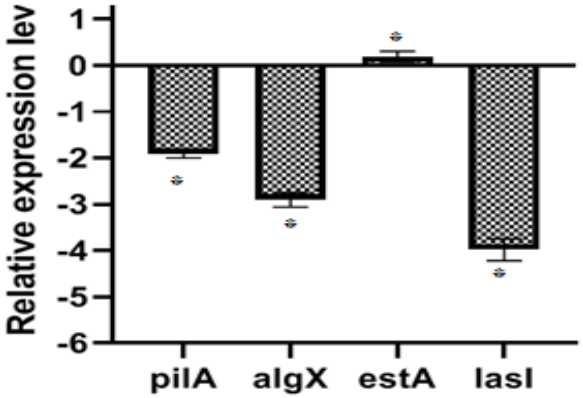

Fig. 4 The expression level of the studied genes.

A study was conducted on 50 strains of *S. aureus* in order to check the presence of norB and norA genes using multiplex PCR and real-time PCR methods. 12 isolates were resistant to ciprofloxacin antibiotic. Moreover, *norA* and *norB* genes were found in 100 % and 83% of ciprofloxacin-resistant isolates, respectively [60].

The expression of *pilA*, *algX*, and *lasI* genes, which are virulence factors in biofilm in *P. aeruginosa*, decreased by 1.91, 2.9, and 3.98 times when exposed to curcumin nanoparticles, but increased by 0.19 for *estA*. The MIC value was a concentration equivalent to 128 µg/ml. Due to the inhibitory effect of nano curcumin on genes involved in the formation of *P. aeruginosa* biofilm, it is possible that this nanoparticle could be used as a therapeutic option against this bacterium and to inhibit the genes involved in its virulence (Fig. 4).

Research results show that curcumin is better than some antibiotics, the process of respiratory explosion in cells infected with *Chlamydia* can be inhibited and the treatment of inflammation of atherosclerosis caused by *Chlamydia pneumonia* is effective [69].

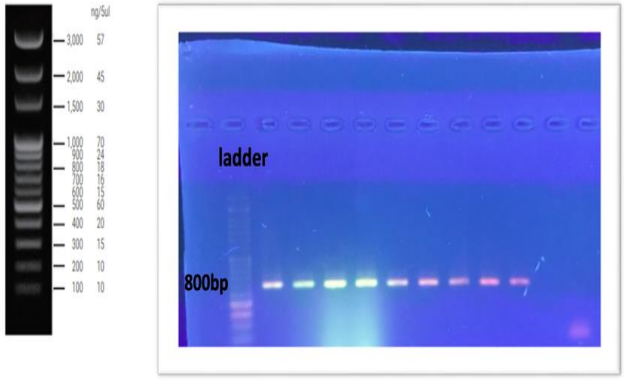

Fig. 5 PCR technique for the *mecA* gene of clinical and standard isolates of *S. aureus*; the band length of 800 bp indicates the *mecA* gene.

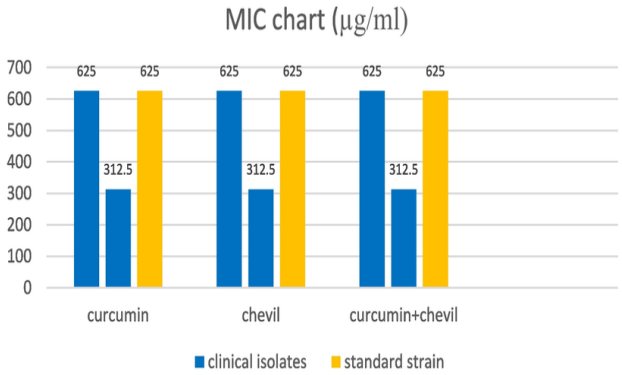

Fig. 6 The minimum inhibitory concentration of nanocurcumin, *Ferulago angulate* (chevil) extract, and mixture of curcumin and extract on MRSA clinical isolates.

Also, curcumin nanoparticles in combination with *Ferulago angulate* extract have shown good antimicrobial effects on methicillin-resistant *S. aureus*, the cause of hospital burns. The presence of the *mecA* gene effective on biofilm was confirmed by PCR. Curcumin nanoparticles and *F. angulate* extract inhibited the growth of MRSA strains at a concentration of 312.5 to 625 µg/ml. According to the obtained results, the combination of curcumin nanoparticles and *F. angulate* can be used as an effective drug combination in the treatment of hospital burns caused by *S. aureus* (Figs. 5 and 6) [62]. In the studies conducted on Helicobacter pylori bacteria, it was shown that curcumin was in the range between 5-50 µg/ml. It can reduce the activity of NF-κB factor and thus reduce the inflammation caused by Heliobacteria. Also, curcumin can prevent the attachment of *H. pylori* to epithelial cells and reduce the expression of cysteine deaminase in bacteria. Of course, bud said that using curcumin alone is not enough, and appropriate antibiotics should also be used in the treatment of *H. pylori* infection, but due to the good antibacterial effect of curcumin, it is hoped that it will be used in auxiliary treatments shortly [63-66]. Curcumin prevents the growth of *E. coli* by inhibiting the FTsZ factor. At a concentration equal to 8 µg/ml, it inhibits the SOS responses caused by levofloxacin by reducing the expression of *blaTEM-1*gene [67,68].

On the other hand, curcumin is a water-soluble compound that has low bioavailability, and its effectiveness can be increased by using nanotechnology. A study was conducted to compare the effectiveness of curcumin and curcumin nanoparticles on *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, and significant differences were observed between curcumin and nano curcumin [70]. The genes studied in the research can be considered prominent in the pathogenicity of this bacterium. By comparing the results of previous studies with the present study, it is concluded that nanotechnology improves the performance of curcumin particles and creates higher sensitivity in bacterial cells because curcumin is less used due to its poor solubility in water, rapid decomposition, and bioavailability.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author Contribution

All authors contributed to data collection, writing, and revision of the manuscript. All authors read and approved the final manuscript.

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