

Original Research Article

Effects of ZnO Nanoparticles on Initial Adhesion and *fimH* Gene Expression in Uropathogenic *Escherichia coli*

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ABSTRACT

Introduction: Uropathogenic *Escherichia coli* (UPEC) strains are the main cause of urinary tract infections. Adhesion is one of the main and primary steps of UPEC pathogenicity. Type 1 fimbriae are bacterial surface structures that play an important role in bacterial adhesion. The aim of this study was to evaluate effects of ZnO nanoparticles on the initial adhesion and *fimH* gene expression in UPEC strains. **Materials and Method:** Four UPEC isolates from patients with urinary tract infection were exposed to sub-minimum inhibitory concentration of ZnO nanoparticles (1250 µg/ml). Expression of the *fimH* gene was evaluated by Real-time PCR. **Result:** Presence of the ZnO nanoparticles reduced *fimH* expression in all four isolates. The highest and lowest rates of down-expression were 1.4-fold and 16.37-fold, respectively. These results were confirmed with phenotypic observations. **Conclusions:** Sub-MIC concentrations of ZnO significantly reduce the expression of the *fimH* gene in strong biofilm forming UPEC strains but cannot inhibit biofilm formation. However, further studies are required to confirm whether ZnO nanoparticles could completely prevent UPEC adhesion.

KEYWORDS: UTI, UPEC, ZnO nanoparticle

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INTRODUCTION

Uropathogenic *Escherichia coli* (UPEC) strains are the main cause of urinary tract infections (UTIs). These bacteria are responsible for over 90% of uncomplicated UTIs [1]. Approximately 40-50% of healthy adult women will experience UTI in their lifetime [2]. UTI has high mortality rates in many countries and its treatment is costly [3]. Patients with urinary catheter have significantly higher risk of developing the infection. According to previous studies, bacterial biofilm are formed on the catheter seven days after using catheter, which can eventually lead to UTI [4]. The first essential steps of developing a lasting UTI are invasion and primary adhesion [5]. Type 1 fimbriae is the most common virulence factor produced by more than 80% of UPEC strains. Type 1 pilus is encoded by the *fim* gene cluster (*fimA-H*). The tip of this hair-like adhesive organelle is composed of subunits FimF, FimG and FimH (mannose-binding adhesive subunit). UPEC strains that express FimH effectively bind to both monomannose and trimannose glycoprotein receptors on the surface of epithelial cells in the urinary tract, which leads to bacterial colonization [6].

Biofilm is a community of growing microorganisms that are attached to a surface and enclosed in a self-produced exopolysaccharide matrix. In biofilms, bacteria are protected from environmental stress such as host immune system, dryness and antibiotics [7]. Due to the increased rate of resistance to antimicrobial agents, infectious diseases remain a public health problem worldwide. Treatment of antibiotic-resistant bacteria require high doses of antibiotics that often causes intolerable toxicity. Therefore, alternative strategies for treatment of bacterial infections are being investigated. Nanostructures (<100 nm) have been introduced as new antimicrobial agents with unique physical and chemical properties. Their exact mechanism of action is not yet fully understood. Metal nanoparticles cause DNA damage and destroy bacteria via various methods including oxidation of membrane lipids, changing the permeability of the bacterial cell wall and releasing the cell content. ZnO nanoparticles are relatively cheap, UV-resistant and have antimicrobial activity in natural pH. These advantages have expanded

the use of these nanoparticles for clinical purposes. Among the metal oxide nanoparticles, ZnO has shown the most toxic effects against *E. coli*. These nanoparticles disrupt the integrity of the membrane by producing reactive oxygen species, and destroy bacteria [8-11]. The aim of this study was to determine the effects of ZnO nanoparticles on the initial adhesion and level of *fimH* gene expression in UPEC strains isolated from UTIs.

MATERIALS AND METHODS

Bacterial strains

Three UPEC isolates from patients with UTI (from 176 samples) in hospitals of Gorgan (Iran) were investigated. *E. coli* PTCC 1399 strain was used as the positive control for biofilm production and presence of the *fimH* gene. Minimum inhibitory concentrations (MICs) and sub-MIC of ZnO (USA, lot number: us3590) for all four isolates were determined (by agar dilution method) as 2500 µg/ml and 1250 µg/ml, respectively.

Biofilm production measurement

The effects of nanoparticles on biofilm of isolates were evaluated according to the method described by Samet et al. [12]. Samples with OD values less than 0.1 and between 0.1 and 0.2 were considered as non-biofilm former and poor biofilm former, respectively. Samples with OD value of 0.2-0.3 and more than 0.3 were considered as moderate biofilm former and strong biofilm former, respectively [13]. All experiments were repeated three times and the mean values were used for the analysis of results.

*Determination of *fimH* gene expression by Real-Time PCR*

All four strains formed biofilm in the test tube in the presence and absence of sub-MIC concentrations of ZnO (1250 µg/ml). RNA extraction was performed from the biofilms formed on the tube wall using RNX-Plus kit according to the manufacturer's instructions (SinaColon). Decontamination with genomic DNA was done using RNase-free DNaseI according to the manufacturer's instructions

(Thermo Scientific). One µg of RNA treated with the DNaseI was used for cDNA synthesis using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). Expression of the *fimH* gene was evaluated using specific primers (Table 1)[14,15]. Real-time PCR was performed by an ABI-7300 thermocycler (Advanced Biosystems, Foster, California, USA) using SYBR Green kit (ampliqon, Denmark).

Table 1. Specific primers used in the study

Name	Sequence (5 to 3)	Size (bp)
<i>FimH(F)</i>	GCTGTGATGTTTCTGCTCGT	168
<i>FimH(R)</i>	AAAACGAGGCGGTATTGGTG	
<i>gapA(F)</i>	ACTTACGAGCAGATCAAAGC	170
<i>gapA(R)</i>	AGTTTCACGAAGTTGTCGTT	

Temperature conditions consisted initial denaturation at 95°C for 15 minutes followed by 35 cycles of 95°C for 15 seconds and 60°C for 1 minute. *GapA* gene encoding glyceraldehyde 3-phosphate dehydrogenase was used as the independent internal control. In this study, CTs obtained from the Real-time PCR were used in the $\Delta\Delta CT$ formula to calculate mRNA expression levels [16].

RESULTS

Type 1 fimbriae are one of the main adhesins necessary for bacterial adhesion. In this study, we examined the effect of sub-MIC concentrations (1250 µg/ml) of ZnO nanoparticles on the expression of *fimH* gene in UPEC isolates using Real-time PCR. All isolates produced strong biofilms, which was later weakened in the presence of sub-MIC concentrations of ZnO nanoparticles. The sub-MIC concentration of ZnO nanoparticles caused 1.4, 2.69, 4.31 and 16.37-fold reduction in *fimH* expression in isolates 1, 2, 3 and PTCC 1399 (Figure 1).

The results of gene expression were consistent with results of the phenotypic observations (biofilm formation and gene expression levels decreased in all four isolates) (Table 2).

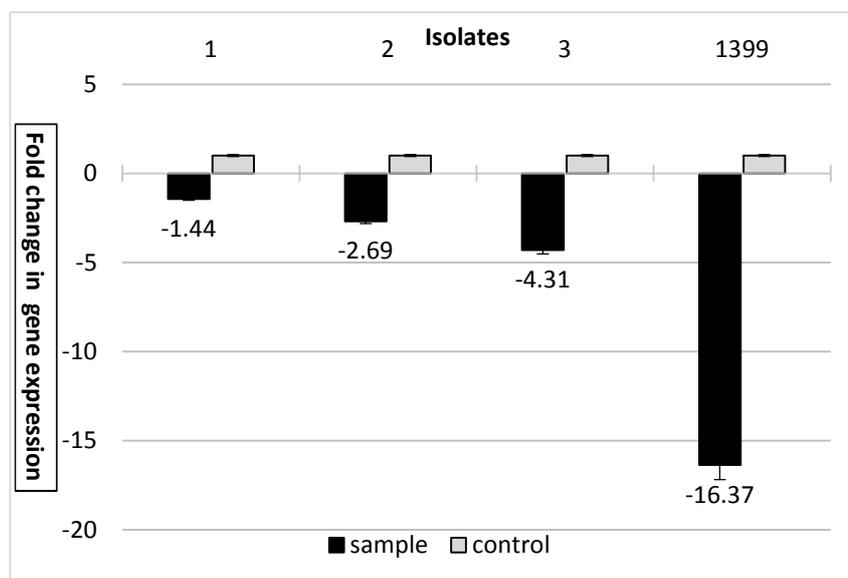


Figure1. Down-expression of the *fimH* gene in the presence of sub-MIC concentrations of ZnO nanoparticles

Table 2. Results of gene expression and biofilm formation

Isolates	Biofilm formation based on OD		Gene expression reduction
	Before treatment with sub-MIC of ZnO nanoparticles	After treatment with sub-MIC of ZnO nanoparticles	
1	0.317(strong)	0.122(weak)	1.44
2	0.404(strong)	0.148(weak)	2.69
3	0.869(strong)	0.150(weak)	4.3
PTCC 1399	0.467(strong)	0.162(weak)	16.37

DISCUSSION

Irreversible adhesion is the first essential step of UPEC pathogenicity. At this stage, majority of bindings are specifically between bacterial adhesins and host cell surface receptors. This highly stable attachment leads to establishment of bacteria on the cell surface and causes infection. Amalaradjou et al. have recently shown that essential oil of cinnamon (Trans-cinnamaldehyde) decreases expression of genes related to bacterial adhesion and invasion (*fimA*, *fimH*, *papG*, *sfaS*, *focA*). They concluded that the down-expression of the mentioned genes also

reduces adhesion (2). Burt et al. showed that the essential oil of thyme prevents motility by inhibiting synthesis of flagella in *E. coli* O157:H7 [17]. In the present study, ZnO nanoparticles decreased the expression of *fimH*, an important gene involved in the irreversible adhesion.

Jin-Hyung Lee et al. reported that zinc ions and ZnO nanoparticles markedly inhibit production of virulence factors (pyocyanin, pseudomonas quinolone signal, pyochelin and hemolytic activity) and biofilm formation in *Pseudomonas aeruginosa* [18].

These results are consistent with our findings. Islam and et al. also showed that biofilm formation in UPEC isolates containing the *flu* genes (coding ag43) is inhibited by nitric oxide, an effect not observed in isolates without the *flu* genes [19]. In a previous study, we showed that ZnO nanoparticles reduce biofilm formation and expression level of the *flu* gene in UPEC [11].

The results of this study and our previous study showed that sub-MIC concentrations of ZnO nanoparticles reduce the expression of *flu* gene and *fimH* gene, which in turn reduces biofilm formation. However, further studies are required on gene expression and adhesion of bacteria to surfaces to determine whether ZnO nanoparticles could completely prevent UPEC adhesion and biofilm formation.

CONCLUSION

Sub-MIC concentrations of ZnO significantly reduce the expression of the *fimH* gene in strong biofilm forming UPEC strains but cannot inhibit biofilm formation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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