The Effect of ZnO NPS and TiO2NPs on Gene Expression of Five adhesion genes fimB - fimH of E. coli Isolated from Clinical Sources in Mousl –Iraq

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Abstract

The study was carried out on (135) various clinical samples collected from patients attended Ibn Sina and Al Salam Hospitals in Mosul city during December 2020 – March 2021. *E.coli* isolated from urine and burns at percentage 20% for each of them shown.where isolation from sputum at 10 % and from stool (control) at 100. The sub-Minimum Inhibition Concentration (sub-MIC) of ZnO NPs for *E. coli* it appeared between (2500–1250) µg/ml, while for TiO_2 NPs appeared between (5000–2500)µg/ml.The present of adhesion genes in (24) isolates of *E. coli* was as follow: *fim*A at percentage 8.3% and *fim*B, *fim*C present at 100% for each one, *fim*E, *fim*I as 95.8% for each one, *fim*H 87.5%.Real Time PCR used to detect the ability of ZnO NPs and TiO_2 NPs on expression of the adherence genes *fim*B ,*fim*C, *fim*E, *fim*I and *fim*H. In *E.coli* show increasing in expression after exposed to sub MIC of ZnO NPs at concentration (2500µg/ml), the most effected gene was *fim*H followed by *fim*B and *fim* I gene while same effect on *fim*C and *fim*E. *E.coli* show increasing in expression after exposed to sub MIC of TiO_2NPs at concentration (5000µg/ml), the most effected gene was *fim*C followed by *fim*H and same effect on *fim*B and *fim*I and less effect on *fim*E

Key word: *E. coli*, Biofilm, ZnO NPs, TiO_2 NPs, Real Time PCR, *fim*H gene.

Introduction

Type 1 pile are important type of pile in *E. coli*, they help bacteria adhesion to biotic and abiotic surface by attaching with mannose receptors present on different host tissues, it is encod by group of genes called *fim* gene cluster that include nine genes: *fim*A, *fim*B, *fim*C, *fim*D,*fim*E, *fim*F, *fim*I ,*fim*G, *fim*H, each gene have its own function (Pusz *etal* .,2014).Pathogenic *E. coli* can causes many disease such as diarrhea, urinary tract infections peritonitis, colitis, bacteremia, infant mortality Hospital acquired pneumonia, surgical sit infection, Hemolytic Uremic Syndrome (Dubreui., 2020). ZnO NPs are inorganic metallic nanoparticles, it is safe, non-toxic materials with stable physical and chemical properties (Ogunyemi *etal.*, 2019). It is characterized a biological activity against many gram-positive and gram-negative bacteria, (Uribe *etal.* 2020) This antibacterial activity due to the large surface area compared with the size, which makes it one materials that have application as a material with antibacterial activity (Albukhaty *etal.*, 2020). The Nanoparticles titanium dioxide TiO_2NPs, is characterized a white powder is insoluble in water, in addition to its have antibacterial activity (Sagadevan *etal.*, 2022).. It is also characterized by antibacterial activity against gram-positive and negative bacteria, aerobic and anaerobic bacteria, fungi and parasites, and mixing nano titanium dioxide with silver particles will increase of its anti-bacterial activity (Abdulkadim *etal.*, 2021). This study aim to investigation effect of ZnONPs and TiO_2NPs of gene expression of adhere gene in *E. coli* by using Real Time PCR.

Materalis and Methods

Isolation: (135) sample were collected from patients on Ibn Sina and Al – Salam hospital in the Mosul city for isolation of *E. coli*. Samples includes (100 urine, 15 burns,10 Sputum), we collected 10 Stool samples as control and 2 stander strain *E. coli* O157:H7ATCC 43888 and *E. coli* (Qc)25922.

Diagnosis of *E. coli*: using Macroscopic and Microscopic examination, biochemical test to identified *E .coli* depending on (Macfaddin ., 2000) .**Determined of Minimum inhibitory concentration(MIC) and subMIC for ZnONPs and TiO_2 NPs.:**The double dilution method used depending on (Saginur *etal* ., 2006), by add 20000 µg/ ml of ZnO NPS with size 20 nm until reach to (10000, 5000, 2500, 1250, 625, 312.5, 156.25, 78.125, 39, 19.5) µg/ml, and add 40000µg/ml of TiO_2 NPs with size 10 nm until reach to (20000, 10000, 5000, 2500, 1250, 625, 312.5, 156.25, 78.125, 39) µg/ml.

DNA extraction: It according to kit from Geneaid and measure purity and concentration by using Nano - drop Spectrophotometer from Act Gen. **Detection of Adhesion genes (*fim*A, *fim*B, *fim*C ,*fim*E, *fim*I, *fim*H) using primer as in Table 1**

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Primer	(´5 -3´)	bp	Length of gene (bp)	Reference
*fim*A-F	GGACAGGTTCGTACCGCATC	20	151	(Blumer*et al.*2005)
*fim*A-R	ACGTTGGTATGACCCGCATC	20		
*fim*B-F	ACTGGAGATTCATCCGCACA	20	179	(Pusz *etal* ., 2014)
*fim*B-R	GTCGTCCTCTGGCTCTATCC	20		
*fim*C-F	CTCGCAATTATCAGCCGCAT	20	182	(Pusz *etal* ., 2014)
*fim*C-R	GCATTTTCAAGAACCCGGGT	20		
*fim*E-F	TATGAATTGGCGGAGCGTGGTG	22	153	(Blumer *et al.* 2005)
*fim*E-R	AAACGAGCAGCATTACTGGCGGTAT	25		
*fim*H-F	GCTGTGATGTTTCTGCTCGT	20	168	(Pusz *etal* ., 2014)
*fim*H-R	AAAACGAGGCGGTATTGGTG	20		
*fim*I-F	GACGGTCAATATGGGGCAAA	20	153	(Blumer *et al.* 2005)
*fim*I-R	TTTTTACCATCCGCGACACC	20		

Real Time PCR: Total RNA was extracted using (kit from addbio), gene expression understudy except for *fim*A gene because it was not found in all isolates and due to mutation, that affected binding of its primer in qPCR experiments. Using of (2500µg/ml) and (5000 µg/ml) of sub-MIC for ZnO NPs and TiO_2 NPs respectively to determined gene expression for isolating *E. coli* and Primer used in Real Time PCR in Table 1.

Result and Discussion

From (135) Samples including urine, burns, sputum, stool (control) 34 isolates of *E. coli* were collected. The high percentage for isolating was from urine and burns at 20% for each one, 10% from sputum, where 100 % from stool as (control) as show in Table 2. *E. coli* consider a normal flora in the intestinal tract of human and animal, and it can transport to other site of body such as urine tract, blood and causes disease (Novella *etal.*, 2015).

Table 2. Number and percentage of *E .coli*			
Type of sample	Number of samples	Number of isolates	Percentage of isolates %
Urine	100	20	20
Wound	15	3	20
Sputum	10	1	10
Stool (control)	10	10	100
Total	135	34	25.1
Stander strain	*E. coli* O157:H7ATCC 43888, *E. coli* (Qc)25922		

E. coli show as pink color colony on MacConkey agar due lactose fermenter (Omolou – Aso *etal* ., 2017) shown as figure1A, *E. coli* appears as green color with metallic shine on Eiosin methylene shown in figure1B, was selective media that allow to growing gram negative bacteria and inhibited gram positive bacteria by Eosin and methylene blue pigment that inhibit gram positive, The capability of bacteria to ferment sugar lead to decrease pH, The acidity of media lead to precipitate pigment and colonies appears as green with metallic shine(Hassain and Ali ., 2022). In Endo agar colony of *E. coli* show as red color with metallic shine as shown in figure 1C, due to lactose fermenter and release fuchsine pigment was give red color of colony (Hassan *etal* ., 2013). Colonies of *E. coil* appears as green – blue on Hi crom agar. This result agree with study researcher (Antony *etal.*,2016) shown as figure1D. The biochemical test were used to Diagnosis of *E. coli* according to(Jawetz *etal* ., 2016).it appears as gram negative bacilli, postive for catalase, oxidase, indol test, methyl red test, nitrate reduction, and show negative test for voges proskauer, citrate utilization.

A B C D

Figure1. Growth of E. coli in different media

A: MacConkey agar B: Eosin Methylene Blue C: Endo agar D: Hi crom agar

The Minimum inhibition concentration of ZnO NPs and TiO_2 NPs: In this study we depended on sub MIC of ZnO NPs (20nm) and TiO_2 NPs(10nm) for inhibition adhesion genes of (24) isolates of *E .coli*. The Minimum inhibition concentration MIC for ZnO NPs(20nm) was (10000, 5000, 2500, 1250, 625,

312.5, 15625, 78.125, 39, 19.5) µg/ml and TiO_2 NPs(10) sub MIC was (20000, 10000, 5000, 2500, 1250, 625, 312.5, 15625, 78.125, 39)µg/ml. subMIC for ZnO NPs(20nm) appeared between (2500– 1250) µg/ml. where sub MIC of TiO_2 NPs(10nm) appears between (5000- 2500) µg/ml. Researchers Nazosri and Karimike reported in 2018 that the minimum inhibitory concentration of zinc oxide nanoparticles for *E. coli* was 2.5 mg/ml, and the researcher (Shakerimoghaddam *etal.*, 2017) refer that the MIC for uropathgeinc *E. coli* was 2500µg/ml. So, the difference in the inhibitory concentration with other studies due to the difference in the size and concentration of nanoparticles in addition to the type of bacteria and the strain. Although metals and metal oxides are considered to have a toxic effect on human cells when they are present in high concentrations, they are not considered toxic if they are in low concentrations,(Dogan and Kocabs., 2020).

Investigation of Adhere Genes by PCR :In this study result show that percentage for adhere genes in *E. coli* isolates was *fim*A at 8.3%, *fim*B, *fim*C appears at100% of each one of them respectively and *fim*E in 95.8%, *fim*I as 95.8% and *fim*H appears at 87.5% shown as table 3.The researcher (Rahder *etal* ., 2015) that *fimH* adhesion gene was presence as 68%, with a higher rate than the rest of the other type1 genes, researcher(Karimian *etal.*,2012) refer to the presence of the *fimH* gene as 79.6%, while the researcher (Jalali *etal.*, 2015) diagnosed the *fimH* gene as 73 % in *E. coli*. Although most of the studies relied to diagnosis of adhesion genes depending on *fimH* gene due to it is the main gene responsible for the adhesion of *E. coli* bacteria, it encoded by fimH protein found at the tip of the filaments. The researcher (Frommel *etal* ., 2013) refer that the *fimC* gene was identify in *E. coli* strains with an extra-intestinal at percentage as 95.3% and it have an essential role in adhesion, while the researcher (Valenski *etal.*,2003) refer that the *fimI* gene has a main role in the biosynthesis of Type1 fimbriae, when occur a mutation of the *fimI* gene, it will result in a change of a normal shape in Type1 capillaries..

Table3. Percentage of adhesion genes in *E. coli*

adhesion genes					
E. coli isolated	*fimB*	*fimC*	*fimE*	*fimI*	*fimH*
	Number and Percentage				
	(24)100%	(24)100%	(23) 95.8%	(23) 95.8%	(21) 87.5%

The effect of ZnO NPs and TiO_2 NPs of gene expression on adhesion genes: In this study was investigate the possibility of inhibiting gene expression of adhesion genes (*fimB – fimH*) by using ZnO NPs and TiO_2 NPs. Real time quantitative polymerase chain reaction (Real Time PCR) was used for measured the gene expression of adhesion genes in isolate *E.coli* under study and comparison with the house keeping gene 16S rDNA after treatment with ZnO NPs and TiO2 NPs. The results showed an increase in the gene expression of adhesion genes (*fim B - fimH*) when treated, ZnO NPs and TiO 2NPs, and compared with 16S rDNA.its mean the ZnO NPs and TiO 2NPs effect of product of adhesion gene. The results showed that TiO2NPs with a size of 10 nm were the most effective substances increasing the gene expression of adhesion genes *fim B*, *fimC*, *fimE*, *fim I*, *fim H*, when compared with each of the ZnoNPs, and with the 16s rDNA gene, as there was an increase in gene expression of adhesion genes, and that the most influential gene for TiO2NPs was *fimC* gene followed by *fimH* gene, and the effect was similar in both *fimB* and *fimI* genes, and the effect was less in *fim E* gene as shown in figure (2). *fim A* is consider as major subunite while *fimB* and *fimE* act as regulatory function and have important role for to switch the *fimS* element un idirectionally, either Phase-ON to Phase-OFF or vice versa, *fimC* is consider as a periplasmic chaperone protein that help translocate the proteins fimbria through the periplasm until the FimC-Fim protein complex reaches the FimD, *fimI* gene help in transport and assembly of type 1 pili with other gene *fimC* and *fimD*. The adhesion, encoded by the *fimH* gene is assist for binding with mannose receptor (Pusz *etal* .,2014 ; schwan *etal* ., 2011). Shawkat and Cherhri in 2021 was indicated that TiO2NPs was characterized by anti-bacterial activity, as it has the ability to inhibit biofilm formation in *E. coli* and thus inhibit disease. As the researcher (Sodagar *etal* ., 2017) was indicated the inhibitory activity of titanium dioxide nanoparticles TiO_2NPs against *E. coli* is due to its ability to create holes in the cell wall, which leads to increased cell permeability to materials and thus causes its death. Also, the nanoparticles of titanium dioxide, TiO_2NPs effect on the DNA and cause its fragmentation and dissolution, thus affecting the activity of genes (Zhukova *etal.*, 2015).. ZnO NPs with a size of 20 nm showed little inhibition of the adhesion genes *fim* B, *fim* C, *fim* E, *fim* I, *fim* H compared with the nanoparticles of titanium dioxide TiO 2NP with a size of 10 nm. The genes were most affected is the *fim*H gene, followed by the *fim*B gene and the *fim*I gene, while the genes *fim*C, *fim*E, had the same effect as shown in Figure (2). (Rutherford *etal* ., 2021) indicated that the inhibitory activity of ZnO nanoparticles depends on the formation of Reactive Oxygen Species (ROS) and releasing the zinc ion Zn^{+2}, which has a toxic effect on the cell, (Hsuen *etal.*, 2015),The results were agree with result of (Jamalan *etal.*, 2019) indicated that the zinc oxide nano-ZnO has an inhibitory activity for *E. coli* O157:H7 bacteria, as well as effective against other strains of *E. coli*, as ZnO NPs works to inhibit the adhesion of *E. coli* through its ability to inhibit the *fim*H gene, which is the main gene responsiblefor bacteria adhesion to the surface and tissues of the host

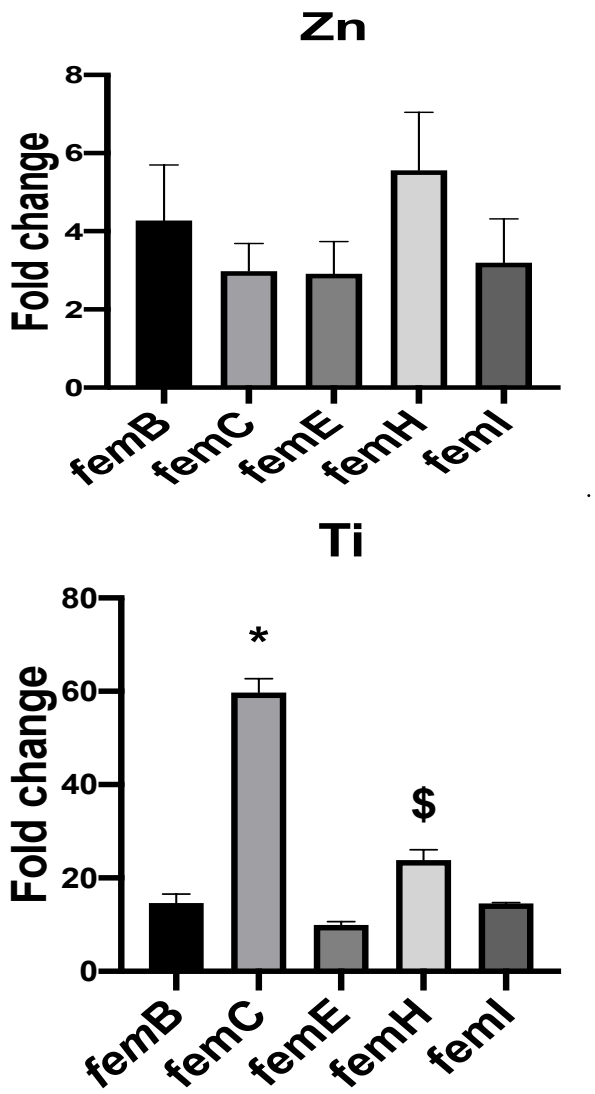

Figure2. Effect of Nanoparticals on gene expression of adherent gene fim B -fim H

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