Comparing *Tsh* and *Pic* Genes in UPEC Strain Isolated from Urinary and Digestive Systems Infections and Examining their Relationship in Biofilm Formation

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Abstract
Tsh gene encodes a protein similar to immunoglobulin A (IgA) proteases in *Neisseria gonorrhoea* and *Hemophylus influenza*. The *pic* gene product is a 116 kilodalton protein with a role in intestinal colonization. The study examined the relationship between the prevalence of *tsh* and *pic* genes and biofilm formation in isolates isolated from digestive and urinary tract infections (UTIs). 250 stool and urine samples (100 stool samples and 150 urine samples) were collected from the patients. The age range of the patients was 10-65 years with a mean age of 42±6. The isolated strains were identified using phenoty*pic* and biochemical tests and commercial antisera. Biofilm formation of pathogenic E .coli strains was examined using the microtiter method. The existence or non-existence of *tsh* and *pic* genes and their frequency were examined using PCR method. In the E. coli strains examined, the *pic* gene with the size of bp147 and the *tsh* gene with the size of bp824 were seen on agarose gel. The *pic* gene was seen in 5 (3.3%) UPEC strains and *tsh* in 2 (2%) EPEC strains. The findings indicated that the *tsh* gene is associated with the ability of the isolated strains to produce biofilm, and with its effect on biofilm production, it could affect the severity of the disease and antibiotic resistance; therefore, it could be used as a genetic tool to detect strains E. coli pathotype. However, regarding the *pic* gene of the strain studied, biofilm production was not seen in them. The study findings indicated that the *tsh* gene is associated with the ability of the isolated strain to produce biofilm and with its effect on biofilm production could affect the severity of the disease and antibiotic resistance.

Keywords: Urinary and digestive system infection, biofilm formation, *tsh* and *pic* genes, UPEC strain.

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1. Introduction

Biofilm is a type of slimy coating on solid surfaces shaped by the growth of bacteria in nature. One or more species of bacteria form the biofilm. Biofilms formed with one species of bacteria are called monoculture, and biofilms formed on mucous surfaces like the intestine, mostly a mixture of various species of bacteria, are called multicultural. Biofilm is a type of bacterial accumulation growing wherever there is water. Ninety-nine of all bacteria live inside the biofilm. A biofilm is a

group of cells fixed on a surface and surrounded by a matrix of organic polymeric materials of microbial origin (extensive glycocalyx exopolymer). These exopolysaccharides, which make up more than 90% of the dry weight of the biofilm facilitate attachment to the surfaces of microcolony formation and resistance to antimicrobial substances (Emtiyazi, 2000). The ability of bacteria to stick to surfaces, especially in pathogenic organisms, is seen as a significant phenomenon for disease onset (Emtiyazi, 2000). In nature, bacteria mostly grow as colonies in tangled groups and are rarely found individually or in pure culture. Mucous biofilms, intestinal surface, and urinary infections, and dental plaques are well-known examples of this phenomenon in humans (Picard *et al.*, 2009). Moreover, in nature, the slippery substance on the river stones and the thin gel-like coating on the inner wall of the pot where the flowers have been kept for nearly a week are also examples of biofilm. Biofilm formation is a strategy for the bacteria survival. Food is used optimally regarding this (West *et al.*, 2007).

E. coli considered a part of the normal flora of the digestive system of humans and warm-blooded animals (Schwan *et al.*, 2002). Although this bacterium is usually harmless and does not cause an infection, some strains of E. coli have acquired virulence genes in different forms like mutation or receiving a plasmid containing a pathogenic gene that gives them pathogenic properties for humans and animals (Ulett *et al.*, 2007). The pathogens are responsible for causing several types of clinical infections like enteric and diarrheal diseases, UTIs, sepsis and meningitis. According to the pathogenic characteristics of each of them and the clinical symptoms of the host, E. coli pathogenic strains are divided into several groups or pathotypes (Raksha *et al.*, 2003).

Uropathogenic Escherechia coli is the primary cause of UTI in developed countries. UTI is considered one of the most prevalent infectious diseases in humans. In America, 1.6 million dollars are annually spent on the treatment of these infections (Sorsa, 2007). It is estimated that 40-50% of healthy adult women will experience a UTI at least once during their lifetime. This infectious disease usually starts with a bladder infection, but it usually develops by covering the kidneys and may eventually lead to kidney failure and even enter the blood (Sivakumar *et al.*, 2010). The ability of UPEC to cause symptomatic UTI of the urinary system is because of the presence of two types of virulence agents: adhesins (like type I fimbriae, P-fimbriae, S-fimbriae, FIC-fimbriae, and so on) and toxins (like hemolysin, tumor necrosis factor) (Snyder *et al.*, 2005). Connecting to the epithelium of the urinary tract is the first stage of UTI, and this makes the bacteria resistant to the hydrodynamic forces of urine flow, and this increases their ability to multiply and invade the kidney tissue (Al-Hasani *et al.*, 2001).

Strains causing intestinal infections are classified as enterotoxigenic E. Coli (ETEC), enteropathogenic E. Coli (EPEC), enteroinvasive E. Coli (EIEC), or Shiga-Toxin Producing E. Coli (STEC), enterohemorrhagic E. Coli (EHEC).

Enteroaggregative E. coli (EAEC) and Diffusely Adherent E. coli (DAEC) cause persistent diarrhea, particularly among the young children in developing countries. ETEC strains are common causes of traveler's diarrhea and cause watery and non-inflammatory diarrhea syndrome. EPEC strains are among the causes of diarrhea among children, particularly in developing

countries. EIEC strains cause dysentery syndromes similar to those caused by Shigella species and are rare in the United States of America. EHEC strains usually form the colitis serotype (O157:H7), which do not show inflammatory cells during laboratory diagnosis and examination of these bacteria in the stool, but the stool of patients with this type of bacteria can be bloody. Subsequently, in a few patients, hemolytic-uremic syndrome (HUS) takes place, where Shiga-like cytotoxins play a role, and these factors cause the symptoms of the disease in these people (Matiuzzi da Costa *et al.*, 2008).

The *tsh* gene was first identified in APEC strain O78:K80, when it was cloned in E. coli K-12. The protein encoded by this gene is 50% similar to immunoglobulin A (IgA) proteases in Neisseria gonorrhoeae and Haemophilus influenzae (Provence and Curtiss, 1994). Stathopoulos et al. found that the *tsh* protein represents a subcategory of the IgA protease family in the autotrans protease that is seen in many species of Shigella and E. coli (Stathopoulos *et al.*, 1999). The *pic* gene is a 116 kilodalton protein identified in EAEC (Henderson *et al.*, 1999). The reason for its name was its role in intestinal colonization and its biological activity (Navarro-Garcia *et al.*, 1998). This protein cannot increase the secretion of ions (Henderson *et al.*, 1999). The *pic* gene is present on the chromosomes of EAEC and UPEC and Shigella flexneri strains. This gene is located on the DNA next to the set1A and set1B genes, these two genes cause the production of ShET1 protein and are involved in the toxicity caused in the intestine (Navarro-Garcia *et al.*, 1998).

The *tsh* gene has been detected in 33% of UPEC clinical samples and 63% of stool species, and the *pic* gene is seen in 31% of pyelonephritis and 7% of stool species. Studies have revealed that these studied groups of E. coli, differ in terms of the distribution of pathogenic genes (Anderson and Dodson, 2004). UTIs are the most common bacterial infections that involve large economic costs and cause a large number of people in the society to be infected. The clinical symptoms of these infections range from asymptomatic bacteriuria to cystitis and pyelonephritis to infectious shock and defects in many organs. UTIs in infants under one year of age are more common in boys than in girls, but at the age of 1-2, girls are more common than boys, and this ratio remains strong until the age of 50. E. coli is the main cause of urinary infections in more than 80% of cases as the cause of UTI among the humans (Jafari *et al.*, 2012).

The necessity of molecular screening diagnosis and timely treatment of patients is of great significance given the prevalence of UTI and diarrhea associated with E. coli and gastrointestinal infections in general. Irreparable problems will occur in the future for the patient and her family because of negligence in the treatment of this infection, while we should not forget the costs imposed on governments and the public health system in this regard (Jafari *et al.*, 2012).

Anderson and Dodson (2004) examined the presence of intracellular bacterial populations of uropathogenic E. coli in UTIs. The studies of this group showed that these bacteria follow a complex molecular process to produce biofilm in epithelial cells and can manifest many biofilm-like characteristics. These formed intracellular biofilms cause high immunity of bacteria against the defense of the body's immune system (Anderson and Dodson, 2004). Henderson et al., indicated that *pic* protein can be isolated from the culture supernatant of enteroaggregative E. coli

(EAEC). Its genetic identification overlapped with the shetAB gene, which encodes the enterotoxin Shet1. This toxin is responsible for the accumulation of fluid in the loops of the rabbit's intestine. Such as other SPATE proteins, *Pic* has serine protease activity that affects mucin (Karimian *et al.*, 2012). Zobisizkansa *et al.* (2012) examined the profiles of pathogenic genes and the phylogenic origin of E. coli in chronic and acute intestinal diseases. The studies by this group determined that various strains isolated from chronic and acute gastroenteritis infections can be distinguished according to their pathogenic genes. The difference in the pathogenic genes of E. coli strengthened the hypothesis that chronic intestinal diseases mostly rely on the pathogenic potential of the strains connected (Anderson and Dodson, 2004).

The prevalence of *tsh* and *pic* genes and biofilm formation in isolates isolated from gastrointestinal and UTIs have been discussed in the present study. The study is based on the hypothesis that strains containing *tsh* and *pic* genes are significantly present in clinical samples and are directly involved in the formation of biofilms created in UTIs and cause more stability of pathogen strains. According to this, the purpose of the study is to compare *tsh* and *pic* genes in UPEC strain isolated from urinary and digestive system infections and examine their relationship in biofilm formation.

2. Materials and Methods

The present study is descriptive-analytical and 250 stool and urine samples (100 stool samples and 150 urine samples) were collected from medical centers in Tehran from August 2014 to June 2015. The age range of these patients was from 10 to 65 years with a mean age of 42±6. Firstly, the stool sample was diluted with physiological serum, then it was cultured on blood agar, EMB and McConkey media, and urine samples were cultured on EMB and McConkey blood agar media. Some lactose-positive and lactose-negative colonies were selected and each one was cultured separately on TSI for 24 hours in a 37°C incubator. Then differential media were used to determine the identity of the isolated strains. The isolated strains were identified using phenoty*pic* and biochemical tests and using commercial antisera.

2.1 Required culture media

The environments needed for growing samples and how to prepare them

2.1.1 Blood Agar

According to the manufacturer's instructions, 40 grams of the relevant powder was weighed and mixed in one liter of distilled water and heated until it completely dissolves and the color of the environment becomes clear to prepare this environment. Then it was autoclaved (121°C for 15 min) and after that, when the culture environment temperature reached 55°C, 5-10% sheep defibrinated blood was slowly added to it and shaken a little to mix well. After these steps, the prepared medium was distributed in sterile plates. The environments were ready for use after quality control and non-contamination. The strains that have hemolysin create colonies with a halo of hemolysis on this medium.

2.1.2 MacConkey Agar culture medium

Lactose fermenting strains can be distinguished from strains that cannot ferment lactose with the help of this environment. Moreover, this environment is suitable for the growth of bacteria in the intestine due to the presence of bile salts. To prepare this medium according to the manufacturer's instructions, 17 grams of the respective powder was weighed and dissolved in one liter of distilled water and heated until it was completely dissolved and the color of the medium became clear, then it was autoclaved (121 °C for 15 min) and after that, when the temperature of the culture medium reached about 55 °C, they were distributed in sterile plates. After examining the quality control and ensuring non-contamination (incubation at 37 °C for 48 hours), these plates can be used. The reagent present in this medium is neutral red, which is purple-red in acidic pH and pale pink in neutral pH. Lactose negative bacteria cannot produce acid from lactose sugar and as a result, the color of the reagent does not change, they create colorless colonies in the environment. However, lactose-positive bacteria turn the environment red by producing acid from lactose fermentation, and the penetration of color into them also causes the formation of purple-red colonies. The existence of bile salts prevents the growth of gram-positive bacteria and some gram-negative bacteria in this environment. This culture medium is a differential and selective medium, so that only gram-negative bacteria grow on it. Moreover, in this culture medium, lactose-fermenting bacteria could be distinguished from bacteria that do not ferment lactose.

2.2 Eosin Methylene Blue (EMB) medium

Isolating and diagnosing lactose fermenting species from other non-fermenting species and the display of metallic luster of some Enterobacteriaceae species such as E. coli and Citrobacter are possible on this medium. Then 36 grams of the relevant powder was weighed and dissolved in one liter of distilled water and heated until it was completely dissolved and the color of the medium became clear, then it was autoclaved (121 °C for 15 min) to prepare this medium according to the manufacturer's instructions. After the temperature of the culture medium reached 55 °C, it could be divided into sterile plates and after quality control (incubation of several plates randomly at 37 °C for 24 hours). A group of coliforms produce stable acetaldehyde from the decomposition of lactose, which by releasing substances in the environment (eosin y and methylene blue) and oxidizing them in the vicinity of air causes the formation of a metallic luster on the surface of the colonies. E. coli and Citrobacter can produce metallic polish. Another group of lactose positive coliforms created unstable acetaldehyde and only the colonies created by these bacteria exist in red-violet color without metallic polish.

2.3 Iron medium - triple sugar Triple Sugar Iron (TSI) Agar

This environment is used to examine the production of gas, H2S, and acid production from the fermentation of glucose and lactose sugars, which helps in the differential diagnosis of Enterobacteriaceae species. According to the manufacturer's instructions, 65 grams was weighed and after dissolving it in one liter of distilled water, it was heated until it was completely dissolved and the color of the medium became clear, then the medium was divided into tubes with cotton lids and autoclaved. (121 °C for 15 min) to prepare this medium. After autoclaving, the mediums

were placed in an inclined way with a little height so that at least 3 cm of the bottom of the tube is completely filled with the medium and the rest of the medium creates a suitable *gradient*. Following the solidification of the medium, quality control and ensuring the absence of contamination (incubation at 37 °C for 48 hours) can be used.

2.4 SIM medium

This environment is used to study sulfur regeneration, indole production, and bacterial movement. According to the manufacturer's instructions, 30 grams was weighed and poured it into a liter of distilled water and heat it until it completely dissolves and the color of the medium becomes clear, then distribute the medium in tubes with cotton lids and autoclave (121 °C for 15 min) to prepare this medium. After solidification, quality control and examining non-contamination (incubation at 37 °C for 48 to 72 hours) can be used. The appearance of red color a few seconds after adding Kovac's reagent is a sign of the presence of indole and the test is positive. The movement of the bacteria is first examined prior to pouring the reagent.

2.5 Designing primer

The primers studied by Karimian et al. were used with a single nucleotide change in the primer sequence to assess the presence of *tsh* and *pic* genes in E. coli strains. The specificity of the primers of this study was examined by blasting in the NCBI database. In the following step, melting point and GC percentage were analyzed using Gene runner software. After confirming the desired primer sequence, its manufacture was given to Metabion Company (Karimian *et al.*, 2012).

2.6 DNA extraction of E. coli strain

Bacterial genome extraction was carried out using a Roche extraction kit. DNA extraction was carried out based on the instructions of the kit.

1- Firstly, cell suspension was prepared from E. coli strain grown on culture medium using Elution buffer for DNA extraction.

2- 200 microliters of binding buffer and 40 microliters of proteinase K were poured into a 1.5 ml microtube without DNase and RNase.

3- 200 microliters of cell suspension were added into a 1.5 ml microtube without DNase and RNase.

4- It was immediately placed at 70 °C for 10 min.

5- The resulting mixture was carefully transferred to the filter installed in the kit and collection tube.

6- 100 microliters of alcohol were added to it and centrifuged at 8000 rpm for 1 min.

7- The tube containing the solution was discarded and the filter was transferred to another tube.

8- 500 microliters of inhibitor removal buffer were added and centrifuged at 8000 rpm for 1 min.

9- The tube containing the solution was discarded and the filter was transferred to another tube.

10- 500 microliters of washing buffer were added and centrifuged at 8000 rpm for 1 min.

11- The tube containing the solution was discarded and the filter was transferred to another tube.

12- 500 microliters of washing buffer were added and centrifuged at 8000 rpm for 1 min.

13- For 10 seconds, it was centrifuged again at a speed of 13,000 revolutions.

14- The tube with the solution was discarded and the filter was transferred to a 1.5 ml microtube without DNase and RNase.
15- 200 microliters of elution buffer were added to the filter containing DNA.
16- It was centrifuged for 1 min at g8000 speed.
17- The extracted DNA was stored at -70 °C.
Using an Eppendorf BioPhotometer, absorb diluted DNA solutions were measured at a ratio of 1:50 (20 microliters of DNA stock solution plus 980 microliters of sterile distilled water or distilled water) at a wavelength of 260 nm (absorption wavelength of nucleic acids) and 280 nm (absorption wavelength of proteins) and ultimately the ratio of optical absorption of DNA solutions at a wavelength of 260 nm to 280 nm (260/280) was obtained, which is an index of DNA purity.

2.7 Amplification stage of *tsh* and *pic* genes by PCR method

To start the PCR method to amplify and detect *tsh* and *pic* genes and to examine the correctness of the reaction components in the stages of the study, the extracted genome prepared from the E. coli reference laboratory was used as a positive control. Distilled water was used as a negative control to indicate the non-transfer of contamination to the reaction tubes.
The sequence of primers for the amplification of *tsh* and *pic* genes presented in Table 1.

Table 1. The sequence of primers for the amplification of *tsh* and *pic* genes (Karimian *et al.*, 2012).

Primer	Sequence	Size
tsh (F)	ACTATTCTCTGCAGGAAGTC	bp 824
tsh (R)	CTTCCGATGTTCTGAACG	
pic (F)	CCTGACAGAGGACACGTTCA	Bp 147
pic (R)	GAGAGACCGTACTGCGTG	

A 2% gel was prepared using 0.5X TBE buffer and agarose to carry out electrophoresis. Then 10X TBE from Roche was used to prepare 0.5X TBE buffer.
The desired strain was cultured on Tryptic Soy Agar with 2% glucose (Tryptic Soy Agar + 2% glucose) TSA + 2% glu and incubated at 37°C for 24 hours. Single colonies grown on tryptic medium of soy agar with 2% glucose were inoculated into tryptic medium of soy broth with 2% glucose to obtain a suspension with light absorption equal to 0.1 at 625 nm wavelength.

3. Results

The results showed that, 250 clinical samples (100 stool and 150 urine samples) were collected in this study. The age range of patients with UTI was 12 to 65 years with a mean age of 45 and 45 (18%) uropathogenic strains were isolated. The *tsh* gene was observed in 5 (3.3%) UPEC strains and the *pic* gene was observed in 5 (3.3%) UPEC strains and 50% of the isolated UPEC strains formed biofilms. The *tsh* gene was seen in all biofilm-producing samples.

A temperature gradient was used to select the best temperature protocol to administer the PCR test, to detect *pic* and *tsh* genes. The study found that the optimal temperature for the PCR method used in the study was 54 °C for the *pic* gene and 56 °C for the *tsh* gene (Figures and (Figure 1 and 2).

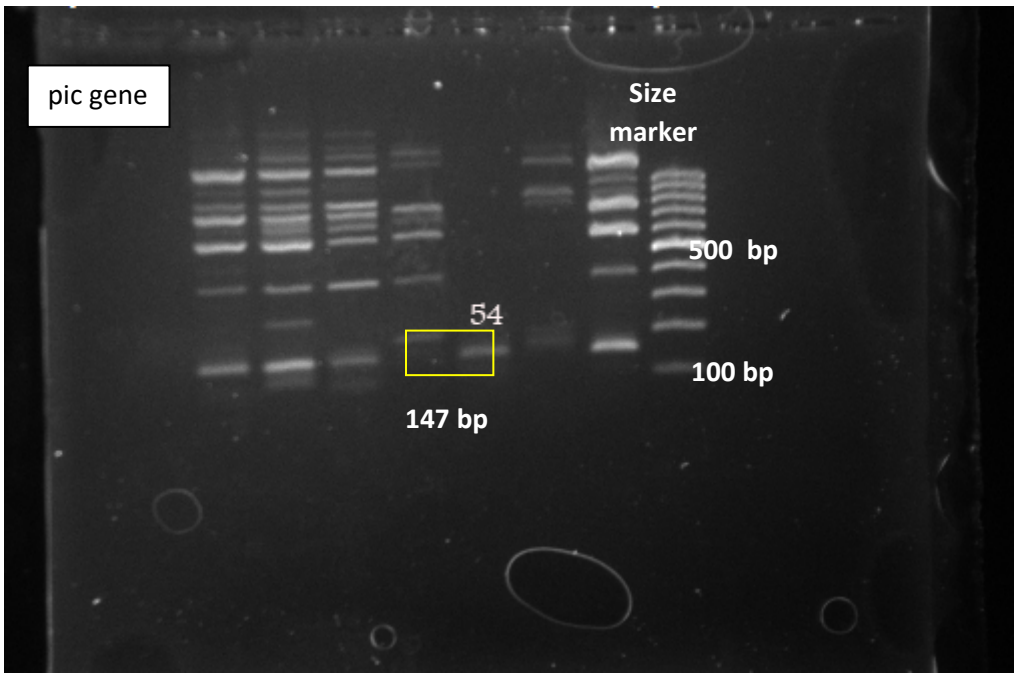

Figure 1. The result of checking the temperature gradient to select the optimal temperature in the PCR test setup for the *pic* gene.

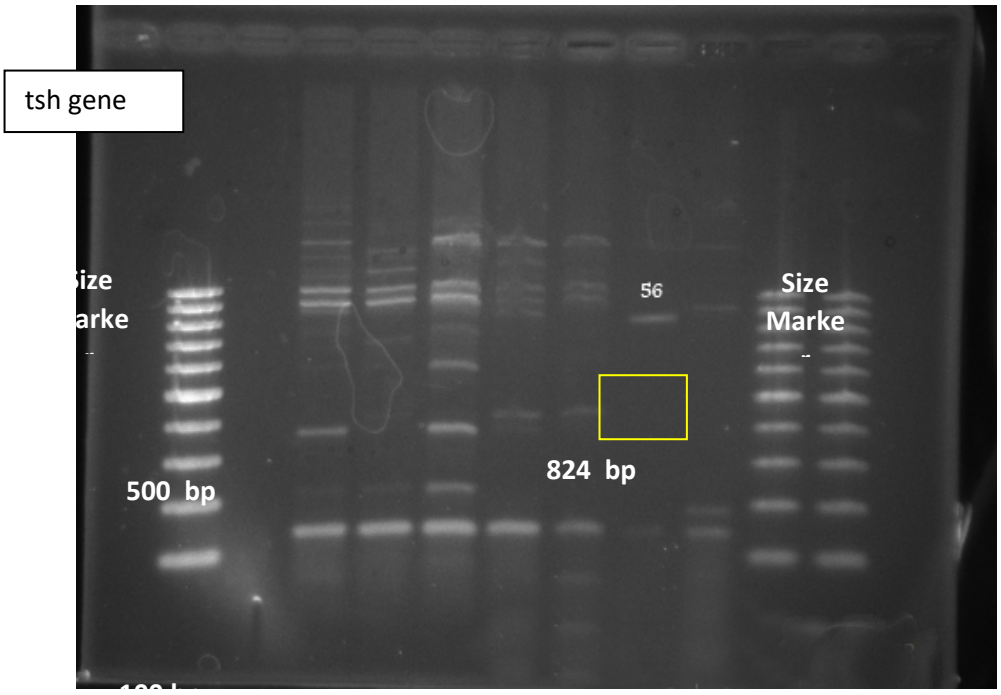

Figure 2. The result of checking the temperature gradient to select the optimal temperature in the PCR test setup for the *tsh* gene.

In the E. coli samples examined, the *pic* gene with the size of 147 bp and the *tsh* gene with the size of 824 bp were seen on the agarose gel. The *pic* gene was seen in 5 (3.3%) UPEC strains and the *tsh* gene was seen in 5 (3.3%) UPEC strains (Figure 3).

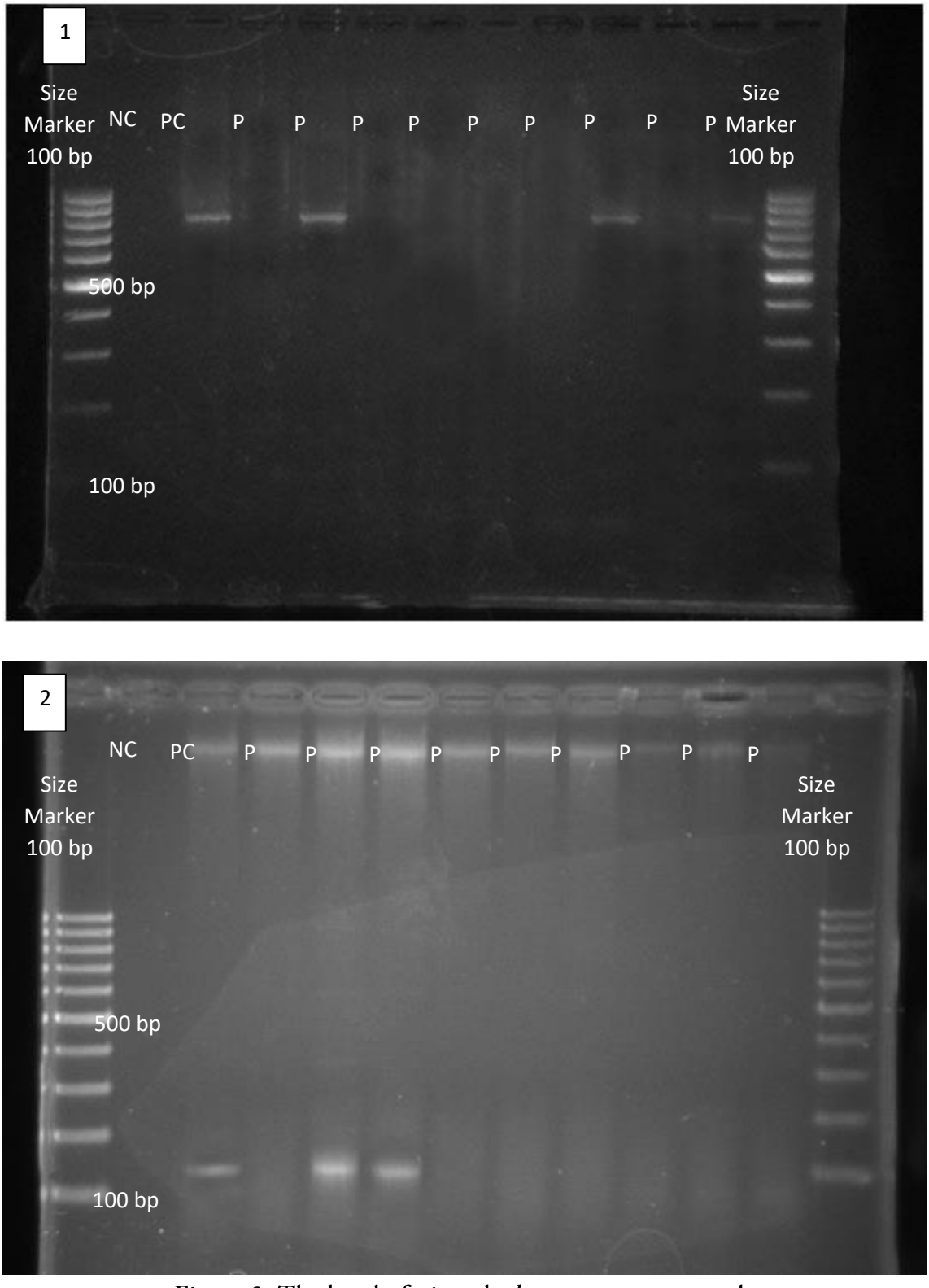

Figure 3. The band of *pic* and *tsh* genes on agarose gel.

1- *tsh* gene with 824 bp size
2- *pic* gene with 147bp size
(NC: Negative Control, PC: Positive Control, P: Patient)
Positive and negative controls were used in all PCRs. Using positive control to ensure the performance of PCR reaction components. Moreover, using negative control (microtube without genetic material) in this series shows the absence of contamination.

3.1 Biofilm formation in UPEC strain

The selection pattern of the samples to examine the biofilm production ability by *tsh* and *pic* genes in the UPEC strain is as follows:
A strain of UPEC where the *pic* gene is positive and the *tsh* is negative.
A strain of UPEC where the *tsh* is positive and the *pic* gene is negative (Table 2).

Table 2. Two-way analysis of variance for UPEC strain (Independent variable: biofilm formation).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.694[a]	3	1.231	67.321	0.000
Intercept	2.756	1	2.756	150.675	0.000
Pic gene	0.056	1	0.056	3.075	0.087
Tsh gene	2.756	1	2.756	150.675	0.000
*pic*gene * *tsh*gene	0.056	1	0.056	3.075	0.087
Error	0.750	41	0.018		
Total	5.000	45			
Corrected Total	4.444	44			
a. R Squared = 0.831 (Adjusted R Squared =0.819)					

P-value or sig. at 1 degree of freedom was formed to examine the effect of *Pic* gene on biofilm formation 0.087. If sig > 0.05, then H_0 hypothesis is accepted, there are no significant relationships between *Pic* gene expression and biofilm formation in UPEC strain.
P-value or sig at 1 degree of freedom to examine the effect of *Tsh* gene on biofilm formation was 0.00. If sig<0.05, the hypothesis H_0 is rejected.
P-value or sig. at 1 degree of freedom to examine the simultaneous effect of both *Tsh* and *Pic* genes on biofilm formation was 0.087. If sig>0.05, hypothesis H_0 is accepted. This shows no significant relationships between *Pic*o and *Tsh* gene expression at the same time and biofilm formation in the UPEC strain.

4. Discussion

Two-way analysis of variance for UPEC and EPEC strains only showed a significant relationship between *tsh* gene expression and biofilm formation, meaning that the *tsh* gene has a significant role in biofilm formation. Moreover, there were no significant relationships between *pic* gene expression and biofilm formation. Accordingly, the simultaneous expression of *pic* and *tsh* genes showed a very little significant relationship with biofilm formation, which could be ignored due to the weakness of this relationship, which approves of the issue that only the *tsh* gene can be effective in biofilm formation and *pic* gene does not affect it.

Zobisizkansa *et al.* (2012) studied the profiles of pathogenic genes and the phylogenic origin of E. coli in chronic and acute intestinal diseases. Their study was carried out on 78 E. coli strains isolated from three groups of patients. Acute diarrhea, Crohn, and inflammatory bowel diseases were the three diseases examined in their study. Genome extraction from the bacteria was carried out using sonication. The technique used in the study is DNA microarray. The studies by this group determined that the various strains isolated from chronic and acute gastroenteritis infections could be distinguished based on their pathogenic genes. The difference in the pathogenic genes of E. coli strengthened the hypothesis that chronic intestinal diseases rely more on the pathogenic potential of related strains.

Concerning the *tsh* gene, as was seen, all the strains with this gene could create biofilm in the present study. The *pic* gene was found in 11 (4.4%) of the strains that did not produce biofilm. The study indicated that the *tsh* gene has an effect on the pathogenicity of the studied strain and the pathogenic potential of the strains associated with the presence of this gene could be connected. Regarding the *pic* gene, the conclusion is not like this – Zobisizkansa's results indicated that the *tsh* gene in pathogenicity is similar to the results of the present study on the *tsh* gene 15 (Anderson and Dodson, 2004).

Heimer *et al.* (2004) examined the proteolactic activity of two proteases produced by *pic* and *tsh* genes initially. Moreover, using the Reverse Transcription PCR technique, they indicated that the expression of *pic* and *tsh* genes increases at 37 °C and is almost 4 times the growth at 25 °C. Then they used dot blot to check the correlation of the *pic* and *tsh* indices with UPEC. The prevalence of *tsh* in fecal isolates was lower than that of uropathogenic isolates, whereas the *pic* index was higher in a uropathogenic sample.

The present study showed that the prevalence of the *pic* gene is lower than that of the *tsh*. The reason for this difference could be using a different microarray technique to detect this gene. Another reason for this difference is the difference of the isolated strains genetically based on the difference in the place and time of the study (Anderson and Dodson, 2004).

Anderson and Dodson (2004) studied the presence of uropathogenic intracellular bacterial populations of E. coli in UTIs. The examinations of this group showed that these bacteria follow a complex molecular process to produce biofilm in epithelial cells and can manifest many biofilm-like characteristics. These intracellular biofilms formed lead to high immunity of bacteria against the defense of the body immune system.

The present study revealed that the pathogenicity of uropathogenic strains is associated with the formation of biofilm and it was completely in line with those of Anderson.

Anderson et al. showed that *Pic* protein can be isolated from the culture supernatant of enteroaggregative E. coli (EAEC). Its genetic identification overlapped with the shetAB gene, which encodes the enterotoxin Shet1. This toxin was responsible for the accumulation of fluid in the loops of the rabbit's intestine. Another example of SPATE proteins is the *pic* gene with serine protease activity which affects mucin (Henderson *et al.*, 1999).

The present study found that the presence of *tsh* gene is associated with biofilm production and pathogenicity of uropathogenic strains and can be effective in the pathogenicity process. The reason for the difference with Anderson's study could be in the methods examined and another reason is the difference of genetically isolated strains according to the difference in the location and time of the study (Henderson *et al.*, 1999).

Provence and Curtiss (1994) identified *tsh* in the temperature-sensitive hemagglutinin factor in avian pathogenic E. coli (APEC) for the first time. This gene is the homologue of the hbp gene, as a binding protein, and has serine protease activity by targeting hemoglobin. The study revealed that the *tsh* gene has a role in pathogenicity by producing biofilm.

5. Conclusions

The study results indicated that the *tsh* gene is associated with the ability of the isolated strain to produce biofilm, and could affect the severity of the disease and antibiotic resistance with its effect on biofilm production; therefore, it could be used as a genetic tool to diagnose E. coli pathotype strains.

Examining the differences between animal and human strains and the pathogenicity of each in its non-specific host is suggested.

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