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Molecular Detection of Siderophore Genes in *Escherichia coli* Among Iraqi Strains

Hassan S. Younus *MSc*, **Nada H. A. Al-Mudallal** *PhD*

Dept. of Medical Microbiology, College of Medicine, University of Al-Iraqia, Baghdad, Iraq

Abstract

Background Bacteria can't grow without iron (Fe), and the majority of them actively seek out Fe ions via specialized proteins called siderophores.

Objective To quantify the prevalence of yersinobactin irp2, salmonellochilin iroN, and aerobactin iucA siderophore genes in the virulent Iraqi strains of uropathogenic *Escherichia coli* (UPEC).

Methods Fourteen *E. coli* strains that were previously isolated and diagnosed from urinary tract infections and biologically active siderophore producers. These strains were analyzed for the presence of three siderophore genes (iucA, iroN, and irp2) using conventional polymerase chain reactions (PCR).

Results The frequencies of such iucA, iroN, and irp2 genes among the UPEC isolates were 3 (21.42%), 10 (71.4%), and 4 (28.6%), respectively.

Conclusion Research suggests that siderophores can successfully reduce the pathogenicity of UPEC bacteria. Moreover, their primary function in infection differs depending on the siderophore type and concentration. Yersinobactin irp2 represented the main siderophore gene, followed by salmochelin iroN and aerobactin iucA.

Keywords Escherichia coli, siderophore, aerobactin iucA, salmochelin iroN, yersinobactin irp2.

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List of abbreviations: dNTP = Deoxy nucleoside triphosphates, Fe = Iron, iucA = Aerobactin, iroN = Salmonellochilin, irp2 = Yersinobactin, LB = Luria-Bertani broth, PCR = Polymerase chain reaction UTIs = Urinary tract infections, UPEC = Uropathogenic *Escherichia coli*

Introduction

The creation and growth of all live microbes, including *Escherichia coli* (*E. coli*), rely on iron. Since iron is a one-of-a-kind catalyst [(1)], it takes at least 6 to 10 M of iron for bacteria to thrive and multiply. Conversely, microbes may exhibit bactericidal or antimicrobial activities at greater iron concentrations, which may be cytotoxic [(2)].

Numerous empirical investigations in people and laboratory animals have proven a correlation between iron content and microbial infection [(3)]. High-affinity iron-chelating molecules, such as lactoferrin and transferrin, are common and effective defensive mechanisms that mammals use against bacterial infections. Mammals release these molecules to scavenge free iron ions, thereby preventing invading microorganisms from obtaining these ions [(4)].

Uropathogenic *E. coli* (UPEC) strains, on the other hand, employ 38 different methods during urinary tract infections (UTIs). One of these techniques is the production of a variety

of siderophores, 39 of which are proteins that have a high partiality but a low sub-atomic weight combined [5]. Siderophores are molecules that chelate iron ions, and they are produced by a wide variety of bacteria, both hazardous and benign respectively [6]. These particles have the ability to chelate iron. 42 particles, each. Siderophores, routinely produced and released into the atmosphere, have a stronger and more definitive connection to magnetic particles than ferrous particles. The bacteria utilize 44 specific receptors on their exterior films to ingest ferric-bound siderophores [1]. Different strains of *E. coli* are capable of producing four distinct siderophores: enterobactin, salmochelin, yersiniabactin, and aerobactin. Several types of *E. coli* that are not harmful to humans only had one thing in common: they could make the enterobactin siderophore, which is encoded by the ent-fes-fep gene cluster [6]. Nevertheless, UPEC strains possess the ability to produce siderophores other than enterobactin. These siderophores aid bacteria in their ability to thrive in environments where iron is scarce, such as the urinary system. The iuc DBAC gene cluster encodes bacterial aerobactin, the iroA gene cluster encodes salmochelin, and the irp2, ybtSETUXPQA, and high pathogenicity island (HPI) gene clusters encode yersiniabactin synthesis [1]. Multiple investigations have shown strong correlations between the production of aerobactin and certain infectious diseases, such as cystitis and pyelonephritis [7].

In women who get recurrent UTIs, researchers have found that certain strains of bacteria express salmochelin and aerobactin siderophores at much higher frequencies. Researchers believe these siderophores significantly contribute to the recurrence of infections and the development of drug resistance [8].

The genetic frequency of producing aerobactin, salmochelin, and yersiniabactin siderophores was determined by carrying out an analysis on *E. coli* virulent strains that were isolated from Iraqi patients who suffered from UTIs and type 2 diabetes mellitus (T2DM) [9].

This study aimed to quantify the prevalence of yersinobactin irp2, salmonellochilin iroN, and aerobactin iucA siderophore genes in the virulent Iraqi strains of UPEC.

Methods

Bacterial strains

Fourteen bacterial standard strains that were previously isolated and identified as UPEC from UTIs with T2DM Iraqi patients. These strains were biologically active siderophore producers, either quantitatively or qualitatively. The researchers cultured the bacteria in Luria - Bertani broth (LB) broth overnight at 37°C for molecular studies. Molecular detection of siderophore genes using conventional polymerase chain reaction (PCR) genomic DNA extraction. The ABIOpureTM Total DNA kit (ABIOpure, USA) was used to extract genomic DNA from growing bacteria.

Quantitation of DNA

The concentration of extracted DNA was detected using a Quantus Fluorometer to assess the quality of samples for subsequent uses. It was combined with 199 µl of diluted Quant fluor Dye for every 1 of DNA. Results for DNA concentration were obtained following 5 min of incubation at room temperature.

PCR amplification

Three distinct sets of primers (Table 1) were utilized and constructed in accordance with the manufacturer's instructions in order to identify the IucA, IroN, and Irp2 genes in *E. coli* isolates in this work (Macrogen, Korea). Table (2) displays PCR programs together with their associated cycle numbers, times, temperatures, and volumes. A DNA thermocycler, such as the Eppendorf Mastercycler, was used for cycling in all PCR operations [Eppendorf-Nethel-Hinz (GmbH, Hamburg, Germany)] was used.

A DNA ladder marker measuring 100 kilobases was used to electrophorese 10 microliters of

the final reaction mixture in 1.5% agarose. After that, the amplified products were stained with ethidium bromide so that they could be seen [10].

Table 1. The siderophore genes in uropathogenic *Escherichia coli* may be detected using specific primers

Gene	Primer name	Primer sequence (5'-3')	Size of product (bp)
Iuc	iuc1-F iuc1- R	ATGAGAATCATTATTGACATAATTG CTCACGGGTGAAAATATTTT	1482
iroN	iroN-F iroN-R	AAGTCAAAGCAGGGGTTGCCCG GACGCCGACATTAAGACGCAG	665
irp2	Irp2-F Irp2-r	AAGGATTCGCTGTTACCGGAC AACTCCTGATACAGGTGGC	413

Table 2. PCR program and volume for detection of each siderophore genes

Gene	PCR program	PCR volume (50 µl)
irp2	• at 95°C for one cycle, the time is 4 minutes • 32 cycles • at 94°C for 60 seconds • at 56°C for 60 seconds • at 72°C for 20 seconds	Volume of 5 µl of PCR buffer; The components are; 10X, 1.5 mM of $MgCl_2$, 200 µM of Deoxy nucleoside triphosphates (dNTP) (Fermentas) micromolecular weight of primers F, 1/2 R and 1.5 units of Taq DNA polymerase (Fermentas) The DNA template is 3 and 1/4 ml
iroN	• Initial cycle: 72°C for 10 minutes • The first cycle: 94°C for 6 minutes • at 94°C for 45 seconds • at 58°C for 60 seconds • at 72°C for 75 seconds	Volume of 5 µl of PCR buffer The components are; Ferretas dNTP (150 µM), 1.25 mM of $MgCl_2$ Primers F and R, each at 1 µM, and 1.2 units of Taq DNA polymerase (Fermentas) The DNA template is 3 and 1/4 ml
IucA	• Cycle: • at 72 °C for 8 minutes • at 95°C for 3 minutes • 40-hour cycle • at 94°C for 60 seconds • at 58°C for 70 seconds • at 72°C for 70 seconds	Volume of 5 µl of PCR buffer The components are; 10X, 1.25 mM of $MgCl_2$, 125 µM of dNTP (from Fermentas) micromolecular weight of primers F and 1/2 R and 1.2 units of Taq DNA polymerase (Fermentas) The DNA template is 3 and 1/4 ml

Statistical analysis

The current study used statistical package for social sciences (SPSS) version 20.0 software to conduct statistical analysis on the occurrence of siderophore genes in *E. coli* isolated from UTIs patients. At a P value ≤ 0.05, statistical significance was considered.

Results

The numbers in figures (1, 2, and 3) show the outcomes of regular PCR tests that were used

to find the amounts of yersinobactin, salmochelin, and aerobactin, which are represented by irp2, irpN, and iucA, respectively. Each gene displays distinct single bands measuring 413 bp, 665 bp, and 1482 bp when compared to the ladder DNA. These bands correspond to the irp2, iroN, and iucA genes of UPEC.

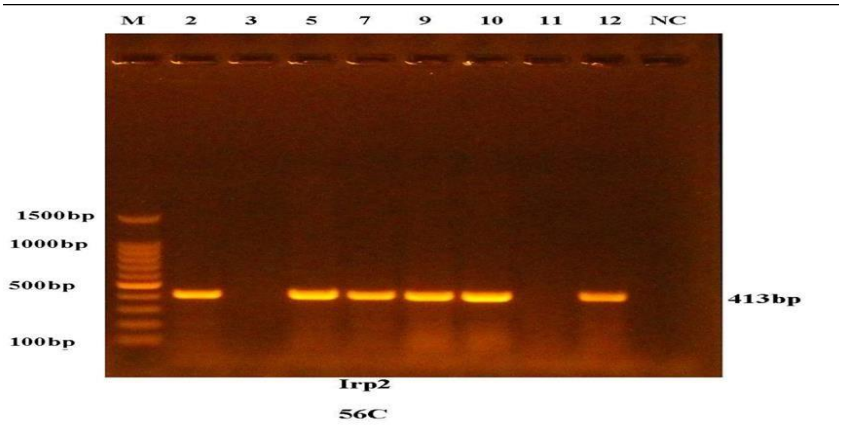

Figure 1. The amplification of Irp2 gene of *E. coli* after separated by gel electrophoresis

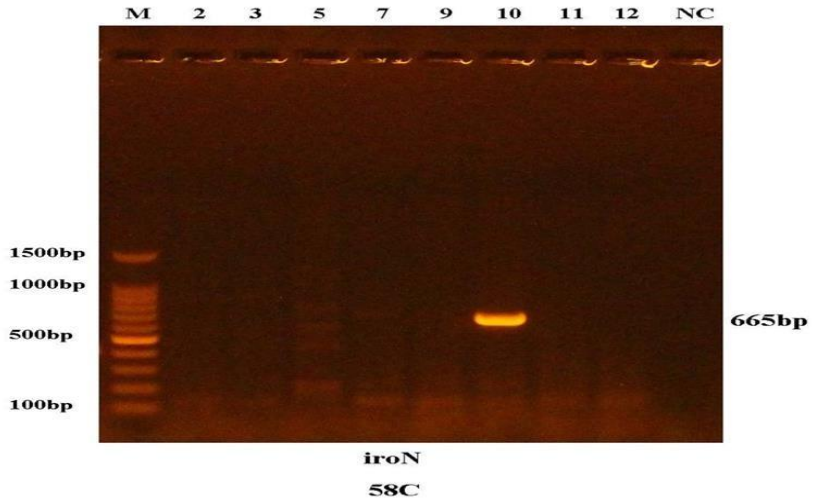

Figure 2. The amplification of iroN gene of *E. coli* after separated by gel electrophoresis

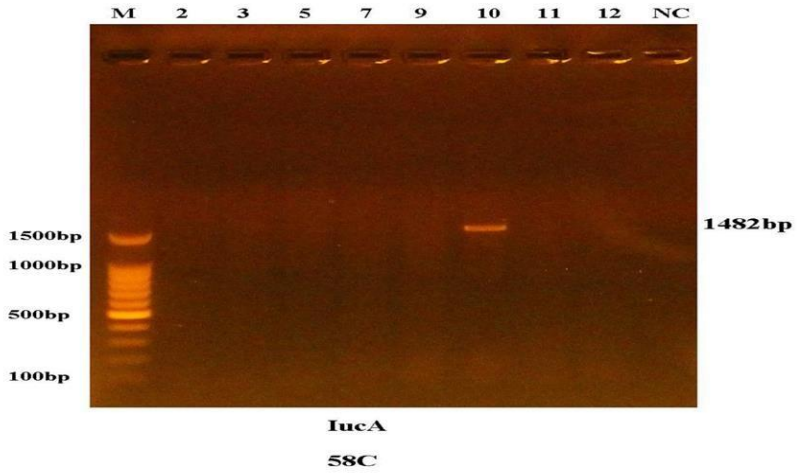

Figure 3. The amplification of iucA gene of *E. coli* after separated by gel electrophoresis

Table (3) summarizes the frequencies, distributions, and percentages of these genes in the fourteen *E. coli* strains. Strains 1, 9, and 12 harbored the three types of genes, while strain number 11 had two: IroN and Irp2. The remaining *E. coli* strains, with the exception of strains numbers 3, 7, 10, and 13, only contain the Irp2 siderophore gene. The highly prevalent siderophore gene among the fourteen strains was irp2, with 10 strains (71.4%), followed by 4 (28.6%) and 3 (21.42%) for IroN and IucA, respectively. With the exception of four strains (4, 8, 11, and 12), the statistical analysis of the data revealed significant differences ($P \leq 0.05$) in these genes across all of the bacteria.

Table 3. The distribution of irp2, iroN and iucA among the fourteen *E. coli* strains

Bacterial strains/*E. coli*	*IucA*	*Irp2*	*IroN*	No.	%	*P value
1	+	+	+	3	21.4%	0.001
2		+		1	7.14%	0.001
3						
4		+		1	7.14%	NS
5		+		1	7.14%	0.05
6		+		1	7.14%	0.05
7						
8		+		1	7.14%	NS
9	+	+	+	3	21.4%	0.05
10						
11		+	+	2	14.2%	NS
12	+	+	+	3	21.4%	NS
13						
14		+		1	7.14%	0.05
No & %	3(21.4%)	10 (71.4%)	4 (28.6%)			

Results were considered significant when P ≤0.05, and statistically nonsignificant when P>0.05 by Chi square test

Discussion

There is a great deal of genetic diversity across *E. coli* strains, and virulence-associated traits are often unique among urinary tract isolates. Stain MG1655, a descendant of *E. coli* UTI89, a model UPEC strain discovered in a patient with acute cystitis, and the original K12 strain, discovered in feces in 1922, are missing approximately 30% of their open reading frames [11]. Siderophores are important virulence factors that help *E. coli* survive in places where the host is iron-deficient. They help the bacteria take in and send iron, which lets them multiply properly [12]. A study has found that the level of pathogenicity and the amount of siderophore that bacteria produce are predictive of prognosis [13]. The biosynthetic differentiation of the siderophores may lead to an increase in the pathogenicity of the bacteria, potentially resulting in the formation of different or improved siderophores. Enterobactin, salmochelin, yersiniabactin, and aerobactin are the four kinds of siderophores that *E. coli* strains may make. Studies have shown that the UPEC strains produce more salmochelin and yersiniabactin than the other siderophores [14,15]. Cavas and Kirkiz et al. [16] discovered that *E. coli* VR50 encompasses gene areas that are accountable for the production of enterobactin and yersiniabactin, according to the genome mining methods employed by antiSMASH. This provides evidence that this strain is liable for UTIs caused by these siderophores. Additionally, the penterobactin and aerobactin biosynthetic gene areas may be found in nonpathogenic *E. coli* Nissle 1917 [17]. Yersiniabactin was recognized in pathogenic *E. coli* VR50 but not in nonpathogenic *E. coli* Nissle 1917, which is an intriguing finding. Furthermore, there is no yersiniabactin or aerobactin-related gene cluster in the nonpathogenic *E. coli* K-12 strain MG1655.

Current findings revealed that the most common siderophore among the 14 *E. coli* strains isolated from UTIs was the gene irp2 (71.4%), which codes for yersiniabactin, followed by salmochelin and aerobactin. These UPEC infections are particularly dangerous due to the presence of three types of siderophore genes, which enable them to produce an abundance of siderophores; in contrast, infections caused by pathogens lacking these genes are less dangerous [13]. Also, pathogens like Gram-positive and Gram-negative bacteria have been found to reduce pathogenicity when the genes that are responsible for siderophore production are deleted [1]. This finding aligns with the published results of Abdi et al., 2014 [18]. Paniagua-Contreras et al. [19] discovered that 89% of recovered UPEC strains had the irp2 gene and originated from UTIs. Consistent with these results, it was found that UPEC isolated from UTI patients most frequently expressed the iron acquisition systems gene irp2 [20]. The study by Karimian et al. [21] found that UPEC strains isolated from patients had varying frequencies for these three siderophore genes. Our results contradict the previously reported frequencies of 21.42, 71.4%, and 28.6% for the iucA, iroN, and irp2 genes, respectively.

In conclusion, when it comes to the pathogenicity of UPEC bacteria, siderophores work well. Moreover, their primary function in infection differs depending on the siderophore type and concentration. Yersinobactin irp2 represented the main siderophore gene, followed by salmochelin iroN and aerobactin iucA.

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Author contribution

Both authors contributed directly to the paper design, data collection, statistical analysis and final revision of the manuscript.

Conflict of interest

The authors declare there is no conflict of interest.

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Correspondence to Hassan S. Younus
E-mail: Hasan.salah.lab@gmail.com
Hasan.salahaldeen@aliraqia.edu.iq
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