Brief Report

J Babol Univ Med Sci
Vol 17, Issu 5; May 2015; P:31-36

The Frequency of Escherichia coli Strain Isolates Causing Urinary Tract Infections in the presence of Pathogenic Aerobactin Genes

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Received: Dec 23th 2015, Revised: Feb 4th 2015, Accepted: Apr 12th 2015

ABSTRACT

BACKGROUND AND OBJECTIVE: Escherichia-coli is one of the major causes of urinary tract infection (UTI). The strains of this bacterium have a variety of virulence factors, such as iron acquisition systems, that provide the energy for iron transport in bacterial membranes. This study aimed to determine the frequency of pathogenic Aerobactin genes among the UTI-causing strains of E. coli isolated from patients referring to Baghiatallah Hospital in Tehran, Iran.

METHODS: This study was conducted on 50 isolates of E. coli provided from UTI patients within the age range of 9 months to 87 years from May 2014 to November 2014. E. coli strains were identified via standardized microbiology and biochemical lab techniques, and the frequency of Aerobactin genes in the isolates of the bacterium were measured via the polymerase chain reaction (PCR) method.

FINDINGS: In this study, PCR was successfully able to create the proper band size of 602 bp, and the presence of aerobactin was confirmed in 44 isolates of E. coli (88%).

CONCLUSION: According to the results of this study, the frequency of virulent aerobactin genes among the UTI-causing strains of E. coli was considerably high, which is an alarming signal of the prevalence of virulent strains among different patients.

KEY WORDS: Escherichia Coli, Urinary Tract Infections, Aerobactin Receptor.

Please cite this article as follows:

Mojabi SM, Ranjbar R. The Frequency of Escherichia coli Strain Isolates Causing Urinary Tract Infections in the presence of Pathogenic Aerobactin Genes. J Babol Univ Med Sci. 2015;17(5):31-6.

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Introduction

Escherichia coli is a gram-negative bacterium from the Enterobacteriaceae family, and is the most important member of the coliform group. E. coli is a normal flora of the digestive tract in humans and other warm-blooded animals. Bacterial infections caused by E. coli are the second most prevalent infections after respiratory tract infections. Furthermore, this bacterium is the prominent cause of referrals due to urinary tract infections in adults. Urinary tract infection (UTI) is the most common bacterial infection in humans, and E. coli is the major cause of this infection (1-3). Although E. coli is normally harmless, it is responsible for 80-90% of community-acquired UTIs and 30-50% of hospital-acquired UTIs. UTI is a major cause of hospitalization leading to several complications and heavy healthcare costs, and the diagnosis and treatment of this disease is a primary health concern. About 150 million people have been diagnosed with UTI around the world, and the treatment of this disease has been estimated to cost six billion dollars every year (4).

According to the literature, UTI is more prevalent among women, and about half of all women in different parts of the world experience UTI at least once in their lifetime (5). E.coli, as a major factor leading to UTI, is normally resistant to antibiotic treatments (6). The severity of UTI depends on the bacterial virulence and the susceptibility of the human host. Bacterial attachment to the cell linings of the urinary tract is the primary stage of the development of UTI; this process allows bacteria to achieve resistance against urine cleaning, bladder emptying, and the activation of signaling pathways in the human host. In addition, iron acquisition systems are another feature of E. coli strains, which contribute to the development of UTI through enabling the absorption of iron from the environment via Siderophores. On the other hand, Aerobactin genes are responsible for encoding siderophore aerobactin; in order to acquire the necessary iron, many types of bacteria tend to secrete iron-chelating agents with high affinity (i.e. intrinsic activity) and low molecular weight, which are referred to as Siderophores. These agents are able to absorb the iron-binding proteins competitively and are categorized into two main groups of Enterobactins and Aerobactins (7).

Iron is an essential element to the survival of bacteria; therefore, bacteria have developed the ability to compete with the iron-binding factors in the host's body in order to thrive and evolve (8). This characteristic results from the ability of bacteria to generate potent iron-chelating agents known as siderophores. Moreover, iron plays a pivotal role in several processes such as photosynthesis, respiration, tricarboxylic acid cycle, oxygen transmission, nitrogen fixation in methanogens, regulation of gene expression and DNA synthesis (7, 8). Iron is poorly soluble in aqueous solutions (9). On average, each bacterial cell needs 105-106 iron ions to initiate biological processes (10, 11), and the amount of iron could occasionally increase by 1.8% of the dry weight of the cell (12). On the other hand, colony-forming bacterial units might face a serious problem in the human body, since more than 99.9% of the iron found in human organs is intracellular, which makes iron virtually inaccessible to the bacteria. In addition, the extracellular iron, which is found in the plasma and lymph fluids, is potently connected to Transferrins (12). Bacteria are able to employ different mechanisms in order to access iron; for instance, in aerobic conditions, bacteria and fungi tend to produce various ligands of ferric iron forms with low molecular weight called siderophores (13). To date, more than 500 different types of siderophores have been chemically identified (14). For the ferric-siderophore passage through the outer membrane, the bacteria need specific receptors and energy; this energy is supplied by the proton propulsion of the cytoplasmic membrane, and it is transferred by the TonB-ExbB-ExbD protein complex into the outer membrane (15, 16).

E. coli and several other species of the Enterobacteriaceae family secrete enterobactin siderophores in the presence of iron deficiency, which are attached to the outer membrane ferric enterobactin protein (FepA) and are eventually released in the periplasmic compartment. In this process, the periplasmic protein (FepB) binds to the ferric siderophore enterobactin, and after passage through the space, delivers it to the ABC transporter in the cytoplasmic membrane. Finally, they actively pass through the cytoplasmic membrane (16). In response to iron stress, E. coli produces enterobactin siderophore, which is a common strategy among different strains of E. coli. Furthermore, FepA plays a key role in the transmission of iron into the cell. This study aimed to determine the frequency of aerobactin virulence factors among different strains of Escherichia coli isolated from UTI patients referring to Baghiatallah Hospital of Tehran, Iran.

Methods

Bacterial Strains: This descriptive study was conducted on patients with suspected UTI referring to Baghiatallah Hospital in Tehran, Iran from May 2014 to November 2014. In accordance with the standard procedures, urine samples were collected from the patients after the washing and drying of the reproductive organs in the laboratory of the hospital. In order to isolate E. coli strains, each sample was separately cultured in MacConkey agar, Chrome agar and eosin methylene blue (EMB) agar and was analyzed by standard biochemical tests of IMViC (Indole, Methyl red, Voges-Proskauer and Citrate). Urinary tract infection was confirmed in case 105 colony-forming units (CFU)/ml grew in the medium. In total, 50 samples containing UTI-causing E. coli were used in this study, and for performing the molecular procedures, all the obtained isolates were preserved in vitro in enriched Skim Milk agar with 15% glycerol at the temperature of -20°C (17).

DNA Extraction of Bacterial Strains: Bacterial DNA extraction was performed by boiling method. Initially, some colonies were removed from the bacteria and boiled in deionized water in water bath for 5 minutes at 95°C. After centrifugation, the supernatant solution was inserted into new micro-tubes as the template DNA. Following that, the amount of DNA was measured by NanoDrop device at 260-280 nm for the uptake of DNA and proteins in order to determine the purity of DNA. Finally, the extracted DNA was preserved at the temperature of -20°C for performing the polymerase chain reaction (PCR).

Polymerase Chain Reaction (PCR): After reviewing similar other studies on this subject (19), proper primer pairs were used for tracking the aerobactin gene (table 1) (purchased from Pishgam Company, Iran). Aerobactin gene amplification was conducted by single PCR, and the PCR was performed in a final volume of 20 micro liters consisting of 7 µl of distilled water, 10 µl of Master Mix, 1 µl of extracted DNA, and 1 µl of each primer (Pishgam, Iran). The temperature cycling of PCR included one cycle at the temperature of 95°C for the initial denaturation (30 sec), followed by 35 cycles consisting of 95°C in order to achieve complete denaturation (30 sec), 55°C for the connection of the primers (30 sec), 72°C for the development of the primers (30 sec) and 72°C for the complete development of the primers (10 min). Gene amplification was performed in a Thermal Cycler manufactured by Eppendorf, Germany.

Electrophoresis of PCR Products: Electrophoresis was conducted on the obtained products of PCR using 1.5% Agarose gel and a marker of specific size (1000 bp DNA ladder). Afterwards, Ethidium bromide was used to stain the gel, and the DNA bands were observed and evaluated using UV irradiation.

Ethical Considerations: In this study, we enrolled UTI patients who volunteered to participate, and the subjects were informed on the results of their laboratory tests. The medical data of the subjects remained confidential.

Result

In this study, PCR was successfully able to identify gene optimization while creating a band size of 602 bp (fig 1). According to the results of PCR, aerobactin virulence genes were present in 88% of all the obtained isolates. Among the positive samples of the aerobactin gene, 32 (64%) cases were female and 12 (24%) were male, and the patients were within the age range of 9 months to 87 years with a mean age of 26.245±0.88. Among these subjects,19 cases (38%) were under 40 years of age, while 31 (62%) were aged 40 years and older. Furthermore, aerobactin gene was not found in 12% (6 samples) of the E. coli, while it was present in 88% (44 samples) of the strains (table 2)

Table 1. Primers used for Detection of Aerobactin genes of Uropathogenic Escherichia coli (UPEC) strains Isolated from Patients

Target gene	Sequencing Primers	Size of product (pair primers)
Aerobactin	F:5`-TACCGGATTGTCATATGCAGACCGT-3`R F:5`-AATATCTTCCTCCAGTCCGGAGAAG-3`R	602

Table 2. Frequency Distribution of E.coli Strains Isolated from Patients by PCR

Effect	Relative cumulative frequency	Cumulative frequency	Relative frequency	Simple frequency
Absence of gene	6	6	6.0	6
Presence of gene	44	44	44.0	44

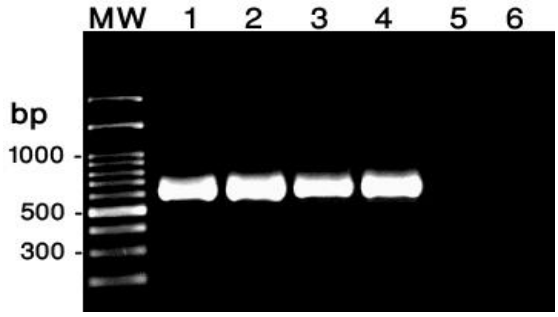

Figure 1. PCR results of some Clinical Samples; Rows 1-4 are samples of positive aerobactin gene, and rows 5 and 6 are negative control samples and samples without aerobactin gene, respectively. MW row is the 1000 bp molecular marker.

Discussion

According to the results of this study, the frequency of aerobactin gene among the isolates of UTI-causing E. coli was observed to be remarkably high. Several studies have indicated E. coli to be the predominant pathogen isolated from urine samples (2, 18). The prevalence of UTI varies in different parts of the world (18, 19); however, in a study by Akoacher et al. in Cameron, no correlation was observed between the prevalence of UTI and the location of the study (20). Moreover, the incidence of UTI was observed to be higher among female patients in the present study; this could be due to the differences in the anatomy of the urinary tract, healthy behaviors, and genetic and microbiological factors. This finding has been confirmed by several other studies in this regard (21, 22). In addition, UTI has been reported to be a major complication in young women in the post-menopausal stage. The risk of infection could increase in this group due to several factors such as the recent history of UTI, delayed bladder emptying during the day, low fluid intake, cleaning after urination, bladder emptying after sexual intercourse, material of underwear and the frequency of weekly sexual activity.

On the other hand, the mean age range of our study population was relatively high, which could be due to the high frequency of UTI micro-factors such as weakened immune system, urinary tract obstruction, diabetes, prostatic hyperplasia and weak bladder emptying among elderly patients (23, 24). In addition to the presence of underlying diseases, the use of catheters and disregarding hygienic measures by the patient could lead to the development of UTI. In a study by Kudinha et al., the aerobactin gene was identified in 87% of the urine samples provided from UTI patients via single PCR (25). Similarly, Oliviera et al. observed the aerobactin gene to be present in 88% of the E. coli strains isolated from the urine samples of the patients, which was consistent with the findings of the current study and another study by Johnson et al. (26). Furthermore, Momtaz et al. and Soto et al. observed E. coli to be the predominant pathogen isolated from the urine samples of UTI patients (1, 28). These two studies indicated the aerobactin gene to be a significant factor in UTI-causing strains of E. coli, and it could appear as an appropriate target for therapeutic intervention due to its unique function. Aerobactin is an iron acquisition system which provides the bacteria with the required iron in iron-poor environments such as the urinary tract; therefore, it could act as a defensive mechanism for the bacteria as well (29). In another study by Mercon et al. on renal transplant patients, the prevalence of aerobactin was reported to be 88% while Santo et al. reported the prevalence to be about 86%, which is similar to the results obtained in the present study (30, 31). In conclusion, the results of this study indicated the prevalence of virulence aerobactin genes to be noticeably higher among different strains of E. coli in patients presented with UTI. This signifies the alarming distribution of these pathogenic strains among patients, emphasizing the need for further research on the new methods of screening, prevention and treatment of UTI in order to reduce the heavy treatment costs.

Acknowledgements

Hereby, we extend our gratitude to the staff of the Laboratory of Molecular Biology Research Center affiliated to Baghiatallah University of Medical Sciences for assisting us in this study. We would also like to thank Mr. Dariush Ghasemi and Ms. Fatemeh Pour Ali for their cooperation in this research project.

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