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Molecular identification of uropathogenic *Escherichia coli*

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Urinary tract infections (UTIs) are among the most frequent infectious diseases in the world. Most urinary tract infections are caused by Uropathogenic *Escherichia coli* (UPEC), which is the main cause of UTIs, and it is resistant to a wide variety of antibiotics. The principal objective of this study is to be identifying uropathogenic *Escherichia coli* (UPEC) strain using molecular identification method to achieve rapid bacterial identification. Samples were collected from fifteen female patients (18-35 years) infected with Uropathogenic *Escherichia coli* (UPEC). The samples were then randomly labeled from (1-15). All samples were obtained from the laboratory of King Fahad Hospital (KFH) in Jeddah city. Urine samples were inoculated on CLED agar, MacConkey, and Blood. Gram staining technique was used with optical microscopy to confirm the bacteria. Identification and susceptibility testing was performed using the VITEK 2 system. Molecular identification method was conducted using the *traT* and 16S rRNA gene. Obtained results showed that all samples were Lactose fermenters except for three samples which were lactose non-fermenting. *E. coli* by the Vitek2 technique system had acceptable, good, very good, or excellent identification after their identities were verified with the reference systems. The *traT* gene is considered the most prevalent virulence gene among UPEC strains. The results of the current study showed that the *traT* gene was detected in all UPEC samples. These results suggest that the *traT* gene can be considered as target for molecular identification of the UPEC. Compared with conventional methods that require few days, this method can make same day reporting possible for therapeutic interventions and consequently allow better patient management.

Keywords: urinary tract infection, antibiotic resistant, PCR, Vitek system, *traT* gene, virulence factors.

INTRODUCTION

Urinary tract infections (UTIs) are among the most frequent infectious diseases in the world, accounting for around 150 million cases every year (Aguiniga et al. 2016 and Siqueira, et al. 2021). In general, 50-60% of adult females will develop at least one UTI over their lives (Medina & Castillo-Pino, 2019). Most urinary tract infections are caused by UPEC, which is the main cause of UTIs (Croxen, et al. 2013; Edelsberg, et al. 2014 and Lo et al. 2017). An epidemic of excessive urinary tract infection caused by *E. coli*, which is resistant to a wide variety of antibiotics, has been reported by the World Health Organization (WHO) (Soltani et al. 2018 and Alam Parvez & Rahman, 2019). Urinary pathogens are becoming more resistant to antibiotics, both in the hospital and in the community infections, resulting in longer hospitalizations, more difficult to treat, and having a negative impact on health and economy (Ashraf, et al. 2015; Esteve-Palau et al. 2015; Parvez & Rahman, 2018 and Aslam, et al. 2018).

The principal objective of this study is to identify the uropathogenic *Escherichia coli* (UPEC) strain using the molecular identification method in comparison to the conventional identification methods for faster identification of the multidrug-resistant bacteria. Sufficient knowledge of the properties of the virulence and its antibiotic resistance pattern helps clinicians to anticipate the development of the infection in patients.

MATERIALS AND METHODS

Collection of Urine samples:

Samples were collected from fifteen female patients (18-35 years) infected with UPEC. The samples were then randomly labeled from (1-15). All samples were obtained from the laboratory of King Fahad Hospital (KFH) in Jeddah city. Urine samples were inoculated on cystine–lactose–electrolyte-deficient agar (CLED agar), MacConkey and blood. The CLED and MacConkey agar were chosen because they are considered differential mediums for the isolation, purification, and identification of Enterobacteriaceae and for detecting the ability of each isolate to ferment lactose and mannitol.

Identification of bacteria using conventional methods:

To confirm the cellular morphology and classification of the bacteria, the Gram staining technique was used with optical microscopy visualization used to determine the shape, arrangement, and classification of Gram-negative

bacteria. Identification and susceptibility testing performed using the VITEK 2 system (bioMérieux) in Jeddah Eye Hospital. Which uses new fluorescence-based technology for testing gram-negative clinical isolations (Naser, 2016).

Molecular identification of bacterial isolates:

Total genomic DNA was isolated using a DNA kit from MOLEQULE-ON company, where 1 colony of pure bacterial culture was grown overnight in 10 ml NA broth, incubated at 37°C in a shaker incubator, then centrifuged the appropriate number of bacteria (about 106~107) at 6,000 x g (8,000 rpm) for 5 minutes at room temperature. The supernatant was to be removed completely and cells were to be resuspended in 200 µl cold TE. Then, 400 µl of digestion solution was added to a 200 µl sample, mixed well, and 3 µl of proteinase K solution (2 mg/150 µl) was added to the sample and incubated at 55°C for 5 minutes. After that, 260 µl of 100% ethanol was added and mixed well. Moreover, the MQ column was placed into a clean 1.5 ml microcentrifuge tube. Then, 30-50 µl Elution Buffer was added to the center part of the membrane in the column. The tube was incubated at room temperature for 2 minutes and then incubated at 37°C for 2 minutes to increase recovery yield. Then, it was centrifuged at 8,000 x g (10,000 rpm) for 2 minutes to elute DNA from the column. For the PCR amplification of the *traT* gene and 16S rRNA gene, the DNA of each bacterial isolation was used as a template. Moreover, universal bacterial primer sets were used. These sets of primers are summarized in (Table 1). The PCR for all bacterial species was performed in a final volume of 25µl containing 2µl of each primer, 1µl of Reverse primer, 1µl of Forwarding primer, and 12.5µl of Master Mix (GoTaq® Green Master Mix, 2X, Promega). About 2µl of DNA was added to PCRs tube. Then, 8.5µl Injection water was added to adjust the final volume to 25 µl. The amplification was performed with a thermal cycler (Applied Biosystems™ Veriti™ 96-Well Thermal Cycler) and was programmed to perform 30 cycles in *traT* gene and 35 cycles in 16S rRNA gene containing four steps which are shown in (Table 2). After the cycles, agarose gel electrophoresis was used to analyze the PCR products. Later PCR products of the 16S rRNA gene were sent to GenaTi in King Fahad Medical Research Center for determining the sequence and identifying the isolated strains. The data were analyzed by using the FinchTV (DNA sequence analysis) program and compared with sequences in the NCBI database (Jahapriya, 2018).

Table 1: Primer Used in *traT* Amplification and 16S rRNA

Primer	Sequence from 5' to 3'	Length	Reference
***traT* R**	**5'-CACGGTTCAGCCATCCCTGAG -3'**	**290bp**	**Johnson & Stell, (2000)**
***traT* F**	**5'-GGTGTGGTGCGATGAGCACAG-3'**		
534R	**5'- ATTACCGCGGCTGCTGGC -3'**	**500bp**	**Smits et al. (2004)**
27F	**5'-GGTGTGGTGCGATGAGCACAG-3'**		

Table 2: Thermal Cycler Program for PCR

traT	**Denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Elongation**
N. cycles	--	30 cycles			--
°C	94°C	94°C	63°C	72°C	72°C
Time	2 min	1 min	30 sec	1.30 min	5 min
16S rRNA	**Denaturation**	**Denaturation**	**Annealing**	**Elongation**	**Elongation**
N. cycles	--	35 cycles			--
°C	94°C	94°C	60°C	70°C	70°C
Time	5 min	30 sec	30 sec	1.30 min	10 min

RESULTS

Identification of bacteria in the urine samples using conventional methods:

Figure (1) declared that all samples were Lactose fermenters led to the changing of the color of CLED media to yellow and the color of MacConkey agar to pink, except for 3 samples in Figure (2) where lactose was nonfermenting. Thus, the CLED media became blue, and it is colorless in MacConkey agar.

Figure 1: An example of identification of Lactose fermenters bacteria by urine samples on (a) MacConkey agar, (b) Blood agar, and CLED media.

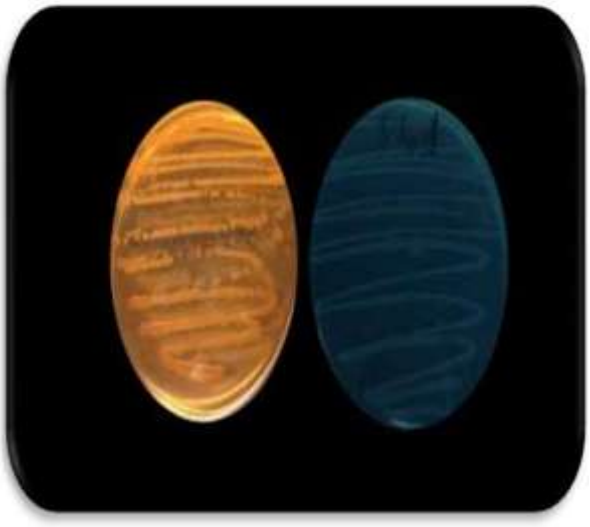

Figure 2: An example of identification of lactose nonfermenting bacteria by urine samples on (a) MacConkey agar, (b) CLED media.

The morphology on all blood agar plates was gray, moist, and beta (β) hemolytic. While figure (3) showed the bacteria organisms revealed gram-negative, pink-colored with rod-shaped appearance, and they were arranged in single or in pairs. Being a Gram-negative bacterium, *E. coli* has an additional outer membrane that is composed of phospholipids and lipopolysaccharides.

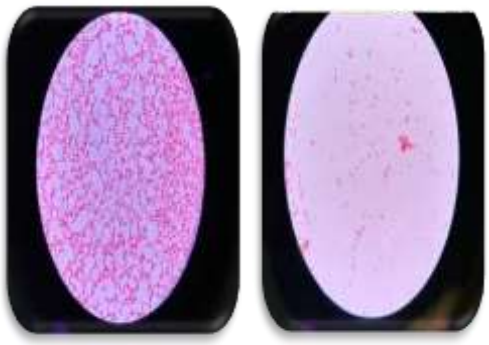

Figure 3: Identification of bacteria using Gram stain

The presence of lipopolysaccharides on the outer membrane of bacteria gives it an overall negative charge to the cell wall. Because of these properties, *E. coli* does not retain crystal violet during the Gram staining process. Identification and susceptibility testing performed were carried out to characterize 6 samples of bacteria isolated from urinary tract infection through the VITEK2 Automated System. Table 3

Table 3: Identification of the bacteria using the VITEK 2 system and probability percentage to be *Escherichia coli*.

Sample	Organism name	Probability	Confidence
1	*Escherichia coli*	99%	Excellent
5		99%	Excellent
6		92%	Good
9		96%	Excellent
10		99%	Excellent
12		93%	Very good

shows that all samples identified as *E. coli* by the Vitek 2 system had acceptable, good, very good, or excellent identification after their identities were verified with the reference systems. The VITEK 2 antimicrobial susceptibility test (AST) results presented in table 4

All samples were almost having similar results except six samples were chosen. Thus, sample 1 was sensitive to all antibiotics except for Ampicillin, samples 6 and 12 were resistant to more than 10 antibiotics, sample 9 was sensitive to all antibiotics, and sample 10 was the only sample resistant to Imipenem.

Table 4: The VITEK 2 Antimicrobial Susceptibility Tests (AST) results

Antibiotic	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10	S 11	S 12	S 13	S 14	S 15
Amikacin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ampicillin	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R
Amoxicillin/clavulanate	S	I	S	S	I	I	S	S	S	S	S	I	N	N	S
Augmentin	S	N	N	N	N	N	N	N	N	N	N	R	R	S	N
Aztreonam	S	N	R	N	N	R	N	R	S	S	S	R	R	S	S
Cefepime	S	R	R	R	R	R	R	R	S	S	S	R	R	S	S
Cefalotin	I	R	N	R	R	N	R	N	N	N	N	N	N	N	N
Cefoxitin	S	R	S	S	S	I	S	S	S	S	S	R	R	S	S
Ceftazidime	S	R	I	R	R	R	R	S	S	S	S	R	R	S	S
Ceftriaxone	S	R	R	R	R	R	R	R	S	S	S	R	R	S	S
+Ceftazidime/avibactam	S	S	N	S	S	N	S	N	N	N	N	N	N	N	N
Cefuroxime	S	N	R	N	N	R	N	R	S	S	S	R	R	S	S
Cephalothin	S	N	N	N	N	N	N	N	N	N	I	R	N	N	N
Ciprofloxacin	S	I	R	S	R	R	S	R	S	R	S	R	S	R	R
Co-trimoxazole	S	N	N	N	N	N	N	N	N	N	N	R	S	R	N
Colistin	S	N	N	N	N	N	N	N	N	N	N	S	S	S	N
Ertapenem	N	N	S	N	N	S	N	S	S	S	S	N	N	N	S
Gentamicin	S	S	S	S	S	R	S	S	S	S	S	S	S	S	R
Imipenem	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
Levofloxacin	N	N	R	N	N	R	N	R	S	R	S	R	S	R	R
Meropenem	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S
Nitrofurantoin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Piperacillin/tazobactam	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S
Tigecycline	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Trimethoprime / Sulfamethoxazole	N	S	S	S	R	R	S	R	S	R	R	N	N	N	R

S: sensitive
R: resistance
I: intermediate
N: not applicable

Molecular identification of bacterial samples isolated from urine samples:

Genomic DNA was extracted from six selected isolates only. Based on the Vitek 2 Antimicrobial Susceptibility Tests (AST) results, the samples were almost similar except for those 6 selected samples. Thus, sample 1 was chosen for its sensitivity to all antibiotics except for Ampicillin, samples 6 and 12 were selected because they were resistant to more than 10 antibiotics, sample 9 was sensitive to all antibiotic, and sample 10 was the only sample resistant to Imipenem. Sample 5 was selected based on the biochemical test results which was lactose non-fermenting in CLED media.

The *traT* gene was amplified using universal primers. According to PCR results, the *traT* gene was sequenced in the selected isolates and it was successfully amplified from a template of DNA that was previously extracted. PCR products of the *traT* gene of these isolates showed bands of about 290bp using electrophoresis as shown in fig. (4). Moreover, the 16S rRNA gene was amplified using universal primers. According to PCR results, the 16S rRNA gene sequence of the selected isolates was successfully amplified from a template of DNA that was previously extracted. PCR products of the 16S rRNA gene of these isolates showed bands of about 500 bp using electrophoresis as shown in Fig (5) & table (5).

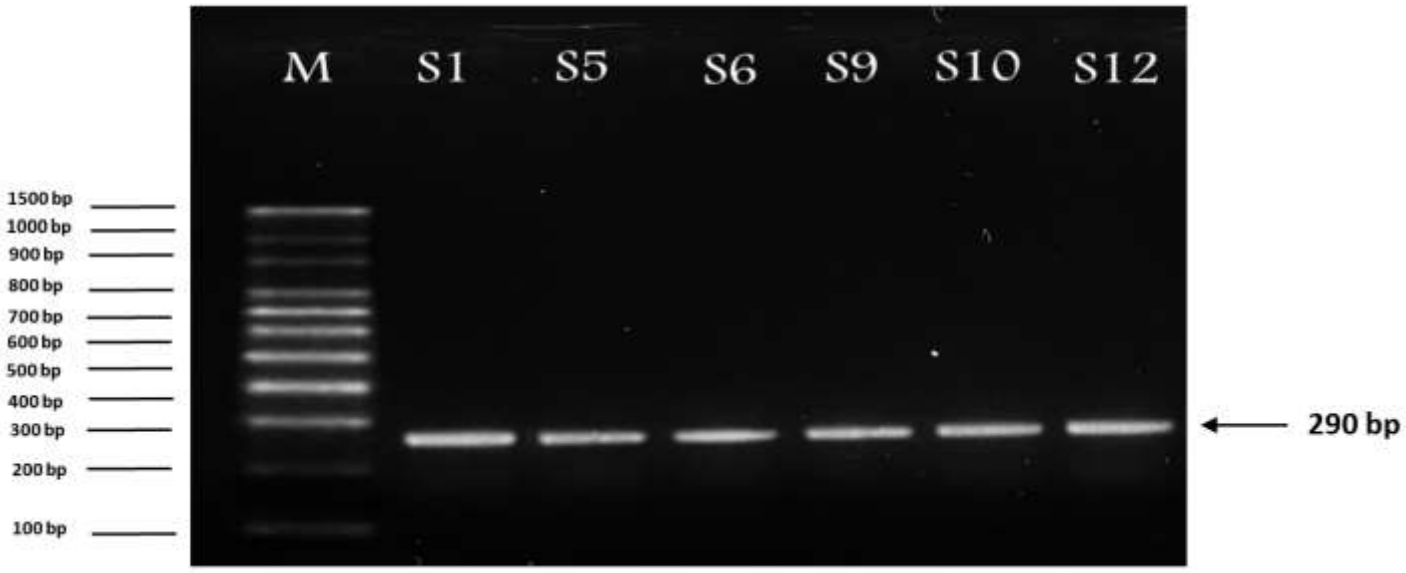

Figure 4: 1% Agarose Gel Showing PCR Products After the Amplification of *traT* of some of The Isolates. Lane 1(M): 100bp DNA Marker.

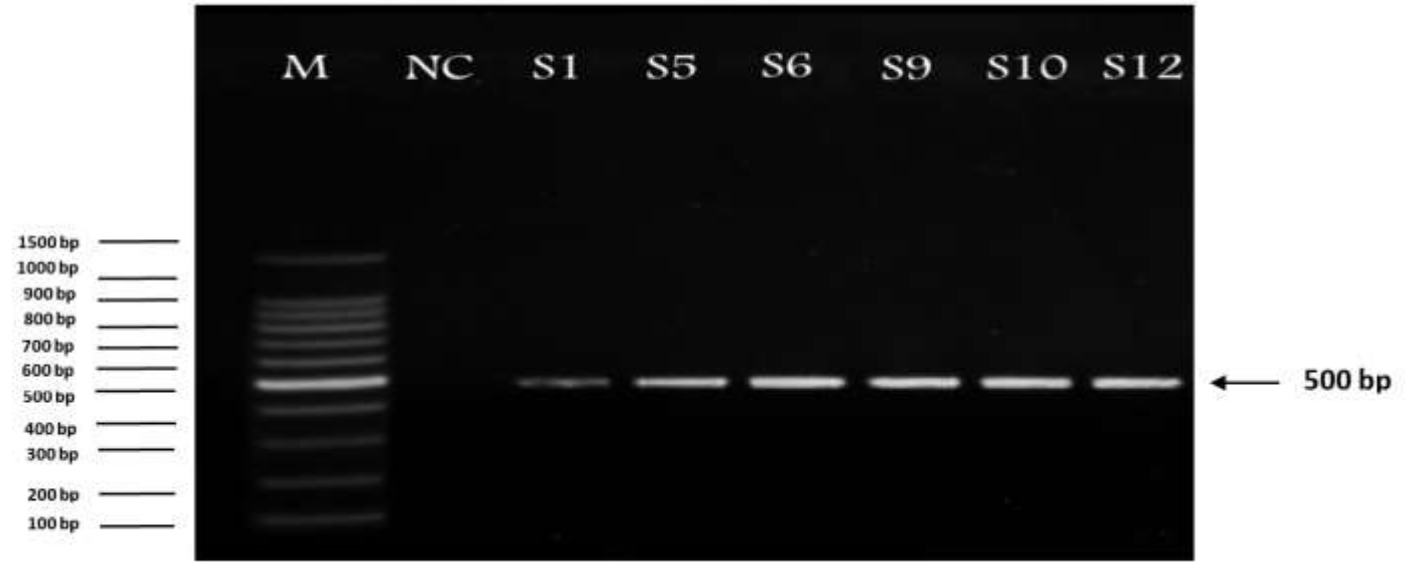

Figure 5: 1% Agarose Gel Showing PCR Products After the Amplification of 16S rRNA of some of The Isolates. Lane 1(M): 100bp DNA Marker.

NG: Negative Control
S1: Sample 1.
S5: Sample 5.
S6: Sample 6.
S9: Sample 9.
S10: Sample 10.
S12: Sample 12.

Table 5: Bacterial identification by 16S rRNA gene

Sample	Bacterial Strain	Identity	Confidence
1	*Escherichia coli* strain NBRC 102203 16S ribosomal RNA, partial sequence	99.38%	NR_114042.1
5		99.38%	
6		98.76%	
9		99.38%	
10		99.37%	
12		99.37%	

DISCUSSION

All samples were Lactose fermenters except for 3 samples lactose nonfermenting Fig. (2). The reason why biochemical identification of *E. coli* is initially based on the bacterium is its ability to ferment lactose. However, some which were *E. coli* strains were not able to metabolize this sugar due to a deficiency in lactose permease encoded by the lacY gene; henceforth, these bacteria are called non-lactose-fermenting *E. coli (*NLFEC) (Firoozeh, et al. 2014; Terlizzi et al. 2017 and Siqueira et al. 2021).

In this study, the identification and evaluation of *E. coli* by the Vitek 2 technique was interpreted as either as excellent, very good or good based on the matching with the *E. coli* reference strain (Kabugo, et al. 2016; Forsyth et al. 2018). The evaluation in the technique of the study concluded that the VITEK 2 identification method is an acceptable automated method for the identification of Gram-negative bacteria which is compatible with previous studies (Flores-Mireles, et al. 2015; Idil, et al. 2016 and Naser, 2016).

Various virulence factors can be attributed to UPEC pathogenicity. Better knowledge of the properties of the virulence and its antibiotic resistance pattern helps clinicians to anticipate the development of the infection in patients (Tan & Chlebicki, 2016). The *traT* gene is considered the most prevalent virulence gene among UPEC strains. The results of the current study showed that the *tra*T gene was detected in all 6 UPEC samples. Further, a study held by (Neamati, et al. 2015 and Wagenlehner et al. 2016) showed that 76 % of the urine samples were identified with multidrug-resistant bacteria carrying the *traT* gene. Another study made by (Behzadi, 2018) showed that 74% of UPEC isolates contained the traT gene. The previous results are compatible with those of the current study which suggests that the traT gene, as a common and important virulence factor, can be considered a s a target for molecular identification of the UPEC.

CONCLUSION

In conclusion, compared with conventional methods that require few days, this method can make same day reporting possible for therapeutic interventions and consequently allow better patient management.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

HAY; conceptualized the study, wrote the manuscript and responsible for the content and similarity index of the manuscript. YFZ; collected, performed the practical study, and reported the results. HE; conceptualized the study and shared in writing the manuscript.

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