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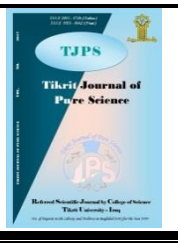

Detection of auto-transporter *Sat* and *Vat* genes among *Escherichia coli* strains isolated from urinary tract infections

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Abstract

This study was designed to detect the presence of genes encoding autotranspoter proteins in *E. coli* that causes UTI by using PCR techniques. Seventy two urine sample were collected from patients infected with UTI whom attended to Salah-AL-deen general hospital in Tikrit city, during three months period (September to November 2016). All samples were cultivated on Blood agar and MacConkey agar. The 47(65.2%) *E. coli* isolates were confirmed using standard biochemical tests for *E. coli*. The results indicate the frequencies of *Sat* gene was 27 strains(57.5%) while *Vat* gene was 12 strains (25.5%) while the Duplex PCR detected 8(17%) strains of *E. coli* contained two genes. With this method, we confirmed that autotransporter genes are pathospecifically distributed among the *E. coli* strains studied.

Introduction

Urinary tract infection (UTI) is a broad term that encompasses both the asymptomatic microbial colonization and symptomatic infection with invasion and inflammation of the urinary tract structures [1]. *Escherichia coli* is a commensal resident of the intestine as well as a pathogen causing various diseases of humans and animals. *E. coli* strains can be grouped into three major categories: diarrheagenic *E. coli*, commensal *E. coli* and extra-intestinal pathogenic *E. coli* (ExPEC)[2].Commensal *E. coli* strains are members of the normal intestinal flora of humans, other mammals, and birds [3].Certain commensal intestinal isolates are capable of causing infections in extra-intestinal tissues, and these pathogenic strains have been collectively termed ExPEC [4]. Among ExPEC, uropathogenic *E. coli* (UPEC) is the most common cause of human urinary tract infections (UTIs)[5]. UPEC isolates typically carry large blocks of genes, called pathogenicity-associated islands which are not found in fecal isolates. Some of these strains are also secrete specific proteins that may contribute to pathogenesis of UTI. Among these proteins are the well-characterized hemolysins and moreover, described secreted autotransporter toxins: *Sat* and *Vat* [6,7]. Autotransporters are a family of secreted proteins from gram-negative bacteria that direct their own secretion across the outer membrane (this secretion mechanism has also been described as type V secretion) [7,8].All autotransporters possess the same general structure, comprised of three domains: an amino-terminal leader peptide (which initiates trans-port of the precursor across the inner membrane),an α or passenger domain (which confers the function of the secreted protein) and a carboxy-terminal domain (the β domain) that mediates secretion through the outer membrane, the passenger domain can either be released at the surface of the outer membrane or remain associated with the bacterial cell surface[7]. The autotransporter group includes a numerous of supposed virulence factors that role as cytotoxins, entero-toxins, immunoglobin proteases, mucinases, heme binding proteins, and adhesions in *E. coli* and other gram-negative bacteria [7,8].Several reports have identified multiple autotransporters in single strains of *E. coli* [9,10]. Hence, they were used in silico analyses of the *E. coli* CFT073 genome sequence to identify uncharacterized autotransporter proteins that might be associated with uropathogenesis, recently documented the presence of 10 members of the autotransporter family in UPEC CFT073, including three SPATEs: Sat, PicU and Vat [9]. Sat is a toxin which has vacuolating cytotoxic activity against bladder and kidney cells [6].Vat the

vacuolating autotransporter toxin recently described for avian pathogenic *E. coli* (APEC), causes vacuolization of chicken embryo fibroblasts in vitro and was shown to be vital for virulence of APEC strain Ec222[11].The virulence factors among UTI agents are really different and clinical isolates could not be detected as UPEC by using traditional methods. Also, some UPEC strains have ascending properties after colonization in bladder and can affect the kidney tissue. Therefore, using molecular detection methods is critical for identification of UPEC strains. The aim of this study was to find out *Sat* and *Vat* virulence gens and there prevalence among *E. coli* strains.

Materials and Methods

Collection of samples and *E. coli* isolates identification

Seventy two urine sample were collected from patients infected with UTI whom attended to Salah-AL-deen general hospital in Tikrit city, during three months period (September to November 2016). All samples were cultured on Blood agar and MacConkey agar. The (47) *E. coli* isolates were confirmed using gram stain and standard biochemical tests for *E. coli* (Oxidase test negative, Catalase test positive, urease test negative and nitrate reduction positive) (with IMVIC test, *E. coli* isolates are identified by positive indole ,methyl red tests, negative for Voges-Proskauer and citrate utilization tests), with (TSI test *E. coli* isolates produce Butt yellow/Slant yellow, gas production positive and H_2S production negative) and in Eosin-Methylene blue agar *E. coli* isolates produce green metallic sheen colonies[12].

Storage of *E. coli* isolates

After identification, each *E. coli* isolate was inoculated into 5ml of nutrient broth and incubated at 37°C overnight. Isolates were stored as a mixture of the broth culture and glycerol(20%-1ml glycerol to 4ml broth culture) at -70°C until PCR assays were performed.

DNA Extraction and PCR

Total DNA was extracted from the 47 isolates using the extraction kit [Promega, USA] according to the manufacturer's instructions and detection their concentration though instrument [Genova- Nano Spectrophotometer, Jenway]. PCR conditions and primers used for *sat* and *vat* genes were described in a (table1) according to *Saraylu et. al*., 2012 PCR was done by using premix (bioneer, South Korea) in a 20 µL reaction mixture containing 5µL template DNA (2 µg/µL), 1.5µl (10pmol) for each of forward and reverse primers, and 12 µl free nuclease H2O. PCR conditions consisted of 35 cycles, having each a denaturation step at 94˚C for 1minute, primer annealing step at 58˚C for 1 minutes, and an extension at 72˚C for 1 minute. The cycles were preceded by a denaturation step at 94˚C for 3 minutes and followed by a final extension step.at 72˚C for 5 minutes. PCR was done for all reactions using the Thermocycler Amplicons of the genes product were subjected to electrophoresis on 1.5%-agarose gels in 1X Tris-borate-EDTA buffer at 80 V for 130 minutes. Gels were stained with ethidium bromide. Amplicons were detected on UV transilluminator [13]. EtBr is the most common reagent used to stain DNA in agarose gels[(11)]. When exposed to UV light, electrons in the aromatic ring of the ethidium molecule are activated, which leads to the release of energy (light) as the electrons return to ground state.

Table 1: Primers used for amplification of *sat* and *vat* genes in this study

Genes name	Primer sequences (5`to 3`)	Amplicon size (bp)
Sat	F: CTACAGCTTGATCACCTATGGC	410
	R: CTCCCTGGTATTTCTTTGTGG	
Vat	F: TTCACGGTACTGTTGTTCGC	217
	R: CAGATAACTCCAGCGTCACG	

Results

Seventy two (72) urine samples were collected and 47 *E. coli* isolates were identified among them. The conventional PCR of *Sat* and *Vat* genes was performed for 47 *E. coli*, and the results showed that 27 strains (57.5%) encompass *Sat* gene and 12 strains (25.5%) comprise *Vat* gene. While the Duplex PCR detected only 8 strains (17%) which contained the two genes (Fig.1 and 2).

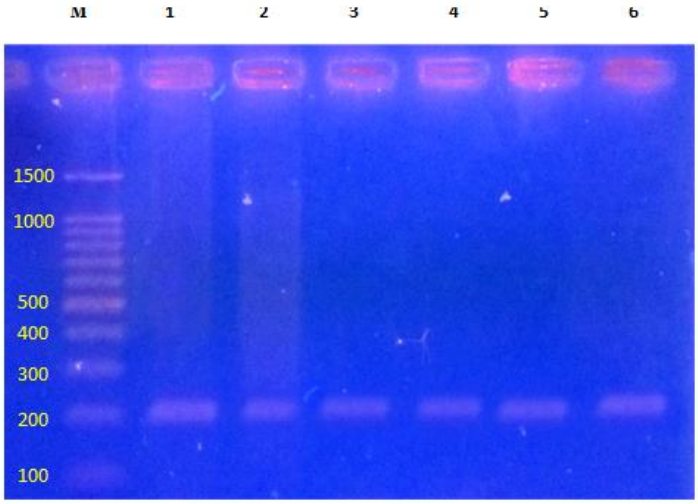

Fig. 1: Based of PCR product of vat gene in *E. coli* isolated from urine samples.
M: DNA ladder 100bp
Lane 1 to 6, Vat amplicon (217 bp).

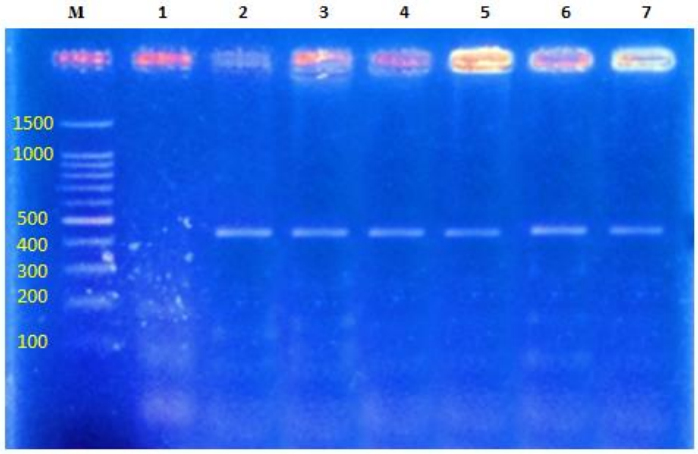

Fig. 2: Based of PCR product of sat gene in *E. coli* isolated from urine samples
M: DNA ladder 100bp
Lane 1 Negative control
Lane 2 to 7, Vat amplicon (410 bp)

Discussion

Urinary tract infections (UTIs) are among the most common bacterial infectious diseases encountered in clinical practice and account for significant morbidity and high medical costs [14].The *E. coli* pathotypes that cause extraintestinal infections are collectively called Extraintestinal Pathogenic *E. coli* (ExPEC) which comprise various virulence factors and could not be determined by conventional diagnostic methods [15].One of these pathotypes is Uropathogenic *E. coli* (UPEC), the most common etiological agent of hospital and community-acquired UTIs [16].Although UPEC has no uniquely defining features, certain serotypes and electrophoretic types are predominate. Uropathogenic virulence factors are found less frequently among commensal strains of fecal *E. coli* than UPEC and often occur together on pathogenicity islands (PAIs),no known uropathogenic virulence factors individually or jointly identify UPEC isolates, uniquely. The present study showed that *E. coli* was the commonest bacterial causative agent of UTI isolated from47(65.2%)of patients. This result was in accordance with other studies in which *E. coli* was isolated from (64.4 % to 75.0%)[17,21].

The presence of Autotransporters (*Sat* &*Vat*) genes among *E. coli* was investigated in this study, during the detection of amplicon, we noticed that the best results were obtained with 1.5% concentration of agarose gel, the percentage of agarose which used depends on the size of fragments to be resolved. Agarose gels are normally in the range of (0.2% to 3%). The lower concentration of agarose will be going to fasten the migration of the DNA fragments. In general, if the aim is to separate large DNA fragments, a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended [22].In present study, the presence of *Sat* gene among *E. coli* isolates was (57.5%),our result agrees with [Guyer *et. al*., 2000] whose found

38 out of 67(55%) pyelonephritis isolates but only 6 out of 27(22%)fecal isolates carried the *Sat* gene [23].In addition [Maroncle *et. al*.,2006]found that *E. coli* strains associated with the clinical symptoms of acute pyelonephritis (68%) of strains than by fecal strains (14%) [24]. These studies indicated that detection of *Sat* gene in UPEC pathotype is very important and could be an indicator for set-up a new diagnostic method. Moreover, it shows that *Sat* gene has a potential value for selecting as one of candidate antigen for designing new vaccine against UTI [13].The prevalence of *Vat* gene in our detection was (25.5%).This result was in accordance with other studies in which *Vat* gen accounted(18.1% to 36%) [13,25]. [*Parreira and Gyles*,2003] identified gene encoding a serine protease autotransporter protein (*Vat*) which was responsible for the vacuolating activity of a strain of *E. coli* (Ec222) that had been isolated from a septicemic chicken, *Vat* is encoded on a PAI which is inserted adjacent to the thrW tRNA gene [11]. The presence of *Vat* at this position has been demonstrated for Uropathogenic *E. coli* strain CFT073and the neonatal meningitis strain *E. coli* RS218 [7]. The vacuolating autotransporter toxin (*Vat*) often expressed by UPEC strains, shows the ability to induce a variety of cytopathic effects in target host cells, including swelling and vacuolation [26]. [Saraylu *et. al.,* 2012]reported that *Vat* gen considered among urine isolates because it required for *E. coli* to enter and survive within the bloodstream and strain that have *Vat* gene is more risky than other *E. coli* pathotype strains [13]. The using of Duplex PCR in this detection indicted 8 strains (17%) of *E. coli* isolates contained *Sat* and *Vat* genes, our result more than [Saraylu *et. al*., 2012]whose found that(4%) of *E. coli* isolates had the two genes. The using of molecular methods like Duplex PCR which detect *Vat* and *Sat* genes Simultaneously may be informative for innovation the best treatment for UTI [13].

The results globally suggest a pathotype-specific distribution of certain SPATEs among the *E. coli* strains tested. Indeed, *Sat* and *Vat* sequences have previously been shown to be associated with UTI isolates compared to commensal fecal isolates [27].While other study documented that no significant differences in the presence of *Sat* or *Vat* gens were found between UTI isolates compared with other clinical isolates belonging to the same phylogenetic groups [28]. A conventional PCR assay to screen for autotransporter genes in *E. coli* was developed and validated, with this method we confirmed that autotransporter genes are patho - specifically distributed among the *E. coli* strains studied. These results are in agreement with [Escobar-Paramo *et. al.*, 2004] whose fond that, in *E. coli*, the occurrence of certain virulence genes is dependent on the genetic background and/or clinical origin of the strain [29]. It is necessary to evaluate the prevalence of virulence factors among clinical isolates for designing a molecular detection method in future and further researches is now required to ascertain the role of many of these autotransporter proteins in *E. coli* pathogenesis [29].

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الكشف عن جينات النقل التلقائي SAT & VAT Autotransporter بين سلالات *Escherichia coli* المعزولة من التهابات المسالك البولية

وصال رؤوف ياسين الحيالي[1] , علاء يونس مهدي الحمداني[2] , محمد عبدالرزاق القطان[3]

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الملخص

صممت هذه الدراسة من اجل الكشف عن وجود جينات الضراوة Sat و Vat ومدى انتشارها بين جينات بكتريا ال *E. coli* المعزولة من عينات الادرار. تم جمع 72عينة بولية من مرضى عدوى المسالك البولية الذين حضروا إلى مستشفى صلاح الدين العام في مدينة تكريت ، خلال فترة ثلاثة أشهر (سبتمبر إلى نوفمبر 2016) .جميع العينات تمت زراعتها على أجار الدم و أجار MacConkey, تم تشخيص 47(٪ 65.2) سلالة من بكتريا *E. coli* باستخدام الاختبارات الكيميائية الحياتية القياسية للإشريكية القولونية. في هذه الدراسة ، تم الكشف عن وجود جينات تشفير البروتينات autotranspoter في عزلات *E. coli* باستخدام تقنية PCR , وقد اظهرت النتائج الى وجود جينات SAT في 27(٪57.5) سلالة, بينما كان الجين VAT في 12(٪25.5) سلالة, في حين اظهرت النتائج باستخدام تقنيةDuplex PCR وجود 8(٪17) سلالات من *E. coli* تحتوي على جينات SAT وVAT .من الضروري اجراء المزيد من البحوث للكشف عن دور العديد من بروتينات autotransporter في امراضية بكتريا *E. coli*