PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

PADRÃO DE PRODUÇÃO DE CÁPSULAS DE *ESCHERICHIA COLI* UROPATOGÊNICAS DE PACIENTES COM INFECÇÃO DO TRATO URINÁRIO EM HOSPITAIS DE KIRKUK

CAPSULE PRODUCTION PATTERN OF UROPATHOGENIC *ESCHERICHIA COLI* OF URINARY TRACT INFECTION PATIENTS IN KIRKUK HOSPITALS

نمط إنتاج الكبسول لبكتيريا الاشريكية القولونية البولية من مرضى التهاب المسالك البولية في مستشفيات كركوك

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Received 16 May 2020; received in revised form 22 May 2020; accepted 17 June 2020

RESUMO

A bactéria *Escherichia coli* é um dos melhores organismos de vida livre estudados em profundidade. É uma espécie surpreendentemente diversificada, já que algumas cepas de *E. coli* vivem no intestino de animais como comensais inofensivos, enquanto outros genótipos distintos, como uma *E. coli* enteropatogênica ou enterohemorrágica, por exemplo, causam morbidade e morte marcadas como patógenos intestinais humanos. Este estudo teve como objetivo desenvolver e validar um ensaio de PCR para uma região gênica conhecida e suspeita de fator de virulência (*kpsMT*) de *E. coli* uropatogênica para determinar a distribuição do gene e seu papel no desenvolvimento de doenças clínicas do sistema urinário. Um total de 25 amostras de urina foram coletadas de pacientes com infecção do trato urinário (ITU) dos hospitais Azadi e Kirkuk, na cidade de Kirkuk, Iraque. Foram coletadas amostras de ambos os sexos e idades diferentes de pacientes com suspeita de infecção do trato urinário de acordo com as manifestações clínicas e sintomas diagnosticados pelo médico examinador. As amostras foram cultivadas e as amostras positivas foram submetidas ao teste IMViC para identificar as bactérias *E. coli* e, posteriormente, identificadas usando o sistema compacto Vitek 2. Entre 25 amostras, 24 (96%) apresentaram resultados positivos para o crescimento cultural bacteriano. Dessas, 17 (68%) foram identificadas como *Escherichia coli*. Do total de 17 isolados, 14 tinham infecção no trato urinário leve e 3 tinham urosepsia. O gene *kpsMT* estava presente em 14 isolados (82,3%), incluindo 11 (78,5%) isolados de infecção no trato urinário leve e 3 (100%) isolados de pacientes com urosepsia. Concluiu-se que *Escherichia coli* é a mais prevalente em amostras de infecção no trato urinário na urina. Devido à abundância do gene kpsMT na *Escherichia coli* uropatogênica (UPEC), esse gene desempenha um papel importante no desenvolvimento de ITU se não for tratado corretamente e rapidamente; casos leves de ITU podem se transformar em urosepsia.

Palavras-chave: *Escherichia coli, uropatogênico, IMViC, Capsule, gene kpsMT.*

ABSTRACT

The bacterium *Escherichia coli* is one of the best free-living organisms studied in depth. It is a surprisingly diverse species, since some strains of *E. coli* live in the intestine of animals as harmless commensals, while other distinct genotypes, such as an enteropathogenic or enterohemorrhagic *E. coli*, for example, cause morbidity and death marked as human intestinal pathogens. The purpose of this study was to develop and validate a PCR assay for a known and suspected uropathogenic *E. coli* virulence factor (*kpsMT*) gene region to determine the distribution of the gene and its role in the development of clinical diseases of the urinary system. A total of 25 urine samples were collected from patients with urinary tract infection (UTI) at Azadi and Kirkuk hospitals in the city of Kirkuk, Iraq. Samples of both genders and different ages were collected from patients with suspected urinary tract infection according to the clinical manifestations and symptoms diagnosed by the examining physician. The samples were cultured and positive samples were subjected to the IMViC test to identify *E. coli* bacteria and subsequently identified using the Vitek 2 compact system. Among 25 samples, 24 (96%) showed positive results for bacterial cultural growth. Of these, 17 (68%) were identified as *Escherichia coli*. Of the total of 17 isolates, 14 from patients with mild urinary tract infection, and 3 from patients with Urosepsis. The *kpsMT* gene was present in 14 isolates (82.3%), including 11 (78.5%) isolates from patients with mild urinary tract infection, and 3 (100%) isolates from patients with Urosepsis. It was concluded that *Escherichia coli* is the most prevalent

in urine tract infection samples. Due to the abundance of the *kpsMT* gene in uropathogenic *Escherichia coli* (UPEC), this gene plays an important role in developing UTI if it is not treated correctly and quickly; mild cases of UTI can turn into Urosepsis.

Keywords: *Escherichia coli, Uropathogenic, IMViC, Capsule, kpsMT gene.*

الملخص

بكتيريا الاشريكية القولونية هي واحدة من أفضل الكائنات الحية التي تمت دراستها بمعق. هذه الانواع تكون متغايرة فيما بينها بشكل مثير للدهشة، حيث أن بعض سلالات الاشريكية القولونية تعيش في الامعاء كعلاقة تبادل منفعة، بينما الانماط الجينية الاخرى، مثل الاشريكية القولونية الممرضة المعوية او الاشريكية القولونية النزفية، على سبيل المثال، تسبب الامراض والوفيات والذي يشار اليها بمسببات الأمراض المعوية البشرية. الغرض من هذه الدراسة هو لتطور وتحقق اختبار تفاعل البوليميراز المتسلسل لمنطقة معلومة ومشتبهة لجين عامل الضراوة *kpsMT* لتحديد معرفة توزيع هذا الجين ودوره في تطوير الأمراض السريرية للجهاز البولي. تم جمع 25 عينة بول من مرضى يعانون من عدوى المسالك البولية في مستشفيات آزادي وكركوك في مدينة كركوك، العراق. تم جمع عينات من كلا الجنسين ومن أعمار مختلفة من المرضى الذين يشتبه في إصابتهم بالتهاب المسالك البولية وفقًا للمظاهر والأعراض السريرية التي تم تشخيصها من قبل الطبيب الفاحص. تم زرع العينات والعينات الوجبة للنمو أجري لها أختبار IMViC لتحديد بكتيريا الإشريكية القولونية ومن ثم شُخصت بإستخدام نظام Vitek 2 compact system. من بين 25 عينة، أظهرت 24 عينة (96٪) نتائج إيجابية للنمو البكتيري، حيث تم تحديد 17 (68٪) منها على أنها بكتيريا الاشريكية القولونية. في هذه الدراسة، من أصل 17 عزلة كان 14 من المصابين بالتهاب مجرى البول البسيط و 3 من المصابين بأنتان المسالك البولية، كان الجين *kpsMT* موجودًا في 14 عزلة (82.3٪) بما في ذلك 11 (78.5٪) من المصابين بالتهاب مجرى البول البسيط و 3 (100٪) من مرضى أنتان المسالك البولية. خُلصت هذه الدراسة إلى أن الإشريكية القولونية هي الأكثر انتشارا في عينات المسالك البولية. بسبب وفرة الجين *kpsMT* في سلالات بكتيريا الاشريكية القولونية البولية (UPEC) حيث يلعب دورًا مهمًا في تطوير التهاب المسالك البولية إذا لم يتم التعامل معها بشكل صحيح وسريع، فقد تتطور الحالات البسيطة من التهاب المسالك البولية إلى أنتان المسالك البولية.

الكلمات المفتاحية: *الاشريكية القولونية، مسببة لأمراض الجهاز البولي، IMViC، الكبسول، جين kpsMT.*

1. INTRODUCTION:

The bacterium *Escherichia coli* is one of the best free-living organisms studied in depth. It's a surprisingly diverse species as well since some *E. coli* strains live in animal intestines as harmless commensals, whilst other distinct genotypes, including enteropathogenic, enterohemorrhagic, enteroinvasive, enterotoxigenic and enteroaggregative *E. coli* causes marked morbidity and death as human intestinal pathogens. Extraintestinal *E. coli* are another diverse category of life-threatening pathogenic bacteria. This latter group of pathogens includes separate clonal groups responsible for sepsis of neonatal meningitis and infections of the urinary tract. The uropathogenic group accounts for 70–90% of the 7 million cases of acute cystitis and 250,000 cases of pyelonephritis reported in the United States annually (Hooton and Stamm, 1997).

The extraintestinal *E. coli* differs from diarrheal pathogens because when they enter the urinary tract, bloodstream, or cerebrospinal fluid, they can act as either harmless human intestinal inhabitants or serious pathogens (Welch *et al.,* 2002). Within each of these broad groups are sets of strains known as pathotypes that share common virulence factors and elicit similar pathogenic outcomes (Marrs *et al.,* 2005). Several pathotypes of diarrheagenic *E. coli* give rise to gastroenteritis, but rarely cause disease outside of the intestinal tract. ExPEC, on the other hand, has maintained the ability to exist in the intestine without consequence but can spread and colonize different host niches, including the blood, central nervous system, and urinary tract, leading to disease (Wiles *et al.,* 2008). Urosepsis refers to severe infection of the urinary tract and/or the male genital tract (e.g., prostate) with features consistent with systemic inflammatory response syndrome (Kalra and Raizada, 2006).

The severity of disease conditions that are associated with UTI depends on multiple UPEC VFs and host susceptibility. A wide range of VFs genes such as adhesins (*fim, sfa, afaI, iha, papC, tsh, and papGI, -II, and also -III*), iron acquisition systems (*irp2*, and *iuc, iroN*), protectins (*kpsMT*, and *iss, ompT*), and genes encoding for toxins (*astA, cnf1, hlyA, usp, set, vat,* and *cva/cvi*) are involved in the pathogenicity conditions of UPEC (Abe *et al*., 2008; Chiou *et al.,* 2010). Pathogenic bacteria produce a thick, mucus-like layer of polysaccharide, called capsule coat antigenic proteins on the bacterial surface, otherwise, induce an immune response and lead to the destruction of the bacteria. Polysaccharides capsules are water-soluble, usually acidic, thermo-stable, and have molecular weights on the order of 100–2000 kDa, linear and consist of regularly repeating subunits of one to six monosaccharides. There is massive structural diversity; nearly two hundred different polysaccharides are produced by *E.coli* alone (Yun *et al.,* 2014). The gene *kpsMT* encodes for the K antigen capsule, which Enables UPEC to

evade the host's innate immune defenses (e.g., the complement system) (Justice *et al.,* 2006).

This study aimed to develop and validate a PCR assay for a known and suspected uropathogenic *E. coli* virulence factor (*kpsMT*) gene region to determine the distribution of the gene and its role in the development of clinical diseases of the urinary system.

2. MATERIALS AND METHODS:

2.1. Sample collections and strain identification assay

A total of 25 midstream urine samples were collected from hospitalized UTI patients of different ages and gender from local hospitals in Kirkuk / Iraq. Permission to conduct this study was issued by the Health institutional, and the collection of samples of individuals was carried out by under public health technician supervision. All participants have agreed to participate in this study, which was conducted from September 2019 to December 2019. All 25 specimens have been cultured on Blood agar, MacConkey agar, and Eosin methylene blue agar. The preparation of biochemical tests to confirm differentiation of *E. coli* from other lactose fermenters among Enterobacteriaceae was done by the following: IMViC test include: indole positive, methyl red positive, negative in the Voges-Proskauer, and, Simmons citrate also urease production, (MacFaddin, 2002). The results of biochemical tests for the final identification of *E. coli* were based on growth morphology on EMB agar and Vitek 2 compact system.

2.2. Primer design

The primers used to amplify *kpsMT* gene were designed using (Pick Primers) tool in the National Center for Biotechnology Information (NCBI) website and were manufactured by the Alpha company (Canada). The primers information is described in Table 1.

2.3. Extraction of Deoxyribonucleic acid

Bacterial chromosomal DNA of *E. coli* isolates was extracted by Geneaid™ DNA Isolation Kit based on the manufacturer's instructions. The Deoxyribonucleic acid has been evaluated by a nanodrop system that tuned to 260/280nm. Then the DNA was preserved at temperature (-20˚C) till further use.

2.4. Polymerase chain reaction analysis

The PCR assay was performed to detect the Virulence gene (Table 1), were primer *kpsMT* gene that encodes for capsule in *Escherichia coli* based on specified primers. The virulence gene was screened by PCR technique. For the detection this gene; The Chromosomal DNA extracted from all isolates were subjected to primers by monoplex PCR. The mixture of PCR for each primer with final volume 20 µl/reaction and The protocol used depends on Master Mix(AccuPower® PCR PreMix (Bioneer, Korea) instructions. Each monoplex PCR reaction mixture consisted of 2µl Forward Primer (10 picomole), 2µl Reverse Primer (10 picomole), 9µl De-ionized water, and 7µl the DNA of the isolates were added into the AccuPower® *Taq* PCR PreMix tubes that contain (*Taq* DNA polymerase, dNTPs, KCl, $MgCl_2$, and buffer). All PCR components were assembled in PCR tube and mixed by micro-centrifuge at 50 rcf (850 rpm) for 10 second. The PCR reactions began with a 94°C Denaturation for 5 minutes and were terminated with 72°C extension for 3 minutes and a 4°C hold and The Condition for other steps for this primer consisted of 25 cycles of a denaturation at 94°C for 2 min annealing at 65°C for 1 min then extension at 72°C for 2 min in the thermal cycler based on the primer design designated Tm and some modifications for optimization (Qadir *et al.,* 2018).

2.5. PCR product analysis

PCR product has been examined via Electrophoresis instrument in a 0.9% agarose gel substance with the use of TBE buffer, which stained by Ethidium Bromide. The product was visualized and documented under ultra-violet trans-illuminator (Mishra *et al.,* 2010).

3. RESULTS AND DISCUSSION:

Characterization of *E. coli* strains is essential for both epidemiological and clinical implications. Pathogenic behavior is predicted both by repertoire of the virulence factor and by phylogenetic background (Duriez *et al*., 2001; Picard *et al.,* 1999). Urinary tract infection can, in time, develop into a real threat, capable of expanding to renal failure. Enhanced knowledge of the virulence characteristics of the causative organism allows the clinician to predict the evolution of infection within the host. In the current study, From 25 urine samples, 18 (72%) were from female, and 7 (28%) from male, 17 (68%) isolates of *E. coli*, 4 (16%) isolates *Klebsiella pneumonia*,

3 (12%) isolates *Proteus mirabilis* and one sample (4%) showed no growth, the profile of the causative agents are listed in Table 2. Among *E. coli* causing infection, out of 17 isolates, 12 (70.5%) were taken from female patients, and 5 (29.4%) were from male patients, and this rate was close to the results of (Qadir *et al.,* 2018) in Wasit/Iraq and (Aljebory and Mohammad, 2019) in Kirkuk/Iraq as they showed that *E. coli* was the most predominant cause of UTI and that females were more susceptible to it than male. Moreover, the female to male ratio concerning Mild-UTI**/**Urosepsis is described in Table 3.

Recently, several different prevalence rates of UPEC strains related to UTIs have been described in different countries (Derakhshandeh *et al.,* 2015), (Mohajeri *et al.,* 2014), (Lee *et al.,* 2013). The capsule-encoding gene, *kpsMT*, is commonly prevalent in UPEC strains associated with pyelonephritis than in strains associated with other UTIs (Sussman, 1997). In the current study, the gene *kpsMT* was investigated due to its role in evading the immune system, hence causing severe conditions of UTI. The study finding of this gene have shown that 14 (82.3%) isolates were carrying *kpsMT* gene including 11 (78.5%) isolates from Mild-UTI, and 3 (100%) isolates from Urosepsis patients which leads to the assumption that even the (Mild-UTI) patients are susceptible to severe UTI conditions or Urosepsis if not managed in a short period. A demonstration of the assay is shown in Figure 1. The distribution of *kpsMT* gene in relation to Mild-UTI**/**Urosepsis is shown in Table 4.

Fever, flank pain, dysuria, frequency, urgency, and suprapubic pain has been the observed clinical symptoms of our study and can be compared with the Bent's report (Bent *et al.,* 2002). Several studies have had an approaching result to this study; there was a report of *kpsMT* gene's existence to be (84.4%) among Uropathogenic *E. coli* isolates revealed by (Yun *et al*., 2014), similar results were shown by (Aljebory and Mohammad, 2019) in Kirkuk/Iraq as they found that (76.4%) of the isolates were carrying *kpsMT* gene, (Qadir *et al.,* 2018) in Wasit/Iraq and (Yamamoto, 2007) in Japan had close results to this study. However, the results of researches done by (Alqasim *et al.,* 2020) and (Johnson and Stell, 2000) did not match with this study as they revealed (51.7%) and (63%) respectively. However, these strains need to be sequenced by 16S rRNA or RAPD-typing for further studies as recommended by Salih and Shafeek, 2019; Banoon *et al.,* 2019; Aldujaili and Banoon, 2020).

4. CONCLUSIONS:

Escherichia coli are the most prevalent among UTI urine samples. Due to the abundance of the gene *kpsMT* in UPEC, there is a role of this gene in developing UTI. For the same reasons above, mild UTI cases can develop into Urosepsis if not treated accordantly and quickly.

5. ACKNOWLEDGMENTS:

I would like to show my appreciation to Karwan Azeez Mohammed Shaexany at Kirkuk University, College of Science, Department of Biology for collecting the samples and designing the PCR primers, due to his support, this research was doable.

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Table 1. *Primers information*

	Primer sequence (5'->3')	Template strand	Length	Primer coordinates		Tm	GC%	GenBank accession number
				Start	Stop			
Forward	GTGTCCCAGCCCAGGTTTTTA	Plus	21	3659	3679	60.48	52.38	AF007777.1
Reverse	CATCACGTAACAAGATGCCCA	Minus	21	4860	4840	58.64	47.62	
Product length (bp)		1202						

Table 2. *The profile of the causative agentes*

Samples	Total	*E. coli* (%)	*Klebsiella pneumonia* (%)	*Proteus mirabilis* (%)	no growth (%)
Total	25	17 (68)	4 (16)	3 (12)	1 (4)
Male	7 (28)	5 (20)	2(8)	0(0)	0(0)
Female	18 (72)	12 (48)	2(8)	3(12)	1(4)

Table 3. *Female to Male ratio in relation to Mild-UTI/Urosepsis caused by E. coli*

Cases	Total (n=17)	Female (n=12)	Male (n=5)
Mild-UTI	14	10	4
Urosepsis	3	2	1

Table 4. *Distribution of kpsMT gene in relation to Mild-UTI/Urosepsis*

Cases	Total n=17	***kpsMT*** **Positive n=14 (%)**
Mild-UTI	14	11 (78.5)
Urosepsis	3	3 (100)

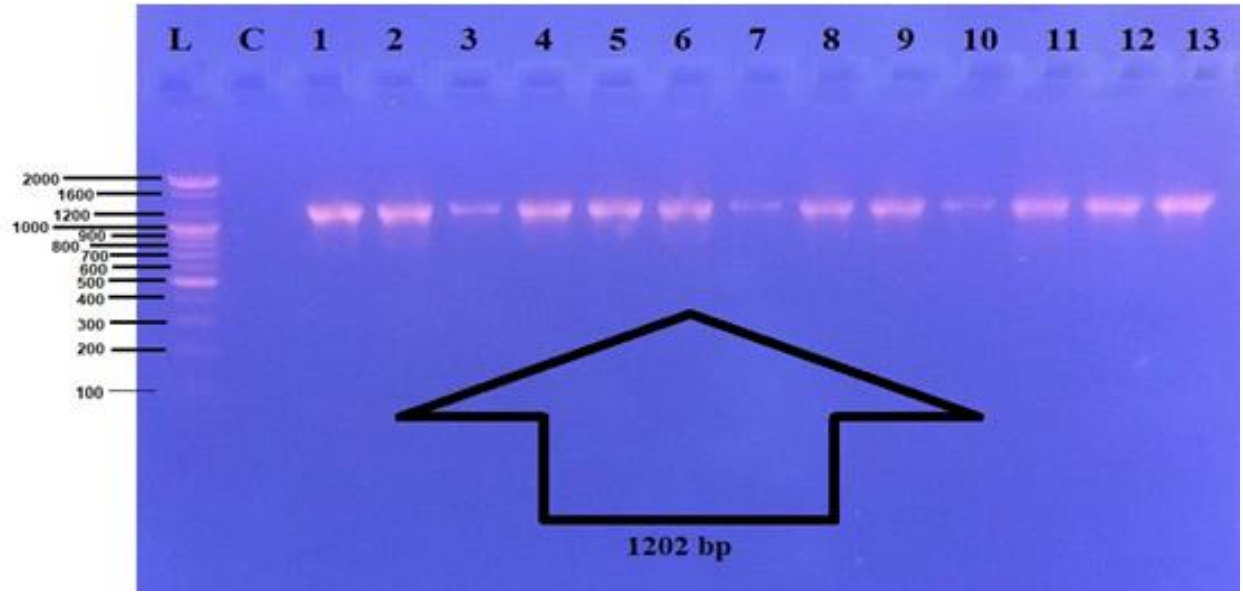

Figure 1. *Electrophoresis of agarose Gel for products of PCR for the inspection of kpsMT gene (1202bp) in 0.9%agarose at 70 volt for 60 minutes, stained via ethidium bromide, L: 100-2000bp Ladder, Lane C: negative control, lanes (1 to 13): Positive for kpsMT gene (1202bp).*