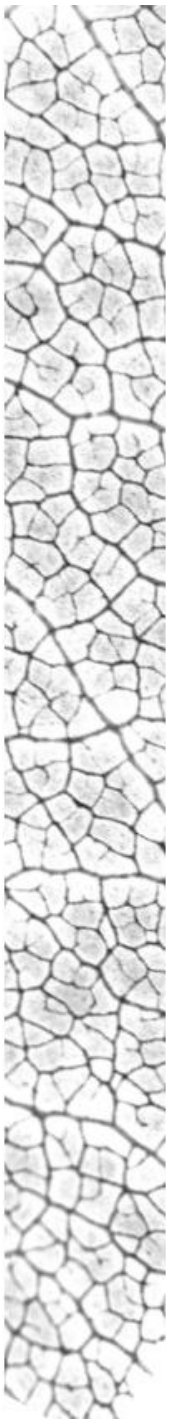

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 7737-7742 (2020)

Identification of pathogenic strains of *Escherichia coli* with *ChuA*, *YjaA*, and *TspE4C2* virulent genetic markers: The source human oral cavity

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Annotation
Background: Although the advances in antibiotic pharmaceuticals, some bacterial members still are making colossal health and economic disasters. *Escherichia coli* represents one of the most annoying microorganisms due to its ever-changing antibiotic susceptibility profile. Pharmaceutical industries keep working to find new antibacterials that can fight this challenging bacterium. **Objectives:** The present study was carried out to identify the virulence profile of *E. coli* previously isolated from the oral cavity of patients and obtained from Al-Diwaniyah General Teaching Hospital, Al-Diwaniyah City, Iraq. **Materials and methods:** In the beginning, identity-based detection steps were performed using cultivation and biochemical analyses and polymerase chain reaction (PCR) and partial gene sequencing (PGS) techniques that both targeted the *16S rRNA* gene of four out of thirteen (total) isolates. Then, all isolates were subjected to a PCR method that targeted the virulence genetic markers of *ChuA* (outer membrane hemin receptor), *YjaA* (stress response protein), and *TspE4C2* (antibiotic resistance fragment). **Results:** The findings of the identity revealed that all isolates were *E. coli*. The PCR of the virulence markers showed the existence of those pathogenic genetic codes in all isolates. The phylogenetic analyses (via the tree) uncovered that the PGS-strains were aligned with global isolates from the United States, China, Algeria, and Brazil that all clustered in one major group. **Conclusion:** The obtained *E. coli* are of pathogenic characteristics due to the detection of the *ChuA*, *YjaA*, and *TspE4C2* virulent genetic markers. These virulence features of the bacterium may indicate a high possibility of causing an infection to the oral cavity.

Key words: *ChuA*, *E. coli*, phylogeny, *TspE4C2*, *YjaA*

Dunia Bahel Gadaan Al-Ghanimy. (2020). Identification of pathogenic strains of *Escherichia coli* with *ChuA*, *YjaA*, and *TspE4C2* virulent genetic markers: The source human oral cavity. Eurasia J Biosci 14: 7737-7742.

INTRODUCTION

The most prevalent gram-negative extraintestinal pathogenic *E. coli* (ExPEC) induces several health conditions in all age groups. ExPEC is the most central cause of bacteremia, particularly in older people and a significant cause of neonatal meningitis. In young, healthy women, ExPEC triggers the bulk of urinary tract infections (UTIs). ExPEC diseases are exhibiting huge and rising global case-infection and case-fatality rates. The Multi-Drug Resistant (MDR) *E. coli* is a global issue to the treatment and protection (Russo et al. 2020; Movert et al. 2013). These groups of bacteria are different from those in the gastrointestinal (GI) tract includes a high number and abundance of *E. coli* that may shield it against pathogens. *E. coli* is one of the first leaders to colonize babies and sets the normal intestinal microbiota. The host is paid back of some advantages such as vitamins K and B12 from some non-pathogenic strains of *E. coli*, while certain strains of *E. coli* may trigger diseases. Enterohemorrhagic *E. coli* (EHec) is a diarrheal Ecoli species that induce moderate to extreme bloody diarrhea in humans and is likely to contribute to hemolytic uremic syndrome (

Received: March 2020
Accepted: September 2020
Printed: December 2020

Croxen et al. 2013; Eggesbø et al. 2020; Blount 2015).

ExPEC can enter several of the tissues of any age group and trigger infection. ExPEC is often recovered from people with infections in the regions of the respiratory tract, skin, and soft tissues. ExPEC is also a significant cause of neonatal meningitis and is a likely trigger of prostatitis, pneumonia, and peritonitis along with Group B Streptococci. ExPEC is a heterogeneous community of *E. coli* pathotypes, such as uropathogenic *E. coli* (UPEC), which characterized by their infections in areas out-patient's GI. ExPEC strains are a large class of disease triggers in humans. No central group of virulence factors can easily differentiate the ExPEC microorganisms from each other; however, several virulence factors are correlated with ExPEC. One significant public health concern is antimicrobial resistance. Substandard usage of antibiotics by individuals, factories, and farms, as well as inadequate sanitation and hygiene, are still known to be significant factors for the production and spread of antibiotic-resistant bacteria. Enzymes that impart tolerance to certain β-lactam antibiotics, namely penicillins and cephalosporins, can be detected in *E. coli* isolates (Pormohammad et al. 2019).

The present study was carried out to identify the virulence profile of *E*. *coli* previously isolated from the oral cavity of patients and obtained from Al-Diwaniyah General Teaching Hospital, Al-Diwaniyah City, Iraq. In the beginning, identity-based detection steps were performed using cultivation and biochemical analyses and polymerase chain reaction (PCR) and partial gene sequencing (PGS) techniques that both targeted the *16S rRNA* gene of four out of thirteen (total) isolates. Then, all isolates were subjected to a PCR method that targeted the virulence genetic markers of *ChuA* (outer membrane hemin receptor), *YjaA* (stress response protein), and *TspE4C2* (antibiotic resistance fragment).

MATERIALS AND METHODS

Sample collection, cultivation, and biochemical analyses

The techniques followed for the sample collection, cultivation, and biochemical analyses of the *E. coli* isolates were described by Blau *et al*. (2020). Briefly, thirteen isolates of *E. coli* were obtained from the oral cavity of patients and obtained from Al-Diwaniyah General Teaching Hospital, Al-Diwaniyah City, Iraq, during October 2019. Those isolates were cultivated on buffered peptone water and incubated at 37°C for overnight. CHROMagar orientation medium (Mast Diagnostica GmbH, Germany) was used to sub-cultivate the growth. The colonies were analyzed by using selective media, biochemical tests, and VITEK GN per methods previously mentioned by Olowe *et al.*, (2019).

Bacterial DNA extraction

Genomic DNA Mini Bacteria Kit, according to its protocol, was employed to run the extraction using an overnight incubated bacterial growth in a 1.5-ml tube 10,000rpm-centrifuged for 1min. Then, the supernatant was removed; the remaining steps were followed. DNA purity and concentration were evaluated using a NanoDrop.

16S rRNA gene PCR

This PCR was recruited to identify the bacterium further. The *16S rRNA* gene was employed to detect the identity of the bacterial isolates using the primer set (F: 5'-ATGCTTAGTGCTGGTTTAGG-3'), which spans at F8-27 and (R: 5'-GCCTTCATCATTTCGCTTTC-3') that spans at 1510-1492 (Eden et al. 1991). In a total PCR reaction volume of 25µl, DNA at 3µl (30ng), each primer direction at 1.5µl (15 pmol), 2X Master Mix at 12.5µl (Kappa Biosystem CO., South Africa), and water for molecular biology at 6.5µl were combined. The mixture-containing tubes were inserted in a thermocycler (Primus 25 peQLab, Germany) utilizing initial denaturation at 5mins-95˚C for, 30 cycles of (denaturation at 1min-94˚C, annealing at 1min-58˚C, and extension at 30s-72˚C), and a final extension at 10mins-72˚C. Then, the PCR products and a ladder (Gene Ruler™ 100bp and 50bp) were run on a 2%-agarose and screened using a UV-illuminated visualizer.

16S rRNA gene sequencing

The PCR products were purified by using PCR Clean UP-kit (Promega Co., USA) and also according to the kit instructions. The purified products were sent out to sequencing using the primer mentioned above and a model 373A automated fluorescent-DNA sequencer (Applied Biosystem Co.,Ltd., USA).

Analysis of the sequencing data

The BioEdit software and BLAST N (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) were used to analyze the nucleotide sequencing data. The phylogenetic tree was generated using MEGA v7.

PCR identification of ChuA, YjaA, and TspE4C2 genetic markers

The occurrence of the *ChuA*, *YjaA*, and *TspE4C2 genetic markers* was investigated using the primers (Table 1) described by Clermont *et al.*, (Clermont et al. 2020) and purchased from (IDT, Canada).

Table 1. *PCR primers of ChuA*, *YjaA*, and *TspE4C2 genetic markers*

Genetic marker	Direction	Sequences (5`-3`)	Product size (bp)
ChuA	**F**	F GACGAACCAACGGTCAGGAT	279
	R	R TGCCGCCAGTACCAAAGACA	
YjaA	**F**	F TGAAGTGTCAGGAGACGCTG	211
	R	R ATGGAGAATGCGTTCCTCAAC	
TspE4C2	**F**	F GAGTAATGTCGGGGCATTCA	152

	R	R CGCGCCAACAAAGTATTACG	

The mixture-containing tubes were entered in a thermocycler (Primus 25 peQLab, Germany) utilizing initial denaturation at 5mins-95˚C for, 30 cycles of (denaturation at 0.5min-94˚C, annealing at 10s-59˚C, and extension at 30s-72˚C), and a final extension at 7mins-72˚C. Then, the PCR products and a ladder (Gene Ruler™ 100bp and 50bp) were run on a 2%-agarose and screened using a UV-illuminated visualizer.

RESULTS

Cultivation and biochemical analyses

The findings revealed that the bacterial isolates were *E. coli.* The VITEK GN further confirmed the identity of the isolates (Table 2).

Table 2. VITEK GN results

Well	Test	Mnemonic
2	Ala-Phe-Pro-ARYLAMIDASE	APPA
3	ADONITOL	ADO
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA
5	L-ARABITOL	lARL
7	D-CELLOBIOSE	dCEL
9	BETA-GALACTOSIDASE	BGAL
10	H2S PRODUCTION	H2S
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG
12	Glutamyl Arylamidase pNA	AGLTp
13	D-GLUCOSE	dGLU
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT
15	FERMENTATION/ GLUCOSE	OFF
17	BETA-GLUCOSIDASE	BGLU
18	D-MALTOSE	dMAL
19	D-MANNITOL	dMAN
20	D-MANNOSE	dMNE
21	BETA-XYLOSIDASE	BXYL
22	BETA-Alanine arylamidase pNA	BAlap
23	L-Proline ARYLAMIDASE	ProA
26	LIPASE	LIP
27	PALATINOSE	PLE
29	Tyrosine ARYLAMIDASE	TyrA
31	UREASE	URE
32	D-SORBITOL	dSOR
33	SACCHAROSE/SUCROSE	SAC
34	D-TAGATOSE	dTAG
35	D-TREHALOSE	dTRE
36	CITRATE (SODIUM)	CIT
37	MALONATE	MNT
39	5-KETO-D-GLUCONATE	5KG

16S rRNA PCR

The PCR revealed the amplification of the *16S rRNA* genetic piece that belonged to the *E. coli* isolates (Fig.1).

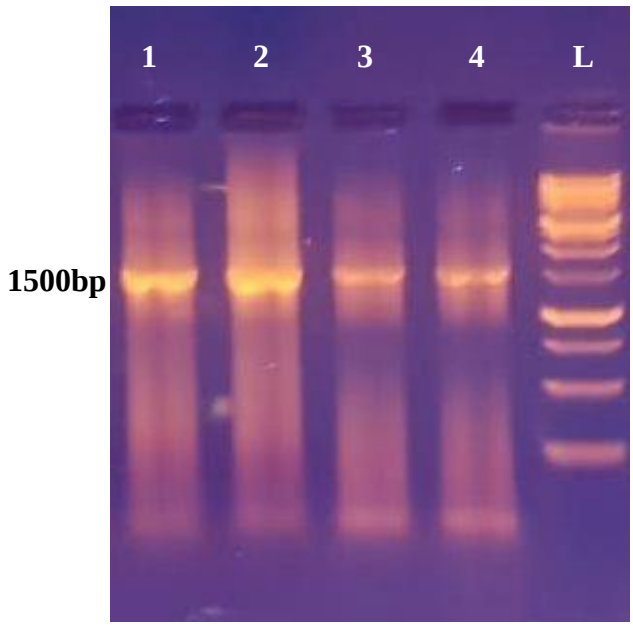

Fig.1. Image of PCR agarose gel of the *16S rRNA* gene that belongs to *E. coli*. Lanes (1-4): Positive amplifications (1500bp). L: The ladder (10K-0.25Kbp).

Phylogenetic analysis

The phylogenetic analyses uncovered that the PGS-strains (under accession Nos. MT784126, MT784125, MT784127, and MT784124) were aligned with global isolates from the United States (NR 114042), China (MT649811), Algeria (MF355370), and Brazil (MK517659) that all clustered in one major group (Fig.2).

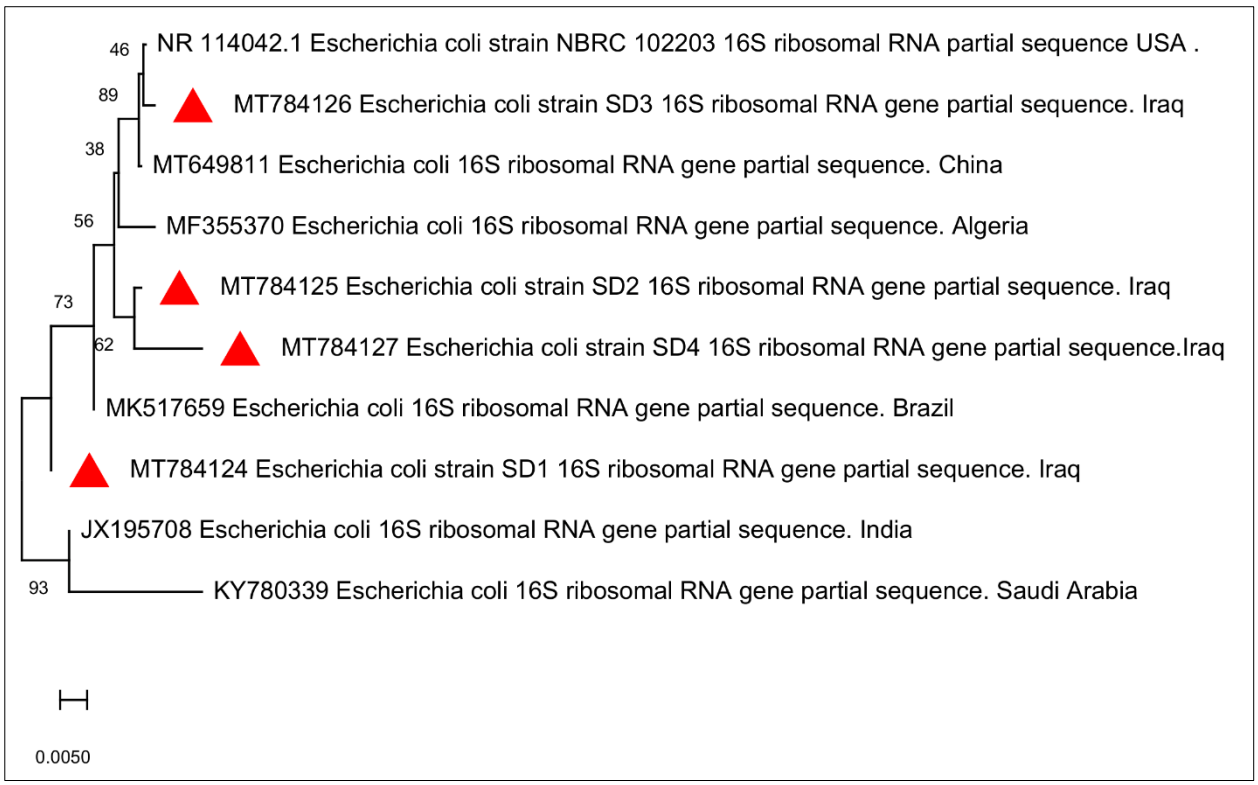

Fig.2. Pylogenetic tree based on the partial *16S rRNA* gene sequencing that belongs to *E. coli*. The phylogenetic analyses uncovered that the PGS-strains (under accession Nos. MT784126, MT784125, MT784127, and MT784124) were aligned with global isolates from the United States (NR 114042), China (MT649811), Algeria (MF355370), and Brazil (MK517659) that all clustered in one major group.

PCR of ChuA, *YjaA*, and *TspE4C2 genetic markers*

The PCR of the virulence markers showed the existence of those pathogenic genetic codes in all isolates (Fig.3).

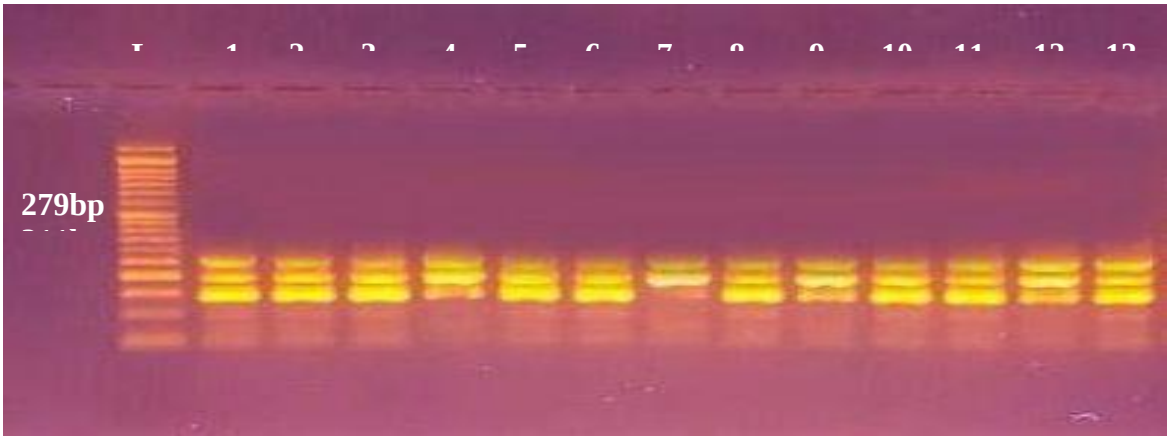

Fig.3. Image of PCR agarose gel of the *ChuA*, *YjaA*, and *TspE4C2 genetic markers* that belong to *E. coli*. Lanes (1-13): Positive amplifications (279, 211, 152bp, respectively). L: The ladder (1.5K-0.05Kbp).

DISCUSSION

The isolates of the ExPEC bacteria have an enormous diversity of genomes with a broad spectrum of virulence-related factors that are encoded by specific mobile or immobile pathogenic islands. Factors such as adhesions, toxins, polysaccharide capsules, lipopolysaccharides, invasins, and proteases are among some virulence factors. Such suppositional virulence factors tend to enhance the bacterium health, resilience, and potential to colonize the human body (e.g., the mechanisms of iron-uptake) rather than to specifically impact the infection like common virulence factors (Mokady et al. 2005; Pitout 2012).

In the present study, the isolation of *E. coli* from the human oral cavities may indicate that these bacteria can cause disease conditions with effects on different oral cavity components such as tooth canal and jawbones. A case report can confirm this hypothesis in which an old adult male aged 61 years old showed non healing tooth extraction site with severe pain at the right maxillary (first molar tooth area) of a three-month duration. Macroscopic and microscopic examinations revealed non-specific infection with a massive infiltration by inflammatory cells. Later, the second molar tooth area was affected. Radiographs displayed diffuse damages to the right maxillary with a loss of the interradicular bone. A swab from the affected bones revealed an antibiotic-resistant *E.coli* isolate (Padhiary et al. 2020).

The current study revealed the presence of pathogenic isolates of *E. coli*. This issue was confirmed by the existence of the virulence *ChuA*, *YjaA*, and *TspE4C2* genetic markers. Mana and Rebecca (Mana et al. 2020) have detected pathogenic strains of *E. coli* in the microbiota of the oral cavity of patients at an age range between 40 to 70 years old. This evidence agrees with the current study findings that indicate the occurrence of pathogenic *E. coli* in the oral cavity of the tested patients. The present virulence factors are characteristics of *E. coli* isolates recovered out of the intestinal compartments. Spurbeck *et al*. (2020) also recognized these features of the pathogenic *E. coli*.

The close nucleotide similarity between the study isolates and some global strains of *E. coli* may indicate an evolutionary history of the local strains. One of the main factors that can help in the distribution of bacterial species is travel. Travel can help to transfer some pathogenic strains of *E. coli* from a country to another, which might explain why such similarty between those isolates is seen (Allocati et al. 2013; Alexander et al.2012).

CONCLUSION

The obtained *E. coli* are of pathogenic characteristics due to the detection of the *ChuA*, *YjaA*, and *TspE4C2* virulent genetic markers. These virulence features of the bacterium may indicate a high possibility of causing an infection to the oral cavity.

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