Molecular characterization and virulence gene markers of *Escherichia coli* strains isolated from different patients in Saudi Arabia

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ABSTRACT

The main objectives of this study were molecular characterization of isolated strains of E. coli collected from diseased patients from Al-Taif, Saudi Arabia, and screening the existence of some virulence genes in these isolated strains. Molecular characterization was carried out through two approaches (16S rRNA and rep-PCR). The existence of Stx1, Stx2, eaeA, hly, KpsII, fimH, UidA and YaiO genes were examined using polymerase chain reaction. Most of the examined isolates were sensitive to amoxicillin/clavulanic acid, cefoxitin, gentamicin, and nitrofurantoin, whereas high resistance to ampicillin, ceftazidime, and cefepime was observed with isolates TU-1 to TU-18. All isolates were sensitive to meropenem and amikacin. BLAST results of the sequenced 16S rRNA gene revealed that 19 isolates belong to E. coli and four isolates belong to other species, clustered into three different clusters. The previous results were attained using fingerprinting based on 16 rep-PCR primers. The rep-PCR primers yielded 302 distinct bands, of which 222 (73.5%) were considered polymorphic and 80 (26.5%) were considered monomorphic. The virulence genes KpsII and YaiO were detected in all E. coli isolates, neither stx2 nor eaeA were detected in all the examined isolates. Stx1, fimH, hly, and UidA were detected in 16.7%, 33.3%, 37.5%, and 66.7% of isolates. In conclusion, the findings of the present work suggested that 16S rRNA is more efficient approach in molecular characterization of bacterial isolates while, PCR is more suitable and rapid method for detection of virulence genes in most bacteria.

Keywords: *Molecular characterization, virulence genes, 16S rRNA, rep-PCR, E. coli.*

INTRODUCTION

Escherichia coli is harmless microorganism which normally lives in humans and animals. However, some *E. coli* isolates are pathogenic, causing human diseases, such as diarrhea, abdominal pain, and fever. Extra intestinal pathogenic *E. coli* strains account for more than 80% of urinary tract infection (UTI) and constitute the second cause of neonatal meningitis and septicemia (Kaper *et al*., 2004). *E. coli* possesses the capacity to acquire and preserve transferable resistance genes found in other organisms and the environment (Da-Costa *et al*., 2013).

Different approaches are available for subtyping of *E. coli* strains, e.g. serological, biochemical and molecular approaches. Biochemical typing methods include fermentation of the isolates on different sugar sources; whereas serological typing is usually conducted by specific kits such as API 20E systems which are suitable methods (Aklilu *et al*., 2013). Genetic fingerprinting based on PCR techniques and the 16S rRNA genes sequencing are used for molecular typing of the *E. coli* (Aklilu *et al*., 2013and Hassan and Belal *et al*., 2016). The 16S rRNA gene sequencing is the standard method for taxonomic classification of different species (Alharthi *et al*., 2016). Therefore, repeated sequence-based PCR (rep-PCR) is a new technique that involves the use of repetitive genomic sequences to characterize the evolutionary relation in bacterial strains within a species (Ateba and Mbewe, 2013; Black *et al*., 2014 and Hassan and Belal, 2016). The rep-PCR technique has been used successfully for the classification of *E. coli* (Gaber *et al*., 2015).

E. coli strains are divided into four groups; A, B1, B2, and D. Most strains belonging to the A and B1 phylogenetic groups have low pathogenic potential (Johnson *et al*., 2005). Whereas, extra intestinal pathogenic strains mainly belong to B2 and D, and intestinal pathogenic strains belong to groups A, B1, and D. Recently, based on *virulence* gene profiles, *E. coli* was classified into four clusters (I-IV) (Johnson and Stell, 2000 and Zhu *et al*., 2017). Shiga toxin-producing *Escherichia coli* (STEC) as virulence factor is a major cause of food-borne infections and is thus a major public health concern (Chapman *et al*., 2006). These strains were produce two types of Shiga toxins (*Stx*), Stx1 and Stx2, which can cause severe diarrhea and hemolytic uremic syndrome (Li *et al*., 2017). Another main virulence factor is STEC (*hlyA*) that is often associated with severe clinical disease in humans, as well as intimin, the product of the *eaeA* gene involved in bacterial attachment and effacing adherence (Chinen *et al*., 2002 and Zhu *et al*., 2017). The virulence factors also include certain capsular antigens (*kpsII* and *K1*), which has been recognized as uropathogenic genes and *FimH*, a major determinant that facilitates colonization and survival in host cells, and has high tropism for urinary tract receptors (Leonard *et al*., 2016). Multiplex PCR has been used elsewhere to detect virulence genes in *E. coli*, particularly *uidA, stx1*, *stx2, eaeA, fimH*, and *hlyA* (Bai *et al*., 2004; Huasai *et al*., 2016 and Li *et al*., 2017). The main objective of this study included molecular characterization of *E. coli* using rep-PCR and 16S rRNA gene sequencing, in addition to screening for the presence of virulence genes in *E. coli* isolates.

MATERIALS AND METHODS

Sample collection and growth

Samples were collected from inpatients at the King Abdul Aziz Specialist Hospital (KAASH) in the Taif area, Saudi Arabia, between August 1, 2015, and June 30, 2016. Clinical samples were derived from various sources; five samples were collected from blood, one from a wound swab, and the rest from urine. In total, 104 bacterial isolates were serialed by a coding system linked to patient identities from the clinical laboratory, 19 of these were identified as *E. coli* isolates.

Antibiotic susceptibility tests

Sixteen antibiotics (Table 1) were used for a disc diffusion bioassay according to Clinical and Laboratory Standards Institute (CLSI) methods (Tajbakhsh *et al*., 2016). A reference strain, *E. coli* (American Type Culture Collection 25922) was used as a control isolate.

Table (1): Antibiotic sensitivity of different bacterial strains isolated in the current study.

Sample Number	Antibiotic sensitivity													
	Amp	**Am/Cla**	**pip/taz**	**Cefo**	**Cefta**	**Cefe**	**Imi**	**Mer**	**Amk**	**Gen**	**Cip**	**Tig**	**Nit**	**Tri/Sulf**
E1	R	R	I	R	R	R	S	S	S	S	R	S	S	R
E2	R	R	I	R	R	R	S	S	S	S	R	S	I	R
E3	R	I	R	S	R	R	S	S	S	S	R	S	S	S
E4	R	S	S	S	R	R	S	S	S	S	R	S	S	R
E5	R	S	R	S	R	R	I	S	S	R	R	S	S	S
E6	R	S	S	S	R	R	S	S	S	S	S	S	S	S
E7	R	I	S	S	R	R	S	S	S	S	R	S	R	R
E8	R	S	S	I	R	R	S	S	S	R	S	S	S	R
E9	R	S	S	R	R	R	S	S	S	S	R	S	S	S
E10	R	S	S	I	R	R	S	S	S	R	S	S	S	R
E11	R	I	R	S	R	R	S	S	S	S	R	S	R	S
E12	R	S	S	S	R	R	S	S	S	S	S	S	S	R
E13	R	S	S	S	R	R	S	S	S	S	S	S	S	R
E14	R	S	S	S	R	R	S	S	S	S	R	S	S	R
E15	R	S	S	R	R	R	S	S	S	S	S	S	S	S
E16	R	I	I	R	R	R	S	S	S	R	S	S	S	S
E17	R	S	S	R	R	R	S	S	S	S	R	S	S	S
E18	R	R	R	R	R	R	S	S	S	R	R	S	S	R
E19	S	S	S	S	S	S	S	S	S	S	S	S	S	S
E20	R	S	S	S	S	S	S	S	S	S	S	S	S	S
E21	R	S	S	S	S	S	S	S	S	S	S	S	S	R
E22	R	S	S	S	S	S	S	S	S	S	S	S	S	R
E23	S	S	S	S	S	S	S	S	S	S	S	S	S	S
E24	R	S	S	S	S	S	S	S	S	S	S	S	S	S

Amp (ampicillin), Am/Clav (amox/clavulinc), Pip/Taz (piperacillin/tazobactam), Cefa (cefalotin), Cefo (cefoxitin), Cefta (ceftazidime), Ceftr (ceftriaxone), Cefe (cefepime), Imi (imipenem), Mer (meropenem), Amk (amikacin), Gen (gentamicin), Cip (ciprofloxacin), Tig (tigecycline), Nit (nitrofurantoin), and Tri/Sulf (trimethoprim/sulfamthoxazole). [3] S (sensitive), R (resistant), I (intermediate).

Molecular characterization of *E. coli* isolates

DNA extraction

DNA was extracted from *E. coli* isolates using a DNeasy Bacterial Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions.

Sequencing of *E. coli* isolates 16S rRNA gene

Two PCR primers were designed (Macrogen, Inc., Seoul, South Korea) to amplify approximately 1465 bp of a consensus 16S rRNA gene as described previously (Hassan and Belal, 2016), forward primer 27f (5′- AGAGTTTGATCMTGGCTCA-3′) and reverse primer 1492r (5′-TACGGYTACCTTGTTACGACTT-3′). PCR amplicons of the 16S rRNA gene were purified from gel bands using a QIAquick PCR Purification Kit (QIAGEN) and sequenced commercially. Raw sequences were edited and assembled using MEGA 5.2. (Tamura *et al*., 2013). The sequences were deposited in GenBank under accession numbers MF179667-MF179690. The nucleotide sequences of the 16S rRNA genes obtained in present study and from GenBank were aligned, sequence identities were calculated, and a phylogenetic tree was generated as previously described (Tamura *et al*., 2013).

Table (2): Primer sequences and amplicon sizes of virulence genes.

Primer Name	Primer Sequence (5′→3′)	Product Size (bp)
Stx1-f	AAA TCG CCA TTC GTT GAC TAC TTC T	370
Stx1-r	TGC CAT TCT GGC AAC TCG CGA TGC A	
Stx2-f	CAG TCG TCA CTC ACT GGT TTC ATC A	283
Stx2-r	GGA TAT TCT CCC CAC TCT GAC ACC	
eaeA-1	CCC GAA TTC GGC ACA AGC ATA AGC	881
eaeA-2	CCC GGA TCC GTC TCG CCA GTA TTC G	
hlyA-1	GGTGCAGCAGAA AAA GTT GTA G	1551
hlyA-2	TCT CGC CTG ATA GTG TTT GGT A	
KpsII-f	GCGCAT TTGCTGATA CTGTTG	272
KpsII-r	CAT CCA GAC GAT AAG CAT GAG CA	
fimH1	GAGAAGAGGTTTGATTTAACTTATTG	559
fimH2	AGAGCCGCTGTAGAACTGAGG	
UidA1	TGGTAATTACCGACGAAAACGGC	162
UidA2	ACGCGTGGTTACAGTCTTGCG	
YaiO1	TGATTTCCGTGCGTCTGAATG	115
YaiO2	ATGCTGCCGTAGCGTGTTTC	

Repetitive sequence analysis

For repetitive sequence analysis, rep-PCR conditions for *E. coli* isolates were standarzed. Thirty repetitive sequence primers were tested. Among them, 16 primers that presented the strongest band resolution were chosen for this study: Rep-01, Rep-02, Rep-03, Rep-05, Rep-06, Rep-07, Rep-08, Rep-09, Rep-10, Rep-12, Rep-13, Rep-18, Rep-26, Rep-27, Rep-28, and Rep-29. DNA amplicons were separated by electrophoresis in a 1.5% agarose gel as previously described (Alharthi *et al*., 2016).

Virulence gene PCR amplification

E.coli isolates were examined by means of duplex PCR using specific primers to determine the presence of *stx1* and *sxt2* genes. Samples were also tested for the presence of *uidA*, *yaiO, eaeA, hlyA, fimH*, and *kpsII* genes according to Molina *et al*. (2015). Primer sequences are indicated in Table 2.

Data analysis

The similarity matrix was subjected to cluster analysis using the program NTSYS-PC version 2.01 (Rohlf, 2000).

Table (3): Polymorphic band patterns and percentages of polymorphism for each rep-PCR primer among pathogenic E. coli isolates.

Primer	Total Bands	No. Monomorphic Bands	No. Polymorphic Bands	% Monomorphic Bands	% Polymorphic Bands
Rep-01	19	4	15	26.3	73.7
Rep-02	22	7	15	31.8	68.2
Rep-03	20	8	12	40.0	60.0
Rep-05	21	5	16	23.8	76.2
Rep-06	20	5	15	25.0	75.0
Rep-07	16	3	13	18.8	81.2
Rep-08	22	6	16	27.2	72.8
Rep-09	16	4	12	25.0	75.0
Rep-10	21	8	11	38.1	61.9
Rep-12	20	5	15	25.0	75.0
Rep-13	21	5	16	23.8	76.2
Rep-18	18	5	12	27.8	72.2
Rep-26	15	4	11	26.7	73.3
Rep-27	15	3	12	20.0	80.0
Rep-28	18	4	12	22.2	77.8
Rep-29	18	4	12	22.2	77.8
Total	302	80	222	26.5	73.5

RESULTS AND DISCUSSION

Isolation of *E. coli* isolates and antibiotic susceptibility examination

The 19 isolates identified as *E. coli* and four isolates as different species were surveyed for the presence of multidrug resistance via the combination disk diffusion examination (Tajbakhsh *et al*., 2016). All isolates were resistant to one or more antimicrobial agents except for isolates TU-19 and TU-23, which were sensitive to all tested antibiotics. The resistance of each of the 24 isolates to the antimicrobial agents tested in this study is summarized in Table (1). Overall, 22 (91.6%) of the isolates were resistant to ampicillin, followed by 18 (75%) bacterial strains resistant to ceftazidime and cefepime. In contrast, all *E. coli* isolates appeared completely sensitive to imipenem, meropenem, amikacin, and tigecycline. The proportions of isolates resistant to trimethoprim / sulfamethoxazole, ciprofloxacin, cefoxitin and piperacillin /tazobactam were 50% (12/24), 45.8% (11/24), 29.2% (7/24), and 16.7% (4/24), respectively.

Antimicrobial resistance of *E. coli* is the extraordinary concern around the world, due to the increasing resistance to several commonly prescribed antibiotics (Moyo *et al*., 2010). In our study, *E. coli* isolates fluctuated in their susceptibility to different antibiotics belonging to different groups. These results are comparable to those of other local and global studies (Sabir *et al*., 2014). The high levels of resistance observed for certain antibiotics might be due to the spontaneous and uncontrollable use of these antibiotics (Sana *et al*., 2011 and Tajbakhsh *et al*. 2016). In contrast, the carbapenems (imipenem and meropenem) are known to be stable against ESBL enzymes and effective in the treatment of infections caused by ESBL-producing bacteria, and the administration routes of these antibiotics (either intravenous or intramuscular) limit their use by most patients (Moyo *et al*., 2010).

16S rRNA of *E. coli* isolates

The 16S rRNA gene from the 24 clinical bacterial isolates was successfully amplified and sequenced. Individual BLAST searches of the 16S rRNA sequences confirmed 19/24 isolates as *E. coli*, whereas 3/24 isolates were found to be *K. pneumoniae* (TU-20, TU-22, and TU-24), and 2/24 isolates were found to be *Enterobacter* (TU-19 and TU-21). All sequences in the current study were deposited in the GenBank under accession numbers MF179667-MF179690. *E. coli* sequences were aligned and compared with the 16S rRNA sequences of selected published strains of the genus *Escherichia* available in the GenBank database, including representative strains of the A, B1, B2, C, D, and E subtypes of *E. coli*. The phylogenic tree of different 16S rRNA sequences was not able to differentiate between different *E. coli* subtypes (Fig. 1). The phylogenetic relationships of the *E. coli* strains revealed four main clusters [Ia, Ib, II, III, and IV]. TU-1, TU-3, TU-4, TU-7, TU-14, TU-15, TU-16, TU-17, and TU-23 were grouped Ia cluster, whereas TU-5, TU-6, TU-8, TU-9, TU-10, TU-12, and TU-18 grouped to Ib, and TU-2, TU-11, and TU-13 grouped to cluster III (Fig. 1). These results are in agreement with those of Clarridge (2004) who reported that the traditional identification of bacteria using phenotypic characteristics is generally not as accurate as identification by genotypic methods.

Table (4): Virulence gene patterns among pathogenic E. coli isolates.

Isolates	Present and absent of virulence genes							
	Stx1	*Stx2*	*eaeA*	*hly*	*KpsII*	*fimH*	*UidA*	*YaiO*
TU-1	–	–	–	–	+	+	–	+
TU-2	–	–	–	–	+	+	+	+
TU-3	–	–	–	+	+	+	–	+
TU-4	–	–	–	+	+	+	+	+
TU-5	+	–	–	–	+	+	+	+
TU-6	–	–	–	+	+	+	+	+
TU-7	–	–	–	–	+	+	–	+
TU-8	+	–	–	–	+	+	–	+
TU-9	–	–	–	–	+	+	+	+
TU-10	–	–	–	+	+	+	+	+
TU-11	–	–	–	+	+	+	+	+
TU-12	–	–	–	+	+	+	–	+
TU-13	+	–	–	–	+	+	–	+
TU-14	–	–	–	–	+	+	–	+
TU-15	–	–	–	–	+	+	–	+
TU-16	–	–	–	+	+	+	+	+
TU-17	–	–	–	+	+	+	+	+
TU-18	–	–	–	+	+	+	+	+
TU-19	–	–	–	–	+	+	+	+
TU-20	–	–	–	–	+	+	+	+
TU-21	–	–	–	–	+	+	+	+
TU-22	–	–	–	–	+	+	+	+
TU-23	–	–	–	–	+	+	+	+
TU-24	–	–	–	–	+	+	+	+

Fig. (1): Phylogeny tree based on 16S rRNA gene sequences of bacterial isolates with 1000 bootstraps. Bacterial isolates in the current study are presented in blue color.

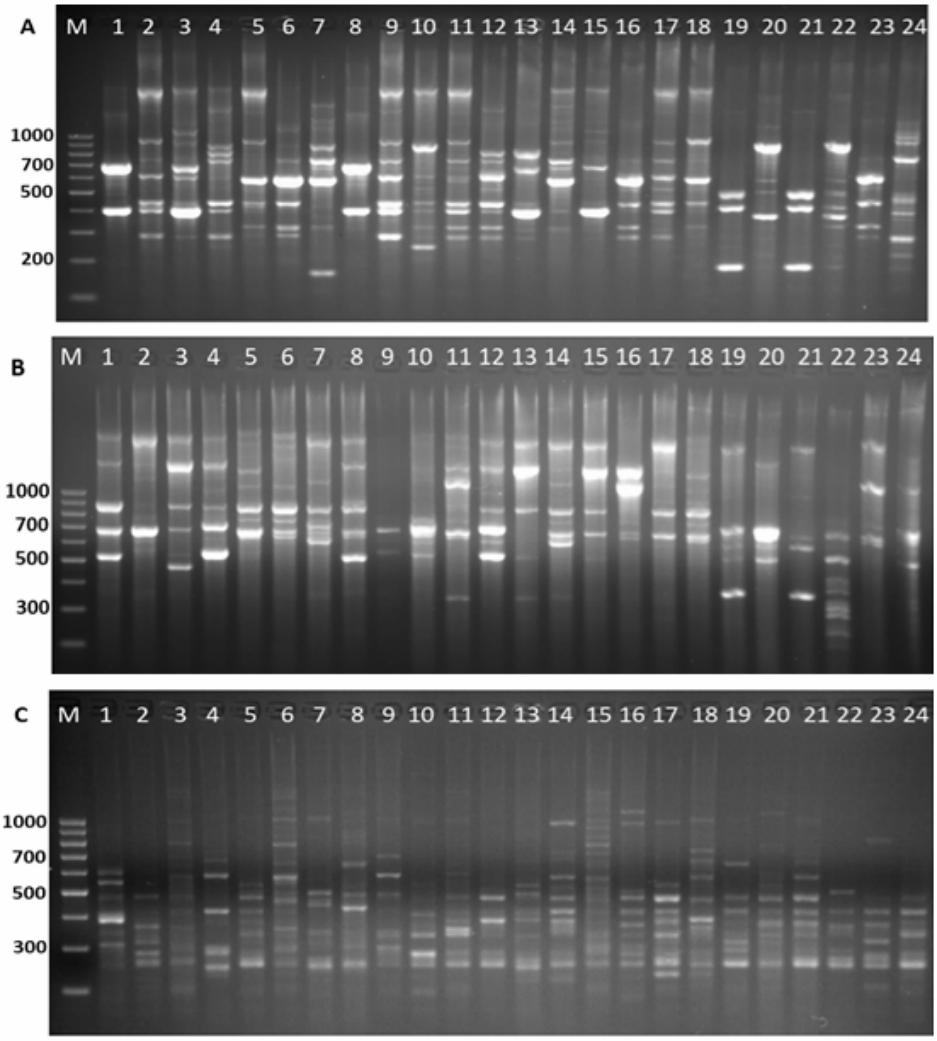

Fig. (2): Rep-PCR profiles of 24 antibiotic-resistant bacterial isolates generated with three primers. A= Rep-2, B= Rep-5, and C= Rep-8 primers. M: 100-bp DNA ladder.

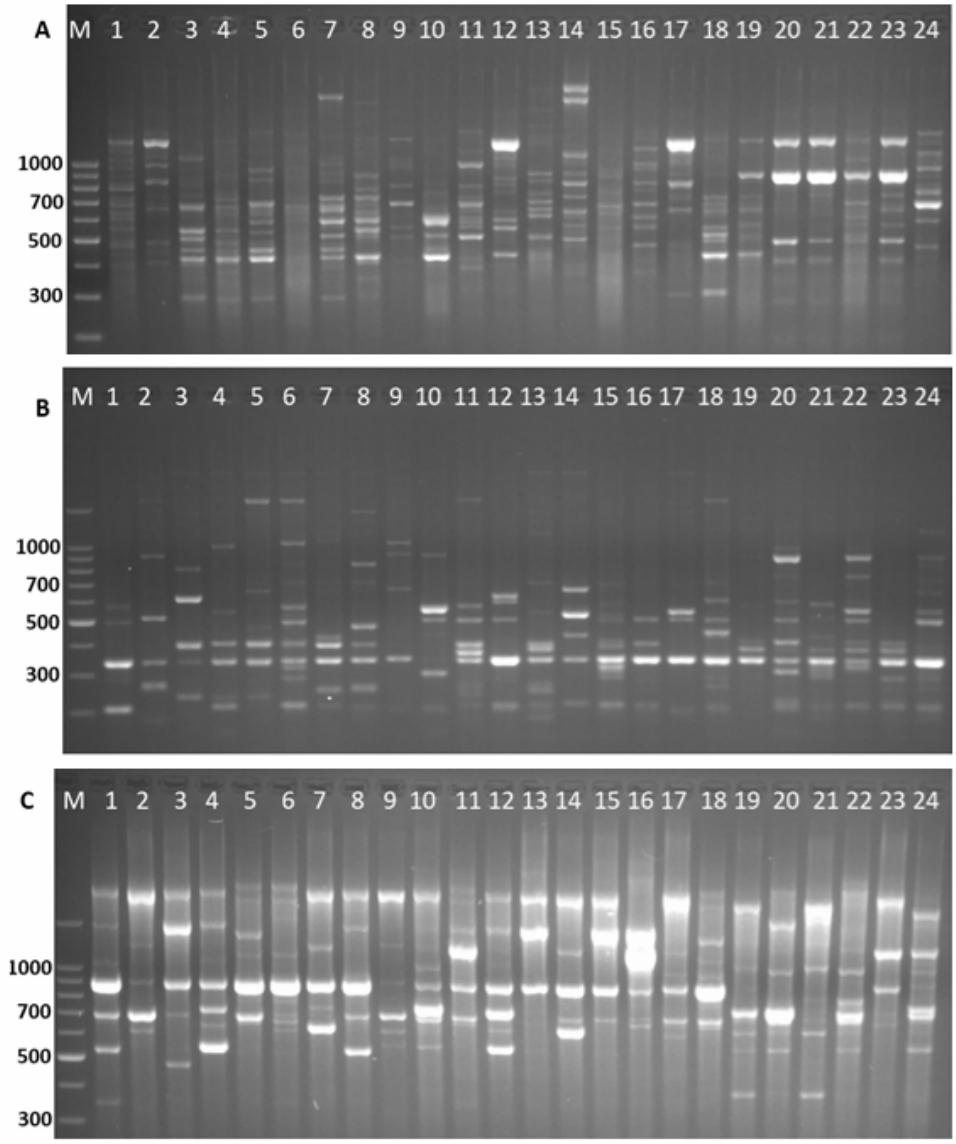

Fig. (3): Rep-PCR profiles of 24 antibiotic-resistant bacterial isolates generated with three primers. A= Rep-10, B= Rep-12, and C= Rep-28 primers. M: 100-bp DNA ladder.

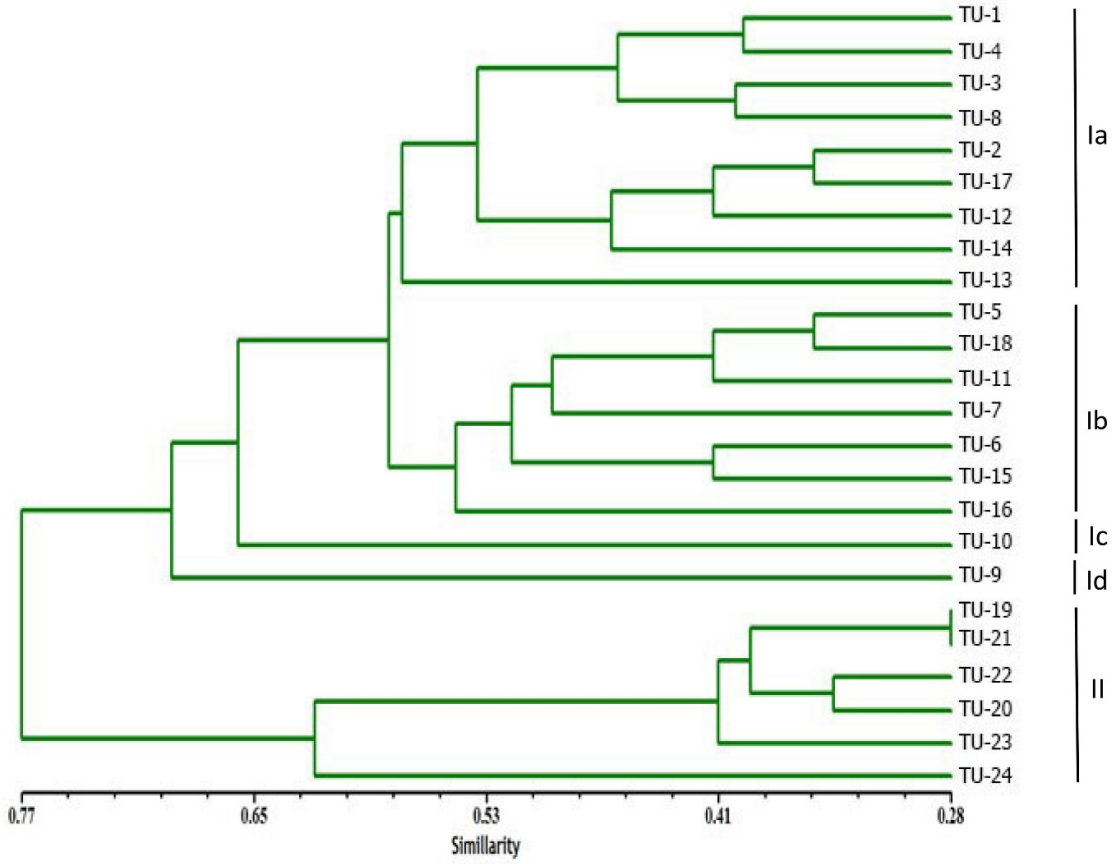

Fig. (4): Dendrogram based on 16 rep-PCR primers of 24 bacterial isolates collected from in-patients at hospitals in Taif, Saudi Arabia. The bacterial isolates were clustered into two main groups.

Repetitive sequence analysis

For DNA fingerprinting, rep-PCR analyses were performed on *E. coli* isolates in this study. Complex fingerprint patterns were obtained for all isolates studied. Although the fingerprint patterns for *E. coli* isolates obtained from the same hospital were similar, they were not always identical. Approximately 156 bands were common to 19 *E. coli* isolates, and 60 bands were shared by more than 85% of the isolates. Individual lanes generally contained 16-22 PCR product bands, although almost 25 bands were obtained for some *E. coli* isolates. The sizes of the PCR products ranged from slightly less than 150 bp to approximately 3,600 bp (Figs. 2 and 3). The initial study was performed with all of the rep-PCR primers. However, 13.6% fewer PCR products were usually present for the fingerprints generated with the rep-1 primers than for the fingerprints obtained with the rep-2 primers (Table 3).

In the rep-PCR, approximately 79% of the isolates (19 isolates) produced high-quality DNA fingerprints with most rep-PCR primers. Some of the isolate templates that were successfully amplified with most rep-PCR primers did not produce a reliable fingerprint

with the rep-26 primer. The rep-PCR primers yielded 302 distinct bands, 222 (73.5%) of which were polymorphic and 80 (26.5%) of which were monomorphic (Table 3). The number of amplified fragments scored for each isolate was recorded. The amplified products among the bacterial isolates (n: 24) in the current study were polymorphic.The rep-PCR results using primer rep-08 showed a total of 22 bands in the 24 bacterial isolates, ranging from 110 to 2,900 bp. Six bands were observed in all isolates, among which 27.2% were monomorphic, whereas the other 16 fragments were 72.8% polymorphic (Table 3). In the case of the rep-27 primer, 15 fragments were 80% polymorphic among the 24 isolates. Despite this variation, the obtained fragments from all analyses were sufficient for the identification and evaluation of genetic similarities and creation of a dendrogram (Figure 4). The first group included most of the bacterial strains and is further sub clustered into four sub clades [Ia (TU-1,2, 3,4,8,12,13,14,17), Ib (TU-5,6,7,11,15,16,18), Ic (TU-10), Id (TU-9)], whereas the second group includes TU-19-24 (Figure 4).

There were completely different clusters of isolated bacterial strains based on the repetitive sequence fingerprinting method using rep-PCR (Fig. 4), in comparison to 16S rRNA sequences (Fig. 1). Although rep-PCR has been reported to be a powerful method for genetic characterization and is useful as a screening or genotyping method (Gaber *et al*., 2015 and Alharthi *et al*., 2016), there is growing evidence regarding accuracy and reproducibility problems (Ishii and Sadowsky, 2009 and Korvin *et al*., 2014). The current results indicate that Rep-PCR fingerprint patterns are misleading, and they are not recommended for molecular typing of bacterial strains.

Virulence gene detection

The characterization of virulence genes can improve the present understanding of the pathogenesis of diseases and minimize resulting complications. All of the bacterial strains in the current study contained at least one virulence gene, among which 4 (16.7%), 8 (33.3%), 9 (37.5%), and 16 (66.7%) were found to harbor *Stx1*, *FimH, hly,* and *uidA* virulence genes, respectively (Table 4). Moreover, 100% of the tested isolates carried the *KpsII* and *YaiO* virulence genes. Conversely, no isolates carried the virulence genes *Stx2* or *eaeA*. The associations among different virulence factors in *E. coli* isolates were documented by Tiba *et al*., (2008). These genes were detected in a high proportion of bacterial strains isolated from mono-microbial cultures. Strains contain the genes *hly* and *FimH* exhibit an interesting relationship with uropathogenic *E. coli* (Qin *et al*., 2013). Intestinal or extra intestinal strains of clinical and commensal *E. coli* isolates from different patients area, with or without clinical disease, have been found to be extremely diverse in their genetic makeup. Over time, this genetic diversity has been exploited by selection and adaptation, such that pathogenic strains have tended to become host-specific or hospital-specific (Firoozeh *et al*., 2014).

CONCLUSIONS

In conclusion, 16S ribosomal RNA was more accurate than rep-PCR for molecular characterization of *E. coli*. All of the isolates in this study carried one or more virulence genes, and the PCR technique is the best method for determining the presence of virulence genes. In addition, increased selective pressure via repeated exposure to antimicrobial agents is a likely factor in the increased frequency of antibiotic resistance observed among pathogenic bacteria.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Ethical approval

The current study has been approved by the Taif University Ethical Committee and by the King Abdul Aziz Hospital, Al-Taif, Saudi Arabia.

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الملخص العربي

التوصيف الجزيئي و معلمات جينات الاصابة في بكتريا القولون المعزولة من المرضي في المملكة العربية السعودية

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الهدف الاساسي لهذه الدراسة هو التوصيف الجزيئي لسلالات بكتريا القولون المعزولة من المرضى في محافظة الطائف، بالمملكة العربية السعودية وكذلك التأكد من وجود بعض جينات الاصابة في هذه السلالات البكتيرية. تم استخدام تقنيات 16S rRNA و rep-PCR وذلك لغرض توصيف العزلات جزيئيا. في بداية البحث تم مسح العزلات محل الدراسة (24 سلالة) باستخدام تفاعل البلمرة المتسلسل وذلك للبحث عن الجينات المسئولة عن الاصابة والتي تم توثيقها من قبل في العديد من الدراسات (*Stx1*, *Stx2*, *eaeA*, *hly*, *KpsII*, *fimH*, *UidA*, *YaiO*). أظهرت العزلات المختبرة احتوائها علي جيني *KpsII* ،*YaiO* في حين لم تحتوي أي منهم علي جيني *stx2* ،*eaeA* كما أظهرت بعض السلالات احتوائها علي بعض الجينات مثل *Stx1, fimH, hly, UidA* بنسب ١٦.٧، ٣٣.٣، ٣٧.٥، ٦٦.٧ ٪ علي التوالي. كذلك أظهرت نتائج الحساسية للمضادات الحيوية العديد من هذه العزلات حساسيتها تجاه الأموكسيسيلين / حمض كلافولانيك، سيفوكسيتين، جنتاميسين، و نيتروفورانتوين، في حين لوحظت مقاومتها العالية للأمبيسيلين، سيفتازيديمي، وسيفيبيمي، في حين ان جميع العزلات كانت حساسة لميروبينيم و أميكاسين. نتائج توالي جين 16S rRNA اظهر عن وجود ١٩ سلالة من اصل ٢٤ سلالة تنتمي لجنس الايكولاي. و نتج عن توصيف العزلات باستخدام الـ rep-PCR عدد ٣٠٢ باند وصفت منها (٢٢٢) بنسبة ٧٣.٥٪ كعديدة الاشكال المظهرية و(٨٠) منها بنسبة ٢٦.٦ ٪ كوحيدة الاشكال المظهرية. وعليه يمكن تلخيص النتائج إلى أهمية استخدام تحديد توالي جين 16S rRNA والذي يعتبر الطريقة ألاكثر كفاءة في التوصيف الجزيئي للعزلات البكتيرية. هذا الى جانب استخدام طريقة تفاعل البلمرة المتسلسل كطريقة ملائمة وسريعة للكشف عن جينات الاصابة في معظم الأنواع البكتيرية.