Fingerprinting and genetic relationship among *Escherichia coli* strains producing B. Lactamase collected from urine using ERIC/PCR Assay

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**ABSTRACT**

This study was conducted on 50 isolates recovered from urine samples of patients with urinary infections admitted to Azadi teaching Hospital/Dohuk City. These isolates were identified and characterized in previous study as *E. coli* producing extended spectrum beta lactamase enzymes, all isolates were subjected to Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC PCR) assay for estimation the fingerprinting and genetic relationship among these isolates. They were successfully producing multiple amplicons ranging from 2-12 bands with different molecular weights ranging from 5212- 213bp. The most frequent band was 724 which was amplified by 31 strains, and the least frequent were 3224, 2424, 1472 and 531bp. observed in only one strain. From the result of dendogram analysis revealed that all isolates were assigned into two main clusters, each cluster was further classified into two subgroups. Similar genotype may indicate clonal spreading of bacteria in hospital wards.

**Keywords:** *Escherichia coli*  
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**1. INTRODUCTION**

*Escherichia coli* is one of a gram negative bacillus belonged to the *Enterobacteriaceae* family, commonly colonize and persist in numerous niches both in the environment, intestines of people and animals (Wiles *et al.*, 2006; Ardakani and Ranjbar, 2016). Strains of uropathogenic *E. coli* (UPEC) are the primary cause of urinary tract infections (Wiles *et al.*, 2006). The Molecular epidemiological study is important for Knowledge of molecular characterization and population genetics of microorganisms (Spratt and Maiden, 1999). Numerous families of short interspersed repetitive sequences with (30–150 bp) length have been described in bacterial genomes, most of these families are restricted to single species or very closely related species, while many other species lack such elements (Tobes and Ramos, 2005). Enterobacterial Repetitive Intergenic Consensus (ERIC) sequence is the sequence length is about 124-127bp, which contain about 44bp highly conservative core sequences in its
center, primarily presents with multiple copies in genomes of *Enterobacteracae* and vibrios (Wilson and Sharp, 2006). These intergenic repetitive units have been widely used as molecular biological markers to assess the clonal variability of many bacterial isolates including *E. coli* (Chansiripornchai et al., 2001). For instance, depending on ERIC-PCR electrophoretic banding pattern Manges et al in 2001 could identified a clonal group named as Clonal Group A (CgA) and he found that more than 50% were Trimethprem-sulfamethoxazole(SXT) resistant *E. coli* were belonged to CgA collected from women with acute uncomplicated UTI (Manges et al., 2001). In our region, different molecular techniques based on PCR technique such as Random Amplified Polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP) have been applied to study the genetic diversity and fingerprinting of different bacterial species such as *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Fadel Al-Deen, 2007; Al-Mizory S., 2007; Merza and Jubrael, 2010). As our known, ERIC-PCR technique hasn't been applied in our region; therefore, the aim of this study is characterize the genetic relationship and fingerprinting of *B. lactamase* producing *E. coli* isolates obtained from urine using ERIC-PCR analysis.

### 2. MATERIALS AND METHODS

#### 2.1. Sample collection and ERIC-PCR typing

From the collected outpatient’s urine samples whom attended Azadi General Hospital in Duhok, 50 *E. coli* isolates were identified and characterized as ESBL producing *E. coli* by Merza et al. (2016). These Samples were subject to PCR amplifications in a final volume of 25μL containing: 2μl of genomic DNA, 25pmol of each primer, and 22μl of master mix (AccuPower PCR premix® (South Korea)) The primers used for ERIC-PCR typing were **ERIC-1** 5’ATGTAAGCTCCTGGGATTCAC3’ and **ERIC2** 5’AAGTAAGTGACTGGGTAGCG3’ (Ramazanzadeh et al., 2013). Each PCR conditions were performed as following: initial denaturation at 95°C for 2min. Then-35 cycles of a denaturation step at 92°C for 30s; annealing at 50°C for 1min; extension at 65°C for 8 min; Then, a final extension step at 65°C for 8min and final storage at 4°C.

#### 2.2. ERIC-PCR DNA Fingerprint Analysis.

ERIC-PCR fingerprints of amplified DNA fragments obtained by agarose gel electrophoresis were recorded. The positions of the bands on each lane and each gel were normalized using Lambda DNA/EcoRI plus HindIII Marker purchased by Thermo Fisher/USA). The molecular weight of amplicons have been determined by Vilber Lourmat-CAPT software. The zero-one manual method was used to count the bands, and, then, the data were entered on the following site: http://insilico.ehu.es/dice_upgma/, and the dendrograms were drawn.

### 3. RESULTS AND DISCUSSION

Fifty isolates of *E. coli* producing ESBLs strains enrolled in Azadi Teaching Hospital / Dohuk City previously identified and characterized by Merza et al., 2016 subjected to genomic DNA diversity analysis using ERIC-PCR fingerprinting method with ERIC-type primer in order to identify the genetic relationship among these isolates. They were produced 42 amplicons with different molecular weights ranging between 5212-213bp. The total amplicons produced by all isolates were 317 bands. These isolates were successfully producing multiple amplicons ranging from 2-12 bands with different molecular weights ranging from 5212-213bp (Figures 3.1; 3.2). It has been found that the most frequent band was 724bp. which was amplified by 21 strains, and the least frequent bands were 3224, 2424, 1472 and 531bp.
observed in only single strain. Through comparing patterns of the amplification products using ERIC primer for each strains, forty five ERIC-types of studies isolates have been identified. Since three isolates (ST1,ST2, and ST3) were shared the same amplification products (1081,622, 724 and 212bp.) and ST22 and ST2345 strains were have the same pattern (4697, 1296, 1166 and 724), and the other isolates have different profiles. Phylogenetic diversity between different E. coli producing ESBLs strains were determined by converting ERIC data into a Jaccard similarity matrix and analyzed by UPGMA to produce a phylogenetic tree. The amplified products were scored as the presence (1) or absence (0) for each individual, and a binary matrix was constructed (Al-Darahi et al., 2008). The results of dendogram analysis showed that ERIC–PCR profiles differentiated of the 50 isolates into two main clusters, Group A accounting 94%, while group B representing only 6%. Group A can be classified into two main subgroups. Nineteen (38%) isolates were belonged to subgroupA1, twenty eight isolates of the studied strains were in subgroup A2 (56%), within each subgroups they were further subdividing into other branches as illustrate in Diagram (3.1). This study may be the first attempt on application of ERIC-PCR assay in Kurdistan Region/Iraq for genotyping and determine the genetic relationship of E. coli isolates (as in our Knowledge). There are many studies have been conducted in neighboring countries as well as the worldwide and applied ERIC-PCR assay for determination of bacterial transmission trace in the hospitals and community (Prabhu et al., 2010; Ardkani and Ranjbar, 2016) and study the diversity of phylogenetic classification of different bacterial isolates such as; Pseudomonas aeruginosa, Haemophilus spp. (Macedo et al., 2011) Vibrio cholera (Shuan et al., 2011) Corynebacterium (Guimaraes et al., 2011), Salmonella spp. (Nath et al., 2010).

da Silveira et al., 2002 genotyped 49 avian E. coli isolates using ERIC-PCR analysis and suggested that this technique could be alternative than other molecular characterization techniques such as Restriction Fragment Length Polymorphism (RFLP) and RAPD-PCR. In other study conducted at the School of Medicine, Kurdistan University/ Sanandaj/ Iran by Ramazanzadeh et al. 2011 typing 230 isolates of E coli isolated from different sources into 205 ERIC-types and grouped into twenty main clusters.

Ardakani and Ranjbar genotyping of 98 isolates of uropathogenic E. coli and classified into 6 clusters. In Turkey, Durmaz, et al; 2015 demonstrated 5 to 6 clonal groups for 42 E. coli producing ESBLS enzymes applying ERIC-PCR analysis. In our study, fifty isolates of E coli producing ESBLs enzymes have been demonstrated into forty five ERIC-types since some of studied isolates shared the same pattern as mentioned in the results, they were classified into two major clusters, most of these isolates were assigned as GroupA representing 94%, only three isolates (6%) were belonged to Group-B. Indeed, within GroupA, the isolates were classified into two subgroups named G-A1 and G-A2 mostly occupied within G-A2. These results refining different ERIC-types of E. coli producing ESBLs in this study into only two cluster groups groupA and groupB may indicating the genetic relatedness among these isolates and dissemination of few clonal groups recovered from patients enrolled in Azadi Teaching Hospital in Duhok City may responsible for community and hospital acquired infections due to rapid dissemination of these groups.

In fact, the precise epidemiological investigations for monitoring and differentiation of strains responsible of outbreak infection need to be applied in long with suitable medication for preventing transmission in along with selection of efficient treatment options for preventing transmission. ERIC PCR analysis could be a good microbial
source tracking (MST) approach (Diab & Al-Turk, 2011). The findings of most studies explicated that the ERIC-PCR is simple, fast with powerful technique to study the genetic diversity and evolution racial structure (Durmaz, et al; 2015).

Figure 3.1. Shows the amplification products of ERIC-PCR method using ERIC primer of some isolates of *E coli* producing ESBLs enzymes, Electrophoresis was performed on (1.2%) agarose gel and run with 5V/Cm, for 2hours. Lane M digested lambda EcoRI-HindIII digested DNA.

Figure 3.2. Shows the amplification products of ERIC-PCR method using ERIC primer of some isolates of *E coli* producing ESBLs enzymes, Electrophoresis was performed on (1.2%) agarose gel and run with 5V/Cm, for 2hours. Lane M digested lambda EcoRI-HindIII digested DNA.
Diagram 3.1. Shows the dendrogram drawn for the *E coli* producing ESBLs strains studied in this research constructed with the use of UPGMA.

4. CONCLUSIONS

We come into a conclusion the ERIC assay was successfully revealed the genetic relatedness among *E. coli* strains producing ESBLs enzymes isolated from urine. The assigning of these isolates into few clusters may indicate the dissemination of distinct clonal groups in Azadi teaching hospital/Dohuk and it may responsible for community and hospital acquired infections.

Conflict of Interest.

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