Genetic heterogeneity of CTX-M type extended-spectrum β-lactamase producing *Escherichia coli* strains from diverse sources in Saudi Arabia

**Abstract**

**Background:** The rise of CTX-M extended spectrum β-lactamase (ESBLs)-producing *E. coli* in non-human sources is a growing concern of public health. Understanding the extent of public health risk attributed to CTX-M type ESBLs-producing strains from different sources is critical for effective control.

**Objective:** This study focuses on detection and molecular typing of CTX-M type ESBL-producing *E. coli* isolated from various sources in Taif, Western Saudi Arabia.

**Material and Methods:** A total of 24 *E. coli* ESBLs-producing isolates from multiple sources were assessed for the presence of CTX-M groups gene by PCR, and subsequently their clonal relatedness by random amplified of polymorphic DNA (RAPD) analysis. Isolates were selected according to a resistance phenotype consistent with production of ESBL-type beta-lactamase using double disk diffusion method.

**Results:** A CTX-M gene was detected in all 24 isolates. RAPD typing of *E. coli* isolates bearing CTX-M gene showed 24 patterns verified into two major clusters (A, B) and three sub-clusters (A1 – A3). Phylogenetic analysis indicating a degree of similarity among clustering isolates from human, sheep and raw milk origins. Identical profile was observed between three isolates obtained from pet bird and chicken.
Introduction

Escherichia coli strains are greatly important in human and animals intestinal and extraintestinal infections. For the treatment of their infections, β-lactam antibiotics are widely used [1]. Production of beta-Lactamases are the commonest cause of bacterial resistance to beta-lactam antibiotics, which are encoded chromosomally or on plasmids and inactivate β-lactams by hydrolyzing the β-lactam ring.

The spread of resistance to extended-spectrum cephalosporins (ESCs) used in human and veterinary medicine causes major therapeutic challenges worldwide [2]. Recently, the rapid dissemination of extended-spectrum β-lactamases (ESBLs) in E.coli is becoming a problem due to the wide spread of the CTX-M enzymes.

The emergence of \( \text{bla}_{\text{CTX-M}} \) has been detected in food animal populations [3], food products [4], and both domestic [5] and wild [6] animal species throughout the world. Saudi Arabia is one of many countries with higher rates of bacterial pathogens with ESBLs-producers in the clinical setting, mostly associated with CTX-M-types [7, 8].

Non-human sources can play an important role in the ecology of antibiotic resistant E. coli, and these may serve as an important reservoir of bacterial resistance. Therefore, a comprehensive understanding of the epidemiology of antimicrobial resistant bacteria and the contribution of different sources to the infections with resistant strains are crucial for effective control [9]. Unfortunately, few data is available for comparisons of the ESBLs-producing E. coli isolates from representative samples of domestic livestock and poultry, wildlife, and humans within the same geographic region.

The aim of this study was to characterize the genetic diversity of E. coli isolates from multiple sources within the same geographic region producing ESBLs and CTX-M using RAPD-PCR.

Materials and Methods

Bacterial Strains: A total of 24 E. coli isolates producing ESBLs were selected from a collection of 103 E. coli isolates obtained from various sources (human & animal ) over a period of one-year (2014). The isolates were identified by biochemical reactions using API 20 E system (bioMerieux, Marcy l’Etoile, France) and confirmed by species-specific PCR amplification of the \( \text{uspA} \) gene [10]. Preliminary antibiotic susceptibility testing done according to the guidelines and recommendations CLSI [11]. The isolates that were characterized had

Conclusion: This study demonstrates occurrence and diversity of ESBL-CTX-M producing E. coli isolates from multiple sources in Saudi Arabia. The study also shows a non-clonal dissemination, despite the fact that many isolates revealed some degrees of genetic relatedness.

Keywords: Molecular typing; E. coli; CTX-M type ESBLs; RAPD-PCR
an ESBLs phenotype with reduced susceptibility to third generation cephalosporin’s, with clavulanic acid synergy [12].

**Genomic DNA preparation:** The DNA of bacterial cells was extracted by a boiling technique that includes heating at boiling of an overnight bacterial culture (200 µl) mixed with 800 µl of distilled water, followed by cooling, centrifugation and the supernatant was used as the DNA template for the PCR.

**Genetic identification of CTX-M ESBLs:** The ESBLs-producer isolates were assessed for the presence of CTX-M type ESBLs gene by multiplex PCR assay using universal primer CTX-M-F: ATGTGCAAGYACCAGTAARGTKATGGC; CTX-M-R: TGGGTRAARTARGTSACCAGAAYCAGCGG, with product size (593 bp) [13]. The cycling parameters of CTX-M specific PCR were as follows: an initial denaturation at 95°C for 15 min; followed by 30 cycles of 94°C for 30 s, 62°C for 90 s, and 72°C for 60 s; and with a final extension at 72°C for 10 min.

**Clonal analysis by RAPD-PCR:** The clonal relatedness was investigated by randomly amplified polymorphic DNA (RAPD-PCR) analysis as described by Williams et al. [14], using primers OPA10 (5’-d GTG ATCGCAG -3’), OPA11 (5’-d CAATCGCCGT-3’) and M13 (5’-dGAGGGTGCGGTTCT-3’). RAPD amplification condition consisted of: initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation (1 minute at 94°C), annealing (1 minute at 36°C) and extension (72°C for 2 minutes), and a final extension step (72°C for 10 minutes). DNA fingerprints of RAPD were compared by visual inspection and considered unique when they differed by at least one band, irrespectively of band intensity. Dendrogram based on RAPD results were constructed by the unweighted pair group method with arithmetic average based on Jaccard’s Similarity Coefficient , and by using Phoretix 1D Advanced V5.20 software (Nonlinear Dynamics, Newcastle upon Tyne, UK). PCR amplification reactions were performed in a volume of 25 µl containing 12.5 µl of 2x GoTaq® Green Master Mix (Promega, Madison, WI, USA) and 2 µl of genomic DNA template. Amplified PCR products were separated using 1.4 % agarose gels, visualized and photographed by G: BOX gel documentation (SYN GENE, Cambridge, UK). A 100 bp DNA ladder (Promega) was used as molecular weight.

**Results**

**Identity of *E. coli* isolates:** All 24 isolates were confirmed as *E. coli* by generating the 884-bp *E. coli*-specific product using species-specific PCR amplification of the universal stress protein A (*uspA*).

**Antimicrobial susceptibility:** The results of antibiogram testing revealed that all *E. coli* 24 isolates were resistant to one or more cephalosporins, but none of them were resistant to cefoxitin / imipenem or both.

**ESBLs and CTX-M- production:** Based on PCR assay of 24 *E. coli* isolates with phenotypically confirmed ESBLs production, yielded a positive amplicon with CTX-M universal primer (Fig. 1).
Figure 2. Random amplified polymorphic DNA patterns obtained by using the primer OPA10 (5’-d GTG ATCGCAG -3’) from different *E. coli* strains isolated from sheep (lanes 1 – 4), chicken (lanes 5-10), wild bird (lanes 11-14), milk (lanes 15-18), human (lanes 19 -22) and cattle (lanes 23 & 24). Lane M is a 100 bp DNA Ladder.

Figure 3. Random amplified polymorphic DNA patterns obtained by using the primer OPA11 (5’-d CAATCGCCGT-3’) from different *Escherichia coli* strains isolated from sheep (lanes 1 – 4), chicken (lanes 5-10), wild bird (lanes 11-14), milk (lanes 15-18), human (lanes 19 -22) and cattle (lanes 23 & 24). Lane M is a 100 bp DNA Ladder.
Clonal analysis: The genomic diversity analysis of 24 ESBLs, CTX-M producing isolates of *E. coli* using the RAPD-PCR fingerprinting method with three-type primers a shown in (Figs. 2-4). The RAPD–PCR profiles allowed differentiation of the 24 isolates and a complex pattern of fingerprints have been obtained for all isolates. Generally, the electrophoretic analysis of the PCR reaction products has revealed that the number of bands ranged from 4-14 bands, and the sizes of the PCR products ranged from 0.2 to 1.4 kb.

The combined results of the three primers were used to construct dendrogram showing the relationships among the studied isolates (Fig. 5). The dendrogram has grouped the 24 strains of *E. coli* into two major clusters (A and B ) and three sub-clusters (A1-A3). Phylogenetic analysis of RAPD profile revealed that isolates from chicken (ST8) and pet birds (ST12, ST13) origins were identical. The remaining of isolates showed mixed RAPD patterns. Even though there was no complete homology among the isolates, two human strains (ST 21 and ST22) were grouped with one isolate from raw milk with a similarity index of 80%, indicating some degree of relatedness. The other human isolates were grouped with isolates from pets and chicken with less similarity index (60%) and (30%), respectively.

**Discussion**

This study presents the epidemiology of *E. coli* isolates harboring CTX-M-group from various sources within one geographic region of Saudi Arabia. All ESBLs-positive isolates were analyzed for *bla*<sub>CTX-M</sub> genes using PCR assay. The results showed that all isolates carried *bla*<sub>CTX-M</sub> group. Obviously, this group is the most common among ESBLs-producing *E. coli* in Saudi Arabia, and similar to most studies reported in the last years [7, 8]. The ESBLs-producing *E. coli* had phenotypes consistent with production of CTX-M enzyme group.

During the last decade, strains of *E. coli* that producing ESBLs-CTX-M have emerged as a cause of serious human infections in many parts of the world [15,16]. CTX-M-type ESBLs have also recently become predominant among ESBLs-positive *E. coli*
human clinical isolates in Middle East countries [17, 18]. Studies from USA, Europe and Asia have shown the recovery of bla\textsubscript{CTX-M} group from farm livestock with increasing frequency [19-21]. The ESBLs-CTX-M type was also reported in faecal samples of broilers [22], and healthy pets [23]. ESBLs-producing E. coli strains have been also identified in food animals [24-25] and wild animals [26].

According to our best knowledge there is no previous study in Saudi Arabia reported on the occurrence of E. coli ESBLs-producing CTX-M type from non-human sources. This study found no evidence of spread of E. coli CTX-M producers between animals and human within the same geographic region of Saudi Arabia. RAPD analysis of the 24 bla\textsubscript{CTX-M} -producing E. coli isolates indicated that there is no common clonal relationship between isolates. In this study, a total of 21 distinct profiles were obtained among 24 CTX-M-producing isolates which are indicating high genetic diversity. In agreement with our results, a recent study of Hu et al. [27], showed that the CTX-M–producing isolates from various sources have great diversity using other molecular typing methods. Additionally, the study of Al-Agamy et al. [28] in Saudi Arabia, indicated a high level of heterogeneity among their CTX-M-producing E. coli isolates from various human clinical samples in Riyadh hospitals. In Korea, Choi et al. [29] found no evidence of the spread of CTX-M-producing E. coli isolates between animal and humans.

On the other hand, some studies have found genotype similarities among CTX-M-producing E. coli isolates from animals, meat and humans [30-32].

**In Conclusion**, this study shows high genetic diversity among isolates of E. coli producing bla\textsubscript{CTX-M} genes suggesting non clonal dissemination among human and animal isolates.

**Acknowledgements**

The authors express their appreciation to the deanship of scientific research at Taif University for funding the work through research project number 1-435-3319.
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