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Full Length Research Paper

Antibiotic resistance in *Escherichia coli* strains isolated from water springs in Al-Ahsa Region

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In Saudi Arabia, water resources are largely limited to groundwater, which is used both for drinking and agriculture. There is no surface water except for a few oases and no permanent streams. The contamination of this source possibly through wastewater, agriculture activities and wild birds as well as amphibians and reptiles that have access to wells and springs. Twenty six strains of *Escherichia coli*, isolated from water springs in Al-Ahsa Region of Saudi Arabia were analyzed for their antimicrobial susceptibility. Fifteen strains, representing 57.74% of the total twenty six strains, showed multidrug resistance phenotypes. RAPD -PCR with two random primers produced different DNA fingerprinting profiles with varied number of bands. The dendrogram obtained from the RAPD-PCR results discriminated the isolates into 26 single isolates and 3 clusters at the level of 40% similarity.

Key words: *Escherichia coli*, antibiotic resistance, water springs, RAPD-PCR.

INTRODUCTION

It is needless to say that the quality of water is of vital importance to public health. Efficient surveillance and check strategies are important for executing a high-quality management of this resource. In water, bacteria from different origins (human, animal, environmental) are able to mix and resistance evolves as a consequence of promiscuous exchange and shuffling of genes, genetic platforms, and genetic vectors (Baquero et al., 2008). Microbial indicators, in particular coliform bacteria and *Escherichia coli*, have been established for assessing the microbiological safety of drinking water. The presence of *E. coli* in water is an indicator of fecal contamination and implies that pathogenic bacteria, viruses, and protozoa may also be present (Environment Agency, 2002). The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. Traditionally these genera included *Escherichia, Citrobacter, Enterobacter* and *Klebsiella*. However, based on the modern taxonomical criteria, the group is heterogeneous and includes non-faecal lactose fermenting bacteria as well as other species which are rarely found in faeces but are capable of multiplication in water (WHO, 1993). These indicators, used to assess the potential public health risk of drinking water, are key elements of most drinking water quality guidelines (WHO, 1997).

Traditionally, the method used to examine water samples for the presence of coliform bacteria and other pathogens is to culture the water sample on nutrient media. However, this method is time consuming and unable to distinguish between the closely related harmful and benign strains of bacteria (Water Research Commission, 2001). It fails to detect viable but nonculturable organisms, where the bacteria lose their ability to grow on agar plates but remain metabolically active (Singh and McFetess, 1992; Hickey and Harkin, 1998). The development of polymerase chain reaction (PCR) technique in 1983 was a major breakthrough in molecular biology. The procedure has been found to be very useful in cloning, DNA sequencing, tracking genetic disorders and forensic analysis (Bitton, 1994). Utilization of PCR in the detection of pathogenic bacteria has been previously reported (McDaniels et al., 1996; Meng et al., 1997; Fratamico and Strobaugh, 1998; Vantarakis et al.,

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Table 1. *E. coli* strains isolated in this study.

Strains code	Localities	Antibiotics resistant
KFUEC[a] 1	Alharah spring	AMP, STR, TET, STR, CIP
KFUEC 2	Alharah spring	AMP, STR, TET, NAL
KFUEC 3	Aljawharyah spring	AMP, CHL, TET, NAL
KFUEC 4	Aljawharyah spring	STR, TET
KFUEC 5	Aljawharyah spring	STR, TET
KFUEC 6	Aljawharyah spring	AMP, CHL, NAL, CIP
KFUEC 7	Aljawharyah spring	AMP, CHL, STR, STX, NAL, CIP
KFUEC 8	Aljawharyah spring	AMP, CHL, STR, TET, NAL
KFUEC 9	Aljawharyah spring	TET, STX
KFUEC 10	Aljawharyah spring	AMP
KFUEC 11	Um Sabaa spring	AMP
KFUEC 12	Um Sabaa spring	AMP, CHL, STR, TET, STX, NAL, CIP
KFUEC 13	Um Sabaa spring	AMP, CHL
KFUEC 14	Um Sabaa spring	STR, STX, CIP
KFUEC 15	Um Sabaa spring	AMP, CHL, STR
KFUEC 16	Um Sabaa spring	MAP, CHL, STR, TET, NAL
KFUEC 17	Um Sabaa spring	AMP, STX
KFUEC 18	Um Sabaa spring	AMP, STR, TET
KFUEC 19	Um Sabaa spring	AMP, CHL, STR, STX, NAL, CIP
KFUEC 20	Albahyela spring	CHL, TET
KFEUC 21	Albahyela spring	AMP, STR
KFEUC 22	Albahyela spring	AMP, CHL
KFEUC 23	Albahyela spring	AMP, CHL, STR, TET, STX, NAL, CIP
KFEUC 24	Albahyela spring	STR, TET, CIP
KFUEC 25	Albahyela spring	AMP, CHL
KFUEC 26	Albahyela spring	AMP, CHL, STR, STX, NAL, CIP

[a] King Faisal University *E.coli* , AMP ampicillin, CHL chloramphenicol, STR streptomycin, TET tetracycline, STX co-trimoxazole, NAL nalidixic acid and CIP ciprofloxacin.

2000; Lindström et al, 2001; Osek, 2002; Ibekwe et al., 2002; Tantawiwat et al., 2005; Ram et al., 2007, 2008; Altalhi et al., 2010).

The overuse of antibiotics in human medicine and agriculture is a growing concern for public health. Overuse combined with inadequate wastewater treatment has led to the presence of antibiotic resistant bacteria and genes encoding antibiotic resistance in surface waters, river sediments, and the feces of wild animals exposed to waste residuals. The multiple resistance of bacteria against antibiotics resulted from the fact that domestic and industrial waste were emitted into threw away without clarifying and so affect the underground water resources. The presence of antibiotic resistant bacteria in water sources throughout the world has been documented (Kelch and Lee, 1978; French et al., 1987; Ogan and Nwiika, 1993; Young, 1993). Exposure to antibiotic resistant bacteria and the corresponding antibiotic resistance genes (ARG) in surface water can occur when the waters are used for drinking or recreation. Infection by antibiotic-resistant strains of pathogenic bacteria can have severe health implications for the sickened individual including more virulent strains and less treatment options (Barza and Travers, 2002).

In Saudi Arabia, investigation of water quality has largely been focused on assessment of water microbial quality through studying the microbial load and detection of coliform bacteria using time-consuming and traditional methods (Hashem, 1992; AlAbdula et al., 1995; Abdel, 1997; Abed and Alwakeel, 2007). Scarcely or seldom investigation was done in relation to quality assessment against pathogenic as well as indicator bacteria utilizing molecular techniques. In this study, however, we examined genotypic characteristics of antibiotic resistant *E. coli* isolates recovered from four different water springs in Al-Ahsa region of Saudi Arabia.

MATERIALS AND METHODS

Study sites description

Thirty different water samples were collected from the four different springs of Alharra, Aljawharyah, Um Sabaa and Albahyela (Table 1). The samples were collected from various locations at various depth from each spring.

Isolation and identification of *E. coli*

The water samples were collected as described elsewhere (Duelge and Unruh, 2002; Alam et al., 2006) and the examination of the water samples was completed within 24 h after collection. Coliform and fecal coliform bacteria were isolated from water samples by multiple tube fermentation and enumerated by most-probable-number (MPN) estimates by using standard techniques (Clesceri et al., 1998). Isolation of fecal *E. coli* out of water samples was achieved by monitoring the acidification and gas production during growth in MacConky broth (Oxoid, UK) at 44 ± 0.5°C for 24 ± 3 h. From the fermentation tube, *E. coli* was restreaked on eosin methylene blue agar (Scharlau, Spain, EU) as metallic sheen. The identification of *E. coli* was confirmed by using API-20E identification kit (bioMerieux-France).

Determination of susceptibility to antimicrobials

The antibiotic sensitivity of isolated pathogens was tested with the disc diffusion method on Muller-Hinton agar (Hi -Media, India) (Bauer et al., 1966) by using commercial antibiotics (100 µg/disc). The isolates were screened for resistance to Ampicillin (AMP 25), Augmentin (AUG 30), Aztreonam (ATM 30), Nalidixic acid (Nal 30), Streptomycin (STR 10), Ciprofloxacin (CIP 10), Chloramphenicol (CHL 30), Cefotaxime (CTX 30), Ceftazidim (CAZ 30), Cefalothin (CEF 30), Gentamicin (GEM 10), Tetracycline (TET 30) and Co-trimoxazol (STX 25). All antibiotic discs were purchased from Hi-Media, India and Mast-Diagnostics, the United Kingdom. Few colonies of each isolate were dispensed in sterile normal saline to match the 0.5 McFarland standards for sensitivity tests as described by National Committee for Clinical laboratory approved Standards (NCCLS, 1999).

DNA extraction

Template DNA was prepared from bacteria grown overnight at 37°C on LB agar. Crude DNA extracts were obtained by suspending 4 colonies in 250 l distilled water and boiling at 94 - 95°C for 15 min. After centrifugation at 15000 rpm for 15 min at 4°C, the supernatant was removed and stored at - 20°C for further assays (Salehi et al., 2008).

RAPD-PCR

GEN15002 (5 -CAATGCGTCT-3) and GEN15009 (5 -AGAAGCGATG-3) are two randomly designed 10-mer primers with 50% gas chromatography (GC) content were employed for fingerprinting the *E. coli* strains, as they provided reproducible and discriminatory patterns. The RAPD-PCR reaction mixtures (25 l) consisted of 2.5 l of 10x PCR buffer, 0.5 l of 10 mM dNTPs, 1.5 l of 25 mM $MgCl_2$, 1 unit of *Taq* polymerase (Promega Co, USA), 5 pmol of primer and 1.0 l (20-30 ng) of DNA template. PCR amplifications were carried out using Techne Thermal Cycler (Model HL-1). The thermal profile consisted of a 3 min at 94°C for predenaturation, followed by 45 cycles of 1 min at 94°C for denaturation, 1 min at 36°C for annealing, 2 min at 72°C for extension, followed by a final extension at 72°C for 5 min. The PCR amplicants were visualized by running 15 l of the amplified products on 1.2% agarose gel, which was stained with ethidium bromide and photographed under UV illumination.

RAPD data analysis

Computer analysis of RAPD patterns were performed as described by Halmschlager et al. (1994) in which the band pattern obtained from agarose gel electrophoresis was digitalized by hand to a two-discrete-character-matrix (0 and 1 for absence and presence of RAPD-bands, respectively). The data of all the samples were combined and analyzed based on the Nei and Lee coefficient (Nei and Lee, 1979). Dendrograms were constructed by the unweighted paired group method of arithmetic average (UPGMA) based on Jaccard's similarity coefficient using Phoretex 1D Software (version 5.2).

RESULTS AND DISCUSSION

Isolation of multi-drug resistance *E. coli* from water sources

In this study, out of thirty samples collected from Al-Ahsa water springs, 26 samples (86.7%) showed contamination with *E. coli*. One strain from each positive sample was used for further analysis. Of the 26 *E. coli* strains, 15 (57.7%) strains were resistant to at least three antibiotics (Table 1). Overall resistance was most frequently observed to ampicillin (76.9%), chloramphenicol (53.8%), streptomycin (65.4%), tetracycline (50%), co-trimoxazole (38.5%), nalidixic acid (42.3%), and ciprofloxacin (34.6%) (Table 2). Most of these antibiotics have been widely used for therapeutic purposes against bacterial infections in humans and animals as well as growth promoters in agriculture and aquaculture (Kruse and Sorum, 1994; Khachatourians, 1998; Aarestrup and Møller, 1999). Similar multidrug resistance phenotypes of *E. coli* isolated from water sources have been reported worldwide (Salvadori et al., 2004; Ramteke and Tewari, 2007; Ozgumus et al., 2007; Ram et al., 2008; Toroðlu and Dinçer, 2008).

Genetic diversity among *E. coli* strains isolated from water samples

The two primers used in this study, GEN15002 and GEN15009, generated a considerable number of amplification products for comparison. A different DNA banding pattern was produced by every isolate. All random primers resulted in robust RAPD fragment patterns (Figures 1 and 2). Primers typically generated between 7 and 14 DNA products for each isolate ranging in size from 400 bp to greater than 2000 bp. Most amplification products were reproducible. For the purpose of epidemiological analysis, it is of great importance to use methods with high discriminatory power, excellent reproducibility and ease of interpretation and use. With the data analysis done with UPGMA (UPGMA) based on Jaccard's similarity coefficient by using Phoretex 1D Software (version 5.2), dendrograms were generated with genetic distance (Figures 3, 4 and 5) . Basically, all 26 *E. coli* strains were divided into 2 main clusters and 4 sub-clusters in the main cluster. The main cluster consisted of about 87% of the total isolates. This showed that most of

Table 2. Antibiotic resistance in *E. coli* strains isolated from water samples.

Antibiotics	No. of samples (n=26)	Resistance (%)
AMP	20	76.9
CHL	14	53.8
STR	17	65.4
TET	13	50
STX	10	38.5
NAL	11	42.3
CIP	9	34.6
Resistance to 1 antibiotic	11	42.3
Multiresistance[a]	15	57.7

[a]Resistance to at least three different antibiotics, AMP ampicillin, CHL chloramphenicol, STR streptomycin, TET tetracycline, STX co-trimoxazole, NAL nalidixic acid and CIP ciprofloxacin.

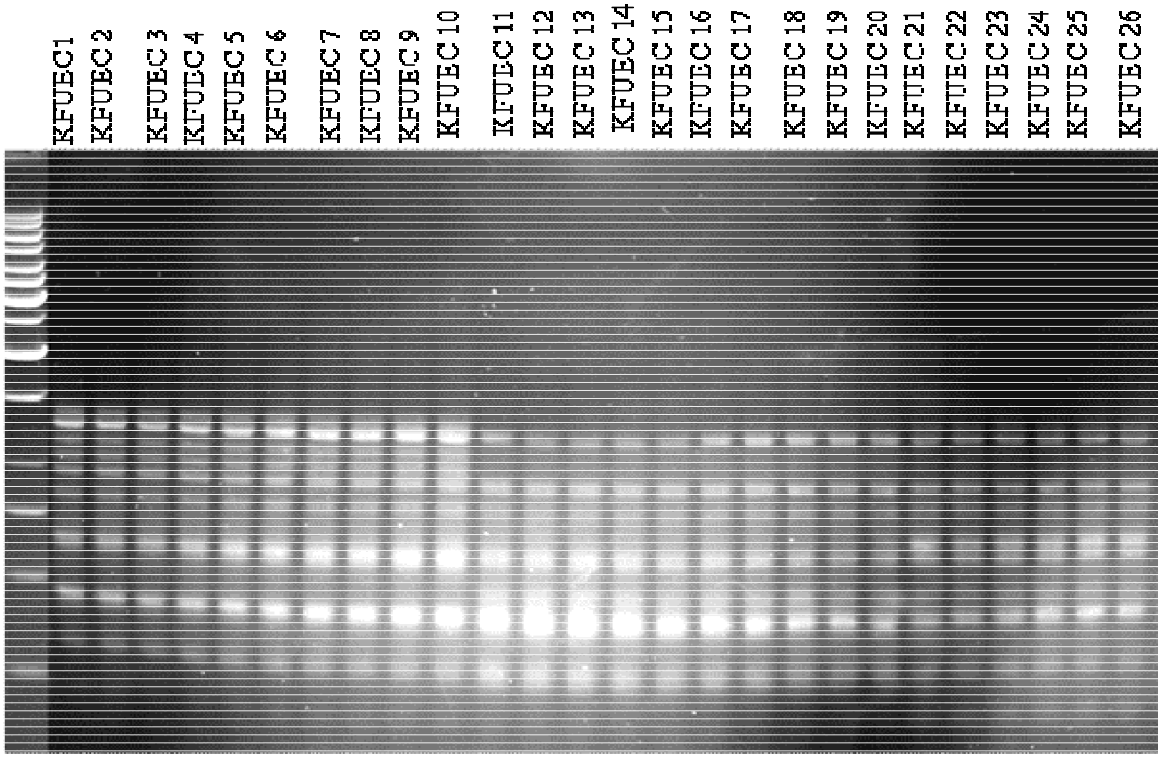

Figure 1. DNA banding patterns from random amplified polymorphic DNA analysis of *E. coli* strains primed by GEN15002 primer .

the isolates might have originated from a similar lineage. For example, 3 strains that showed close relatedness were KFUEC 22, KFUEC 25 and KFUEC 26. Those strains shared their source of isolation, whereas they were all isolated from Albahyela spring. Also, strains isolated from Alharah spring, KFUEC 2 and KFUEC 1, clustered together away from the rest of strains.

The results of this study indicated that the *E. coli* strains were highly diverse, despite the fact that many of the isolates shared the same antibiotic resistance patterns. Indirectly, this might suggest that the resistance patterns were not associated with major genetic alterations, and that the diversity observed could be attributed to other factors. This results were in agreement with Fei et al. (2003), who studied antibiotic resistance, plasmid profile and RAPD- PCR analysis of enteropathogenic *E. coli* (EPEC) clinical isolates and found that the EPEC strains were highly diverse although

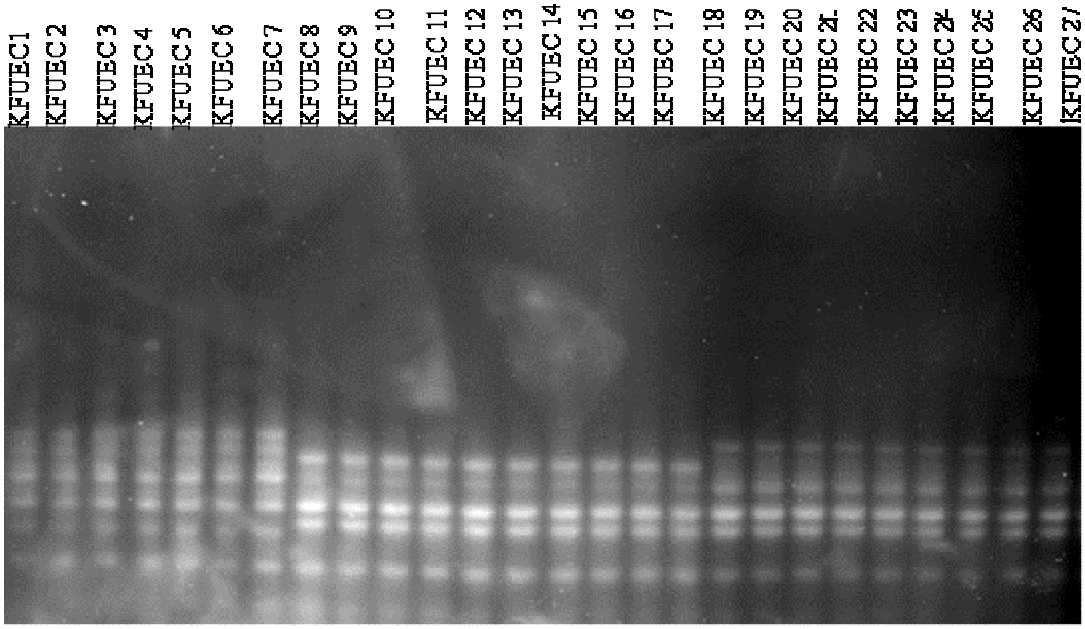

Figure 2. DNA banding patterns from random amplified polymorphic DNA analysis of *E. coli* strains primed by GEN15009 primer.

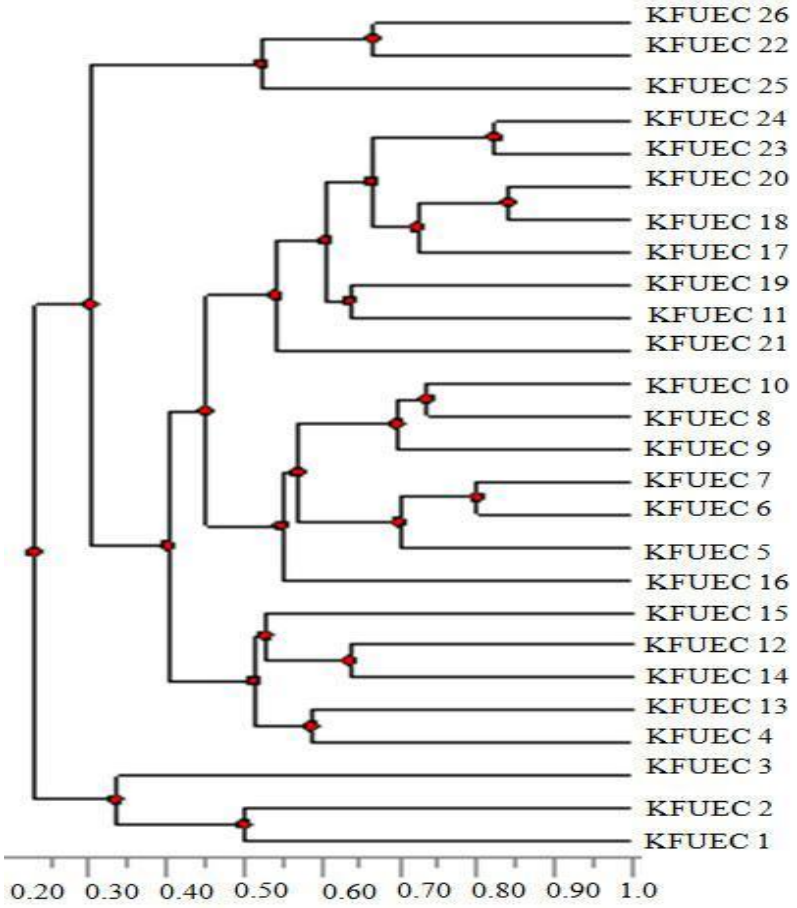

Figure 3. Dendrogram showing genetic relationship of some *E. coli* strains produced by RAPD with primer GEN15002.

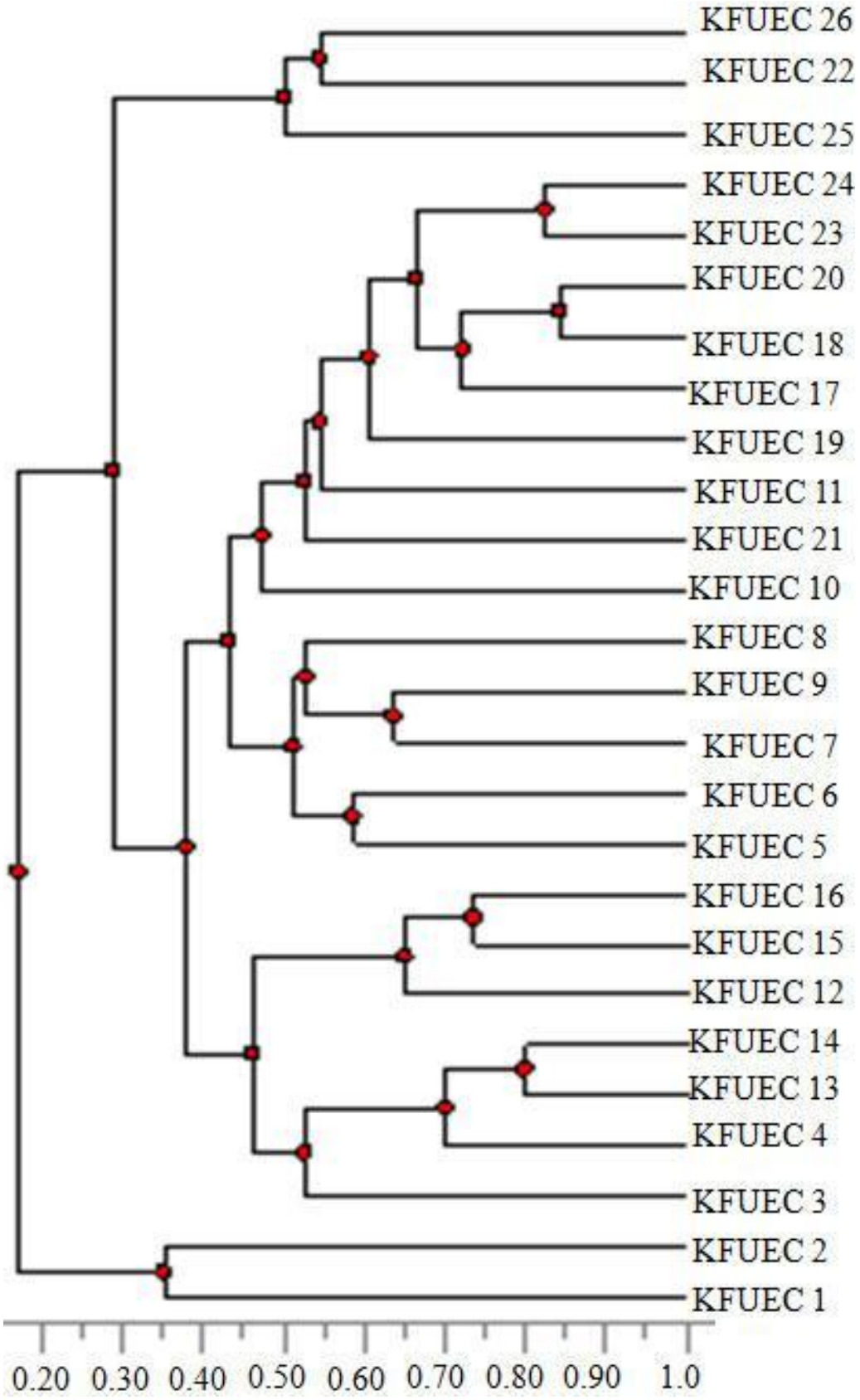

Figure 4. Dendrogram showing genetic relationship of some *E. coli* strains produced by RAPD with primer GEN15009.

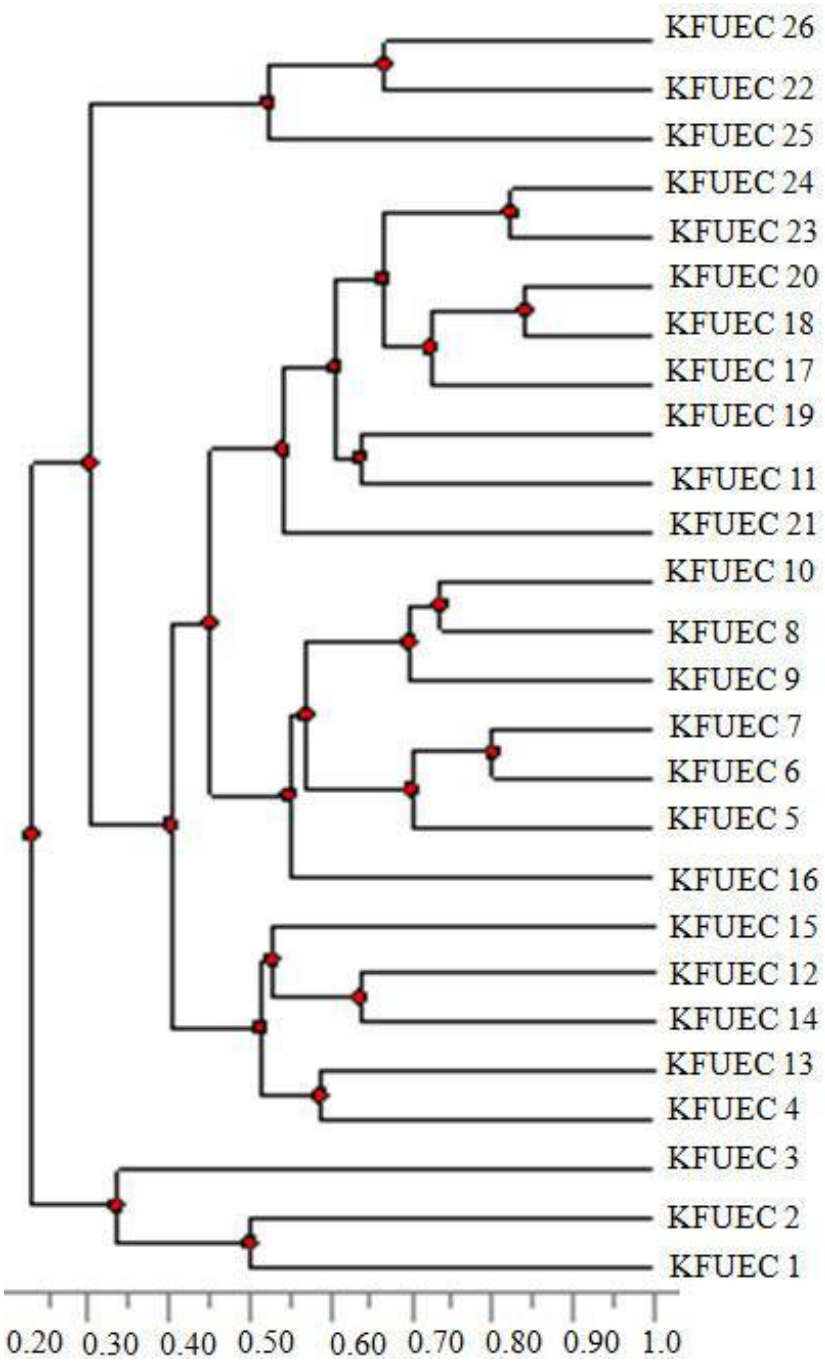

Figure 5. Dendrogram showing genetic relationship of some *E. coli* strains produced by RAPD with primers GEN15002 and GEN15009.

they shared the same antibiotic resistance patterns.

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