Saudi Journal of Biological Sciences 25 (2018) 928–931

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Molecular characterization of antibiotic resistant *Escherichia coli* isolates recovered from food samples and outpatient Clinics, KSA

Hassan A. Hemeg

Department of Clinical Laboratory Sciences, Faculty of Applied Medical Science, Taibah University, Medina 30001, Saudi Arabia

A R T I C L E   I N F O

Article history:
Received 21 December 2017
Revised 28 January 2018
Accepted 30 January 2018
Available online 2 February 2018

Keywords:
Multidrug-resistant
Escherichia coli
Shiga toxogenic
Aminoglycoside resistance
β-lactamases

A B S T R A C T

Multidrug-resistant *Escherichia coli* is one of the most important public health concern worldwide that can be transferred through the food of animal origin to human being causing serious infection. The genetic responsibility of such resistant genes (Plasmids, integrons, and transposons) can be easily transmitted from the resistant strain to another. Therefore, the main objectives of the study is the molecular characterization of the resistant *Escherichia coli* isolates recovered from food samples and human isolates collected from outpatient clinics, KSA especially the resistance strains against aminoglycoside resistance genes which are responsible for the resistance against gentamicin and the resistance caused β-lactamases genes. Examination of food samples revealed 120 *Escherichia coli* isolates (22.22%) (30 strains O26: K60, 28 strains O128: K67, 20 strains O111: K58, 18 strains O126: K58, 10 strains O55: K59, 9 strains O86: K61 and 5 strains O157: H7). All the strains were highly resistance to penicillin, amoxicillin-clavulanic and erythromycin with a percentage of 100%, while the resistance to gentamicin, ampicillin, oxytetracycline, chloramphenicol, norfloxacin, trimethoprim, and nalidixic acid were 83%, 75%, 65.3%, 55.8%, 36.5%, 30.7% and 26.9% respectively. On the other hand, 59.6% of tested strains were sensitive to ciprofloxacin. Positive amplification of 896 bp fragments specific for *aacC2* genes were observed by PCR designated for the detection of the aminoglycoside resistance genes. Meanwhile, multiplex PCR designed to detect the ampicillin and amoxicillin-clavulanic acid resistant *E. coli* isolates revealed positive amplification of 516 bp fragments specific for *BlaTEM* gene with all the resistant strains to ampicillin and amoxicillin-clavulanic acid. Moreover, positive amplification of 392 bp fragments specific for *BlaSHV* resistant gene were observed with (60.52%) of *E. coli* isolate. While all the tested strains were negative for amplification of *BlaOXA_1*.

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1. Introduction

Escherichia coli enclose diverse group of strains inhabit the human intestinal tract and formulate a part in the gut flora, in addition to that Shiga-like toxin *Escherichia coli*, (STEC) are of zoonotic importance foodborne pathogen as they can cause severe economic losses and serious intra and extra intestinal diseases by consumption of contaminated food like hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC), food poisoning and diarrhea (Cordesmeyer et al., 2011; Kuijper et al., 2011; Newell and La Ragione, 2018). The extensive use of antimicrobial therapy to control the diseases caused by *Escherichia coli* may emerge Multi-resistant *Escherichia coli* isolates that become a worldwide public health problem (Frank et al., 2011; Jacoby et al., 2006; Ukah et al., 2018).

The incidence of urinary tract infections especially with multidrug-resistant *Escherichia coli* have been increased (Frank et al., 2011), particularly in women and young children and the recurrence of infection is common (Hu et al., 2008). Moreover, *Escherichia coli* is one of the most important uropathogen that associated with uncomplicated cystitis (Chattaway et al., 2011).

The resistance to co-trimoxazole, ampicillin, and the fluoroquinolones in some uropathogenic *E. coli* was suggested to be originated from food of animal origin through the horizontal transfer of virulence and antibiotic resistance genes (Manges et al., 2007; Johnson and Nolan, 2009; Ramchandani et al., 2005; Rebbah et al., 2017). Moreover, the aminoglycoside- resistance genes found in uropathogenic *E. coli* isolates recovered from human have been

E-mail address: hhamag@taibahu.edu.sa
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https://doi.org/10.1016/j.sjbs.2018.01.016
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suggested to be isolated from food of animal origin which considered being a reservoir of such resistant strains (Taylor et al., 2008).

The resistance of *Enterobacteriaceae* to third-generation cephalosporin is mediated by TEM- and SHV-type L-lactamases. While, the most incriminated plasmid-borne beta-lactamases in amoxicillin-clavulanic acid resistance *Escherichia coli* isolates is TEM type and OXA-1enzymes (Ahmed et al., 2014; Hamed et al., 2017).

The objective of this study was to evaluate the resistance of *E. coli* strains from food samples and human isolates, collected from KSA on molecular basis specially β-lactamases resistance genes (blaTEM, blaOXA_1, and blaSHV genes) and gentamicin the resistant gene (*aacC2* gene).

2. Material and methods

2.1. Samples

A total of 540 beef meat samples "fresh meat samples from the abattoirs, minced meat were collected from different retail establishment markets and abattoirs collected during the period of April 2015 and March 2016 each sample weighted approximately 100 g. 25 fresh meat samples from healthy farms continuously monitored for *E. coli* were collected as negative control samples.

During the period of August 2015 and December 2016, 180 strains of *E. coli* isolates recovered from patients suffering from food poisoning and or diarrhea were collected from outpatients clinics located in Jeddah, Medina, Riyadh, and Makah, Kingdom of Saudi Arabia as a source of human isolates. All the human isolates were identified with standard microbiological techniques, serotyping and molecular techniques.

2.2. Isolation and identification of E. coli

Isolation of *E. coli*, from poultry and meat product, enrichment broth were used "followed by subculturing on CLED agar, MacConkey agar and Eosin methylene blue agar (Cetinkaya et al., 2014). The recovered *E. coli* isolates by standard microbiological techniques were serologically identified using polyvalent and monovalent *E. coli* antisera.

2.3. Antimicrobial susceptibility test

The resistance to wide range of antibiotics were carried out according to the recommendations of Clinical Laboratory Standards Institute (CLSI, 2013), using the Kirby-Bauer disk diffusion method and the following antibiotics disks (nalidixic acid NA30, ampicillin AMP10, gentamicin CN10, ceftazidime CAZ30, erythromycin E15, norfloxacin NOR 10, ciprofloxacin CIP5, amoxicillin-clavulanic acid AMC 30, vancomycin VA 30, penicillin G P10, chloramphenicol C 30, oxytetracycline OT30, sulphamethoxazole-trimethoprim SXT 25.

2.4. Molecular detection and characterization of the recovered E. coli

2.4.1. Extraction of DNA of E. coli

All the recovered *E. coli* isolates were inoculated on 10 ml tryptic soy broth at 37 °C for 24 h and the genomic DNA was extracted after centrifugation of the overnight culture at 3000 RPM for 5 min by the same method reported by (Frank et al., 2011). 5 µl from extracted DNA were used as a template DNA for PCR.

2.4.2. Molecular detection and characterization of the recovered E. coli

2.4.2.1. Molecular detection and characterization of the E. coli and its virulence genes. Molecular characterization of the recovered *E. coli* were carried out by multiplex-PCR using specific primers for the detection of shiga toxin type 2 (*stx2*) and intimin gene (*eaeA*) reported by (Moussa et al., 2010). Moreover, The extracted DNA were tested by using PCR primer pairs specific for *uid A* gene of *E. coli* (Heininger et al., 1999), encoding b-glucuronidase specific for *E. coli*, the increased serum survival *iss* gene of *E. coli* (Yaguchi et al., 2007), aerobactin *iutA* of *E. coli* (Delicato et al., 2003) and *cvaC* gene (Rocha et al., 2008).

2.4.2.1.1. Molecular detection of the resistance genes. PCR for the detection of the resistant gene specific for gentamicin resistance (*aacC2*) were carried out for all of the recovered strains using specific primers (Aleisa et al., 2013). Moreover, multiplex PCR for the molecular detection of resistant genes specific for ampicillin and amoxicillin-clavulanic acid (*BlaTEM*, Bla*SHV* and *BlaOXA_1*) genes were carried out using three primer pairs (Aleisa et al., 2013).

3. Results

3.1. Standard bacteriological examination of the examined herbal samples

120 *E. coli* isolates (22.22%) were isolated from the examined food samples. All of the strains were typed as follows; 30 strains O26: K60, 28 strains O128: K67, 20 strains O111: K58, 18 strains O126: K58, 10 strains O55: K59, 9 strains as O86: K61 while the other 5 strains were O157: H7.

The shiga toxin type 2 (*stx2*) and the intimin (*eaeA*) genes were detected in all strains *E. coli* serovar O157: H7 using multiplex PCR, the incidence of such two genes in the *E. coli* serovar O111 were 30 (53.5%) and 15 (26.79%) for *stx2* and *eaeA* respectively and in the *E. coli* serovar O128 were 22 (40.74%) and 10 (18.52%) for *stx2* and *eaeA* respectively, while the presence *stx2*and *eaeA* decreased to 30% and 20% in O126: K58, O55: K59, and O26: K60 respectively as shown in Fig. 1. Meanwhile, *stx2* and eaeA genes were absent with *E. coli* serovars O119, O86: K61, O78: K80 and O158: K-. It is clear from the obtained result that *cvaC* gene showed the highest percentage (96.9%), followed by *iutA* gene and *iss* gene (80.8% and 62.99%, respectively).

3.2. Antibiotic sensitivity test of the recovered strains of E. coli by disc diffusion method

All the recovered strains from food samples and from patients suffering from food poisoning and or diarrhea were resistant to penicillin, amoxicillin-clavulanic, and erythromycin 100%. The resistance of the recovered strains to gentamicin and ampicillin were 83% and 75% respectively, while the resistance to trimethoprim, oxytetracycline, chloramphenicol, norfloxacin, and nalidixic acid were 65.3%, 55.8%, 36.5%, 30.7% and 26.9%, respectively. On the other hand, 62.8% of tested strains were sensitive to ciprofloxacin.

3.3. Molecular detection of gentamicin-resistant gene

All the isolates recovered from food samples and from human were tested with PCR for molecular detection of the aminoglycoside resistance genes. Positive amplification of 896 bp fragments specific for *aacC2* gene was observed with all the tested strains as shown in Fig. 3.

3.4. Molecular detection of ampicillin and amoxicillin-clavulanic acid resistance genes by multiplex PCR

All the resistance *E. coli* isolates to ampicillin and amoxicillin-clavulanic were tested by multiplex PCR specific for *BlaSHV* (392

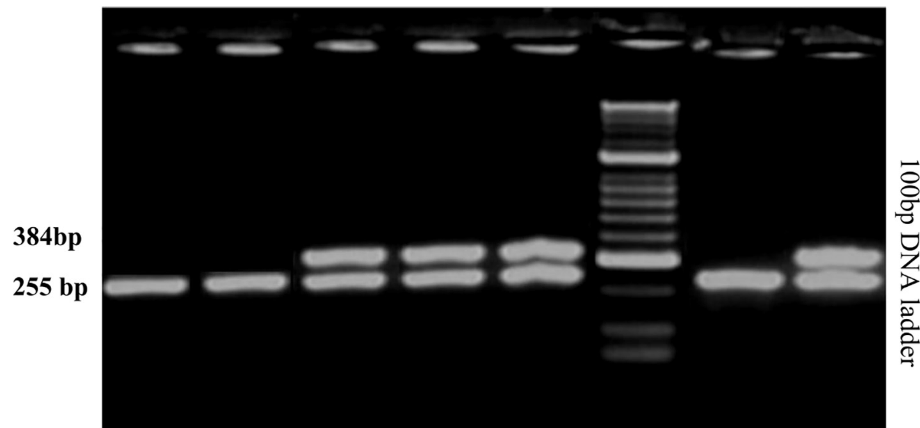

Fig. 1. Multiplex PCR showing positive amplification of 384 bp fragments of intimin (*eaeA*) gene and 255 bp fragments of shiga toxin type two (*stx2*) genes of shiga toxogenic.

bp fragments), *BlaTEM* (516 bp fragments, and *BlaOXA_1* genes. *BlaTEM* gene was observed in all the tested strains (100%). At the same time, positive amplification of 392 bp fragments specific *BlaSHV* gene was detected with 75 of the resistant *E. coli isolate* (62.5%). Meanwhile, all the recovered strains were negative for *BlaOXA_1* gene Fig. 2.

4. Discussion

Urinary tract infections affecting women four times more than men, and more than 50% of women will be infected at least once in their life. Multidrug-resistant *E. coli*, especially the extended-spectrum beta-lactamase (ESBL) *E. coli* is responsible for more than 80% of these infections (Ahmed et al., 2014). Recent studies found that there are genetic similarity between the ESBL *E. coli* isolates recovered from urinary tract infections and those isolated from chickens and food samples which indicated the ability of such strains to be transferred from food of animal origins to the human being causing serious urinary tract infections (Cetinkaya et al., 2014).

Standard Microbiological techniques for isolation and molecular characterization of *E. coli* revealed a total of 120 *E. coli* isolates (22.22%). All of the strains were typed as follows; 30 strains O26: K60, 28 strains O128: K67, 20 strains O111: K58, 18 strains O126: K58, 10 strains O55: K59, 9 strains as O86: K61 while the other 5 strains were O157: H7. Most of the recovered strains from food samples were similar to the strains recovered from serious urinary tract infections in humans (Cetinkaya et al., 2014; Yaguchi et al., 2007).

The shiga toxin type 2 (*stx2*) and the intimin (*eaeA*) genes were detected in all strains *E. coli* serovar O157: H7 using multiplex PCR, the incidence of such two genes in the *E. coli* serovar O111 were 30 (53.5%) and 15 (26.79%) for *stx2* and *eaeA* respectively and in the *E. coli* serovar O128 were 22 (40.74%) and 10 (18.52%) for *stx2* and *eaeA* respectively, while the presence *stx2*and *eaeA* decreased to 30% and 20% in O126: K58, O55: K59, and O26: K60 respectively as shown in Fig. 1. Serious complication such as Haemolytic Uremic Syndrome mostly occurs due to the infection with STEC that harboring the shiga toxin type 2 (*stx2*) gene than STEC that harboring the shiga toxin type 1 (*stx*1) (Moussa et al., 2010). The intimin (*eaeA*) gene which is the marker gene of enterohemorrhagic *E. coli* was detected in all strains of *E. coli* serovar O157: H7 using multiplex PCR (Kuijper et al., 2011).

All the isolates recovered from food samples and from human were tested with PCR for detection of the aminoglycoside resistance genes. Positive amplification of 896 bp fragments specific for *aaC2* gene was observed with all the tested strains as shown in Fig. 3. The obtained results indicated the harboring of the gentamicin-resistant strains to the aaC2 gene responsible for aminoglycoside resistance (Ho et al., 2009). All the resistance *E. coli* isolates to ampicillin and amoxicillin-clavulanic were tested

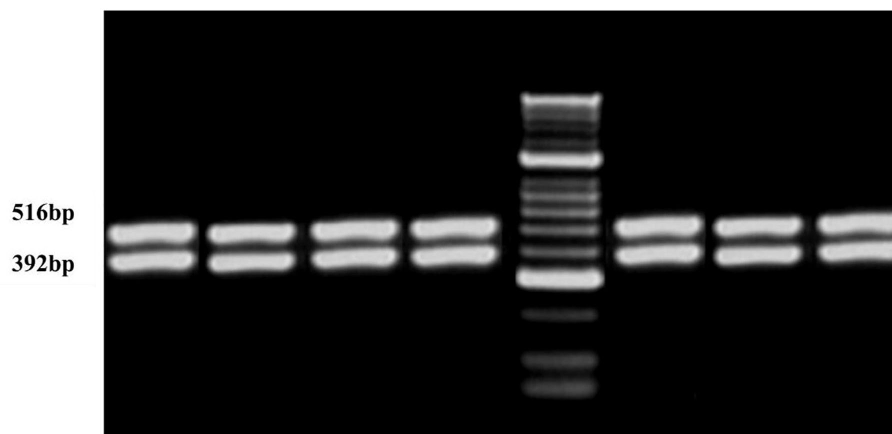

Fig. 2. Agarse gel electrophorsis showing amplification 392 base pair fragments specific for *BiaTSHV* gene and amplification of 516 base fragments specific for *BiaTEM* gene specific for ampicillin and amoxicillin-clavulanic acid resistance.

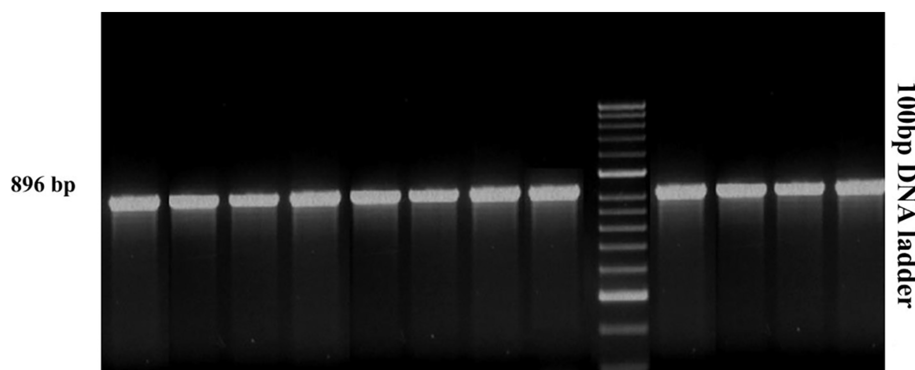

Fig. 3. Positive amplification of 896 bp fragments of aacC2 gene of aminoglycoside resistant gene responsible to gentamicin.

by multiplex PCR specific for *BlaSHV* (392 bp fragments), *BlaTEM* (516 bp fragments, and *BlaOXA_1* genes. *BlaTEM* gene was observed in all the tested strains (100%) indicated that the predominant resistant gene for ampicillin and amoxicillin-clavulanic is the *BlaTEM* gene (Taylor et al., 2008). At the same time, positive amplification of 392 bp fragments specific *BlaSHV* gene was detected with 75 of the resistant *E. coli isolate* (62.5%). Meanwhile, all the recovered strains were negative for *BlaOXA_1* gene Fig. 2. From this study, controlling of resistant strains should not focused only in patients but should include the environment, this raises the questions related to the classification of hospital and community-acquired infections.

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