

Existence of *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM} genes encoding ESBLs among *Escherichia coli* isolates from sheep meat products

Abdolmajid Ghasemian¹, Dhawaa mohammed alkateeb², Mohammad Reza Mohammadi³, Mais Emad Ahmed⁴, Wafaa sadiq alwazni¹, Muneer abdrasul altuoma⁵, Yasemin Khudiar Alghanimi^{6,*}

¹Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran, ²Karbala University, Faculty of Science, Department of Life Sciences, Iraq, ³Islamic Azad University Shahr-e-Qods Branch, Iran, ⁴Department of Microbiology-pathology-Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq, ⁵Ministry of Health, laboratory Hussein Teaching Hospital, Iraq, ⁶College of Education for Pure Sciences, Biology Department, Karbala University, Karbala, Iraq

*Corresponding author: Yasemin Khudiar Alghanimi, College of Education for Pure Sciences, Biology Department, Karbala University, Karbala, Iraq. Email: bacteriology94@gmail.com

DOI: 10.22034/HBB.2019.11

Received: December 30, 2018; Accepted: May 5, 2019

ABSTRACT

The aim of this study was investigation of *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM} ESBLs among *Escherichia coli* (*E. coli*) isolates from meat products. Fifty sheep meat samples were prepared from some slaughter houses in Tehran, Iran. The isolates were also confirmed by conventional biochemical tests. The antibiotic susceptibility of isolates was performed by disc diffusion method according to the clinical and laboratory standards institute (CLSI) 2016 guidelines. The polymerase chain reaction (PCR) was performed for detection of *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{TEM} genes. Among 50 meat samples, 30 *E. coli* isolates were identified. The resistance rate to co-amoxiclav (86 %), doxycycline (10 %), tetracycline (6.7 %), cephamandole (10 %), cefazolin (6.7 %) and ceftazidime (6.7 %) were determined. The PCR screening clarified that all isolates were positive for *bla*_{CTX-M} (100 %) and 23.3 % were positive for *bla*_{TEM}, but none of them amplified the *bla*_{SHV} gene. Among *E. coli* isolates from meat, the antibiotic resistance rate was low, except for co-amoxiclav. High rate of *bla*_{CTX-M} production among *E. coli* from meat is concerning owing to encoding resistance to B-lactam antibiotics and spread to human population.

Keywords: Meat products, *Escherichia coli*, Antibiotic resistance, *bla*_{CTX-M}

INTRODUCTION

Escherichia coli (*E. coli*) is a common and normal inhabitant in the intestinal tract of most animals [1,2]. Extended spectrum beta-lactamases (ESBLs) encoding multiple antibiotic resistance determinants are increasing among gram-negative bacteria worldwide mainly due to no-controlled consumption of cephalosporins. Furthermore, *E. coli* plays role in the transmission of resistant genes such as ESBLs to other species playing as a reservoir. It has been reported 300 subtypes of ESBLs, among which those with clinical importance include bla_{CTX-M}, bla_{TEM} and bla_{SHV} and bla_{AMPC} genes [3]. ESBLs genes in the Enterobacteriaceae are mostly encoded by plasmids that are in various types. Moreover, these genes may be carried by integrons facilitating their transmission. ESBL-producing genes (that mainly belong to the CTX-M type) have been reported among *E. coli* isolates collected from food-producing animals. This issue is a concern fact because causes the dissemination of drug resistant species from animals to human and difficulty in treatment of patients [4]. Antimicrobial therapy should be performed promptly

E. coli isolates from sheep meat

against gram-negative and gram-positive bacteria. Selection of antibiotics should be fulfilled after bacterial evaluation in a medium after 48-72 h. Maximum recommended doses should be administered to prevent liver and kidney failure. Rapid and effective antibiotic treatment improves the condition and improper treatment with antibiotics will even increase mortality in severe infections [5]. The bla_{CTX-M} type ESBL has spread everywhere and the predominant type. Various elements may interfere with the transfer of bla_{CTX-M} genes. Insertion sequence (IS) elements play an important role in this regard. IS elements usually range from 0.8 to 1.8 kb (less than 2.5 kb), with a simple genetic organization with ability to insert multiple locations in the target DNA sequence. In addition to small size, these sequences are genetically truncated [6,7]. Livestock plays a role as reservoir of ESBL-producing pathogens. Hence, antibiotic consumption in veterinary fields must be limited. The aim of this study was determination of prevalence of clinically important ESBLs genes among *E. coli* from sheep meat products and their antibiotic susceptibility pattern.

and ceftazidime (30 µg) disks (ROSCO company).

MATERIALS AND METHODS

Bacterial isolates

Fifty meat samples were collected from meat products in Tehran, Iran. The samples were enriched in EC broth by inoculation of 5 g of each meat sample in the medium and incubated in 37 °C. The bacterial isolates were identified by inoculation of 1×10^7 number of them on agar medium. Additionally, by samples culture on blood agar and Macconkey media (MERK, Germany), the bacterial isolates were identified by conventional biochemical tests including CHROM agar medium and IMVIC (standing to Indole production, motility, methyl-red and citrate fermentation) tests [8].

Antibiotic susceptibility of isolates

The antibiotic susceptibility testing was performed according to the CLSI 2014 guidelines using Kirby Bauer method, and on Mueller Hinton agar medium. The antibiotics used in this study included of aztreonam (30 µg), cefotaxime (30 µg), co-amoxiclav (20/10 µg), gentamicin (10 µg), tetracycline (30 µg), doxycycline (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), cephamandole (30 µg), ertapenem (10 µg), piperacillin-tazobactam (100/10 µg), cefazolin (30 µg)

PCR detection of CTX, TEM and SHV genes

The PCR of isolates for detection of CTX-M, TEM and SHV genes was performed by using specific primers (5'--3') including CTXM1: F: RGMAGYGYRMCGCTKYATGCSC, R: ARTARGTSACCAGAAAYVAGCGG, TEM: F: TCAACATTTCCGTGTGCG, R: CTGACAGTTACCAATGCTTA and SHV: F: ATGCGTTATATTCGCCTGTC, R: ACATAAATCACCACAATGCGC to amplify 590 bp, 1074 bp and 1007 bp products, respectively.

The thermal cycle for the amplification of ESBL-encoding genes in this study included 94 °C for 3min, followed by a 30 cycles of 94 °C for 30s, 60°C for 30s, 72 °C for 45 sec, and a final extension temperature of 72 °C for 10 min.

RESULTS

Of 50 meat samples, 30 *E. coli* isolates were identified. The resistance rate to co-amoxiclav (86 %), doxycycline (10 %), tetracycline (6.7 %), cephamandole (10 %) and cefazolin (6.7 %), aztreonam (6.7 %), cefotaxime (3.3 %), co-amoxiclav (3.3 %), gentamicin (0 %),

Alghanimi et al.

ceftriaxone (6.7 %), ciprofloxacin (6.7 %), nitrofurantoin (0 %), ertapenem (0 %), piperacillin-tazobactam (1.7 %) and ceftazidime (3.3 %) disks were observed.

In the PCR test, all the isolates were positive for CTX-M (100 %) and 23.3 % were positive for TEM, but none of them amplified the SHV gene. The PCR amplified 590 bp and 1074 bp for CTM-M and SHV genes, respectively but not 1007 bp product.

DISCUSSION

ESBL-bearing *E. coli* is a ubiquitous agent and a major contributor to the establishment of various infections, in particular septicemia and UTI, lower respiratory tract infections, visceral ulcers, liver abscesses, cholecystitis and pancreatic abscesses. These drug-resistant agents have also been isolated from livestock origins. Therefore, the distribution of these drug-resistant strains in livestock infections and the pattern of antibiotic resistance of *E. coli* in the community was the major aim of this study. In this study, 30 isolates of *E. coli* from 50 meat samples were identified. The resistance to co-amoxiclav (86 %), doxycycline (10 %), tetracycline (6.7 %),

E. coli isolates from sheep meat

cephamandole (10 %) and cefazolin (6.7 %) was observed. aztreonam (6.7 %), cefotaxime (3.3 %), co-amoxiclav (3.3 %), gentamicin (0 %), ceftriaxone (6.7 %), ciprofloxacin (6.7 %), nitrofurantoin (0 %), ertapenem (0 %), piperacillin-tazobactam (1.7 %) and ceftazidime (3.3 %) disks was observed. The low resistance profile in this study shows low antibiotic use in the veterinary field. In the PCR assay, all isolates were positive for CTX-M (100 %) and 23.3 % were positive for TEM, but none of them exhibited the SHV gene.

Several other studies have outlined various results on livestock investigation. Similar to this study in the Netherlands, a low drug resistance among chicken and human samples was observed and genotype CTXM-15 was predominant [9]. Although there have been extensive campaigns for controlling the safe handling of meat products during processing, enteric pathogenic species are usually transferred to humans and confer a continuous public health threat [10]. In another study in Spain, the most prevalent ESBL gene was CTX-M type [11]. In a study by Aliasadi in Urmia, 60 % of *E. coli* isolates from sheep samples were ESBL positive, and 27.2 %, 18.2 % and 14.5 % of them were positive for CTX-M, TEM and CTX-M plus TEM genes [12]. The level of antibiotic consumption in veterinary

Alghanimi et al.

source was high in a report by Aalipour [13]. The major concern with regard to the antibiotic resistance spread is high-level consumption of various antibiotics in the veterinary field. These phenomenon lead to gradual enhance in the rate of antibiotic resistance and spread to human populations. Therefore, proper control measures seem essential [14,15].

CONCLUSION

Among meat-originated *E. coli* isolates, the resistance to the majority of antibiotics was low, except for co-amoxiclav. PCR screening of ESBLs genes unraveled that all were positive for CTXM gene and 23.3% were positive for TEM gene. The rate of antibiotic resistance among livestock has increased during a decade ago, thus there is a need for more exact surveillance and control of drug consumption in veterinary field.

ACKNOWLEDGMENT

This study was supported by Department of Biology, Central Tehran Branch of Islamic Azad University.

REFERENCES

[1]. Sørnum H, Sunde M. Resistance to antibiotics in the normal flora of animals. *Vet Res*, 2001; 32: 227-41.

E. coli isolates from sheep meat

[2]. Nojoomi F, Ghasemian A. Effect of overgrowth or decrease in Gut microbiota on health and disease. *Arch Pediatr Infect Dis*, 2016; 4.

[3]. Bush K, Jacoby GA. Updated functional classification of β -lactamases. *Antimicrob Agents Chemother*, 2010; 54: 969-76.

[4]. Hammerum AM, Heuer OE. Human health hazards from antimicrobial-resistant *Escherichia coli* of animal origin. *Clin Infect Dis*, 2009; 48: 916-21.

[5]. Rodriguez-Bano J, Navarro M, Romero L, Muniain M, Cueto Md, Galvez J, *et al.* Risk-factors for emerging bloodstream infections caused by extended-spectrum β -lactamase-producing *Escherichia coli*. *Clin Microbiol Infect*, 2008; 14: 180-83.

[6]. Bonnet R. Growing group of extended-spectrum β -lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother*, 2004; 48: 1-14.

[7]. Boyd DA, Tyler S, Christianson S, McGeer A, Muller MP, Willey BM. Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agents Chemother*, 2004; 48: 3758-3764.

[8]. Mahon CR, Manuselis G, Lehman DC. *Textbook of diagnostic microbiology*: Saunders Philadelphia, Pennsylvania; 2000.

Alghanimi et al.

- [9]. Overdeest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P. Extended-spectrum β -lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg Infect Dis*, 2011; 17: 1216-22.
- [10]. DuPont HL. The growing threat of foodborne bacterial enteropathogens of animal origin. *Clin Infect Dis*, 2007; 45: 1353-61.
- [11]. Ojer-Usoz E, González D, Vitas AI, Leiva J, García-Jalón I, Febles-Casquero A. Prevalence of extended-spectrum β -lactamase-producing Enterobacteriaceae in meat products sold in Navarra, Spain. *Meat Sci*, 2013; 93: 316-21.
- [12]. Aliasadi S, Dastmalchi Saei H. Fecal carriage of *Escherichia coli* harboring extended-spectrum beta-lactamase (ESBL) genes by sheep and broilers in Urmia region, Iran. *Iranian J Vet Med*, 2015; 9: 93-101.

E. coli isolates from sheep meat

- [13]. Aalipour F, Mirlohi M, Jalali M. Determination of antibiotic consumption index for animal originated foods produced in animal husbandry in Iran, 2010. *J Environ Health Sci Eng*, 2014; 12: 1.
- [14]. Ghasemian A, Shafiei M, Hasanvand F, Mostafavi SKS. Carbapenem and colistin resistance in Enterobacteriaceae: worldwide spread and future perspectives. *Rev Med Microbiol*, 2018; 29: 173-76.
- [15]. Ghasemian A, Mobarez AM, Peerayeh SN, Abadi ATB, Khodaparast S, Nojoomi F. Report of plasmid-mediated colistin resistance in *Klebsiella oxytoca* from Iran. *Rev Med Microbiol*, 2018; 29: 59-63.