Original Article

Isolation and identification of extended spectrum β-lactamases (ESBLs) *Escherichia coli* from minced camel meat in Eastern province, Saudi Arabia

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Abstract

Antimicrobial resistance is an increasingly serious threat to global public health that requires action across all government sectors and society. The aim of this study was to determine the rate of extended-spectrum β-lactamases (ESBL)-producing *E. coli* isolation from minced camel meat and identify the phenotype and genotype of the ESBL. A total of 150 samples were collected randomly from butchers' shops in Al-Ahsa, Saudi Arabia. The results indicated that, overall, 17 (11.3 %) *E. coli* isolates were recovered from the minced meat samples. The isolates were classified biochemically at the species level using the VITEK 2 system. The antibiotic susceptibility of *E. coli* isolates was determined based on their MIC profile. The highest resistance was determined to be ampicillin (64.7%), doxycycline (23.5%), cefotaxime (23.5%) and ciprofloxacin (17.6%). Multidrug resistance (MDR) was determined in four isolates. Screening of the 17 isolates for ESBLs revealed that, four strains were resistant to cefotaxime and ceftazidime. A combination disk test (CDT) was used for ESBL phenotype conformation. The ESBL-encoding genes were characterized by PCR. The four isolates produced CTX-M group- 1 ESBLs. The *blaSHV* gene was detected in one isolate and *blaTEM* in two isolates. The *eaeA* gene was detected in 3 isolates, *stx*2 gene in two isolates with the h*lyA* gene in one isolate. It can be concluded that there is clear evidence of the circulation of ESBLs producing *E. coli* in the minced camel meat. A high resistance was determined to ampicillin and doxycycline. The molecular detection of virulence genes may suggest the transmission of foodborne illness to consumers.

Keywords: *E. Coli*, β-lactamases, antibiotic resistance, genes, camel meat

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Introduction

Inadequate use of antimicrobials in food-producing animals has been documented to be the main source of antimicrobial resistance (AMR) in commensal bacteria and foodborne pathogens (Friedman, 2015; ICF, 2017). Camel production in the KSA is considered as one of the most popular national animal productions over the last decades. In 2015, the total number of live camels in the KSA was estimated to be about 301717 head (MAWE, 2015; FAO, 2018). The concentration of amino acids and inorganic minerals in camel meat is higher, with less fat and higher moisture content than in beef. Moreover, Camel meat has reduced production costs because camels are usually reared by nomads in arid regions which makes camel meat available to consumers at a relatively low price (Kadim *et al.*, 2014).

Escherichia coli is a common bacteria among the intestinal microbiome of both humans and animals. *E. coli* had been isolated from healthy and diseased camels (Moore *et al* 2002, Salehi *et al.*, 2012, Al-Ruwaili *et al* 2012) moreover, the potential role of camels as a reservoir for ESBL E.coli has been investigated (Fadlelmula *et al.*, 2016). Multidrug resistant bacteria have been isolated from camels in the KSA due to overuse or prophylactic use of antibiotics (Fayez *et al.*, 2020). Depending upon the disease's location, pathogenic *E. coli* are mostly divided into two groups: extra intestinal (ExPEC) and intestinal pathogenic *E. coli* (InPEC). Whereas, ExPEC strains are linked mainly with both neonatal meningitis (NMEC) and urinary tract infections (UPEC) in adults, InPEC strains are linked to diarrheal disease. InPEC strains are subdivided into at least 6 well-known path types: enter pathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enter invasive E. coli (EIEC), enter aggregative E. coli (EAEC), diffusely adherent *E. coli* (DAEC) and enter toxigenic *E. coli* (ETEC). The emergence of antimicrobial resistance in *E. coli* has spread worldwide and their treatment has been greatly complicated by resistance to most first-line antimicrobial agents (Sabate *et al*., 2008).

It has been reported that the ability of *E. coli* to produce broad spectrum beta lactamase is the main reason for the development of multidrug resistance (Zowawi *et al.,* 2013). Extended-spectrum-lactamases (ESBLs) which hydrolyze penicillins and expanded-spectrum cephalosporins appeared in the early 1980s and, recently, beta lactamase that hydrolysise carbapenems have become prominent (Kliebe *et al*., 1985; Paterson, 2006**)**. TEM, SHV, and CTX-M are the 3 main types of ESBLs. Depending on the dissimilarities of the amino acid sequence, the CTX-M enzymes have been subdivided into five groups (group 1: CTX-M-1, group 2: CTX-M-2, group 8: CTX-M-8, group 9: CTX-M-9, and group 25: CTX-M-25).

In Saudi Arabia, a number of surveys have investigated the prevalence of ESBLs producing bacteria in intensive care units, the community and medical wards in various locations, including Jeddah, Riyadh, Dhahran, Al Khobar and Al-Qassim **(**Al-Agamy *et al* ., 2009, Tewfik *et al*., 2011, Zowawi *et al* 2013). Few reports in Saudi Arabia have investigated the microbiological quality of beef meat and poultry (Hemeg, 2018) and to the best of the author's knowledge, there is no available literature on the prevalence ESBLs producing *E. coli* in minced camel meat. Therefore, the aim of this study was to determine the rate of extended-spectrum β-lactamases (ESBL)-producing *E. coli* isolation from minced camel meat and to identify the phenotype and genotype of the ESBL in Al Ahsa city, eastern province, Saudi Arabia.

Materials and Methods

Sample collection: Between January and September 2018, one hundred and fifty minced camel meat samples were randomly collected from 10 butchers' shops in Al Ahsa city, eastern province, Saudi Arabia. The Samples were collected in sterile, separate plastic bags and labeled. Then they were carried in an ice box within the hour to the Meat Hygiene laboratory, College of Veterinary Medicine, King Faisal University for bacteriological investigation. The samples were stored in a refrigerator at a temperature between 4° C and 8° C until examination within 24 h.

Isolation and identification of E. coli: Twenty-five grams (25g) of minced camel meat were weighted and suspended into 225 ml of 0.1% sterilized buffered peptone water (Hi Media, India) in a sterile stomacher bags and homogenized by shaking for 5 minutes. For enrichment purposes, the stomacher bags were incubated at 37 ˚C for 24 hours. A loop-full (10 µl) from the overnight growth was streaked on MacConkey agar (Oxoid, England) and incubated at 37 ˚C for 24 hours. Permissive identification was performed based on colony morphology and gram staining. Five suspected colonies from each sample were purified on brain heart infusion agar (Oxoid, England) and examined for their oxidase activity by oxidase discs (Hi Media, India). Oxidase negative isolates were subjected to further biochemical identification by VITEK 2 compact system using ID-GN cards (bioMérieux, France).

Screening and confirmatory tests for extended-spectrum β-lactamases (ESBLs) producing *E. coli*: Screening for ESBLs was performed by the standard disc diffusion methods according to Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2014) using cefotaxime (30 µg) and ceftazidime (30 µg) discs. The bacterial inoculum was adjusted to be equivalent to 0.5 McFarland's standard and inoculated on to Muller-Hinton agar (Oxoid, England) by sterile cotton swabs then the two discs were placed on the inoculated plates and incubated at 35°C for 18 h. The measurements recommended by (CLSI) for isolates inhibition zone is ≤ 27 mm for cefotaxime and ≤ 22mm for ceftazidime were considered as ESBLs producer. The combination disk test (CDT) method (using both cefotaxime and ceftazidime, separately and in combination with clavulanate) recommended by CLSI was used for phenotypic conformation. The results were interpreted according to CLSI criteria (CLSI, 2014).

Antimicrobial susceptibility testing: The minimum inhibitory concentration (MIC) for nine antimicrobial agents, ampicillin, amoxicillin-clavulanate, cefotaxime,

imipenem, gentamicin, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole and chloramphenicol was determined to isolated bacteria. Dilutions and cut off values were performed according to recommendations of (CLSI, 2014). Multidrug resistant (MDR) was considered when a strain demonstrated resistance to three or more antibiotic classes (Schwarz *et al*., 2010).

Molecular detection of 16SrRNA, virulence and antibiotic resistance genes: Total DNA was extracted from phenotypic identified ESBLs producing strains. QIAamp DNA mini-kit (Qiagen SA, France) was used for isolation and purification of DNA according to the manufacturer's instructions. For molecular conformation of the isolates, the 16SrRNA was amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′TACGGYTACCTTGTTACGACTT-3′) as described previously by (Lane, 1991). Virulence genes including *eaeA*, *hlyA, stx1, stx2* genes encoding intimin, enterohemolysin and shiga toxins were detected after the methods of (Gannon *et al.,* 1997; Wang *et al.,* 2002; Gallien, 2003). Screening for *blaCTX-M*, *blaTEM* and *blaSHV* genes was established by PCR using primers and reaction conditions previously described by (Pitout *et al.,* 1998, 2004). The PCR products were purified for partial sequencing using a QIAquick PCR Purification Kit (Qiagen SA, Courtaboeuf, France). Sequencing was conducted by Genetic Analyzer 3500, (Applied Biosystems). Partial nucleotide sequences were analyzed using the BLAST program https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Results

Bacterial isolation and screening for ESBLs: Overall, 17 (11.3%) *E. coli* isolates were recovered from the minced meat samples. The isolates were classified biochemically to the species level based on ID-GN database of the VITEK 2 compact system. Screening of the 17 isolates for ESBLs revealed that, four strains had an inhibitory zone less than 27 and 22 for cefotaxime and ceftazidime, respectively. Phenotypical confirmation was performed for all suspected isolates and a synergy effect when tested with the double disk synergy method was noticed. A ≥ 5 mm increase in zone diameter was determined for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent or when tested alone.

Antimicrobial susceptibility: The antibiotic susceptibility of *E. coli* isolates was determined based on their MIC profile. The percentage of resistance against the 9 antimicrobials is presented in Table (1). Six isolates (35.3%) were susceptible to all antimicrobials. The highest resistance was determined to ampicillin (64.7%), doxycycline (23.5%), and cefotaxime (23.5%). Three isolates (17.6) showed resistance to ciprofloxacin. The resistance profile of all isolates is shown in Table (2). Multidrug resistance (MDR) was determined in four isolates two of which were identified as ESBLs producer.

Molecular detection of 16SrRNA, virulence and antibiotic resistance genes: The 16SrRNA sequence of the 4 ESBLs producer strains showed ≥ 98 % similarity with sequences of their corresponding strains from GenBank. The sequences were submitted to GenBank as accession numbers (GenBank MN658514.1, MN658513.1, MN658485.1 and MN658479.1).

The ESBL-encoding genes were characterized by PCR. The four isolates produced CTX-M group- 1 ESBLs. Sequences from PCR products were submitted to Gene Bank with accession numbers (GenBank MN690604, MN690605, MN690606 and MN690607). Fig (1) shows the molecular phylogenetic analysis by maximum Likelihood method. *blaSHV* gene was detected in one isolate whereas *blaTEM* was in two isolates. The *eaeA* gene was detected in 3 isolates, *stx2* gene in two isolates with the *hlyA* gene in one isolate. The distribution of different genes in the 4 ESBLs producer isolates is presented in Table (2). The different virulence gene sequences were submitted to GenBank with accession numbers (MN708336, MN708337, MN708338, MN708339, MN708340 and MN708341).

Table 1 Resistance proportion, MIC50 and MIC90 of *E. coli* isolates in minced camel meat in Al Ahsa province, Saudi Arabia.

Antibiotic	Total R (%)	MIC (g/ml)		
		Range	50%	90%
AMC	5.90% [95% CI, 0.3-30.8]	1 - 64	2	4
Ampicillin	64.70% [95% CI, 38.6-84.7]	1 - 128	64	128
Chloramphenicol	5.90% [95% CI, 0.3-30.8]	1 - 64	2	4
Ciprofloxacin	17.60% [95% CI, 4.6-44.1]	0.25 - 4	0.5	4
Cefotaxime	23.50% [95% CI, 7.8-50.2]	0.25 - 16	0.5	16
Doxycycline	23.50% [95% CI, 7.8-50.2]	0.5 - 32	2	32
Gentamicin	17.60% [95% CI, 4.6-44.1]	1 - 64	2	32
Imipenem	0.00% [95% CI, 0.0-22.9]	0.125 - 0.5	0.25	0.5
SXT	11.80% [95% CI, 2.1-37.8]	0.5 - 16	1	8

Table 2 Antimicrobial resistance profile, virulence genes and ESBL genotype of E. *coli* isolates in minced camel meat in Al Ahsa province, Saudi Arabia.

		ESBL genotype					Virulence genes			
Isolate ID	**ESBL phenotype**	***blaCTX-M***	***blaTEM***	***blaSHV***	**Antimicrobial resistance profile**	**MDR**	***eaeA***	***hlyA***	***stx1***	***stx2***
CM1	Positive	Positive	Negative	Negative	AMP CTX CIP CHL	MDR	Negative	Negative	Negative	Negative
CM2	Positive	Positive	Negative	Positive	AMP AMC CTX SXT	MDR	Positive	Positive	Positive	Negative
CM3	Positive	Positive	Positive	Negative	AMP CTX DOX		Positive	Negative	Negative	Negative
CM4	Positive	Positive	Positive	Negative	AMP CTX DOX		Positive	Negative	Positive	Negative
CM5	Negative	Negative	Negative	Negative	AMP GEN DOX CIP	MDR	Negative	Negative	Negative	Negative
CM6	Negative	Negative	Negative	Negative	AMP GEN CIP SXT	MDR	Negative	Negative	Negative	Negative
CM7	Negative	Negative	Negative	Negative	AMP GEN DOX		Negative	Negative	Negative	Negative
CM8	Negative	Negative	Negative	Negative	AMP		Negative	Negative	Negative	Negative
CM9	Negative	Negative	Negative	Negative	AMP		Negative	Negative	Negative	Negative
CM10	Negative	Negative	Negative	Negative	AMP		Negative	Negative	Negative	Negative
CM11	Negative	Negative	Negative	Negative	AMP		Negative	Negative	Negative	Negative
CM12	Negative	Negative	Negative	Negative			Negative	Negative	Negative	Negative
CM13	Negative	Negative	Negative	Negative			Negative	Negative	Negative	Negative
CM14	Negative	Negative	Negative	Negative			Negative	Negative	Negative	Negative
CM15	Negative	Negative	Negative	Negative			Negative	Negative	Negative	Negative
CM16	Negative	Negative	Negative	Negative			Negative	Negative	Negative	Negative
CM17	Negative	Negative	Negative	Negative			Negative	Negative	Negative	Negative

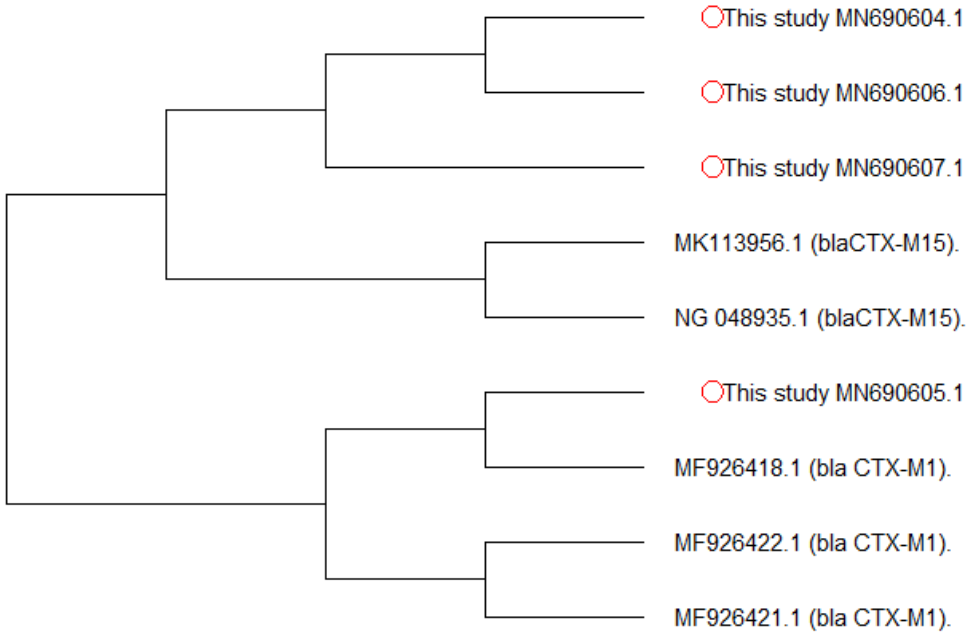

Figure 1 Maximum likelihood tree of BlaCTX-M ESBLs nucleotide sequences. A phylogenetic tree of CTX-M group 1

Discussion

The health of humans, animals and ecosystem is interconnected, pointing to the need for a "One Health" approach. Food-borne diseases and antimicrobial resistance are prominent examples of these close inter-linkages. Food becomes an important vehicle for the transmission of commensal as well as pathogenic micro-organisms including zoonotic agents. Pathogen transmission between food and humans occurs during the processing of raw materials as well as cross contamination at production and distribution (MacDonald *et al.*, 2015).

During this work, *E. coli* was isolated from 17 minced camel meat samples (11.3%). The high proportion of protein and water in fresh red meat provides a suitable condition for growth and multiplication of bacteria (ICMSF, 2005). *E. coli* was previously isolated from fresh beef meat and minced beef meat in the KSA (Iyer *et al.*, 2013; Hemeg, 2018). The variation in the isolation frequencies may be attributed to the sample size, isolation techniques, use of selective media animal species, meat handling and sanitary standards of meat retailer shop premises.

In this study, the MIC of nine antibiotics were determined for all isolates. High resistance to ampicillin and doxycycline was observed and the findings are consistent (Hemeg, 2018, Abayneh *et al.*, 2019). Penicillins and tetracycline are widely used in the treatment of animals in the study area. Three isolates (17.6%) showed resistance to ciprofloxacin. The World Health Organization has classified fluoroquinolones as critically important drugs for human medicine due to a strong link between their use and increased resistance, and has advised careful use of fluoroquinolones in both human and veterinary medicine (WHO, 2012). The possibility of transmitting antibiotic resistance genes from commensals to pathogens or between pathogens has been documented elsewhere (Friedman, 2015; ICF, 2017). The isolates showed 7 resistance profiles and 4 profiles showed MDR. Excessive and inappropriate antimicrobials could be factors enhancing the positive selection of antimicrobial resistance bacteria as a part of commensal flora with an accumulation of antimicrobial resistance genes that encode multiple resistance traits (Nikaido, 2009).

Screening for ESBLs revealed identification of 4 (2.6%) ESBLs producer *E. coli* from minced camel meat. In enterobacteriaceae, the production of beta lactamase is the main mechanism for antibiotic resistance that is a major health issue in the world. The frequency of isolation in this study was lower than other studies elsewhere (Ye *et al.*, 2018, Abayneh *et al.*, 2019, Le *et al.*, 2015). This variation may be attributed to the inappropriate use of fourth and third generations of cephalosporins and the difference in methodology mentioned above.

The molecular characterization of the BlaCTX-M gene revealed that it belongs to the CTX-M group 1. This group includes six plasmid-mediated enzymes; CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-12, CTX-M-15, and FEC-1 (Bonnet 2004). Since there tend to be regional differences in the occurrence of various ESBL variants (Cantòn and Coque, 2006), the current study provides further data relating to minced camel meat. The CTX-M group 1 variants (CTX-M-1 and CTX-M-15) were previously identified in hospitalized people in the Riyadh and Al-Qassim regions (Al-Agamy *et al.*, 2009, Tewfik *et al.*, 2011). In this work, the *eaeA*, *hlyA* and *stx2* genes encoding intimin, enterohemolysin and shiga toxins of *E coli* were identified by PCR in three ESBLs producer isolates, Table. (2). Intimin is a virulence factor on the outer membrane of pathogenic *E coli* and is responsible for attachment and adhesion of the bacteria to host cells. Enterohemolysin is a potential virulence factor for pathogenic *E coli* that is often connected with severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome. Shiga toxin producing *E. coli* is considered one of the most crucial causes of food-borne illness that can lead to life-threatening complications such as hemolytic-

uremic syndrome (Liptakova *et al.*, 2002). Domestic ruminants, mainly cattle, sheep and goats, have been reported to be major natural reservoirs for shiga toxin producing *E.coli* and play a significant role in the epidemiology of human infections (Islam *et al.*, 2008 and Darwish *et al.*, 2018). Isolation of shiga toxin producing *E.coli* from feces of camels has been reported elsewhere (Martin, and Beutin 2011; Baschera *et al.*, 2019). Contamination of minced meat with pathogenic *E coli* may occur during handling in abattoirs or botcher markets (Kaper *et al.*, 2004; Vincent *et al.*, 2010). Good, widespread sanitation and hygiene is recommended during food processing to prevent fecal contamination of foodstuffs. Clean and convenient restroom facilities and routine cleaning and sanitation of the distribution and transport environment, processing sites and food contact surfaces are essential for preventing cross contamination.

In conclusion, from the obtained results, it can be concluded that there is clear evidence of the circulation of ESBLs producing *E. coli* in the minced camel meat. A high resistance was determined to ampicillin and doxycycline. Resistance of non ESBLs isolates to fluoroquinolones is a serious public health problem. Isolation of multidrug resistance bacteria from minced meat may be a reflection of the improper and excessive use of antibiotics in food producing animals. The ESBLs producing E. coli isolates were genotyped as blaCTX-M group 1. The molecular detection of virulence genes may suggest the transmission of foodborne illness to consumers. Therefore, approaches to enhance the knowledge and practice of butchers in the handling and storage of meat should be prepared and enforced. In addition, monitoring the prevalence of antimicrobial resistance among isolates from healthy animals and their food products provides evidence of the need for the design of a strategy for the prevention and control of the spread resistance strain in the community.

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