Austral Journal of Veterinary Sciences
ISSN: 0719-8000
ISSN: 0719-8132
australjvs@uach.cl
Universidad Austral de Chile
Chile

Detection of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from diarrhoiec dogs in Iran

Torkan, S; Bahadoranian, MA; Khamesipourc, F; Anyanwu, MU

Detection of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from diarrhoiec dogs in Iran
Austral Journal of Veterinary Sciences, vol. 48, no. 2, 2016
Universidad Austral de Chile, Chile
Available in: http://www.redalyc.org/articulo.oa?id=501752375007

Detection of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from diarrhoiec dogs in Iran

Detección de virulencia y genes de resistencia antimicrobiana en aislados de Escherichia coli provenientes de perros en Irán

S Torkan
Islamic Azad University, Irán
MA Bahadoranian
Islamic Azad University, Irán
F Khamesipourc Dr_Faham@yahoo.com
Sabzevar University of Medical Sciences, Irán
MU Anyanwu
University of Nigeria, Nigeria

Austral Journal of Veterinary Sciences, vol. 48, no. 2, 2016

Universidad Austral de Chile, Chile

Accepted: 19 November 2015

Redalyc: http://www.redalyc.org/articulo.oa?id=501752375007

Abstract: This study was conducted to investigate the presence of some virulence and antimicrobial resistance genes in E. coli isolates from diarrhoiec dogs in Iran. Seventy dogs were randomly selected by direct sampling. Rectal swabs were collected and cultured for isolation and identification of E. coli following standard methods. Polymerase chain reaction (PCR) was used to detect 5 virulence genes and 12 antibacterial resistance genes in 14 of the isolates. From the 70 rectal swabs cultured, 33 (47.1%) gave positive growth of E. coli. Out of 14 isolates tested for the presence of virulence genes, 9 (64.3%) were positive for PCR of stx (1), 5 (35.7%) were positive for stx (2), 7 (50%) were positive for eae, and 1 (7.1%) isolate was positive for cnf (1). Out of the 14 isolates tested for the presence of antibacterial resistance genes, 9 (64.3%) were positive for CITM gene, 6 (42.9%) were positive for aad (A1) and bla (SHV), 5 (35.7%) were positive for tet (A), dfr (A1) and cat (1), 4 (28.6%) were positive for aac (3)-IV, 3 (21.4%) were positive for both tet (B), sul (1) and cml (A), while 1 (7.1%) of the isolate was positive for ere. The results showed that enterohaemorrhagic E. coli (EHEC), shiga toxigenic E. coli (STEC) and necrotoxic E. coli (NTEC) strains harboring several antibacterial resistance genes could be involved in canine diarrhoea in Iran.

Keywords: antimicrobial resistance, diarrhoea, dogs, Eschericha coli, gene, virulence.

Resumen: El objetivo de este studio fue investigar la presencia de algunos genes de virulencia y resistencia a los antimicrobianos en E. coli aislados de perros diarreicos en Irán. Setenta perros fueron seleccionados al azar y muestreados directamente. Se recogieron hisopos rectales y se cultivaron para el aislamiento e identificación de E. coli siguiendo métodos estándar. Se utilizó la reacción en cadena de la polimerasa (PCR) para detectar cinco genes de virulencia y 12 genes de resistencia a antibacterianos en 14 de los aislamientos. De 70 hisopos rectales cultivados, 33 (47,1%) dieron positivos al crecimiento de E. coli. De 14 cepas analizadas para detectar la presencia de genes de virulencia, nueve (64,3%) fueron positivas para PCR de stx (1), cinco (35,7%) fueron positivos para stx (2), siete (50%) fueron positivos para eae, y uno (7,1%) fue positivo para aislar cnf (1). De los 14 aislamientos probados para determinar la presencia de genes de resistencia antibacterial, nueve (64,3%) fueron positivos para el gen CITM, seis (42.9%) fueron positivos para aad (A1) y bla (SHV), cinco (35,7%) fueron positivos para tet (A), dfr (A1) y cat (1), cuatro (28,6%) fueron positivos para aac (3) -IV, tres (21,4%) fueron positivos para ambos tet (B), sul (1) y cml (A), mientras que uno (7,1%) del aislado fue positivo para ere. Los resultados mostraron que cepas de E.

coli enterohemorrágica (EHEC), E. coli shiga toxigénica (STEC) y E. coli necrotóxica (NTEC) que albergan varios genes que codifican para la resistencia antimicrobiana podrían estar involucrados en la diarrea canina en Irán.

Palabras clave: resistencia antimicrobiana, diarrea, perros, Escherichia coli, genes, virulencia.

INTRODUCTION

Escherichia coli, a member of the family Enterobacteriaceae, constitute part of normal commensal bacterial flora of animals and humans (Nataro and Kaper 2003, Rahimi et al 2012, Puno-Sarmiento et al 2013, Tajbakhsh et al 2016). E. coli have been implicated severally in clinical cases of diarrhoea in dogs (Beutin 1999, Morato et al 2009, Paula and Marin 2009, Puno-Sarmiento et al 2013). But mere isolation of E. coli from diarrhoiec faeces is not enough to regard such isolate as a diarrhoeagenic strain. Diarrhoeagenic E. coli isolate may belong to the enteroinvasive E. coli (EIEC), enterohaemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), entroaggregative E. coli (EAEC), necrotoxic E. coli (NTEC), enterotoxigenic/shiga-like toxin producing E. coli (STEC) or diffusely adherent E. coli (DAEC) strain/ pathotypes, depending on the type of virulent factor(s) elaborated and the type of lesion produced (Bien et al 2011, De Rycke et al 1999, Puno-Sarmiento et al 2013, Salvadoris et al 2003). Nevertheless, canine diarrhoea may not primarily be caused by E. coli, although pathogenic strains of E. coli has been widely incriminated in cases of diarrhoea in humans and animals (Aslani et al 2008, Salvadoris et al 2003, Shahrani et al 2014). In many clinical conditions of dogs such as canine distemper, parvoviral enteritis, coronavirus infection, helminthosis, etc and a myriad of non-infectious and toxic conditions, the integrity of intestinal mucosa is altered resulting in enteritis and diarrhoea (Hammermueler et al 1995, Torkan et al 2015). In these conditions, secondary opportunistic infections by pathogenicE. coli following immune depression and their subsequent discharge in diarrhoeic faeces may occur. DiarrhoeagenicE. coli strains have been reported to harbour genes which encode virulent factors responsible for their pathogenicity (Aslani et al 2008, Bien et al 2011, Shahrani et al 2014). Virulent factors often possessed by pathogenic E. coli strains and used for their classification into pathotypes include: Shiga-like/Shiga toxin (stx) encoded by Shiga toxinogenic (stx) genes 1 and 2 (stx1 and stx2), cytotoxic necrotizing factor (cnf) encoded by cytotoxic necrotizing factor genes 1 and 2 (cnf1 and cnf2), and intimin encoded by E. coli attaching and effacing (eae) gene (De Rycke et al 1999, Landraud et al 2000, Salvadoris et al 2003, Bentancor et al 2007, Puno-Sarmiento et al 2013). These virulent factors have been widely reported to be associated with diarrhoea in humans and animals (Randall et al 2004, Aslani et al 2008, Kavitha et al 2010).

Treatment of companion animals especially dogs with antibacterial agents such as β-lactams, fluoroquinolones, potentiated sulfonamides, etc., in suspected cases of bacterial infection, is often practiced by

veterinary clinicians and non-veterinarians, especially in countries where there are no strict regulations for the use of these drugs in animals (Bradford 2001, Guardabassi et al 2004, Abatcha et al 2014, Torkan et al 2015). This resulted in increased detection of antibacterial-resistant E. coli both pathogenic and non-pathogenic strains, in companion animals worldwide (Hammermueler et al 1995, Bradford 2001, Guardabassi et al 2004, Ewers et al 2012). E. coli develop resistance following prolonged exposure to an- tibacterial agents especially in sub-therapeutic doses by acquisition of antibacterial resistance genes from other resident commensal or transient pathogens colonising the individual or the environment. Various antimicrobial resistance determinants including multidrug resistance genes encoding for extended-spectrum β-lactamases have been described in E. coli isolates from companion animals (Bradford 2001, Costa et al 2008, Ewers et al 2010, Shaheen et al 2011, Tajbakhsh et al 2015). Antimicrobial resistance genes spread easily among bacterial organisms by mobile genetic elements like plasmids, and transposons (Salvadoris et al 2003, Randall et al 2004).

Faecal shedding of E. coli by companion animals con- stitutes an important source of environmental contamination (Morato et al 2009). Animals with clinical conditions such as diarrhoea usually have immune suppression which favours increased faecal shedding of E. coli (de Almeida et al 2012). Diarrhoeic animals defecate frequently and uncontrollably, thus they tend to spread E. coli more than the non-diarrhoeic ones. Because both pathogenic and non-pathogenic E. coli isolates are potential reservoirs of antimicrobial resistance genes, their presence in diarrhoeic faeces of dogs pose serious threat to public health following zoonotic transmission; dog owners/handlers, children and veterinarians, are more at risk since they have direct close contact with these animals (Hammermueler et al 1995, Paula and Marin 2009). In many parts of the world, compromise/ complications during antibacterial therapy in dog owners were traced to acquisition of antibacterial resistance genes from E. coli colonizing companion animals (Warren et al 2001, Abatcha et al 2014).

Isolation of diarrhoeagenic antimicrobial-resistant E. coli from dogs with or without diarrhoea and/or their handlers have been reported in countries such as Italy (Carattoli et al 2005), Portugal (Costa et al 2008, Bien et al 2011), Poland (Rzewuska et al 2015), Brazil (de Almeida et al 2012, Paula and Marin 2008, Paula and Marin 2009, Siqueira et al 2009, Puno-Sarmiento et al 2013), the Netherlands (Ewers et al 2010, Ewers et al 2012), Argentina (Bentancor et al 2007), America (Shaheen et al 2011), and Egypt (Ali and Metwaly 2015, Yunis et al 2015). In the available literature, studies on pathogenic E. coli in diarrhoeic and/or healthy dogs in Iran include the reports of Zahrei Salehi et al (2011) and Koochakzadeh et al (2014). These studies detected STEC and EPEC strains in dogs with or without diarrhoea, but neither of them assessed antimicrobial resistance genotypes of the isolates. Other E. coli pathotypes have been isolated from diarrhoeic and non diarrhoeic animals elsewhere (Bentancor et al 2007, Kavitha et al 2010). Zahrei

Salehi et al (2011) only determined the phenotypic resistance profile (antibiogram) of the isolates. But phenotypic resis- tance is determined by the genotype (Morrison et al 2015). Moreover, Aslani et al (2008) characterised the virulence genes and antibiogram of E. coli isolates from diarrhoeic humans in Iran. The findings of the study showed that the E. coli isolates are diarrhoeagenic strains that can cause zoonotic infections. Therefore, further investigations are needed regarding the pathogenic potential of E. coli isolates from dogs reared in Iran and their capacity as reservoirs of antimicrobial resistance genes. Characterisation of the virulence and antibacterial resistance determinants in the E. coli isolates is necessary for empirical treatment of infections associated with these organisms. The objective of this study was to isolate and detect some virulence and antimicrobial resistance genes in E. coli isolates from dogs with diarrhoea presented to the Islamic Azad University Veterinary Teaching Hospital (IAUVTH), Iran.

MATERIAL AND METHODS

SAMPLING

This cross-sectional study was conducted between February and April, 2014. By directed sampling, a total of 70 diarrhoeic dogs of varied breeds, sex and ages (puppies and adults) presented to IAUVTHI for diagnosis and treatment were randomly selected. Prior to administration of any drug, rectal swab was collected from the dogs using sterile swab sticks. The swabs were transported aseptically in ice-packs to Microbiology Laboratory, Islamic Azad University of Shahrekord Branch, Iran and processed within 6 hours of collection.

Table 1
PCR primers used for detection of virulence genes

Virulence factor	Target virulence gene	Primers Sequence	Amplicon size (base pair)	Annealing temperature (ºC)	Reference
Shiga-like toxin	*Stx* (1)	F: 5´- CAGTTAATGTGGTGGCGAAGG- 3´ R: 5´- CACCAGACAATGTAACCGCTG- 3´	348	56	(Cebula *et al* 1995)
	Stx (2)	F: 5´- ATCCTATTCCCGGGAGTTTACG- 3´ R: 5´- GCGTCATCGTATACACAGGAGC- 3´	584		
Attaching and effacing factor	eae	F: 5´- TGCGGCACAACAGGCGGCGA- 3´ R: 5´- CGGTCGCCGCACCAGGATTC- 3´	629	56	(Heuvelink *et al* 1995)
Cytotoxic necrotizing factor	*Cnf* (1)	F: 5´- GGGGGAAGTACAGAAGAATTA- 3´ R: 5´- TTGCCGTCCACTCTCACCAGT- 3´	1111	56	(Toro *et al* 2005)
	Cnf (2)	F: 5´- TATCATACGGCAGGAGGAAGCACC- 3´ R: 5´- GTCACAATAGACAATAATTTTCCG- 3´	1240		

Cebadores de PCR utilizados para la detección de genes de virulencia

ISOLATION AND IDENTIFICATION OF E. coli ISOLATES

The rectal swabs were cultured on Mac Conkey agar [1] and incubated at 37 °C for 24 hours aerobically. On each plate that produced growth, three lactose-fermenting (pinkish) colonies were purified by sub-culturing

on fresh Mac Conkey agar and incubated at 37 °C for 24 hours. Characterization and identification of the isolates as E. coli was done by subjecting the purified isolates to Gram staining, oxidase, indole, citrate, urease, methyl-red and triple sugar iron tests and they were further evaluated for production of characteristic greenish metallic sheen by inoculating on eosin methylene blue agar [2] following standard procedures.

ANTIMICROBIAL RESISTANCE AND VIRULENCE GENOTYPE OF THE E. coli ISOLATES

DNA of 14 isolates was extracted using bacteria DNA extraction kit [3] following the manufacturer's instructions. Using Ependorf Mastercycler [4] , the presence of the following 5 virulence genes: Shiga-like toxin genes stx (1) and stx (2), attaching and effacing gene eae, and cytotoxic necrotizing factor genes cnf(1) and cnf(2) was investigated in the E. coli isolates using primers that have been described by other authors (table 1).

Table 1 shows the list of primers, annealing temperatures and predicted sizes used for the detection of virulence genes of E. coli isolated. Positive controls from the collection of the Islamic Azad University of Shahrekord Branch, Iran were included in each PCR reaction. Sterile distilled water was used as the negative controls. The analysis of the PCR products was performed in 1.5% horizontal agarose gel electrophoresis stained with ethidium bromide under UV light. The isolates were categorised based on the virulence genes they carried. The isolate that carried both stx and eae genes was considered as enterohaemorrhagic E. coli (EHEC) strain. The one that was PCR positive for only cnf gene was regarded as necrotoxic E. coli (NTEC) strain, while those that were PCR positive for only stx gene was considered Shiga-like toxin producing E. coli (STEC) strain. The presence of the following 12 antimicrobial resistance genes: streptomycin – aad (A1), tetracycline – tet (A), tet (B), trimethoprim – dfr (A1), fluorquinolone - qnr, genta- micin – aac (3)-(IV), sulfonamide – sul (1), cephalothin and chloramphenicol – cat (1) and cml (A) was investigatedin 14 of the E. coli isolates by PCR using primers that havebeen described by other authors (table 2), annealing temperaturesand predicted sizes of amplified products for primers(table 2). The positive and negative controls were sourcedfrom and used as aforementioned in each PCR reaction.

Analysis of the PCR products was performed as above.

STATISTICAL ANALYSIS

Data generated were subjected to descriptive statistics using Microsoft Excel version 2010 (Microsoft, USA) and expressed in percentages.

RESULTS

OCCURRENCE OF VIRULENCE GENES IN E. coli ISOLATES FROM DIARRRHOIEC DOGS

Out of 70 rectal swabs cultured, 33 (47.1%) gave positive growth of E. coli. Out of 14 isolates tested for the presence of virulence genes, 9 (64.3%) were positive for PCR of stx (1), 5 (35.7%) were positive for stx (2), 7 (50%) were positive for eae, and 1 (7.1%) isolate was positive for cnf (1) (figure 1). None of the isolate was positive for PCR of cnf (2). Among the 14 isolates, 7 (50%) were positive for both stx and eae (EHEC), 6 (42.9%) were positive for only stx (STEC) while 1 (7.1%) was positive for cnf (1) (NTEC) (figure 2).

Table 2
PCR primers used for detection of antimicrobial resistance genes.

Antimicrobial agent	Target resistance gene	Primers Sequence	Amplicon size (base pair)	Annealing temperature (°C)	Reference
Streptomycin	*aad* (A1)	F: 5´- TATCCAGCTAAGCGCGAACT- 3´ R: 5´-ATTTGCCGACTACCTTGGTC- 3´	447	58	(Puno-Sarmiento *et al* 2013)
Tetracycline	*tet* (A)	F: 5´- GGTTCACTCGAACGACGTCA- 3´ R: 5´-CTGTCCGACAAGTTGCATGA- 3´	577	57	
	tet (B)	F: 5´- CCTCAGCTTCTCAACGCGTG- 3´ R: 5´-GCACCTTGCTGATGACTCTT- 3´	634	56	(Puno-Sarmiento *et al* 2013)
Trimethoprim	*dfr* (A1)	F: 5´- GGAGTGCCAAAGGTGAACAGC- 3´ R: 5´- GAGGCGAAGTCTTGGGTAAAAAC- 3´	367	45	(Torkan *et al* 2015)
Fluoroquinolone	qnr	F: 5´- GGGTATGGATATTATTGATAAAG- 3´ R: 5´-CTAATCCGGCAGCACTATTTA- 3´	670	50	(Li 2005)
Gentamicin	*aac (3)*- (IV)	F: 5´- CTTCAGGATGGCAAGTTGGT- 3´ R: 5´-TCATCTCGTTCTCCGCTCAT- 3´	286	55	(Van *et al* 2008)
Sulfonamide	*sul* (1)	F: 5´- TTCGGCATTCTGAATCTCAC- 3´ R: 5´-ATGATCTAACCCTCGGTCTC- 3´	822	47	(Van *et al* 2008)
Cephalothin	*bla* (SHV)	F: 5´- TCGCCTGTGTATTATCTCCC- 3´ R: 5´-CGCAGATAAATCACCACAATG- 3´	768	52	(Van *et al* 2008)
Ampicillin	CITM	F: 5´- TGGCCAGAACTGACAGGCAAA- 3´ R: 5´-TTTCTCCTGAACGTGGCTGGC- 3´	462	47	(Van *et al* 2008)
Erythromycin	ere	F: 5´- GCCGGTGCTCATGAACTTGAG- 3´ R: 5´-CGACTCTATTCGATCAGAGGC- 3´	419	52	(Van *et al* 2008)
Chloramphenicol	*cat* (1)	F: 5´- AGTTGCTCAATGTACCTATAACC- 3´ R: 5´- TTGTAATTCATTAAGCATTCTGCC- 3´	547	55	(Van *et al* 2008)
	cml (A)	F: 5´- CCGCCACGGTGTTGTTGTTATC- 3´ R: 5´-CACCTTGCCTGCCCATCATTAG- 3´	698	55	

Cebadores de PCR utilizados para la detección de genes de resistencia a los antimicrobianos

ANTIMICROBIAL RESISTANCE GENOTYPES OF E. coli ISOLATES FROM DIARRHOEIC DOGS

Out of 14 isolates tested for the presence of antimicrobial resistance genes, 9 (64.3%) were positive for CITM gene, 6 (42.9%) were positive for aad(A1) and bla(SHV), 5 (35.7%) were positive for tet(A), dfr(A1) and cat(1), 4 (28.6%) were positive for aac (3)-IV, 3 (21.4%) were positive for both tet(B), sul(1) and cml(A), while 1 (7.1%) of the isolate was positive for ere (figure 3).

DISCUSSION

In this study, the presence of some virulence and antimicrobial resistance genes in E. coli isolates from dogs with diarrhoea in Iran was investigated. The presence of virulence and antimicrobial resistance genes in E. coli strains harbored by companion animals is of public health concern because humans are in close contact with these animals (Puno-Sarmiento et al 2013). The presence of pathogenic E. coli strains in diarrhoeic companion animals is of greater importance because of high possibility of zoonotic transmission following widespread environmental contamination with these organisms (Geser et al 2011, Nguyen and Speradio 2012). In this study, isolation of 33 (47.1%) E. coli strains from 70 diarrhoeic dogs suggested the involvement of E. coli in a sizeable percentage of canine diarrhoea in Iran. The fact that virulence genes were detected in the isolates investigated indicates that they were pathogenic E. coli strains (Bentancor et al 2007, Shahrani et al 2014). Although the serotypes of the isolates in this study were not determined, they could belong to the serogroups capable of causing zoonotic infections (Morato et al 2009, de Almeida et al 2012, Tramuta et al 2014). The 47.1% pathogenic E. coli prevalence in this study is higher when compared with 44.4, 25 and 37.1% faecal pathogenic E. coli prevalence among 45, 68 and 70 dogs with diarrhoea reported in Canada (Hammemermulaer et al 1995), Brazil (Puno-Sarmiento et al 2013) and Egypt (Ali and Metwaly 2015), respectively. In Iran, Zahraei Salehi et al. (Zahraei Salehi et al 2011) reported 10% faecal pathogenic E. coli prevalence among 100 apparently healthy/diarrhoeic dogs while Koochakzadeh et al (2014) reported 36.64% pathogenic E. coli prevalence among 79 faecal E. coli isolates from 252 equidae/canidae. In Argentina, Bentacour et al (2007) reported 15.5% faecal pathogenic E. coli prevalence among 450 dogs. These findings are also lower than the result (47.1%) of the present study. The 47.1% pathogenic E. coli prevalence in this study is however lower when compared with 66.6% pathogenic E. coli prevalence among 51 dogs with diarrhoea reported in Egypt (Yunis et al 2015). Variations in prevalence of pathogenic E. coli strains in these studies may be due to differences in the level of contamination of dogs' environment, food and drinking water, age, immune status, stage of infection and number of samples analysed (Shaheen et al 2011, Yunis et al 2015). The focus of this study however was not on predisposing factors for faecal E. coli shedding but on isolation of E. coli from dogs with diarrhoea.

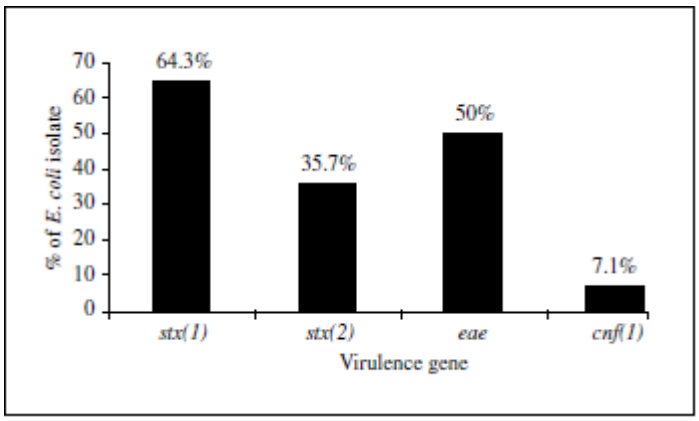

Figure 1
Frequency of occurrence of tested virulence genes in 14 E. coli isolates from dogs with diarrhoea
Frecuencia de ocurrencia de genes probados de virulencia en 14 aislados de E. coli de perros con diarrea.

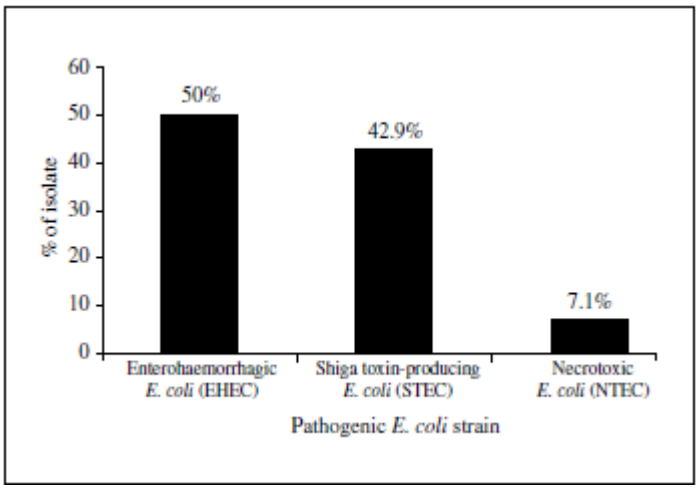

Figure 2
Distribution of pathogenic E. coli strains (pathotypes)
among 14 strains isolated from dogs with diarrhoea
Distribución de cepas patogénicas de E. coli (patotipos) entre 14 cepas aisladas de perros con diarrea

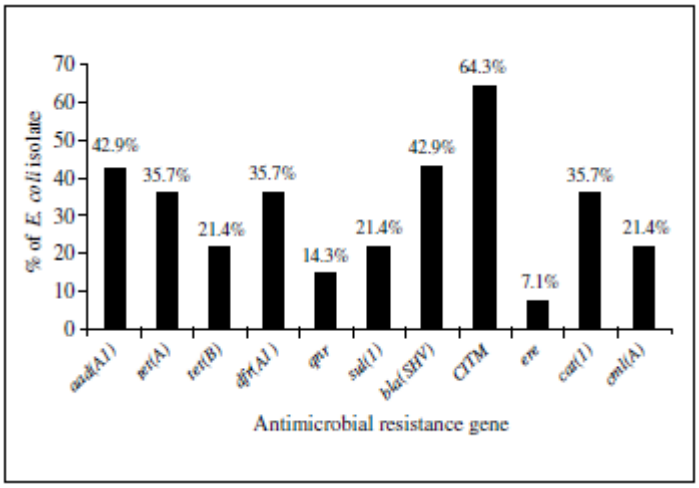

Figure 3
Frequency of antimicrobial resistance genes in 14pathogenic E. coli isolates from dogs with diarrhoea.
Frecuencia de genes de resistencia a los antimicrobianos en14 aislados patogénicos de E. coli en perros con diarrea.

In this study, the presence of 3 important virulence genes stx, eae and cnf often haboured by pathogenic E. coli were investigated in 14 isolates which were categorised into pathotypes based on the virulence genes detected. It is noteworthy that 13 (92.8%) of the 14 isolates examined haboured stx gene. The stx genes encode shiga-like toxin (stx) also called verocytotoxin/verotoxin, a putative virulent factor involved in the pathogenicity of STEC also known as verocytotoxin-producing E. coli (VTEC) and EHEC strains (Paton and Paton 1998, Goldwater et al 2012, Nguyen and Speradio 2012, Shahrani et al 2014). The stx inhibits protein synthesis and allows invasion of the intestinal mucosa similar to what is observed in human shigellosis (Nguyen and Speradio 2012). The 92.8% stx gene prevalence in this study is higher when compared with 40 and 44.4% stx gene prevalence among 92 (from 25 diarrhoeic dogs) and 20 (from 45 diarrhoeic dogs) faecal E. coli isolates reported in Brazil (Paula and Marin 2008, Paula and Marin 2009) and Canada (Hammermueler et al 1995), respectively. In Iran, Zahraei et al (2011) reported 4% stx gene prevalence among 10 pathogenic E. coli isolates from 100 apparently healthy/diarrhoeic dogs while Koochakzadeh et al (2014) reported 18.9% stx gene prevalence among 79 pathogenic E. coli isolates from a population of 252 canidae/equidae. Their findings are also lower when compared with the results (92.8%) of the present study. Detection of stx (1) in 64.3% of the isolates as against eae (50%), stx(2) (35.7%) and cnf(1) (7.1%) in this study, suggested that stx (1) may be the dominant virulence gene harbored by E. coli strains isolated from dogs with diarrhoea in Iran. The 63.4% stx(1) gene prevalence recorded in this study is higher when compared with 8.9 and 7.6% stx(1) gene prevalence among 20 and 92 E. coli isolates from dogs with diarrhoea reported in Canada (Hammermueler et al 1995) and Brazil (Paula and Marin 2008, Paula and Marin 2009), respectively. It is also higher than 12.3% stx(1) gene prevalence among 57 faecal E. coli isolates

from healthy dogs reported in Canada (Hammermueler et al 1995), and 18.9% prevalence among 79 faecal E. coli isolates from canidae/equidae reported in Iran (Koochakzadeh et al 2014). On the other hand, 35.7% stx(2) gene prevalence in this study is higher than 1.1, 22.2 and 5.4% stx(2 gene prevalence among faecal E. coli isolates from dogs reported in Argentina (Bentancor et al 2007), Canada (Hammermueler et al 1995) and Brazil (Paula and Marin 2008, Paula and Marin 2009), respectively. But it is lower when compared with 60% stx(2) gene prevalence among 34 E. coli isolates from dogs with diarrhoea reported in Egypt (Yunis et al 2015). Thus, the result of this study suggested that stx especially the stx1, may be associated with majority of canine diarrhoea in Iran in which E. coli is isolated. This finding corroborates previous reports in Iran (Zahraei et al 2011, Koochakzadeh et al 2014). The differences in the prevalence of stx genes in the aforementioned studies indicate variation in the rate of contamination and infection by E. coli strains habouring these genes in the study areas.

In the present study, detection of stx and eae in 7 (50%) of the investigated isolates enabled their placement in the EHEC group (Bentancor et al 2007, Aslani et al 2008, Goldwater and Bettelheim 2012, Nguyen and Speradio 2012, Shahrani et al 2014, Ali and Metwaly 2015). The eae gene encodes intimin which enables adhesion of the E. coli isolates to the intestinal epithelial cells resulting in the classical histopathological attaching and effacing (A/E) lesions (Nataro and Kaper 2003, Goldwater and Bettelheim 2012, Shahrani et al 2014). Interestingly, none of the isolates in this study was positive for the eae gene only. This nullifies possible involvement of EPEC/ AEEC strains in diarrhoeal disease in the sampled dogs (Bentancor et al 2007, Shahrani et al 2014). EPEC strains are defined as eae-harbouring diarrhoeagenic E. coli that possess the ability to form A/E lesions on intestinal cells and that do not possess shiga-like toxin encoding genes (Moxley and Smith 2010, Shahrani et al 2014). EPEC strains habouring the plasmid-encoded bundle forming pilli (bfp) gene, are regarded as typical EPEC (tEPEC) while bfp non-habouring strains are atypical EPEC (aEPEC) (Moxley and Smith 2010, Ali and Metwaly 2015). Since this study did not detect EPEC strains, the presence of bfp gene in the isolates was not investigated. Nonetheless, the EHEC pathotypes in this study may habour bfp gene and this needs to be further verified. On the contrary, Zahraei Salehi et al. (Zahraei Salehi et al 2011) reported that 6 (6%) isolates among 10 pathogenic E. coli isolates from dogs without diarrhoea in Iran were EPEC strains. The 50% eae gene (combined with stx gene) prevalence noted in this study is higher when compared with 13, 17.6 and 20% eae gene prevalence among 19, 12 and 34 E. coli isolates from 146, 68 and 51 dogs with diarrhoea reported in Canada (Nakazato et al 2004), Brazil (Puno-Sarmiento et al 2013) and Egypt (Ali and Metwaly 2015), respectively. It is also higher than 8 and 10.5% eae gene prevalence among 36 and 86 E. coli isolates from dogs without diarrhoea reported in Canada (Nakazato et al 2004) and Brazil (Puno-Sarmiento et al 2013), respectively. This finding further

suggests higher rate of environmental contamination and doginfection with pathogenic E. coli strains in Iran than the other study areas.

In this study, the prevalence (50%) of EHEC patho- type is higher compared against 1 (7.1%) of the isolates which haboured cnf (1) only and was regarded as NTEC (De Rycke et al 1999; Landraud et al 2000, Salvadoris et al 2003, Shahrani et al 2014), and 6 (42.9%) which haboured stx only and were grouped as STEC (Aslani et al 2008, Shahrani et al 2014). This result suggested that EHEC strains may be the predominant diarrhoeagenic E. coli pathotype isolated from dogs with diarrhoea in Iran. EHEC strains habouring highly conserved plasmid families encoding for multiple virulence have been described (Wood et al 1986, Hales et al 1992, Nataro and Kaper 2003). EHEC are diarrhoeagenic strains incriminated in different types of diarrhoea in humans (Aslani et al 2008, Amisano et al 2011, Goldwater and Bettelheim 2012, Nguyen and Speradio 2012). Thus, isolation of EHEC from dogs with diarrhoea in this study, portends public health risk particularly to individuals that could have direct or indirect contact with these dogs (Nguyen and Speradio 2012). The 50% EHEC prevalence recorded in the present study is higher than 0.22% EHEC prevalence among 70 pathogenic E. coli isolates from 450 dogs reported in Argentina (Bentancor et al 2007). However, lack of EHEC detection in previous studies (Zahraei et al 2011, Koochakzadeh et al 2014) in Iran is attributed to the fact that the authors classified isolates which haboured both stx and eae genes as STEC strains. The stx is a major virulent factor involved in pathogenicity of the EHEC and STEC/ VTEC pathotypes (Paton and Paton 1998, Nguyen and Speradio 2012, Shahrani et al 2014). STEC strains have been associated with diarrhoea in dogs (Paton and Paton 1998, Paula and Marin 2008, Zahraei et al 2011). The 42.9% STEC prevalence observed in the present study is higher when compared with 13% STEC prevalence among 92 E. coli isolates from 25 dogs with diarrhoea reported in Brazil (Paula and Marin 2008, Paula and Marin 2009). It is also higher than 6% STEC among 10 pathogenic E. coli isolates from 100 healthy/diarrhoeic dogs reported in Iran (Zahraei et al 2011). In Turkey, Sancak et al (2004) reported a lower STEC prevalence of 24.6 and 28% among 57 and 82 dogs with acute and chronic diarrhoea, respectively. Thus, higher prevalence of STEC in this study suggested that the environment and/ or food and drinking water of dogs in the present study could have been contaminated with STEC strains more than in the other study areas (Nguyen and Speradio 2012). The health status of the dogs and duration of infection (Sancak et al 2004) might also have affected the reported prevalence in the various studies. The finding of high STEC (42.9%) and EHEC (50%) prevalence in this study, portends serious threat to public health since STEC and EHEC strains causes highly fatal and untreatable infections such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) which causes renal failure in humans especially in children (Bentancor et al 2007, Amisano et al 2011, Goldwater et al 2012, Nguyen and Speradio 2012, Shahrani et al 2014). Although the EHEC isolates in this study were not serotyped, their zoonotic significance

cannot be ruled out since both EHEC O157 and non-O157 EHEC strains are known causes of HC and HUS (Goldwater and Bettelheim 2012).

The 7.1% NTEC strains observed in this study suggested that it may be the least predominant E. coli pathotype isolated from dogs with diarrhoea in Iran. Pathogenicity of NTEC strains is based on elaboration of cnfs as well as other virulent factors (Kavitha et al 2010, Shahrani et al 2014). The cnf(1) gene encodes cnf1, a toxin which interfere with the pahagocytic activities of polymorphonuclear cells thereby facilitating blood stream invasion by E. coli with subsequent apoptosis of intestinal epithelial cells (Emödy et al 2003, Kavitha et al 2010, Koochakzadeh et al 2014, Shahrani et al 2014). None of the isolate investigated in this study haboured cnf(2) gene which encodes cnf2 (Kavitha et al 2010). This suggested that all the NTEC strains obtained in this study belonged to the NTEC-1 pathotype (Kavitha et al 2010). Based on the type of cnf gene haboured, NTEC strains are grouped into two distinct homogenous categories NTEC-1 and NTEC-2, each of them being genetically linked to several other specific virulence markers (Kavitha et al 2010). The 7.1% cnf (1) gene prevalence in this study is lower when compared with 16.4% cnf(1) gene prevalence among 55 faecal E. coli isolates from healthy dogs reported by Siqueria et al (2009) in Brazil. Variation in NTEC-1 strain prevalence in these studies could also be due to differences in level of environmental, food and/or drinking water contamination by NTEC-1 strains in the study areas. Therefore, the environment of dogs in the present study could have been contaminated more with the organisms which resulted in higher infection and isolation rate.

Resistance to antimicrobial agents is encoded by chromosomal and plasmid genes haboured by bacterial organisms (Tenover 2006). These genes may be inherent or acquired via vertical or horizontal transfer (transformation, conjugation and transduction) mechanisms (Tenover 2006). Phenotypic resistance is determined by the genotype (Morrison and Rubin 2015). The aad(A1) and aac3-(IV) genes encode aminoglycoside adenyltransferases and acetyltranferaseses which mediate resistance to streptomycin and gentamicin, respectively (Szczepanowski et al 2009). These genes were detected in this study indicating that the isolates are aminoglycoside-resistant strains. Detection of aad(A1) gene in 6 (42.9%) of the investigated isolates as against 4 (28.6%) for aac3-(IV) gene, suggested acquisition of streptomycin resistance gene more than gentamicin resistance gene. The high acquisition of aad(A1) gene may be a result of selection pressure due to frequent use of streptomycin which is often combined with penicillin to elicit broad-spectrum action, in treating bacterial infections in companion animals. In this study, the presence of tetracycline resistance genes tet(A) and tet(B), showed that the isolates possessed multiple tetracycline determinants. The tet(A) and tet(B) genes are among several tetracycline determinants in E. coli which encode energy-dependent membrane-associated efflux proteins (Roberts 2005). Detection of tet(A) in 5 (35.7%) of the examined isolates as against 3 (21.4%) for tet(B) suggested that tet(A) may be

the predominant tetracycline resistance gene haboured by pathogenic E. coli colonising dogs in Iran. Other tetracycline-resistant genes which are thought to confer resistance through ribosomal protection and enzymatic inactivation (Nde and Logue 2008, Torkan et al 2015) may also be haboured by the tetracycline-resistant gene-positive isolates in this study. However, the presence of these other genes was not verified in this study.

The emergence of β-lactam-resistant bacteria in companion animals and their transfer to humans pose serious risk to public health (Hammermueler et al 1995, De Rycke et al 1999). In this study, the presence of two determinants (CITM gene cluster and bla(SHV) gene) for β-lactam resistance in the isolates was investigated. Detection of CITM gene cluster in 9 (64.2%) of examined isolates suggested that among all the resistance genes tested, it is the most predominant. The high prevalence of CITM gene cluster may be a result of selection due to frequent exposure to β-lactams especially ampicillin. Beta-lactams are widely used in veterinary medicine for treating infections caused by E. coli in companion animals (Li et al 2007). In E. coli, the CITM gene cluster encodes AmpC β-lactamase which hydrolyses β-lactams (Van et al 2008). Detection of bla(SHV) in 6 (42.9%) of the examined isolates, suggested high prevalence of this SHV β-lactamase-encoding gene (Feria et al 2002, Ojdana et al 2014). The bla(SHV) gene encodes β-lactamase which mediates resistance to cephalothin, a first-generation cephalosporin. However, some variants of bla(SHV) encode extended-spectrum β-lactamase which hydrolyses third-generation cephalosporins (extended-spectrum β-lactams) (Bradford 2001, Bush and Jacoby 2010, Ojdana et al 2014), these variants have been reported in faecal E. coli isolates from dogs (Rocha-Gracia et al 2015, Schmidt et al 2015). Therefore, the 42.9% bla(SHV) detection rate in this study suggested that many dogs with diarrhoea in Iran may harbor extended-spectrum β-lactam (ESBL)-resistant E. coli. This finding is a cause for concern because extended-spectrum β-lactams are critical for treatment of bacterial infections in humans and animals (Bradford 2001) and E. coli iso- lates habouring bla(SHV) have been reported to exhibit multidrug resistance (Branger et al 2005, Bush and Jacoby 2010, Geser et al 2011). Thus, the presence of bla(SHV) gene in the examined isolates in this study, pose serious threat to public health as well as that of the examined dogs since compromise in antibacterial therapy may result following zoonotic transmission of the organisms (Warren et al 2001). In America, bla(SHV) was also detected in E. coli isolates from companion animals but with a lower 17% prevalence (Shaheen et al 2011).

The detection rate (14.3%) of fluoroquinolone determinant qnr gene in this study is surprising because fluoroquinolones are not known to be used in canine medicine in Iran. Nevertheless, the isolates could have acquired the gene from bacterial organisms from other sources. The presence of qnr gene in isolates in this study poses threat to public health. This is because qnr-plasmids are often associated with integrons and they carry multiple resistance determinants, thus providing resistance to several classes of antimicrobials including β-lactam and aminoglycoside

(Kang et al 2005, Li 2005). In this study, the trimethoprim determinant dfr (A1) gene was haboured by 5 (35.7%) of the examined isolates. This rate of trimethoprim resistance gene acquisition is high, and may be due to selection resulting from frequent use of sulfonamide/ trimethoprim combination (due to its broad-spectrum activity) in small animal medicine (Antunes et al 2005, Torkan et al 2015). This reason may also explain the 28.6% prevalence of sul(1) gene in the examined isolates. The dfr(A1) gene is one of the variants of dfr gene.; in E. coli it encodes dihydrofolate reductase (DHFR), thus countering the inhibitory effect of trimethoprim (Szczepanowski et al 2009). The sul(1) gene is among the sulfonamide determinants encoding dihydropteroate synthase (DHPS) which is not inhibited by sulfonamide in E. coli (Enne et al 2001). Detection of erythromycin determinant ere gene in 1 (7.1%) of the examined isolates, suggested that the gene was acquired at a low rate by the isolates. The low ere gene prevalence in this study may be related to the fact that erythromycin is not used for treatment of infections caused by Gram- negative organisms. Therefore, there may not have been selection pressure to necessitate acquisition of ere gene which encodes erythromycin methylases, the mediators of resistance to macrolides (Landraud et al 2000, Gaynor and Mankin 2003). In the current study, detection of cat(1) gene in 5 (35.7%) and cml(A) gene in 3 (21.4%) of the examined isolates, suggested that the isolates haboured different chloramphenicol determinants. The prevalence of these genes suggests that cat(1) gene may be the predominant chloramphenicol determinant haboured by E. coli isolates from dogs with diarrhoea in Iran. The high prevalence of these genes may be due to use selection pressure which resulted in acquisition of the genes at a high rate. In E. coli, the cat(1) gene is a variant of cat genes encoding chloramphenicol acetyltranferases, the major mediators of chloramphenicol resistance (Schwarz et al 2004, Torkan et al 2015) while the cml(A) is among the genes encoding chloramphenicol efflux proteins (exporters) (Schwarz et al 2004).

It is concluded that E. coli isolates from dogs with diarrhoea presented to IAUTH, Iran haboured various virulence and antimicrobial resistance genes. The isolates belonged to the EHEC, STEC and NTEC pathotypes with the EHEC strain being the most prevalent. The CITM gene cluster is the predominant antimicrobial resistance determinant haboured by the examined isolates. The bla(SHV) gene which confers resistance to β-lactams including extended-spectrum β-lactams was detected in some of the examined isolates. Thus, antibacterial-resistant diarrhoeagenic E. coli strains are possible offenders in diarrhoeal diseases of dogs reared in Iran. This poses serious threat to public health following zoonotic transmission. However, further molecular studies to detect other virulent and antimicrobial resistance genes in the isolates obtained in this study is recommended. This study is the first report on detection of cnf(1) gene in E. coli isolates from companion animals in Iran.

Acknowledgements

The authors would like to acknowledge the valuable contribution of Dr. Hassan Momtaz and Dr. Elahe Tajbakhsh.

References

Abatcha MG, Z Zunita, Dk Gurmeet, KL Thong 2014. Occurrence of antibiotic resistant Salmonella isolated from dogs in Klang Valley, Malaysia. Malays J Microbiol 10, 219-224

Ali DA, A Metwaly. 2015. Characterization of enteropathogenic E. coli and antibiotic resistance properties in diarrheic pets. Alex J Vet Sci 45, 99-104.

Amisano G, S Fornasero, G Migliaretti, S Caramello, V Tarasco, F Savino. 2011. Diarrheagenic Escherichia coli in acute gastroenteritis in infants in North-West, Italy. New Microbiol 34, 45-51.

Antunes P, J Machado, JC Sousa, L Peixe. 2005. Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese Salmonella enteric strains and relation with integrons. Antimicrob Agents Chemother 49, 836-839.

Aslani MM, S Salmanzadeh-Ahrahbi, YM Ahlikani, F Jafari, RM Zali, M Mani. 2008. Molecular detection and antimicrobial resistance of diarrheagenic Esherichia coli isolated from diarrheal cases. Saudi J Med 29, 388-392.

Bentancor A, MV Rumi, MV Gentilini, C Sardoy, K Irino, A Agostini, A Cataldi. 2007. Shiga toxin-producing and attaching and effacing Escherichia coli in cats and dogs in a high hemolytic uremic syndrome incidence region in Argentina. FEMS Microbiol Lett 267, 251-256. Beutin L. 1999. Escherichia coli as a pathogen in dogs and cats. Vet Res 30, 285-298.

Beutin L. 1999. Escherichia coli as a pathogen in dogs and cats. Vet Res 30, 285-298.

Bien J, O Sokolova, P Bozko. 2011. Role of uropathogenic Escherichia coli virulence factors in development of urinary tract infection and kidney damage. Int J Nephrol 2012, 681473. doi:10.1155/2012/681473

Bradford PA. 2001. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14, 933-951.

Branger C, O Zamfir, S Geoffoy, G Laurans, G Ariet, HV Thien, S Gouriou, B Pichard, E Denamu. 2005. Genetic background of Escherichia coli and extended spectrum beta-lactamase type. Emerg Infect Dis 11, 54-61.

Bush K, GA Jacoby. 2010. Updated functional classification of beta- lactamases. Antimicrob Agents Chemother 54, 969-97

Carattoli A, S Lovari, A Franco, G Cordaro, P Di Matteo, A Battisti. 2005. Extended-spectrum betalactamases in Escherichia coli isolated from dogs and cats in Rome, Italy, from 2001 to 2003. Antimicrob Agents Chemother 49, 833-835

Cebula TA, WL Payne, PI Feng. 1995. Simultaneous identification of strains of Escherichia coli serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J Clin Microbiol 33, 248-50.

Costa D, P Poeta, Y Saenz, AC Coelho, S Matos, L Vinue, J Rodrigues, C Torres. 2008. Prevalence of antimicrobial resistance and resistance genes in faecal Escherichia coli isolates recovered from healthy pets. Vet Microbiol 127, 97-105.

De Almeida PMP, LR Arais, JRC Andrade, EHRB Prado, K Irino, A deMF Cerqueira. 2012. Characterization of atypical enteropathogenic Escherichia coli (aEPEC) isolated from dogs. Vet Microbiol 158, 420-424.

De Rycke J, A Milon, E Oswald. 1999. Necrotoxic Escherichia coli (NTEC): two emerging categories of human and animal pathogens. Vet Res 30, 221-233.

Emödy L, M Kerényi, G Nagy. 2003. Virulence factors of uropathogenic Escherichia coli. Int J Antimicrobial Agents 22, 29-33.

Enne VI, DM Livermore, P Stephens, LMC Hall. 2001. Persistence of sulfonamide resistance in Escherichia coli in the UK despite national prescribing restriction. Lancet 357, 1325-1328.

Ewers C, M Grobbel, I Stamm, PA Kopp, I Diehl, T Semmler T, A Fruth, J Beutlich, B Guerra, LH Wieler, S Guenther. 2010. Emergence of human pandemic O25:H4-ST131 CTXM-15 extended-spectrum beta-lactamase-producing Escherichia coli among companion animals. Antimicrob Agents Chemother 65, 651-660.

Ewers C, A Bethe, T Semmler, S Guenther, LH Wieller. 2012. Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective. Clin Microbiol Infect 18, 646-655.

Feria C, E Ferreira E, JD Correia, J Goncalves, M Canica. 2002. Patterns and mechanisms of resistance to beta-lactams and beta-lactamase inhibitors in uropathogenic Escherichia coli isolated from dogs in Portugal. Antimicrob Agents Chemother 49, 77-85.

Gaynor M, AS Mankin. 2003. Macrolide antibiotics: binding site, mechanism of action, resistance. Curr Top Med Chem 3, 949-960.

Geser N, R Stephan, P Kuhnert, R Zbinden, U Kaeppeli, N Cernela, H Haechler. 2011. Fecal carriage of extended-spectrum beta- lactamase-producing enterobacteriaceae in swine and cattle at slaughter in Switzerland. J Food Prot 74, 446-449.

Goldwater PN, KA Bettelheim. 2012. Treatment of enterohemorrhagic Escherichia coli (EHEC) infection and hemolytic uremic syndrome (HUS). BMC Med 10, 12.

Guardabassi L, S Schwarz, DH Lloyd. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. Antimicrob Agents Chemother 54, 321-332

Hales BA, CA Hart, RM Batt RM, JR Saunders. 1992. The large plasmids found in enterohemorrhagic and enteropathogenic Escherichia coli constitute a related series of transfer-defective Inc F-IIA replicons. Plasmid 28, 183-193.

Hammermueler J, S Kruth, J Prescott, C Gyles. 1995. Detection of toxin genes in Escherichia coli isolated from normal dogs and dogs with diarrhea. Can J Vet Res 59, 265-270.

Heuvelink AE, NC van de Kar, JF Meis, LA Monnens, WJ Melchers. 1995. Characterization of verocytotoxin-producing Escherichia coli O157

isolates from patients with haemolytic uraemic syndrome in Western Europe. Epidemiol Infect 115, 1-14.

Kang HY, YS Jeong, JY Oh, SH Tae, CH Choi, DC Moon, WK Lee, YC Lee, SY Seol, DT Cho, JC Lee. 2005. Characterization of antimicrobial resistance and class 1 integrons found in Escherichia coli isolates from humans and animals in Korea. J Antimicrob Chemother 55, 639-644.

Kavitha K, K Prabhakar, S Rajendran, B Uma, YL Sarayu. 2010. Isolation of necrotoxigenic Escherichia coli from paediatric patients with acute diarrhoea. J Med Microbiol 59, 503-504.

Koochakzadeh A, T Zahraei Salehi, B Nayeri Fasaei, M Askari Badouei. 2014. Detection of verotoxin (Shiga-like toxin)-producing and eae harboring Escherichia coli in some wild captive and domestic equidae and canidae. Arch Razi Inst 69, 157-163.

Landraud L, M Gauthier, T Fosse, P Boquet. 2000. Frequency of Escherichia coli strains producing the cytotoxic necrotizing factor (CNF1) in nosocomial urinary tract infections. Lett Appl Microbiol 30, 213-216.

Leclercq R. 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis 34, 482-492.

Li XZ. 2005. Quinolone resistance in bacteria: emphasis on plasmid- mediated mechanisms. Int J Antimicrob Agents 25, 453-463.

Mammeri H, M Van De Loo, L Poirel, L Martinez-Martinez, P Nordmann. 2005. Emergence of plasmidmediated quinolone resistance in Escherichia coli in Europe. Antimicrob Agents Chemother 49, 71-76

Morato EP, L Leomil, L Beutin, G Krause, RA Moura, AF Pestana de Castro. 2009. Domestic cats constitute a natural reservoir of human enteropathogenic Escherichia coli types. Zoonoses Pub Health 56, 229-237.

Morrison BJ, JE Rubin. 2015. Carbapenemase producing bacteria in the food supply escaping detection. PloS ONE 10(5), e0126717. doi:10.1371/journal.pone.0126717

Moura RA, MP Sircili, L Leomil, MH Matte, LR Trabulsi, WP Elias, K Irino, AF Pestana de Castro. 2009. Clonal relationship among atypical enteropathogenic Escherichia coli strains isolated from different animal species and humans. Appl Environ Microbiol 75, 7399-7408

Moxley RA, DR Smith. 2010. Attaching-effacing Escherichia coli infections in cattle. Vet Clin North Am Food Anim Pract 26, 29-56.

Nakazato G, C Gyles, K Ziebell, R Keller, LR Trabulsi, TA Gomes, K Irino, WD Da Silveira, AF Pestana De Castro. 2004. Attaching and effacing Escherichia coli isolated from dogs in Brazil: characteristics and serotypic relationship to human enteropathogenic E. coli (EPEC). Vet Microbiol 101, 269-277.

Nataro P, JB Kaper. 2003. Diarrheagenic Escherichia coli. Clin Microbiol Rev 11, 142-201

Nde CW, CM Logue. 2008. Characterization of antimicrobial susceptibility and virulence genes of Salmonella serovars collected at a commercial turkey processing plant. J Appl Microbiol 104, 215-223.

Nguyen Y, V Speradio. 2012. Enterohaemorrhagic E. coli pathogenesis. Front Cell Infect Microbiol 2, 90.

Ojdana D, P Sacha, P Wieczorek, S Czaban, A Michalska, J Jaworowska, A Jurczak, B Poniatowski, E Tryniszewska. 2014. The Occurrence of blaCTX-M, blaSHV, and blaTEM genes in extended-spectrum β-lactamase-positive strains of Klebsiella pneumoniae, Escherichia coli, and Proteus mirabilis in Poland. Int J Antibio 2014, Article ID 935842.

Paton JC, AW Paton. 1998. Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. Clin Microbiol Rev 11, 450-479.

Paula CJS, JM Marin. 2008. Occurrence of non-O157 Shiga toxin-producing Escherichia coli in dogs with diarrhea. Cienc Rural 38, 1682-1686.

Paula CJS, JM Marin. 2009. Multidrug-resistant Shiga toxin-producing Escherichia coli in dogs with diarrhea. Arq Bras Med Vet Zootec 61, 511-514.

Puno-Sarmiento J, L Medeiros, C Chiconi, F Martins, J Pelayo, S Rocha, J Blanco, M Blanco, M Zanutto, R Kobayashi, G Nakazato 2013. Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. Vet Microbiol 166, 676-680

Rahimi E, F Khamesipour, F Yazdi, H Montaz. 2012. Isolation and characterization of Enterohaemorragic Escherichia coli O157: H7 and EHEC O157:NM from raw bovine, camel, water buffalo, caprine and ovine milk in Iran. Kafkas Univ Vet Fak Derg 18, 559-564.

Randall LP, SW Cooles, MK Osborn, LJ Piddock, MJ Woodward. 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty five serotypes of Salmonella enterica isolated from humans and animals in the UK. J Antimicrob Chemother 53, 208-216.

Roberts MC. 2005. Update on acquired tetracycline resistance genes. FEMS Microbiol Lett 245, 195-203.

Rocha-Gracia RC, G Cortés-Cortés, P Lozano-Zarain, F Bello, Y Martínez-Laguna, C Torres. 2015. Faecal Escherichia coli isolates from healthy dogs harbour CTX-M-15 and CMY-2 β-lactamases. Vet J 203, 315-319.

Rzewuska M, M Czopowicz, M Kizerwetter-Świda, D Chrobak, B Błaszczak B, M Binek. 2015. Multidrug resistance in Escherichia coli strains isolated from infections in dogs and cats in Poland (2007-2013). Scientific World J 2015, 408205.

Salvadoris MR, GF Valadares, DS Leite, J Blanco, T Yano. 2003. Virulence factors of Escherichia coli isolated from calves with diarrhea in Brazil. Braz J Microbiol 34, 230-235.

Sancak AA, HC Rutgers, CA Hart, RM Batt. 2004. Prevalence of enteropathic Escherichia coli in dogs with acute and chronic diarrhea. Vet Rec 154, 101-106

Schmidt VM, GL Pinchbeck, T Nuttall, N McEwan, S Dawson, NJ Williams. 2015. Antimicrobial resistance risk factors and characterisation of faecal E. coli isolated from healthy Labrador retrievers in the United Kingdom. Prev Vet Med 119, 31-40

Schwarz S, C Kehrenberg, B Doublet B, A Cloeckaert. 2004. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. FEMS Microbiol Rev 28, 519-542.

Shaheen BW, R Nayak, SL Foley, O Kweon, J Deck, M Park, F Rafii, DM Boothe. 2011. Molecular characterization of resistance to extended-spectrum cephalosporins in clinical Escherichia coli isolates from

companion animals in the United States. Antimicrob Agents Chemother 55, 5666-5675

Shahrani M, FS Dehkordi, H Momtaz H. 2014. Characterization of Escherichia coli virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biolog Res 47, 28.

Siqueira AK, MG Ribeiro, DS Leite, MR Tiba, C de Moura, MD Lopes, NC Prestes, T Salerno, AV Silva. 2009. Virulence factors in Escherichia coli strains isolated from urinary tract infection and pyometra cases and from feces of healthy dogs. Res Vet Sci 86, 206-210.

Szczepanowski R, B Linke, I Krahn, KH Gartemann, T Gutzkow, W Eichler, A Puhler, A Schluter. 2009. Detection of 140 clinically relevant antibiotic resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. Microbiol 155, 2306-2319

Tajbakhsh E, F Khamesipour, R Ranbar, IC Ugwu. 2015. Prevalence of class 1 and class 2 integrons in multidrug resistant Escherichia coli isolated from aquaculture water in Chaharmahal Va Bakhtiari province, Iran. Ann Clin Microbiol Antimicrob 14, 37.

Tajbakhsh E, P Ahmadi, E Abedpour-Dehkordi, N Arbab-Soleimani, F Khamesipour. 2016. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic E. coli isolated from clinical samples in Iran. Antimicrob Resist Infect Control 5, 11.

Tenover FC. 2006. Mechanisms of antimicrobial resistance in bacteria. Am J Med 119, 3-10.

Torkan S, F Khamesipour, MU Anyanwu. 2015. Detection of virulence and antibacterial resistance genes in Salmonella isolates from diarrhoeic dogs in Iran. Revue Méd Vét 166, 221-228.

Toro CS, M Farfán, I Contreras, O Flores, N Navarro, GC Mora, V Prado. 2005. Genetic analysis of antibiotic-resistance determinants in multidrugresistant Shigella strains isolated from Chilean children. Epidemiol Infect 133, 81-86

Tóth I, F Hérault, L Beutin, E Oswald. 2003. Production of cytolethal distending toxins by pathogenic Escherichia coli strains isolated from human and animal sources: establishment of the existence of a new cdt variant (Type IV). J Clin Microbiol 41, 4285-4291.

Tramuta C, P Robino, D Nucera, S Salvarani, G Banche, A Malabaila, P Nebbia. 2014. Molecular characterization and antimicrobial resistance of faecal and urinary Escherichia coli isolated from dogs and humans in Italy. Vet Italiana 50, 23-30.

Van TT, J Chin, T Chapman, LT Tran, PJ Coloe. 2008. Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes. Int J Food Microbiol 124, 217-223.

Warren A, K Townsend, T King, S Moss, D O'Boyle, R Yates, D Trott. 2001. Multi-drug resistant Escherichia coli with extended-spectrum β-lactamase activity and fluoroquinolone resistance isolated from clinical infections in dogs. Aust Vet J 79, 621-623.

Wood PK, JG Morris, PL Small, O Sethabutr, MR Toledo, L Trabulsi, JB Kaper. 1986. Comparison of DNA probes and the Sereny test for identification of invasive Shigella and Escherichia coli strains. J Clin Microbiol 24, 498-500.

Yunis K, M Badour, MS Ibrahim. 2015. Detection of diarrheagenic Escherichia coli in pet animals and its antibiotic resistance in Alexandria governorate. Alex J Vet Sci 45, 113-118

Zahraei Salehi T, MA Badouei, IM Gohari. 2011. Molecular detection and antibacterial susceptibility of enteropathogenic Escherichia coli (EPEC) and shigatoxigenic Escherichia coli (STEC) strains isolated from healthy and diarrhoeic dogs. Comp Clin Path 20, 585-589.

Notes

1 MCA; Oxoid Basingstoke, United Kingdom
2 EMB; Basingstoke, United Kingdom
3 Cinagen, Tehran, Iran
4 Ependorf, Hamburg, Germany

Author notes

Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran. Dr_Faham@yahoo.com