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RESEARCH

Genetic Characterization of *Escherichia coli* O157:H7 Strains Isolated from the One-Humped Camel *(Camelus dromedarius)* by Using Microarray DNA Technology

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Abstract From the Camelidae family members, several serotypes of *Escherichia coli* (*E. coli*) have recently been isolated from diarrhoeic and non-diarrhoeic faecal samples. To date Shiga toxin-producing *E. coli* (STEC) strains have never been typed in one-humped camel (*Camelus dromedarius*). In the present study, two *E. coli* O157:H7 strains isolated from sick dromedaries were investigated. Virulence gene profiles were determined using a custom *E. coli* virulence DNA microarray, composed of 70-mer oligonucleotide probes targeting 264 virulence or related genes of known *E. coli* pathotypes. Both strains displayed positive hybridization signals for the Locus of enterocyte effacement (LEE) gene probes (*ler, eae, espA, espB, tir* genes), two Shiga toxin probes (*stx1* and *stx2*), the O157 O-antigen specific probe, various virulence plasmid (*pO157*) probes like *katP* in addition to other accessory virulence genes characterized in STEC.

Keywords Camelidae · *Camelus dromedarius* · *Escherichia coli* O157:H7 · EHEC · Microarray · STEC

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Introduction

Escherichia coli (*E. coli*) strains fall into four phylogenetic groups, namely A, B1, B2 and D. Group B2 and, to a lesser extent, group D, house the majority of virulent extra-intestinal *E. coli*, whilst groups A and B1 primarily represent commensal, low-pathogenic *E. coli* or enteropathogenic *E. coli* only for animal species [1].

Enterohemorrhagic *E. coli* (EHEC) represent an important pathotype of Shiga toxin-producing *E. coli* (STEC) group and can cause severe disease in humans ranging from diarrhoea and haemorrhagic colitis (HC) to haemolytic uremic syndrome and thrombocytopenic purpura [2, 3]. Although various EHEC serotypes like O26:H11, O91:H21, O111:H8, O113:H21, O157:H−, are frequently associated with human disease, *E. coli* O157:H7 is arguably the most important member of this group since this serotype is the more frequently detected during human disease outbreaks [4, 5].

Domestic ruminants like sheep, goats and particularly cattle, are considered important reservoirs for *E. coli* O157:H7 and play a role in the spread of the infection to man and other animals. Cattle are usually healthy carrier lacking any overt clinical symptoms related to the presence of this serotype in their gastrointestinal tract [6, 7]. In Iran, a large number of investigations about the presence of STEC have recently concerned both hospitalized humans

in Tehran [8, 9] and other areas of the country [10, 11], diarrhoeic and healthy calves in Tehran [12] and slaughtered sheep in Shiraz [13]. Results of these studies reported the mostly prevalence of non-O157 Shiga toxin-producing *E. coli*. However, *E. coli* O157 was found in stool specimens of children [8] and carcasses during slaughtering of sheep [13].

In Camelidae, many serotypes of *E. coli* have been isolated and their pathogenic character was investigated by rabbit ileal loop assay [14] or haemolytic activity [15]. Specific survey studies have excluded the presence of STEC in camels populations, in several countries of Eastern Africa [16] and United Arab Emirates [17]. More recently, a survey of *E. coli* O157 in camelids from public open farm in Great Britain showed instead a low prevalence of Shiga and non-Shiga toxin-producing *E. coli* O157 in Llama and Alpaca species [18].

The main genetic characteristics of virulent *E. coli* O157:H7 is the presence of one or more Shiga toxins encoded genes as well as the Locus of enterocyte effacement (LEE), a pathogenicity island essential for attachment/effacement of bacterial cells to the intestinal epithelium and the presence of plasmid pO157 [19, 20].

Microarray technology has recently been applied for phylogenetic studies of *E. coli* O157:H7 [21, 22]. An oligonucleotide microarray to characterize the virulence potential of *E. coli* isolates was developed and validated by a collection of well-characterized reference *E.coli* strains [23, 24] and applied for the pathotyping of *E. coli* isolates from environmental and animal origin [25, 26].

This communication reports, for the first time, the isolation from dromedaries with hemorrhagic diarrhoea of two strains of *E coli* O157:H7 and their genetic characterization using a custom designed virulence gene microarray. The importance of DNA microarray technology in practice diagnostic is also discussed.

Materials and Methods

Strains Isolation and Identification

Faecal samples from 3 and 4 years old dromedaries, located in Gonbad-Qabus, northeast of Tehran, both with haemorrhagic diarrhoea, were collected and *E. coli* isolation was performed according to the protocol described by Alonso et al. [27]. The serotype identifications were done using the MAST ASSURE™ pathogenic *Escherichia coli* antisera kit (Mast Diagnostics, EA). Genomic DNA was extracted from isolated strains with the AccuPrep® Genomic DNA extraction kit (BIONEER, Korea) according to the manufacturer's protocol.

DNA Labelling

Purified genomic DNA was quantified using a Nanodrop Spectrophotometer (Nanodrop Technologies, Thermo Scientific, USA) and approximately 300 ng of DNA was subjected to fluorescent labelling using the Bioprime DNA labelling system (Invitrogen Life Technologies, Burlington, Canada). Labelling efficiency and the percentage of dye incorporation was then determined by scanning the DNA sample in the Nanodrop spectrophotometer from 200 to 700 nm. Cy3 dye incorporation was calculated using a web-based percent incorporation calculator (available on web page http://www.pangloss.com/seidel/Protocols/percent_inc.html).

Virulence Oligonucleotide Microarray

The microarray version used in the present study, originally developed by Bruant et al. [24], was composed of 70-mer oligonucleotide probes targeting 264 virulence or virulence-related genes covering all known *E. coli* pathotypes.

Hybridizations and Data Acquisition

For each hybridization 500 ng of labelled DNA was dried under vacuum in a rotary desiccator without heating (Savant SpeedVac, ArrayIt, USA). Dried labelled DNA was resuspended in hybridization buffer (DIG Easy Hyb Buffer, Roche Diagnostics, Laval, Canada). Microarrays were pre-hybridized for at least one hour at 50 °C with a pre-heated pre-hybridization buffer containing 5× SSC, 0.1% SDS and 1.0% BSA. After pre-hybridization, the microarrays were hybridized with a solution that consisted of 25 µl of hybridization buffer, 20 µl of Bakers Yeast tRNA (10 mg/ml) (Sigma Aldrich, St. Louis, USA) and 20 µl of sonicated Salmon Sperm DNA (10 mg/ml) (Sigma Aldrich), mixed together with the labelled DNA which had previously been denatured. Microarrays were hybridized overnight at 50 °C in a SlideBooster (model SB800; Advalytix, Germany). After hybridization, stringency washes were performed with Advawash (Advalytix) using 1× SSC, 0.02% SDS preheated to 50 °C. Microarray slides were scanned with a ScanArrayLite fluorescent microarray analysis system (Perkin-Elmer, Mississauga, Canada) using with ScanArrayGx software (Perkin-Elmer, Foster City, USA). Fluorescent spot intensities were quantified with QuantArray Version 3.0 (Packard Bioscience, Boston, USA). All the microarrays were normalized using the same method. For each sub array, the mean value for each set of duplicate spotted oligonucleotides was divided by the correction factor taken from the negative controls spots. This value was then divided by the average of the empty spots to create a signal-to-noise ratio. Oligonucleotide

spots with a signal-to-noise fluorescence ratio greater than the established threshold (3 in this case), were considered positive. These ratios were then converted into binary data where a value of 0 indicates a negative probe and a value of 1 a positive probe. A threshold of 3 was chosen because it best represented spot quantification. At least three arrays were hybridized to each strain and the six technical replicate points (two per array) were pooled. At least five probes of the six gene probes had to be positive before a positive score was considered.

Results

Strains Identification

The microarray data analysis of isolates confirmed the phenotypic features observed in the cultural and serotyping essays (lack of β-glucuronidase activity and delayed D-sorbitol fermentation, agglutination with O157:H7 specific monovalent antisera). The categorized virulence genes listed in Table 1 provide further support for the EHEC O157:H7 serotypes as both strains displayed positive hybridization signals for the complete locus of enterocyte effacement (LEE) gene probes (*ler, eae, espA, espB, tir* genes), two Shiga toxin probes (*stx1* and *stx2*), the O157 O-antigen specific probe, various virulence plasmid (pO157) probes like *katP* in addition to other accessory virulence genes characterized (e.g. the type II secretion pathway gene *etpD*) in *E. coli*.

Genetic Characterization

The *E. coli* isolates fell into two different phylogenetic groups. Strain 1 was from the D phylogroup (*chuA* positive and *yjaA* negative) whilst the strain 2 was from phylogroup B2 (*chuA* and *yjaA* positive). Although both isolates are O157 EHECs, strain 2 has approximately eight times the number of total positive virulence scores on the microarray (98 positive probe signals vs. 12, data not shown) and is just short of possessing a complete uropathogenic or meningitis-associated pathotype profile. The missing markers genes for these pathotypes are *kpsM(II)* or *kpsM(III)*.

Discussion

The microarray technology applied to genetic characterization of *E. coli* proved suitable for the purpose of pathotyping field strains of camelid origin, confirming that the one-humped camel is a susceptible host of STEC infection, similar to other no-camel camelid species as Llama and Alpaca. The two isolates did belong to phylogenetic groups B2 and D, which have also been detected in cases of human infection. In fact, according to the classification established by Zhang et al. [22], the examined *E. coli* strains belong to the lineage I (presence of *stx2* and *nle* genes), which are most commonly associated with human disease.

Comparative and epidemiological studies indicate that *E. coli* O157:H7 descends from the enteropathogenic *E. coli* O55:H7 (EPEC pathotype) by acquisition, in different evolutionary steps, of *stx2* and *stx1*-encoding bacteriophages, plasmid pO157 and loss of their ability to ferment D-sorbitol and beta-glucoronidase activity [21, 28]. The production of Shiga toxins and ability to induce attaching and effacing lesions are considered essential in the pathogenesis of *E. coli* O157:H7 infection but, for inducing severe disease in humans, the possession of pO157 is required. Both strains examined in the present study have a complete set of Shiga toxin genes and the locus of enterocyte effacement genes. They also possess plasmid genes related to pO157 like the EHEC haemolysin gene (*ehx*) required for haemolysin synthesis, the EHEC-catalase/peroxidase gene (*katP*) that may help O157:H7 to colonize the host intestine in deprived oxygen conditions, the type II secretion system (*etp*), and the serine protease gene (*espP*) that influences the adherence to bovine intestinal epithelial cells and the degradation of human coagulation factor V, contributing to intestinal haemorrhages observed in HC patients [28].

No specific research has been performed in Iran on the presence of STEC strains in camels before the research described in this article. However, the STEC infections are present in Iran in humans and domestic animals, and O157:H7 serotype has been isolated in humans and sheep. The same factors (related to the STEC, to the environment and to the camel themselves) identified as possible explanations of the absence of STEC isolations (including *E. coli* O157:H7) in camels in northeast African countries [16], could be at work in Iran as well. However, our results can provide new evidence on the role of the camel in the human Shiga toxin-producing *E. coli* outbreaks.

Concerning the advantages of the use of microarrays-based technology, the recent outbreak of haemolytic uraemic syndrome in Germany and France, where a typical enteroaggregative *E. coli* had acquired the bacteriophage encoding *stx2* gene [29], encourages the adoption of DNA microarrays-based technology as rapid method for the determination of virulence genes of *E. coli* isolates, before molecular sequencing. In addition, during health alert, the characterization of the virulence profiles of *E. coli* strains by DNA microarray technology can facilitate the activity of trace back investigations following STEC infections.

Table 1 List of main microarray positive virulence genes for E. coli O157:H7 strains isolated from two dromedaries in Teheran province[a]

Group of genes	Gene	Description
Common genes to all *E. coli* pathotypes	*csgE*	Assembly/transport component in curli production
	fimA	Major fimbrial subunit of type 1 fimbriae
	fimH	Adhesin of type 1 fimbriae
	hlyE	Silent haemolysin, haemolytic phenotype when over expressed
	fliC	*E. coli* flagellin, major subunit
	gad	Glutamate decarboxylase A, isozyme (amino acid catabolism and metabolism)
	ompA	Outer membrane protein (OMPA or OMPII)
	ompT(2)	Outer membrane protein 3b, other name: protease VII
	ompT	Outer membrane protein 3b, other name: protease VII
	artJ	L-Arginine periplasmic binding protein, supposed to be involved in virulence
	mviM	Putative virulence factor
	mviN	Putative virulence factor
	iha	Adhesin (IrgA homologue adhesin)
	espG	T3SS effector protein
	map(3)	Methionine aminopeptidase
	nleE	T3SS secreted effector
	nleH	Non-LEE-encoded effector
	eprJ	Type III secretion apparatus protein
	eivG	Type III secretion apparatus protein
	astA	Enteroaggregative *E. coli* heat-stable enterotoxin 1
	fliC(H7)	*E. coli* H7 flagellin gene
	chuA	Outer membrane receptor protein, heme utilization/transport protein
	TspE4-C2	DNA fragment of unknown function
	YjaA[b]	Conserved stress-induced protein
Common genes to EHEC and EPEC pathotypes	*Efa-1*	EHEC factor for adherence
	espP	Extracellular serine protease, autotransporter
	eae	Intimin (attaching and effacing lesions)
	eae(gamma2)	Intimin, gamma 2 variant
	espA-1	EspA protein secreted by the type III secretion system of the LEE, involved in formation of surface appendages, group I
	espA-2	EspA protein secreted by the type III secretion system of the LEE, involved in formation of surface appendages, group II
	espB-1	EspB protein secreted by type III secretion system of the LEE, groupe I
	ler	LEE transcription regulator
	escJ	T3SS structure protein
	escN	Translocator
	nleD	Non-LEE-encoded type III secreted effector
	nleF	Non-LEE-encoded type III secreted effector
	paa	Porcine attaching and effacing associated protein
	paa(ETEC)	Porcine attaching and effacing associated protein from ETEC

Table 1 continued

Group of genes	Gene	Description
EHEC genes	*stx1A*	Shiga-like toxin I, subunit A
	stx1B	Shiga-like toxin I, subunit B
	stx2A	Shiga-like toxin II, subunit A, various variants
	stx2B-1	Shiga-like toxineII,subunit B, various variants
	csgA	Cryptic curlin major subunit
	ECs1282	Probable filamentous hemagglutinin-like protein
	rtx	Putative RTX family exoprotein
	set	Probable enterotoxin (ShET homologue of S. flexneri enterotoxin)
	nleA	Non-LEE encoded effector A (type III secreted effector)
	fepC	Ferric enterobactin transport ATP-binding protein
O157 genes	*rfbE*	Perosamine synthetase
	lpfA(O157)	Major fimbrial subunit of fimbriae LPF (long polar fimbriae)
	wzy(O157:H7)	O antigen polymerase, O157 antigen
	ureD	Urease-associated protein
	nleb(O157)	T3SS secreted effector NleB homologue
	nlec(O157)	Non-LEE-encoded type III secreted effector
	nleg(O157)	T3SS secreted effector NleG-like protein
Plasmid encoded genes	*ccdB*	Cytotoxic protein, F-plasmid-encoded toxin
	etpD	Type II secretion pathway
	katP	EHEC-catalase/peroxidase
	ehxA	EHEC haemolysin gene
	L7095	Putative cytotoxin, similar to Toxin B

[a] This table lists only EHEC related genes. All other positive genes probes representing controls (16S, iudA), non-EHEC and antibiotic resistance genes are not shown

[b] *yjaA* gene was only detected in strain 2

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References

1. Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Applied and Environmental Microbiology, 66*, 4555–4558.
2. Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. *Nature Reviews Microbiology, 2*, 123–140.
3. Karpac, C. A., Li, X., Terrell, D. R., Kremer Hovinga, J. A., Lammle, B., Vesely, S. K., et al. (2008). Sporadic bloody diarrhoea-associated thrombotic thrombocytopenic purpura-haemolytic uraemic syndrome: an adult and paediatric comparison. *British Journal of Haematology, 141*, 696–707.
4. Naylor, S. W., Gally, D. L., & Low, J. C. (2005). Enterohaemorrhagic E. coli in veterinary medicine. *International Journal of Medical Microbiology, 295*, 419–441.
5. Karmali, M. A. (2004). Infection by Shiga toxin-producing *Escherichia coli*: an overview. *Molecular Biotechnology, 26*, 117–122.
6. Sheng, H., Lim, J. Y., Knecht, H. J., Li, J., & Hovde, C. J. (2006). Role of *Escherichia coli* O157:H7 virulence factors in colonization at the bovine terminal rectal mucosa. *Infection and Immunity, 74*, 4685–4693.
7. Dunn, J. R., Keen, J. E., & Thompson, R. A. (2004). Prevalence of Shiga-toxigenic *Escherichia coli* O157:H7 in adult dairy cattle. *Journal of the American Veterinary Medical Association, 224*, 1151–1158.
8. Salmanzadeh-Ahrabi, S., Habibi, E., Jaafari, F., & Zali, M. R. (2005). Molecular epidemiology of *Escherichia coli* diarrhoea in children in Tehran. *Annals of Tropical Paediatrics, 25*(1), 35–39.
9. Jafari, F., Garcia-Gil, L. J., Salmanzadeh-Ahrabi, S., Shokrzadeh, L., Aslani, M. M., Pourhoseingholi, M. A., et al. (2009). Diagnosis and prevalence of enteropathogenic bacteria in children less than five years of age with acute diarrhea in Tehran children's hospitals. *Journal of Infection, 58*(1), 21–27.
10. Bonyadian, M., Momtaz, H., Rahimi, E., Habibian, R., Yazdani, A., & Zamani, M. (2010). Identification and characterization of Shiga toxin-producing *Escherichia coli* isolates from patients with diarrhea in Iran. *Indian Journal of Medical Research, 132*, 328–331.
11. Aslami, M. M., & Bouzari, S. (2009). Characterization of virulence genes of non-O157 Shiga toxin-producing *Escherichia coli* isolates from two provinces of Iran. *Japanese Journal of Infectious Diseases, 62*(1), 16–19.
12. Badouei, M. A., Salehi, T. Z., Khorasgani, M. R., Tadjbakhsh, H., Brujeni, G. N., & Nadalian, M. G. (2010). Virulence gene profiles and intimin subtypes of Shiga toxin-producing *Escherichia coli* isolated from healthy and diarrhoeic calves. *Veterinary Record, 167*(22), 858–861.
13. Tahamtan, Y., Hayati, M., & Mehdi Namavari, M. (2010). Contamination of sheep carcasses with verocytotoxin producing *Escherichia coli* during slaughtering. *Transboundary and Emerging Diseases, 57*(1–2), 25–27.

14. Shuchismita Chatterjee Kashyap, S. K. (2005). Pathogenicity testing of *Escherichia coli* isolates of livestock and poultry. *Veterinary Practitioner, 6*(2), 105–108.
15. Seleim, R. S., & El-Sebaey, W. A. F. (2004). Camel-calves diarrhoea instigated by *Escherichia coli* and detection of some virulence-associated markers. *Veterinary Medical Journal Giza, 52*, 153–163.
16. El-Sayed, A., Ahmed, S., & Awad, W. (2008). Do camels (*Camelus dromedarius*) play an epidemiological role in the spread of Shiga Toxin producing *Escherichia coli* (STEC) infection? *Tropical Animal Health and Production, 40*, 469–473.
17. Moore, J. E., McCalmont, M., Jiru, Xu, Nation, G., Tinson, A. H., Crothers, L., et al. (2002). Prevalence of faecal pathogens in calves of racing camels (*Camelus dromedaries*). *Tropical Animal Health and Production, 34*, 283–287.
18. Featherstone, C. A., Foster, A. P., Chappell, S. A., Carson, T., & Pritchard, G. C. (2011). Verocytotoxigenic *Escherichia coli* O157 in camelids. *Veterinary Record, 168*, 194–195.
19. Lim, J. Y., Hong, J. B., Sheng, H., Shringi, S., Kaul, R., Besser, T. E., et al. (2010). Phenotypic diversity of *Escherichia coli* O157:H7 strains associated with the plasmid O157. *Journal of Microbiology, 48*, 347–357.
20. Lim, J. Y., Yoon, J., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology, 20*, 5–14.
21. Wick, L. M., Qi, W., Lacher, D. W., & Whittam, T. S. (2005). Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. *Journal of Bacteriology, 187*, 1783–1791.
22. Zhang, Y., Laing, C., Steele, M., Ziebell, K., Johnson, R., Benson, A. K., Taboada, E., & Gannon V. P. (2007). Genome evolution in major *Escherichia coli* O157:H7 lineages. *BMC Genomics*, *8*, 121. http://www.biomedcentral.com/1471-2164/8/121.
23. Afset, J. E., Bruant, G., Brousseau, R., Harel, J., Anderssen, E., Bevanger, L., et al. (2006). Identification of virulence genes linked with diarrhea due to atypical enteropathogenic *Escherichia coli* by DNA microarray analysis and PCR. *Journal of Clinical Microbiology, 44*, 3703–3711.
24. Bruant, G., Maynard, C., Bekal, S., Gaucher, I., Masson, L., Brousseau, R., et al. (2006). Development and validation of an oligonucleotide microarray for detection of multiple virulence and antimicrobial resistance genes in *Escherichia coli*. *Applied and Environmental Microbiology, 72*, 3780–3784.
25. Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T. A., Bekal, S., et al. (2006). A virulence and antimicrobial resistance DNA microarray detects a high frequency of virulence genes in *Escherichia coli* isolates from great lakes recreational waters. *Applied and Environmental Microbiology, 72*, 4200–4206.
26. Tonelli, A., Badagliacca, P., Bruant, G., Letizia, A., Di Provvido, A., Harel, J, & Scacchia, M. (2008). Genetic characterization of rabbit *Escherichia coli* strains with the use of microarray technology. In Proceedings 9th World Rabbit Congress, Verona, Italy, June 10–13, 2008. World Rabbit Science Association, Valencia, Spain, 1097–1101.
27. Alonso, J. L., Soriano, A., Carbajo, O., Amoros, I., & Garelick, H. (1999). Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5 °C. *Applied and Environmental Microbiology, 65*, 3746–3749.
28. Lim, J. Y., La, H. J., Sheng, H., Forney, L. J., & Hovde, C. J. (2010). Influence of plasmid pO157 on *Escherichia coli* O157:H7 Sakai biofilm formation. *Applied and Environmental Microbiology, 76*, 963–966.
29. Scheutz, F., Møller Nielsen, E., Frimodt-Møller, J., Boisen, N., Morabito, S., Tozzoli, R., Nataro, J.P, & Caprioli, A. (2011). Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. *Euro Surveillance, 16(24),* 19889. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19889.