Pathotyping of diarrhoeagenic cattle Escherichia coli strains isolated in the Province of Tehran, Iran

Hamid Staji¹, Pietro Badagliacca²*, Taghi Zahraei Salehi³, Federica Lopes³, Mariangela Iorio², Alfreda Tonelli² and Luke Masson⁴

¹ Department of pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.  
² Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise ‘G. Caporale’, Campo Boario, 64100 Teramo, Italy.  
³ Veterinary Microbiology department, Faculty of Veterinary Medicine, Tehran university, Tehran, Iran.  
⁴ National Research Council of Canada, 6100 Royalmount, Montreal, QC, Canada H4P 2R2.

* Corresponding author at: Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise ‘G. Caporale’, Campo Boario, 64100 Teramo, Italy.  
Tel.: +39 0861 332415, Fax: +39 0861 332251, e-mail: p.badagliacca@izs.it.

Keywords  
Cattle,  
Escherichia coli,  
ExPEC,  
Iran,  
InPEC,  
Microarray.

Summary  
An oligonucleotide DNA microarray targeting 348 virulence factors and genetic markers was used in the pathotyping, serotyping and phylogrouping of 51 Escherichia coli strains isolated from faecal samples. The samples were collected from diarrhoeic 1 to 30 days old calves located at 14 farms in the Tehran province, Iran. Positive microarray signals for genes encoding the Locus of Enterocyte Effacement (LEE), the Type III Secretion System (TTSS), and the absence of EPEC adherence factor (EAF) permitted the pathotyping of 25 strains as atypical Enteropathogenic (aEPEC) or Enterohaemorrhagic Escherichia coli (EHEC). The lack of LEE and TTSS‑associated genes distinguished the remaining 26 strains, which were classified as Extraintestinal pathogenic E. coli (ExPEC). Atypical EPEC belonged to phylogroup B1 and possessed a LEE profile tir‑1, eae(betα), espA‑1, espB‑3. The EHEC strains primarily belonged to the B1 phylogroup type‑O26 and possessed either a LEE profile tir‑1, eae(betα), espA‑1, espB‑3, or a B1 type‑O111, LEE tir‑3, eae(gammα), espA‑1, espB‑2. ExPEC‑typed strains generally harboured genes localised to the constant region of Colicin V plasmid (pColV), including increased serum survival factor (iss), complement resistance protein (traT), aerobactin operon (iucD), and the siderophore receptor (iroN). The microarray platform used in this study is well suited to accurately and rapidly type attaching and effacing E. coli (AEEC‑types), thus providing a database for the meta‑analysis of ExPEC‑typed strains.

Veterinaria Italiana 2017, 53 (4), 345‑356. doi: 10.12834/VetIt.728.3521.1  
Accepted: 17.11.2015  |  Available on line: 29.12.2017

Patotipi di Escherichia coli isolati da vitelli diarroidi della Provincia di Teheran, Iran

Pietro Badagliacca*  
Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise ‘G. Caporale’, Campo Boario, 64100 Teramo, Italy.  
Tel.: +39 0861 332415, Fax: +39 0861 332251, e-mail: p.badagliacca@izs.it.

Parole chiave  
Bovino,  
Escherichia coli,  
ExPEC,  
Iran,  
InPEC,  
Microarray.

Riassunto  
Nel presente lavoro è stato utilizzato un DNA microarray disegnato con sonde oligonucleotiche nei confronti di 348 fattori di virulenza e marker genetici per determinare l’appartenenza al patotipo, al sierotipo e al filogruppo di 51 ceppi di Escherichia coli isolati da campioni di fegato. I campioni sono stati raccolti da vitelli con diarrea, di età compresa tra 1 e 30 giorni, provenienti da 14 allevamenti situati nella provincia di Teheran, Iran. La positività dell’ibridizzazione nei confronti di geni codificanti il Locus di Enterocyte Effacement (LEE) e il Type III Secretion System (TTSS), e l’assenza di segnale nei confronti del fattore di adherenza di E. coli (EAF), ha permesso di determinare l’appartenenza di 25 ceppi al patotipo eEPEC (atypical Enteropathogenic E. coli) o EHEC (Enterohaemorrhagic E. coli). L’assenza di segnale nei confronti di geni del LEE e di geni TTSS-associati hanno caratterizzato i restanti 26 ceppi, classificati come ExPEC (Extraintestinal Pathogenic E. coli). I ceppi eEPEC possedevano i marker genetici del filogruppo B1 e il loro profilo LEE è risultato caratterizzato dalle seguenti varianti genetiche: tir‑1, eae(betα), espA‑1, espB‑3. I ceppi EHEC sono risultati prevalentemente classificati nel filogruppo B1 e il loro profilo LEE è risultato caratterizzato dalle varianti tir‑1, eae(betα), espA‑1, espB‑3, in sierotipi O26, o dalle varianti tir‑3, eae(gammα), espA‑1, espB‑2, in sierotipi O111. I ceppi classificati come ExPEC in generale ospitavano geni localizzati nella regione costante del plasmide Colicin V (pColV), comprendenti i geni iss (increased serum survival factor), traT (complement resistance protein), iucD (aerobactin operon) e iroN (siderophore receptor). La tecnologia microarray utilizzata in questo studio si è dimostrata
DNA microarray to pathotype Escherichia coli

Staij et al.

adatta nel tipizzare con precisione e facilità ceppi di E. coli appartenenti ai patotipi AEEC (Attaching and Effacing E. coli). Inoltre essa ha fornito un database genetico utile alla meta-analisi di ceppi ExPEC.

**Introduction**

Pathogenic *Escherichia coli* (PEC) has evolved by acquiring virulence factors through transfer of foreign DNA mediated by mobile genetic elements, such as conjugative plasmids, transposons, bacteriophages, pathogenicity islands (PAI), as well as DNA recombination. Based on histological characters and target organs, where they exert their pathogenic effects, PECs can be grouped into intestinal and extraintestinal types, also called InPEC and ExPEC (Schmidt and Hensel 2004, Johnson and Nolan 2009).

Due to their particular virulence gene content and overt diarrhoeic symptoms, InPECs are classified as enteropathogenic (EPEC), enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), and enteropathogenic *E. coli* strains differing from each other in the colonization factors and effectors normally involved in InPEC pathogenesis. Nonetheless, they share similarities with EHEC and ETEC, respectively the genetic determinants defining ExPECs. Since these strains normally colonize the gut of healthy hosts, like commensal *E. coli*, this poses the question as to whether ExPECs are essentially opportunistic pathogens. The ExPEC pathotypes lack LEE-encoding genes as well as plasmid or chromosomal-bearing colonization factors and effectors normally involved in InPEC pathogenesis. Nonetheless, they have been classified into uropathogenic (UPEC), neonatal-meningitis associated (MNEC), and septicemic *E. coli* (SEPEC) pathotypes (Russo and Johnson 2000, Köhler and Dobrindt 2011). The

**DNA microarray to pathotype Escherichia coli**

Staij et al.

adatta nel tipizzare con precisione e facilità ceppi di *E. coli* appartenenti ai patotipi AEEC (Attaching and Effacing *E. coli*). Inoltre essa ha fornito un database genetico utile alla meta-analisi di ceppi ExPEC.
ExPEC-associated virulence factors include fimbral and pilum adhesins-encoding genes (F/S fimbriae, sfa, foc; pyelonephritis-associated pili, pap; Dr family adhesins, afa, dra), invasins (ibeAB), toxins (cytolethal distending toxin, cdt; cytotoxic necrotizing factor, cnf; hemolysin, hly), surface antigens (capsule synthesis, KpsM, neuAC; enterobactin receptor, inya), iron uptake (chuA, fepC, irp, fyuA, iroN, iucD), and ColV plasmid-encoded PAI (aerobactin receptor, iutA; iron transport system, sitABCD; outer membrane, ompT; increased serum survival, iss; temperature-sensitive hemolysin, tsh; and avian hemolysin, hlyF).

Although some gene patterns have been proposed to define the UPEC (papA/C/G, hlyA, cnf1, iron acquisition and specific PAIs), SEPEC (cdtB, cnf, F17A, F165-1A, gatD, iucD), and MNEC (ibeA, neuA, neuC) pathotypes, any classification scheme should assume that a common set of virulence factors is undoubtedly required to generate a symptomatic response (Hill 2013). A possible key to understand the virulence gene composition of ExPECs comes from studies using sequenced Avian Pathogenic E. coli (APEC) genomes. Avian colibacillosis predominantly occurs in extra intestinal sites, inducing septicemia, airsacculitis-polyserositis, yolk sac infection, and salpingitis, cellulitis, resulting in acute, sub-acute and chronic diseases. The APEC genomes show remarkable similarities with the ExPEC genomes, especially UPEC strains possessing the pap fimbrial operon, yersiniabactin siderophore gene (fyuA), salmochelin siderophore gene (iroN), iron transport gene (sitABCD), and the aforementioned ColV plasmid-encoded PAI (Rodriguez-Siek et al. 2005, Johnson et al. 2007, Johnson and Nolan 2009, Kabir, 2010).

A separate classification scheme for pathogenic E. coli involves the clustering of strains into 4 phylogenetic groups based on the presence/absence of the heme transport gene chuA, the genetic marker yjaA, and the putative lipase esterase DNA fragment TspE4.C2, as proposed by Clermont and colleagues (Clermont et al. 2000). Groups A and B1 are primarily composed of commensal, low-pathogenic or enteropathogenic E. coli mainly associated with animals, while group B2 and, to a lesser extent, group D includes the majority of virulent human extra-intestinal E. coli (Carlos et al. 2010).

In Iran, the relatively few epidemiological studies and the absence of a surveillance system for diarrhoeagenic E. coli limit our understanding of the impact of pathogenic E. coli on animal and human health in this country (Jafari et al. 2012). However, data from the Iranian Nosocomial Infection Surveillance System demonstrate a high instance of urinary tract and bloodstream infections in children resulting in hospitalization (Zahraei et al. 2012). Furthermore, some recent investigations about diarrhoeagenic E. coli have focused on hospitalized human patients in Tehran and other areas of the country (Jafari et al. 2009, Bonyadian et al. 2010), diarrhoeic and healthy calves in Tehran (Badouei et al. 2010), and slaughtered sheep in Shiraz (Tahamtan et al. 2010). In these studies, non-O157 Shiga toxin-harbouring E. coli was prevalent. In contrast, E. coli O157:H7 was found in stool specimens of children (Salmanzadeh-Ahrabi et al. 2005), cattle carcasses, diarrhoeic calves (Hashemi et al. 2010, Shahrai et al. 2014), raw milk from ruminants (Rahimi et al. 2012), and camel faeces (Salehi et al. 2012). Finally, some recent studies on E. coli isolated from healthy and/or diseased poultry and classified as APEC showed virulence gene patterns composed of iss-hlyF-ompT-iroN and iss-tsh genes (Arabi et al. 2013, Kafshdouzan et al. 2013).

An oligonucleotide microarray, initially developed and validated as previously described (Bekal et al. 2003, Bruant et al. 2006, Jakobsen et al. 2011), allows for the detection of an exhaustive list of E. coli virulence genes as well as gram-negative antimicrobial resistance genes. The array, or its upgraded version, has been useful in assessing the virulence gene composition of E. coli isolated from coastal and waste waters (Frigon et al. 2013, Hill 2013), camels (Salehi et al. 2012), poultry (Bonnet et al. 2009), and rabbits (Badagliacca et al. 2016).

The present study expands our knowledge on the circulation of pathogen-associated virulence genes in the most populated area of Iran through the molecular typing of E. coli isolates by DNA microarray from outbreaks of calf colibacillosis.

**Materials and methods**

**Strains, isolation, and DNA extraction**

A collection of 51 E. coli strains was isolated from faecal samples collected from diarrhoeic 1 to 30 days old calves from 14 farms located in the Tehran province, Iran (Figure 1). Escherichia coli isolation was performed according to the protocol described by Alonso and colleagues (Alonso et al. 1999). Briefly, faecal samples were cultured on CHROMagarECC (CHROMagar Microbiology, Paris, France) and incubated at 41°C. All β-galactosidase (LAC) and β-glucuronidase positive isolates (blue colonies) were selected. Biochemically confirmation was obtained via commercial miniaturized kit. Confirmed samples were considered to be E. coli. DNA was extracted using Maxwell 16 Cell DNA Purification Kit (Promega, Milano, Italy) according to manufacturer’s protocol, and stored at -20°C until use.

**Microarray design**

The microarray version used in the present study was

DNA microarray to pathotype Escherichia coli

Staji et al.

Canada) using ScanArray Gx software (Perkin-Elmer, Foster City, CA). Fluorescent spot intensities were quantified with QuantArray Version 3.0 (Packard Bioscience). 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 (2 in this study) were considered positive. These ratios were then converted into binary data, where 0 indicates a negative probe and 1 indicates a positive probe.

Pathotype, serogroup, and phylogroup assignment rules

According to the relevant literature (Rodriguez-Siek et al. 2005, Hamelin et al. 2006, Johnson et al. 2008, Johnson and Nolan 2009, Kohler and Dobrindt 2011, Schouler et al. 2012, Hill 2013), assignment to attaching and effacing E. coli (AEEC) type was based on possessing the LEE functional domain-encoding genes (ler, tir, eae, espA, espB) and LEE or non-LEE effector protein-encoding genes (map, espG, nle family genes). To subdivide further AEEC-specific composed of 70-mer oligonucleotide probes printed in duplicate on Corning Ultra GAPS slides (Corning Canada, Whitby, Ontario, Canada), targeting 348 E. coli virulence factors, and covering all known E. coli pathotypes, including 95 antimicrobial resistance genes. The tryptophanase (tnaA), beta-glucuronidase (uidA), lactose permease (lacY), and beta-galactosidase (lacZ) genes were included as positive controls; whilst negative controls included empty buffer spots as well as genes for the green fluorescent protein of Aequoria victoria (gfp) and the chlorophyll synthase gene of Arabidopsis thaliana (At3g). Only data related to virulence or virulence-related gene hybridizations were considered in this study, since the antimicrobial resistance profiles of the same strains have been analysed separately (Badagliacca et al. 2014).

DNA labeling, hybridization and microarray data acquisition

The purified genomic DNA was quantified using a Nanodrop Spectrophotometer (Nanodrop Technologies, Celbio Srl., Milan, Italy), labelled and submitted to microarray hybridization as previously described (Badagliacca et al. 2014, Badagliacca et al. 2016). Microarray slides were scanned with a ScanArray Lite fluorescent microarray analysis system (Perkin-Elmer, Mississauga, Ontario, Canada) using ScanArray Gx software (Perkin-Elmer, Foster City, CA). Fluorescent spot intensities were quantified with QuantArray Version 3.0 (Packard Bioscience). 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 (2 in this study) were considered positive. These ratios were then converted into binary data, where 0 indicates a negative probe and 1 indicates a positive probe.
pathotypes, tEPEC or aEPEC strains either possessed or lacked the bundle forming pilus bfp family genes, respectively. Strains possessing Shiga toxin-encoding stx1 and/or stx2 family genes were classified as EHEC. The AEEC-negative InPEC strains were identified on the basis of the presence of colonization factors and toxin-encoding genes related to ETEC (coli surface antigen CS family genes, K88 F4 fimbrial-encoding gene, heat-stable enterotoxins, heat-labile enterotoxins), to EAE, DAEC (genetic markers agg, aaf, aap for pAA plasmid, genetic marker virK, capU, shf for pAA2 plasmid and daa Dr family adhesins, respectively), and to EIEC (marker for the invasion plasmid antigen, ipaH). Since characterizing ExPECs strains into specific pathotypes could be ambiguous, assignment was done on the basis of prevalent patterns related to positive signals for fimbrial factors (paa family genes, F/S fimbriae), toxins (hly, hra, cif, senB), adhesins, and invasins (afa, nfa, tib, ipaH), iron acquisition (chuA, fepC, itoN, fyuA, iut, sit, irp), capsule synthesis (kpsM, neuAC), and pColV plasmid determinants (iss, traT, ompT, tsh, and colicin structural genes).

*Escherichia coli* isolates were assigned to a serogroup based on their wzy gene for O7, O15, O22, O24, O26, O28, O45, O53, O55, O56, O59, O66, O86, O91, O98, O103, O104, O113, O114, O117, O121, O123, O126, O127, O128, O138, O139, O141, O145, O146, O147, O148, O149, O155, O157, O172, O174, and O177. Assignment to other serogroups was based on their phylogenetic group assignment was done on the basis of prevalent patterns related to positive signals for fimbrial factors (paa family genes, F/S fimbriae), toxins (hly, hra, cif, senB), adhesins, and invasins (afa, nfa, tib, ipaH), iron acquisition (chuA, fepC, itoN, fyuA, iut, sit, irp), capsule synthesis (kpsM, neuAC), and pColV plasmid determinants (iss, traT, ompT, tsh, and colicin structural genes).

*Results*

Strain screening using a complete genetic set of the LEE functional domains, the positive signals for genes encoding structural proteins of TTSS, the LEE and the non-LEE effector proteins involved in enteritis pathogenesis, and the absence of EAF plasmid determinants showed that 25 of 51 isolates were AEEC or more specifically, 4 aEPEC, and 21 EHEC/STEC pathotypes. The absence of complete LEE and TTSS-associated genes distinguished the remaining 26 strains. These strains showed genetic patterns related to the presence of fimbrial factors, toxins, adhesins, iron uptake, capsule synthesis, and colicin plasmid markers, thus classifying them as ExPEC strains. Among the ExPECs, a cluster of 13 primarily UPEC/APEC strains was differentiated from 2 other groups of APEC and undefined ExPEC strains, respectively.

**Genetic profiles of InPEC strains**

Table I and II summarize the genetic characterization of aEPEC and EHEC found in the AEEC cluster, respectively. The 4 aEPEC strains all belonged to phylogroup B1 and showed a common genetic core consisting of a LEE profile (tir-1, eae(beta), espA-1, espB-3 variants), the adhesion factor long polar fimbriae (lpfA), the lymphocyte activation inhibitor (liFA), and the TTSS-translocated effectors espG, map and/or cif, attesting to their ability to induce intestinal lesions. No correlations were found between the genetic profile and serogroup or farm of origin.

The EHEC-typed isolates, including both LEE and Shiga toxin-harbouring *E. coli* strains, were primarily from phylogroup B1, with the exception of 2 strains belonging to the B2 and 4 strains belonging to the D phylogroups. These strains all possessed the A and B subunits of stx1, and 6 of them were also positive to the A and/or subunits B of stx2. They also showed 1 or more pO157 plasmid markers (katP, ehxA, espP, iha, ureD). Their genetic set of colonization factors included the above mentioned pattern lpfA-liFA, in addition to the porcine A/E associated protein, paa. The LEE profiles concerning the prevalent B1-phylogroup O26 (7 strains) and O111 (8 strains) serogroup were tir-1, eae(beta), espA-1, espB-3, and tir-3, eae(gamma), espA-1, espB-2, respectively. The B2 or D-phylogroup O157 (2 strains), the D-phylogroup O155 (1 strains),

**Table I. Prevalent virulence factors characterizing atypical EPEC strains.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sero-type</th>
<th>Phylo-type</th>
<th>LEE functional domain profile</th>
<th>LEE and non-LEE effector</th>
<th>Colonization factors and adhesin</th>
<th>Plasmid virulence gene</th>
</tr>
</thead>
</table>

*Strain identification was coded by Farm number (from Figure 1) and letter (strain isolate); NT = non-typeable.
DNA microarray to pathotype Escherichia coli

Table II. Prevalent virulence factors characterizing EHEC strains.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sero-type</th>
<th>Phylo-type</th>
<th>LEE functional domain profile</th>
<th>Shiga toxin</th>
<th>LEE and non-LEE effector</th>
<th>Colonization factors and adhesin</th>
<th>Plasmid virulence gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-B</td>
<td>026</td>
<td>B1</td>
<td>ler, tir-1, eae(beta), espA-1, espB-3, escl, eprl</td>
<td>stx1A, stx1B</td>
<td>cif, espG, map(T1), nleA, nleB, nleC, nleD, nleE, nleF, nleG, nleH</td>
<td>paa, efa1(lfIA), lpfA</td>
<td>iha, ureD, set</td>
</tr>
<tr>
<td>3-B</td>
<td>026</td>
<td>B1</td>
<td>ler, tir-1, eae(beta), espA-1, espB-3, escl, eprl</td>
<td>stx1A, stx1B</td>
<td>cif, espG, map(T1), nleA, nleB, nleC, nleD, nleE, nleF, nleG, nleH</td>
<td>paa, efa1(lfIA), lpfA</td>
<td>espB, ehaA, katP, etp0, L7095, iha, rfbE, ureD</td>
</tr>
<tr>
<td>4-A</td>
<td>NT</td>
<td>D</td>
<td>eae, eae(gamma2), espA-2, espB-1, escl, eprl</td>
<td>stx1A, stx1B</td>
<td>map(3), nleA, nleB, nleC, nleD, nleE</td>
<td>paa, lpfA, espB, etp0, iha, rfbE, ureD</td>
<td>set</td>
</tr>
</tbody>
</table>

*Strain identification was coded by Farm number (from Figure 1) and letter (strain isolate); NT = nontypeable.

and the D-phylogroup O-antigen nontypeable (2 strains) possessed a similar LEE profile, tir-2 or tir-3, eae(gamma2), espA-2, espB-1. Furthermore, LEE- and non-LEE-TTS effector overlapping the above mentioned pattern for aEPEC strains, were also detected in the EHEC group.

Genetic profiles of ExPEC strains

In order to pathotype the 26 ExPEC strains found in our E. coli collection, Tables III to VI show the genetic profiles related to fimbrial factors, toxins, adhesins, and invasins, as well as iron acquisition, capsule
synthesis, and pColV plasmid markers encoding genes. Hybridization to the outer membrane protein gene involved in the pyelonephritis-associated pili assembly (papC), the variants of fimbrial subunit of P pili (papA), and/or the pili adhesin (papG) were used to identify a cluster of 13 strains having the genetic potential to colonize urethra, bladder, and kidney. These strains harboured genes related to the constant region of pColV plasmid, including the increased serum survival factor (iss), the complement resistance protein (traT), the aerobactin operon, iucD (in 10 of 13 strains), and the siderophore receptor,

### Table III. Virulence factors characterizing ExPEC. Prevalent profiles of UPEC/APEC strains.

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Sero-type</th>
<th>Phylo-type</th>
<th>Fimbrial factors</th>
<th>Toxins</th>
<th>Adhesins, invasins</th>
<th>Iron acquisition</th>
<th>Capsule synthesis</th>
<th>pColV determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-A</td>
<td>O121</td>
<td>B1</td>
<td>papA(11), papGI, papC, F165(1A)</td>
<td>vat, senB, ccbB</td>
<td>hra1, tibc, iheB</td>
<td>fepC, fyuA, iroN, iucD, sitAD, iutA</td>
<td>kpsM-III, neuA, neuC</td>
<td>iss, traT, tsh, cvaC, cba, cci, cma, collS</td>
</tr>
<tr>
<td>1-C</td>
<td>O111</td>
<td>B2</td>
<td>papA(11), papGI, papC, F165H(1A)</td>
<td>hlyE, senB, tibc</td>
<td>hra1, iheB</td>
<td>chuA, fepC, iroN, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-II, neuA, neuC</td>
<td>iss, traT, ompT, tsh, cvaC, cia</td>
</tr>
<tr>
<td>1-D</td>
<td>NT</td>
<td>B2</td>
<td>papA(11), papGI, papC, F165A</td>
<td>hlyE, vat</td>
<td>hra1, iheB, tibc</td>
<td>chuA, fepC, iroN, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-II, neuA, neuC</td>
<td>iss, traT, ompT, tsh, cvaC, cia</td>
</tr>
<tr>
<td>4-B</td>
<td>NT</td>
<td>B1</td>
<td>papA(11), papGI, papC, F165(1A), fmaAS4</td>
<td>hlyE, ccbB</td>
<td>hra1, iheB, tibc</td>
<td>iroN, iucD, iutA, sitAD</td>
<td>kpsM-III</td>
<td>iss, traT, sh, cvaC, collS, cba, cci, cma</td>
</tr>
<tr>
<td>9-A</td>
<td>O86</td>
<td>B1</td>
<td>papA(7-2, 11), papC, F17a, F165(1A), fmaAS4</td>
<td>hlyE, ccbB</td>
<td>hra1, iheB, tibc, fmaA, fmaB, fmaC, fmaD</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, cteA, cci</td>
</tr>
<tr>
<td>10-C</td>
<td>O8</td>
<td>B1</td>
<td>papA(12), papC, papGI, F17c</td>
<td>hlyE, ccbB</td>
<td>hra1, iheB, tibc</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-III</td>
<td>iss, traT, sh, cvaC, collS, cba, cci, cma</td>
</tr>
<tr>
<td>12-D</td>
<td>O56</td>
<td>D</td>
<td>papA(7-2, 14, 15), papGI, F17a, fmaF41a, fmaAS4</td>
<td>cdBC(2, 3, 4), cdB</td>
<td>hra1, iheB, tibc, fmaA, fmaB, fmaC, fmaD</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, collS, coll, cda, cie</td>
</tr>
<tr>
<td>12-E</td>
<td>O104</td>
<td>D</td>
<td>papA(7-2, 11, F17a, fmaF41a, F165(1A)</td>
<td>hlyE, sibB</td>
<td>hra1, iheB, tibc, fmaA, fmaB, fmaC, fmaD</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, collS, coll, cda, cie</td>
</tr>
<tr>
<td>12-F</td>
<td>O155</td>
<td>B1</td>
<td>papA(7-2, 14, 15), papC, F17b, fmaF41a, fmaAS4</td>
<td>cdBC(2, 3, 4), cdB</td>
<td>hra1, iheB, tibc, fmaA, fmaB, fmaC, fmaD</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, collS, coll, cda, cie</td>
</tr>
<tr>
<td>13-A</td>
<td>O55</td>
<td>B1</td>
<td>papA(11), papC, papGI, F165(1A)</td>
<td>hlyE, ccbB</td>
<td>hra1, iheB, tibc, fmaA, fmaB, fmaC, fmaD</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, collS, coll, cda, cie</td>
</tr>
<tr>
<td>14-A</td>
<td>O155</td>
<td>B1</td>
<td>papA(7-2, 8, 9, 14), papC, F17d</td>
<td>hlyE, ccbB</td>
<td>hra1, iheB, tibc, fmaA, fmaB, fmaC, fmaD</td>
<td>fepC, fyuA, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, collS, coll, cda, cie</td>
</tr>
</tbody>
</table>

*Strain identification was coded by Farm number (from Figure 1) and letter (strain isolate); NT = nontypeable.

### Table IV. Virulence factors characterizing ExPEC. Prevalent profiles of APEC strains.

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Sero-type</th>
<th>Phylo-type</th>
<th>Fimbrial factors</th>
<th>Toxins</th>
<th>Adhesins, invasins</th>
<th>Iron acquisition</th>
<th>Capsule synthesis</th>
<th>pColV determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-C</td>
<td>O86</td>
<td>B1</td>
<td>papA(7-2, 14), F17a</td>
<td>hlyE, senB</td>
<td>iheB, fmaDE, tibc, fmaE</td>
<td>fepC, fyuA, iroN, iucD, iutA, sitAD</td>
<td>kpsM-I</td>
<td>iss, traT, cteA, cci</td>
</tr>
<tr>
<td>7-E</td>
<td>NT</td>
<td>D</td>
<td>hlyE, iheB, fmaDE, tibc, fmaE</td>
<td>fepC, fyuA, iroN, iucD, iutA, sitAD</td>
<td>kpsM-II, neuA, neuC</td>
<td>iss, traT, sh, cvaC, cia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-B</td>
<td>NT</td>
<td>B1</td>
<td>papA(7-2, 14), fmaAS4</td>
<td>hlyE, ccbB</td>
<td>iheB, fmaDE, tibc, fmaE</td>
<td>fyuA, iutA</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, cia</td>
</tr>
<tr>
<td>11-C</td>
<td>O146</td>
<td>NT</td>
<td>papA(7-2, 14), F17b</td>
<td>hlyE, ccbB</td>
<td>hra1, iheB, fmaDE, fmaE</td>
<td>fepC, fyuA, iroN, iucD, iutA</td>
<td>kpsM-I</td>
<td>iss, traT, sh, cvaC, cia</td>
</tr>
</tbody>
</table>

*Strain identification was coded by Farm number (from Figure 1) and letter (strain isolate); NT = nontypeable.
Conclusions

A new set of virulence profiles for atypical APEC strains, in particular, for EHEC strains, is provided. The results of this study will be useful for epidemiological studies on EHEC and APEC.

Discussion

The microarray used in this study is a useful tool to sort E. coli strains into various classifications (serogroup, phylogenetic group, and pathotype), including identifying common virulence plasmids. Stool sampling only partially limited the diagnostic value of this technique with respect to direct isolation from intestinal contents. In fact, only the LEE and TTSS-harbouring strains (about 50%) could be considered as the cause of diarrhoea in calves.

In contrast, the variability and abundance of virulence patterns in a large group (at least 23 strains) of ExPEC-typed strains revealed the epidemiological value of our microarray technology when diarrheal stools are investigated. In general, the virulence gene profile of these strains was confirmed a previous study on gene profile patterns in diarrhoeagenic calves in the province of Tehran (Badouei et al. 2010). All but 2 InPEC strains contained genetic markers related to colonization (efa1/lifA); while cell effectors (nleB, nleE, nleF, nleG) have been recently considered markers in the molecular risk assessment for virulent EPEC and EHEC in human colibacillosis (Zang et al. 2007, Afset et al. 2008, Bugarel et al. 2011). The microarray used in the present study did not detect the arpA gene, encoding the ankyrin repeat protein, considered in the revised Clermont phylogenotyping method. This gene, common in avirulent E. coli strains, could have helped to better differentiate the 4 phylogroups considered in this study, including the possible assignment to phylogroup E (mostly O157:H7), F (a sister group of B2), and C, closely related to B1 strains (Clermont et al. 2013).

Table V. Virulence factors characterizing ExPEC. Prevalent profiles of atypical APEC strains.

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Sero-type</th>
<th>Phylo-type</th>
<th>Fimbrial factors</th>
<th>Toxins</th>
<th>Adhesins, invasins</th>
<th>Iron acquisition</th>
<th>Capsule synthesis</th>
<th>pColV determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-C</td>
<td>NT</td>
<td>B2</td>
<td>hlyE, ccdB</td>
<td>hla1, ileAB, tibC</td>
<td>chuA, fepC, iop2, iyuA, iucD, iutA, sitA, sitD</td>
<td>traT, ompT, tsb, cvaC, cba, ce1a, cia, cma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-A</td>
<td>O26</td>
<td>B1</td>
<td>papA(11), papC, F165(1)A</td>
<td>hlyE, ccdB</td>
<td>hla1, ileB, tibC</td>
<td>iucD, iutA, sitA, sitD</td>
<td>traT, ompT, cda, ce1a, cci</td>
<td></td>
</tr>
<tr>
<td>10-A</td>
<td>NT</td>
<td>D</td>
<td>papA(12), papGl, papGIV, focG, F17bc</td>
<td>hlyE, ccdB</td>
<td>hla1, afaDE</td>
<td>chuA, fepC, iop2, iucD, iutA, sitA, sitD</td>
<td>caa, cma</td>
<td></td>
</tr>
<tr>
<td>10-B</td>
<td>O45</td>
<td>D</td>
<td>papA(7-2, 14), papGl</td>
<td>hlyE, senB, cdb</td>
<td>ileB</td>
<td>chuA, fepC, iucD, iutA, sitA, sitD</td>
<td>kpsM-II, neuA</td>
<td></td>
</tr>
<tr>
<td>11-D</td>
<td>O155</td>
<td>B1</td>
<td>papA(7-2, 14)</td>
<td>hlyE, ccdB</td>
<td>ileB, afaE</td>
<td>fyuA, iutA</td>
<td>traT, ompT</td>
<td></td>
</tr>
<tr>
<td>13-B</td>
<td>O155</td>
<td>NT</td>
<td>tibC</td>
<td></td>
<td></td>
<td>sitA</td>
<td>kpsM-III, neuA</td>
<td>col5, cba, ce1a, cda, cna, csa, ce1a</td>
</tr>
</tbody>
</table>

* Strain identification was coded by Farm number (from Figure 1) and letter (strain isolate); NT = nontypeable.

Table VI. Virulence factors characterizing undefined ExPEC strains.

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Sero-type</th>
<th>Phylo-type</th>
<th>Fimbrial factors</th>
<th>Toxins</th>
<th>Adhesins, invasins</th>
<th>Iron acquisition</th>
<th>Capsule synthesis</th>
<th>pColV determinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-E</td>
<td>O8</td>
<td>NT</td>
<td>F17ac</td>
<td>hlyE</td>
<td>hla1, ileB, afaD</td>
<td>iucD</td>
<td>iss</td>
<td></td>
</tr>
<tr>
<td>11-A</td>
<td>NT</td>
<td>NT</td>
<td>tibC</td>
<td></td>
<td>ileB</td>
<td></td>
<td>iss</td>
<td></td>
</tr>
<tr>
<td>11-B</td>
<td>NT</td>
<td>NT</td>
<td>papA(14)</td>
<td>hlyE</td>
<td>senB</td>
<td>ileB</td>
<td>traT</td>
<td></td>
</tr>
</tbody>
</table>

* Strain identification was coded by Farm number (from Figure 1) and letter (strain isolate); NT = nontypeable.
pVir plasmid products known to cause human and bovine septicemia (Lopez-Alvarez and Gyles 1980, El Mazouari et al. 1994), including genes coding for cytotoxic necrotizing factor 2 (cnf-2), type III cytotoxic necrotizing factor 2 (cdtB-1,2,2/3,3,4), and F17abcd fimbriae. Furthermore, tibAC adhesin/invasin genes, involved in bacterial aggregation and biofilm formation, are diffusely present in UPEC/APEC-typed strains. Moreover, several of these strains also harboured the Dr family adhesins (daaE, nfaAE and drbE genes), the Prs-like fimbriae (f165 and focAG genes), and the genetic markers of pAA or pAA2 plasmids (strains 9B, 10A, 10C, 12D, and 12E; data not shown), reflecting a high level of genomic plasticity among these strains (see below).

The diffuse distribution of pColV markers, among the UPEC/APEC strains (Table III, IV and V) could reflect their avian origin. However, the prevalent avian colibacillosis-associated O1, O2 or O78 serotypes (Kabir 2010, Schouler et al. 2012) were not found in the examined strain collection. The pColV-harbouning strains 1A, 12D, 12E, and 13B showed multidrug resistance (MDR) plasmid patterns comprising aminoglycosides, sulfonamides, quinolones, and the Extended Spectrum Beta-Lactamase (ESBL) CTX- and TEM-types (Badagliacca et al. 2014, data not shown). The linkage of MDR-encoding regions with the pColV plasmid was investigated, since it provided a mean for the selection of virulent strains through the use of antibiotics (Johnson and Nolan 2009, Brzuszkiewicz et al. 2011).

The inherent genomic plasticity of E. coli was suggested as a key to understand the thin interface between commensal and human/animal (avian) pathogenic strains in the pathotyping of ExPEC isolates (Escobar-Paramo et al. 2006, Rendón et al. 2007, Clermont et al. 2011, Schouler et al. 2012). The ‘pangenome’ of E. coli is considered a genomic mosaic between a pathogen and a commensal, which evolves by gene acquisition and diversification as revealed by Rasko and colleagues (Rasko et al. 2008) following the genomic sequence of a commensal E. coli. These findings further support the idea that this dynamic genome plasticity, caused essentially by the dynamic exchange of virulence genes through mobile genetic elements like plasmids or pathogenicity islands, may highlight an evolutionary strategy for E. coli, namely the creation of a mixed assortment of virulence factors coming from various pathogenic strains (Schubert et al. 2009). This strategy could lead to serious problems in public health through the emergence of highly virulent new strains as it was dramatically illustrated by the 2011 Shiga toxin-producing E. coli O104:H4 in German and France outbreaks (Denamur et al. 2011, Scheutz et al. 2011, Rasko et al. 2011, Brzuszkiewicz et al. 2011, Zhang et al. 2013).

In conclusion, the microarray method has proven to be an easy, robust tool for the pathotyping of E. coli isolates. In particular, it was suited to accurately type attaching and effacing E. coli (AEEC-types).

Moreover, the microarray platform has provided a database for the meta-analysis of ExPEC-typed strains from diarrheal stools of calves. This database should prove useful for further analysis of gene expression (transcriptomics, RNA-seq) or for the development of predictive computational models of the biological or pathogenic significance of gene products found in commensal strains of E. coli. As mentioned above, due to rapid virulence gene movement among E. coli strains, the emergence of new pathotypes (Denamur et al. 2011, Brzuszkiewicz et al. 2011) or multiple pathotypes (Bekal et al. 2003) can occur. Simultaneously screening for such a large, comprehensive number of virulence and virulence associated genes through microarray platforms could allow for detecting novel pathogens that are easily missed by more conventional PCR methods.
References


Sheng H., Lim J.Y., Knecht H.J., Li J. & Hovde C.J. 2006. Role of *Escherichia coli* O157:H7 virulence factors in...


