Escherichia coli DERIVED FROM DIFFERENT SOURCES SHARE ANTIGENIC CHARACTERISTICS WITH Shigella boydii 18 AND VIRULENCE FACTORS WITH ENTEROTOXIGENIC E. coli

Armando Navarro¹, Carlos A. Eslava-Campos², Enrique Melendez-Herrada³ and Alejandro Cravioto⁴

¹. Department of Public Health, Faculty of Medicine, Universidad Nacional Autónoma de México.
². Laboratorio de Patogenicidad Bacteriana, Unidad de Hemato-Oncología e Investigación; Hospital Infantil de México Federico Gómez/División de Investigación, Facultad de Medicina, Universidad Nacional Autónoma de México.
³. Department of Microbiology and Parasitology, Faculty of Medicine, Universidad Nacional Autónoma de México.
⁴. Precision Global Health, Seattle, WA, USA.

Manuscript Info

Abstract

Genotypic studies have shown that enteroinvasive Escherichia coli (EIEC) and Shigella form a single pathotype sharing a common composition of carbohydrates in the lipopolysaccharide (LPS). In this study 23 E. coli strains isolated from different sources, were characterized. Serotyping was performed with 187 O and 53 H E. coli and 46 Shigella sera samples. PCR assays to lacY, lacZ, uidA, cyd, ipaH, wzx, wzy, ltA, stp, cfaI, sc3 and phylogenetic groups were performed. A serum against the E. coli 44037 strain was obtained. Serotyping of 23 strains only showed reaction against S. boydii 18 and 44037 antisera and were positive for lacY, lacZ, uidA and cyd, but negative for ipaH. The same gene analysis of S. boydii 18 strain showed positive reaction for ipaH, uidA and cyd but negative for lacZ and lacY. Both E. coli and S. boydii 18 strains amplified wzx and wzy genes. The genes ltA, stp, cfaI and sc3 were detected in 14 (61%), 6 (26%), 21 (91%) and 13 (57%) of E. coli strains. The phylogenetic analysis included the E. coli strains in: clade I (35%), and A (22%), B1 (22%), B2 (13%) and D (4%) groups. The 23 E. coli strains isolated from children with diarrhea and dairy cattle reacted against S. boydii 18 but that amplified ETEC genes, constitute a new serogroup (44037) with the serotypes 44037:NM, H2 and H16 in children and 44037:H3, H9 and H48 from dairy cattle.

Introduction:

Diarrheal diseases are an important public health problem around the world with more than 2 million related deaths being reported each year with children under 5 years of age in developing countries being particularly affected (Kosek, 2003). Escherichia coli is one of the most common etiological agents responsible for childhood diarrhea and is one of the most important public health problems in developing countries (Davidson, 2002). The traditional Shigella genus consists of four species: S. dysenteriae; S. flexneri, S. boydii and S. sonnei, or Shigella subgroups A, B, C and D (Ewing, 1986). However, recent genotyping studies have shown that the four...
species are closely related to enteroinvasive E. coli (EIEC), which suggests that distinct ancestral lineages of E. coli gave rise to EIEC and Shigella through a process of convergent evolution by acquiring of similar pathogenic characteristics (Pupo, 2000; Peng, 2006; Yang, 2007; Peng, 2009). According to a study based on analyzing housekeeping genes, both genera constitute one single pathotype of E. coli (Lan, 2004).

The identification of somatic (O) surface antigens and flagellar (H) antigens using specific antibodies has been used for many years to characterize enterobacteria antigenically (Orskov, 1984). Shigella and E. coli present a wide diversity in terms of the combination of O and H antigens with each of these combinations forming the bacterial serotype, that is to say, the serological identity of these microorganisms. For over 20 years, our laboratory has been serotyping E. coli isolated from different sources for a multitude of studies. Among these were epidemiologic studies investigating the etiology of childhood diarrhea and the colonization of dairy cattle by E. coli. Serotyping for these studies used 187 sera developed in rabbits against somatic antigens and 53 against flagella antigens, as well as 46 sera against the O antigens of the four Shigella sub-groups.

In a previous study carried out by our laboratory, we isolated E. coli strains of infantile diarrhea from different geographical regions and the results showed that these strains presented a somatic antigen similar to that of S. boydii 16. The presentation of this somatic antigen was demonstrated by agglutination reactions and sera absorption with heterologous antigens. The presence of wzx and wzy genes related to the biosynthesis of the S. boydii 16 somatic antigen corroborated the results derived from the previously mentioned tests, as did the RFLP generated profiles of the rfb gene cluster, which were similar to those of S. boydii 16 (Navarro, 2010). These strains contain genes of the ETEC pathotype suggesting that they belong to a new serogroup of E. coli, which contain somatic antigens identical to S. boydii 16. Considering the aforementioned findings in E. coli and S. boydii 16 strains, the current study aims to discover if E. coli strains that have an antigenic cross-reaction against S. boydii 18 present characteristics related to Shigella or to E. coli.

Materials and Methods:-

Strains. Of the 23 E. coli strains, 9 were isolated from children under the age of 2 years (6 with diarrhea and 3 with no diarrhea) living in a rural area of Mexico during a longitudinal study in 1986 (Cravioto, 1990); 2 strains were obtained from 2 children under 5 years of age suffering from diarrhea and being treated in the Mexican Institute of the Social Security (IMSS) in Mexico City; 4 strains were from children under 5 years of age living in Egypt (collected by Dr. Stephen Savarino); and for comparison, 8 strains were isolated in 2007 from rectal swabs from a dairy cattle herd in the State of Jalisco, Mexico.

Biochemical Identification. Identification of the 23 strains was carried out using standard tests that included fermentation of glucose, lactose, maltose, raffinose, sorbitol and xylose, the production of indole and the decarboxylation of lysine as previously described (Barrow, 1993).

Serotyping. Serotyping was carried out by microagglutination (Orskov, 1984) in 96-well plates using rabbit sera (SERUNAM) against somatic antigens (O1 to O187) and flagellar (H1 to H53) from E. coli and from 46 Shigella somatic antigens. In addition, a serum against the somatic (O) antigen from one of the strains in the study (E. coli 44047) was obtained using an immunization model reported previously (Ewing, 1986).

Absorption tests. The rabbit sera prepared against S. boydii 18 and E. coli 44037 were absorbed with heterologous antigens (E. coli 44037, S. boydii 18E10163) according to the method described by Ewing (1986). In brief, the absorption test was carried out as follows: smooth colonies of S. boydii 18 and E. coli 44037 grown on blood agar plates were selected, the colonies were inoculated into plates of trypticase soya agar (TSA) and incubated at 37°C for 18-24 hours. The bacteria were harvested in 10 mL of 0.15 M NaCl (saline solution). The bacterial suspension was heated using saturated vapor (100°C) for 1 h after which the concentration was adjusted to 9 X10⁸ bacteria/mL. Anti-O serum from S. boydii 18 was incubated at 50°C for 2 h in a suspension of E.coli 44037 while anti-E. coli 44037 serum was incubated in a suspension of S. boydii 18. These suspensions were then centrifuged at 6000g for 10 min at 4°C. Finally, the sera were used in agglutination tests against homologous antigens.

DNA Extraction. Extraction of DNA from the strains was carried out using the boiling method reported by Islam (2006). Briefly, the strains were inoculated into Luria-Bertani (LB) broth and incubated at 37°C for 18-24 h. One mL of the bacterial suspension was centrifuged at 13000g for 5 mins. The supernatant was decanted and the pellet was homogenized with 200 µL ultrapure water. The resulting suspension was boiled for 10 mins and placed in an ice
bath for 5 mins. The suspension was mixed by mechanical shaking with a vortex and centrifuged at 13000g for 10 mins before taking 100 μL of the supernatant to be preserved frozen (-20°C) until use.

**Genes to differentiate between E. coli and Shigella.** As reported by Horáková (2006), primers were used in a multiplex PCR form (Table 1) to determine the lacZ,uidA and cya genes. The primers for ipaH and lacY were designed in the laboratory with the nucleotide sequence for the ipaH gene being obtained from the complete Shigella genome analyzed by the ShiBase database (http://www.mgc.ac.cn/ShiBase/). With regards to the primers for lacY, the complete E. coli O157:H7 Sakai genome was generated with the Genbank access number BA000007.2 (Makino, 1999; Ohnishi, 2000). The size of the amplicon was analyzed and compared against ShiBase BLAST. This analysis corresponded to the ipaH-5 from S. sonnei strain 046, ipaH-1 from S. boydii 4 strain 22, ipaH-7 from S. flexneri 2a strain 301, ipaH-6 from S. dysenteriae 1 strain 197, and finally ipaH-2 (a pseudogene). All of the PCR primers mentioned here were designed by the free software program PRIMER3 (http://primer3.ut.ee/). For the pair of primers lacY and ipaH, a duplex PCR was used with the following parameters: 35 amplification cycles with initial denaturation at 94°C for 30 sec, annealing at 60°C for 25 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 mins. In addition, the primers were used to detect the ItA, sth, sfaI, cfaI, cs1, cs3 y cs2I genes from ETEC employing the previously described conditions (Bekal, 2003; Rodas, 2009; Mazariiego-Espinosa, 2010; Chattopadhyay, 2012).

The same PCR technique was used to determine the presence of the wzt (flippase) and wzy (polymerase) genes, which relate to the biosynthesis of the somatic antigen of S. boydii 18. The nucleotide sequences of the wzt and wzy primers (Table 1) were obtained from the complete S. boydii 18 deposited in the GenBank with access number AY948196 (Feng, 2005). For this PCR, the following parameters were used: 30 amplification cycles with denaturing at 95°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 30 sec and final extension at 72°C for 5 min (Table 1).

**Phylogenetic Groups.** Primers and conditions for quadruple PCR reported by Clemont (2013) (Table 1) were used to define the phylogenetic group of E. coli strains. In each case, the amplification products of the DNA obtained by PCR were analyzed by electrophoresis in an agarose gel at 1.8% with 100 Volts and the DNA was stained with GelRed (BioLabs). The amplicons were viewed using UV light in an image reader (Biosens SC805, Gel Imagine Sistems). Amplicons were considered to be positive if they presented the same size of pair bases as those presented by the positive controls.

**Antimicrobial Sensitivity.** Using the diffusion method in agar (Kirby-Bauer) and taking into account the recommendations reported in the 10th Edition of the Handbook of the National Committee of Clinical Laboratory Norms (CLSI, 2010), the sensitivity of the following antimicrobials against E. coli was evaluated: cefoxitin (FOX) 30 μg, ceftriaxone (CRO) 30μg, ceftazidime (CAZ) 30 μg, cefotaxime (CTX) 30 μg, cefepime (FEP) 30 μg, ofloxacin (OFX) 5 μg, norfloxacin 10 μg (NOR), nalidixic acid (NA) 30 μg, ciprofloxacin (CIP) 5 μg, imipenem (IMP) 10 μg, aztreonam (ATM) 30 μg Trimethoprim/sulfamethoxazole (TMP/SMX) 1.25/23.73 μg and tetracycline (TE) 30 μg.

**Results:-

**Biochemical Identification.** All 23 strains presented a biochemical profile typical of E. coli in terms of fermentation of glucose, lactose, maltose, raffinose, sorbitol, xylose and sucrose; lysine decarboxylation; and gas and indole production.

**Serological Typing.** E. coli strains only showed agglutination reaction against S. boydii 18 serum and rabbit serum immunized with the E. coli 44037 strain isolated from one of the children in the study. The following flagellar antigens were detected: H2 in 8 (34.8%) strains, H3 in 6 (26.1%) strains, and H9, H16 and H48 in 1 strain (13%). Meanwhile, 6 (26%) strains were non-motile (H-).

**Absorption Tests.** In order to confirm that the reaction against S. boydii 18 was specific, the sera against S. boydii 18 and E. coli 44037 antigens were absorbed with heterologous antigens. The absorption tests also included an antiserum against E. coli O152 that reacted against the S. boydii 18 antigen at a low titer level (1:100). However, antiserum against S. boydii 18 and E. coli 44037 did not react against the E. coli O152 antigen. Absorption of the S. boydii 18 serum with the E. coli O152 antigen did not change the reaction against the antigens from S. boydii 18 and E. coli 44037. In contrast, when the S. boydii 18 serum was absorbed with the 44037 antigen, agglutination against E. coli

---

utra-jrap-004-4024 (2012) 123-128.
44037 and S. boydii 18 was eliminated completely. In a similar way, absorption of the E. coli 44037 antiserum with the S. boydii 18 antigen, completely eliminated the reaction against the previously mentioned antigens (Table 2).

**Virulence Genes to Differentiate Shigella and E. coli.** PCR detection of the wzx and wzy genes was positive for both genes in all 23 (100%) strains of E. coli and S. boydii 18, while the same test for E. coli O152 was negative. The PCR test to detect lacZ, uidA, cyd, lacY, and ipaH was positive for the first four genes in 23 strains and for E. coli O152 but negative for the ipaH gene (Table 3). The same test for S. boydii 18 showed a positive PCR reaction for the uidA, cyd and ipaH genes and a negative reaction for the lacZ and lacY genes. The PCR test to determine the presence of the ETEC ItA and stp genes was positive in 14 (61%) and 6 (26%) of strains, respectively. With regards to the colonization factors cfal and cs3, 21 (91%) and 13 (57%) of the strains were positive, respectively (Table 3).

**Phylogenetic Groups.** Analysis to define the phylogenetic group of the strains showed that 5 (22%) corresponded to group A, 5 (22%) to Group B1, 8 (35%) to Clade I, 1 (4%) to Group D1 and 3 (13%) to Group B2. The phylogenetic group was unable to be determined for 4 (17%) strains. Both the S. boydii 18 and E. coli O152 strains corresponded to group A.

**Antimicrobial Resistance.** Of the E. coli strains in the study, 6 showed resistance to one and three antimicrobials (Table 4). Of these six, two (33%) of the strains, both from Egypt, showed resistance against one antimicrobial (NA or TE), 1 (17%) was resistant to FOX, NA and TE, and another one (17%) was resistant to CAZ, NA and TE. Finally, only 2 (33%) of the isolated strains from dairy cows were resistant to NA or TE.

**Discussion:**
This study characterized the phenotypic and genotypic profiles of 23 E. coli strains isolated in different years from children with and without diarrhea in Egypt and Mexico. In addition, E. coli strains isolated from dairy cattle from a herd in Jalisco State, Mexico were also analyzed. All of the strains presented a biochemical profile characteristic of E. coli that reacted specifically against a rabbit serum prepared against the somatic antigen of S. boydii 18. However, in contrast to S. boydii 18, which is a non-motile strain, the majority of the E. coli strains isolated in Egypt, presented the flagellar antigen H2, while the strains from Mexico presented the H2, H3, H9, H16, H48 antigens or were non-motile (H-).

PCR tests used to identify the wxy and wza genes, which are related to the biosynthesis of the O antigen of S. boydii 18, showed that these two genes were present in all E. coli strains. Together with the microagglutination tests, these results suggest that the 23 strains form a new sero-variety of E. coli. These results were similar to those from other studies that reported the presence of Shigella antigens in strains of E. coli (Navarro, 2010; Iguchi, 2011; Iguchi, 2015). Recently, Iguchi (2015) reported antigenic relationships between E. coli O38 and S. dysenteriae 8; E. coli O169 and O183 with S. boydii 6 and 10. Although 21 shared O antigens were recognized between E. coli and Shigella (Iguchi, 2015), in the case of S. boydii 18 no antigenic cross-reaction was observed with any other E. coli or Shigella somatic antigen (Liu, 2008). Previously, our laboratory reported E. coli strains isolated from three geographic zones with antigens from S. boydii 16 showing characteristics of ETEC (Navarro, 2010). In addition, antigenic relationships between S. dysenteriae 10 and E. coli have been found that presented characteristics of E. coli strains producing the Shiga toxin (STEC) (Iguchi, 2011). The presence of the wzz and wzy genes in the E. coli strains confirm the existence of common epitopes similiar to those found in the linear pentasaccharide of the repeat units of the S. boydii 18 O antigen. This linear pentasaccharide consists of three carbohydrate residues made up of rhamnose, a residue of alpha-d-galacturonic (D-GalA) acid and a residue of N-acetylgalactosamine (N-GalNAc) (Feng, 2005).

The PCR results to determine the E. coli pathotype showed that a significant number of strains belong to the ETEC group due to the fact that they contained ItA and stp genes. The presence of both ItA and biosynthesis of the S. boydii 18 O antigen suggests the acquisition of these genes by a horizontal transfer system (Reid, 2000; Gogarten, 2002). The E. coli strains with S. boydii 18 antigens were found in fecal samples from children and dairy cattle. These cows could provide natural reservoirs of this bacteria and be related to the transmission of pathogens associated with diarrheal diseases.

Since the E. coli strains in this study presented an antigen identical to S. boydii 18, further investigation was made to see if these strains presented the uidA, cyd, lacY, lacZ and ipaH genes in order to establish whether the strains contained Shigella genes. The results showed that all the strains were positive for uidA, cyd, lacY and lacZ genes.
but negative for ipaH. The presence of these four genes in the strains indicated that their genotypic identity was E. coli. In contrast, S. boydii 18 presented ipaH, uidA and cyd genes but lacked the lacY and lacZ genes. Horakova (2008) reported that the lacY gene, which is responsible for lactose fermentation, is a molecular marker to identify Shigella strains, and in this study, the strains lacked this gene. However, the lacY gene is present in E. coli as well as in Enterobacter cloacae and Citrobacter freundii. The other important marker that differentiates between Shigella and E. coli is the uidA (β-glucuronidase) gene that is present in both types of strains, including enteroinvasive E. coli (Pavlovic, 2011).

Analysis of the phylogenetic groups showed that as many strains isolated from children in Mexico and Egypt, as well as strains from dairy cattle, belonged to Clade I. Reports indicate that Clade I group strains that present microbiological characteristics similar to those of E. coli that making them indistinguishable from this bacteria. However, genotypic analysis shows that they are considered as a divergent group of typical E. coli but that finally they are classified as phylogroups of E. coli (Luo, 2011; Clermont, 2013).

Further to the Clade I strains, our study identified strains belonging to groups A and B1. These groups comprised mainly of intestinal type E. coli strains that form part of the commensal microbiota of the human intestine, while groups B2 and D are recognized as comprising strains originating from outside the intestine with both pathogenic capacity and more virulence factors than those in groups A and B1 (Duriez, 2001; Nowrouzian, 2005). However, group B1 is found more frequently in herbivorous animals, such as cows, sheep and goats (Baldy-Chudzik, 2008; Carlos, 2010; Ziebell, 2008). In addition, group B2 is found in the human intestine (Carlos, 2010) forming part of the resident microbiota that can colonize the intestine of humans for anumber of weeks (Nowrouzian, 2005). The presence of B2 strains in the human intestine could indicate that these strains are more adapted to the human intestine.

The lack of resistance to the antimicrobials that was found in the majority of the strains in this study was interesting. Only one of the strains from Mexico presented resistance (CAZ, NA and TE) to the antimicrobials used, in contrast to three strains from Egypt that showed resistance. Of these three, two were resistant to one antimicrobial (NA or TE) and the other to three antimicrobials (FOX, NA and TE). Of the strains isolated from dairy cows, only two presented resistance to NA or TE. This lack of resistance overall correlates with the results from a previous study of E. coli with an S. boydii 18 antigen (Navarro, 2010) but different from other results (Estrada-García, 2005; Amábile-Cuevas, 2010) that arose from studies in Mexico in which resistance to ciprofloxacin, ampicillin, trimethoprim/sulfamethoxazole and tetracycline was reported in E. coli strains isolated from environmental and clinical samples. An important note with regards to the Mexican strains is that these were isolated in the mid-1980’s in a rural region (Cravioto, 1990).

The results from this study suggest that E. coli 44037:H−, E. coli 44037:H2, E. coli 44037:H3, E. coli 44037:H9, E. coli 44037:H16 and E. coli 44037:H48 strains belong to a serogroup with at least 5 serotypes that have a somatic antigen identical to that of S. boydii 18 with some exhibiting characteristics of ETEC strains with a wide geographic distribution. The presence of this type of strain opens up a new discussion as to whether these strains represent a variant of S. boydii 18 or if they are actually ETEC strains with an S. boydii 18 antigen that acquired the genes for the biosynthesis of this antigen through a horizontal transfer mechanism. These results need to be confirmed with strains from other geographical regions from similar sources.

Acknowledgements:-
We thank Delia Licona, Liliana N. Cortes, Gabriel Pérez Soto and Luis Antonio León (Facultad de Medicina, UNAM) for their technical assistance in the laboratory.

Funding:-
This work was supported by grants from “Fondo Sectorial de Investigación para la Educación SEP-CONACYT”, project CB-2012-01/179000.
### Table 1: PCR Primers.

| Genes | CODE | Nucleotide Sequence | Molecular Weight (pb) | Reference |
|-------|------|---------------------|-----------------------|-----------|
| ipaH  | EM_Sh_ipaH_F/EM_Sh_ipaH_R | ATCTAATAACCTTGGATGTGGTCCGA/TTAAGATGGAAACCTGCCAGATGGAA | 187 | Current study |
| lacY  | EM_LacY_F/EM_LacY_R | CAATAATCGITCGTTTCTGTC | 241 | |
| lacZ  | LacZ-F/LacZ-R | ATGAAAGCTGTCACAGGAAGGCC/GGGTATGCAGCAACCTGCCAGATGGAA | 264 | Horákova(2006) |
| uidA  | UidA-F/UidA-R | ATCGGCGAAATTCATACCTG | 319 | |
| cyd   | Cyd-F/Cyd-R | CGTATCATGTTGCCGTTTCTGGG | 393 | |
| yjaA  | yjaA-F/yjaA-R | CAAACGTTGAAGTTCGAGGAG | 211 | Clermont (2013) |
| TspE4 | TpsE4-F/TpsE4-R | CAATATCGTTACCTACCTGTC | 152 | |
| arpA  | arpA-F/arpA-R | AAGGCTATGCACGACCTG | 400 | |
| chuA  | chuA-F/chuA-R | ATGGTACCGGACGAACCAAC/TCGCCCATACGATAACCGACCA | 288 | |
| wzx   | EM-WZX-L60/EM_WZX-R399 | GCAGGGGACCAAACCTTCTG/GACATATACCCATACCAACCGACCA | 340 | Current study |
| wzy   | EM-WZY-L70/EM-WZY-R606 | GCTTTTATGCGTTATGCG/GACATATACCCATACCAACCGACCA | 537 | |
| cfaI  | CFaI-F/CFaI-R | GGTCGAAATCGCTGGCAGA/AGTATGCAGCAGAAGAAGA | 479 | Bekal (2003) |
| cs1   | CS1-F/CS1-R | GCTCAGACCAACACACCGT/GCTTACGGAAGAAGAAGA | 321 | |
| cs3   | CS3-F/CS3-R | GGCGTCACTGACCAACCGACCA | 401 | |
| cs21  | CS21-F/CS21-R | ATGAGCCTGGGCAATCGGATATGGG | 608 | Mazariego-Espinosa (2010) |
| lta   | LTa-F/LTa-R | ACGGCCGTTACTATCCTCACCT/TGGTTCGGGAATGATCTTGGG | 273 | Rodas (2009) |
| sth   | Sth-F/Sth-R | ATGAAAGCTGTCACAGGAAGGCC/GGGTATGCAGCAACCTGCCAGATGGAA | 181 | Chattopadhyay (2012) |
| stp   | Stp-F/Stop-R | TCTTTTCCTTCTTTTAATCTTCT/TACGCCAGATTACACCAACACAA | 166 | Rodas (2009) |

### Table 2: Agglutination Titers of Absorbed and Non-absorbed *E. coli* O152, 44037 and *S. boydii* 18 Sera.

| Antigens | Titers of non-absorbed sera | Titers of absorbed sera |
|----------|------------------------------|-------------------------|
|          | O152 | 44037 | *S. boydii* 18 | O152 | 44037 | *S. boydii* 18 |

| Antigens | Titers of non-absorbed sera | Titers of absorbed sera |
|----------|------------------------------|-------------------------|
|          | O152 | 44037 | *S. boydii* 18 | O152 | 44037 | *S. boydii* 18 |
| *E. coli* O152 | 1:3200 | - | - | 1:3200 | 1:3200 | - | - | - | - |
| *E. coli* 44037 | - | 1:800 | 1:800 | - | - | 1:1600 | - | 1:1600 | - | - |
| *S. boydii* 18 | 1:100 | 1:1600 | 1:1600 | - | - | 1:1600 | - | 1:1600 | - | - |
Table 3:- Genotypes and phylogenetic groups of *E. coli* strains with *S. boydii* 18 antigens

| Year of isolation  | No. of strains | Serotype | ETEC genes | Biosynthesis of *S. boydii* 18 O antigen† | Genes to differentiate between *Shigella* and *E. coli* | Phylogenetic groups | N (%) |
|-------------------|----------------|----------|------------|------------------------------------------|------------------------------------------------------|-------------------|-------|
| Mexico            |                |          |            |                                          |                                                      |                   |       |
| 1986              | 3              | 44037:H- | 3          | –            | –                                    | –                  | 3 (13) |
|                   | 3              | 44037:H- | 2          | –            | –                                    | –                  | 3 (13) |
|                   | 1              | 44037:H2 | –          | –            | –                                    | 1                  | –                  | A     |
|                   | 2              | 44037:H2 | –          | –            | 1                                    | 1                  | 1                  | B1    |
| 1999              | 1              | 44037:H2 | –          | –            | –                                    | 1                  | 1                  | I     |
| 2000              | 1              | 44037:H1 | 1          | 1            | 1                                    | 1                  | –                  | I     |
| Egypt             |                | 44037:H2 | 1          | –            | 2                                    | 1                  | 1                  | D     |
| 1999              | 2              | –        | –          | 1            | –                                    | –                  | 1                  |       |
|                   | 1              | –        | –          | –            | 1                                    | 1                  | 1                  |       |
| 2005              | 1              | –        | –          | –            | 1                                    | 1                  | 1                  |       |
| Dairy cattle herd, Mexico | | 44037:H3 | 1          | 1            | 1                                    | 1                  | 1                  |       |
| 2007              | 1              | –        | –          | 1            | 1                                    | –                  | 1                  |       |
|                   | 2              | –        | –          | 2            | 1                                    | –                  | 2                  |       |
|                   | 1              | 44037:H9 | 1          | 2            | 1                                    | –                  | 2                  |       |
|                   | 2              | 44037:H3 | 1          | 1            | 2                                    | 1                  | 1                  |       |
|                   | 1              | –        | –          | –            | 1                                    | –                  | 1                  |       |
|                   | 1              | –        | –          | –            | 1                                    | 1                  | 1                  |       |
| Total             | 23             | 14       | 6          | 21           | 13                                   | 23 (100)           | 23 (100)           | 23 (100) |

†Primers for the biosynthesis of *S. boydii* 18 somatic antigen; wzx and wzy genes code for a flippase and a polymerase.
‡ Primers designed in the laboratory for PCR duplex.
Table 4: Resistance to antimicrobials by *E. coli* strains with an *S. boydii* 18 O Antigen

| FMU Number | Year of isolation | Identification | Serotypes | FOX | CAZ | NA | TE | Number of antimicrobials to which the strain is resistant |
|------------|-------------------|----------------|-----------|-----|-----|----|----|--------------------------------------------------------|
| 100238     | Egypt1999         | *E. coli*      | 44037:H2  | R   |     |    |    | 1                                                      |
| 110072     | Egypt2005         | *E. coli*      | 44037:H2  | R   |     |    |    | 1                                                      |
| 100324     | Egypt1999         | *E. coli*      | 44037:H2  | R   | R   | R  |    | 3                                                      |
| 101356     | México1999        | *E. coli*      | 44037:H2  | R   | R   | R  |    | 3                                                      |
| 108709     | México 2004       | *E. coli*      | 44037:H9  |     |     |    |    | 1                                                      |
| 113778     | *E. coli*         | 44037:H3       | R   |     |    |    |    | 1                                                      |
| Total      |                   |                |           | 1 (4)| 1 (4)| 4 (17)| 4 (17)|[

FOX: cefoxitin.
CAZ: ceftazidime.
NA: nalidixic acid.
TE: tetracycline.
R: resistant.

References:
1. Amábile-Cuevas, C.F., Arredondo-García, J.L., Cruz, A. and Rosas, I. (2010): Fluoroquinolone resistance in clinical and environmental isolates of *Escherichia coli* in Mexico City. J. Appl. Microbiol., 108: 158–162.
2. Baldy-Chudzik, K., Mackiewicz, P. and Stosik, M. (2008): Phylogenetic background, virulence gene profiles, and genomic diversity in commensal *Escherichia coli* isolated from ten mammal species living in one zoo. Vet. Microbiol., 131: 173-184.
3. Barrow, G.I. and Feltham, R.K.A, Cowan and Steel’s Manual for the Identification of Medical Bacteria, 3rd edn. Cambridge 1993; Cambridge University Press.
4. Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J. and Harel, J. (2003): Rapid identification of *Escherichia coli* pathotypes by virulence gene detection with DNA microarrays. J. Clin. Microbiol., 41: 2113-2125.
5. Carlos, C., Pires, M.M., Stoppe, N.C., Hachich, E.M., Sato, M.I., Gomes, T.A., Amaral, L.A. and Ottoboni, L.M. (2010): *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol., 10:161.
6. Chattopadhyay, S., Tchesnokova, V., McVeigh, A., Kisiela, D. I., Dori, K., Navarro, A., Sokurenko, E.V. and Savarino, S.J. (2012): Adaptive evolution of class 5 fimbrial genes in enterotoxigenic *Escherichia coli* and its functional consequences. J. Biol. Chem., 287:6150-8
7. Clermont, O., Christenson, J.K., Denamour, E. and Gordon, D.M. (2013): The Clermont *Escherichia coli* phylotyping method revisited: improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep., 5: 58-65.
8. Clinical and Laboratory Standard Institute. Approved standard M2-A10. (CLSI 2010): Performance standards for antimicrobial disk susceptibility test, 10th ed. Wayne, Pa.
9. Cravioto, A., Reyes, R.E., Trujillo, F., Uribe F., Navarro, A., De La Roca, J.M., Hernández, J.M., Pérez, G. and Vázquez, V. (1990): Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. Am. J. Epidemiol., 131, 886–904.
10. Davidson, G., Barnes, G., Bass, D., Cohen, M., Fasano, A., Fontaine, O. and Guandalini, S. (2002): Infectious diarrhea in children: working group report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. J. Pediatr. Gastroenterol. Nutr., 35 (Suppl. 2):S143–S150.
11. Duriez, P., Clermont, O., Bonacorsi, S., Bingen, E., Chaventré, A., Elion, J., Picard, B. and Denamur, E. (2001): Commensal *Escherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. Microbiology., 147: 1671-1676.
12. Estrada-García, T., Cerna, J.F., Pacheco-Gil, L., Velázquez, R.F. and Ochoa, J.T. (2005): Drug-resistant Diarrheogenic *Escherichia coli*, Mexico. Emerg. Infect. Dis., 11: 1306–130.
13. Ewing W H. Edwards and Ewing’s identification of Enterobacteriaceae. (1986): Elsevier Science Publishing Inc., New York.
14. Feng, L., Senchenkova, S.N., Wang, W., Shashkov, A.S., Liu B., Shelev, S.D., Liu, D., Knirel, Y.A. and Wang L. (2005): Structural and genetic characterization of the *Shigellaboydii* type 18 O antigen. Gene., 355: 79–86.

15. Gogarten, J.P., Doolittle, W.F. and Lawrence, J.G. (2002): Prokaryotic evolution in light of gene transfer. Mol. Biol. Evol., 19: 2226–2238.

16. Horaková, K., Mlejnková, H. and Mlejněk, P. (2008): Specific detección de *Escherichia coli* isolated from water samples using PCR targeting four genes: cytochrome bd complex, lactose permease, β-D-glucoronidase, and β-D-galactosidase. J. Applied. Microbiol. 105: 1364-5072.

17. Horáková, K., Mlejnková, H. and Mlejněk, P. (2006): Direct detection of bacterial faecal indicators in water samples using PCR. Water Sci. Technol., 54: 135-140.

18. Iguchi, A., Iyoda, S., Kikuchi, T., Ogura, Y., Katsura, K., Ohnishi, M., Hayashi, T. and Thomson, N.R. (2015): Complete view of the genetic diversity of the *Escherichia coli* O-antigen biosynthesis gene cluster. DNA Res., 22: 101-107.

19. Iguchi, A., Iyoda, S., Seto, K., Ohnishi, M. and EHEC Study Group. (2011): Emergence of a novel Shiga toxin-producing *Escherichia coli* O serogroup cross-reacting with *Shigellaboydii* type 10. J. Clin. Microbiol. 49: 3678-3680.

20. Islam, M.A., Heuvelink, A.E., Talukder, K.A., Zwiertering, M.H. and De Boer, E. (2006): Evaluation of immunomagnetic separation and PCR for the detection of *Escherichia coli* O157 in animal feces and meats. J. Food Protect., 69: 2865-2869.

21. Kosek, M., Bern, C. and Guerrant, R.L. (2003): The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organization, 81: 197-204.

22. Lan, R., Alles, M.C., Donohoe, K., Martínez, M.B. and Reeves, P.R. (2004): Molecular evolutionary relationships of enteroinvasive *Escherichia coli* and *Shigella* spp. Infect. Immun., 72: 5080-5088.

23. Liu, B., Knirel, Y.A., Feng, L., Perepelov, A.V., Senchenkova, S.N., Wang, Q., Reeves P.R. and Wang, L. (2008): Structure and genetics of *Escherichia coli* O157:H7 derived from the Sakai outbreak. J. Appl. Microbiol., 105: 5072.

24. Luo, C., Walk, S.T., Gordon, D.M., Feldgarden, M., Tiedje, J.M. and Konstantinidis, K.T. (2011): Genome sequencing of environmental *Escherichia coli* expands understanding of the ecology and speciation of the model bacterial species. Proc. Natl. Acad. Sci. USA, 108: 7200-7205.

25. Makino, K., Yokoyama, K., Kubota, Y., Yutsudo, C.H., Kimura, S., Kurokawa, K., Ishii, K., Hattori, M., Tatsuno, I., Abe, H., Iida, T., Yamamoto, K., Ohnishi, M., Hayashi, T., Yasunaga, T., Honda, T., Sasakawa, C. and Shinagawa, H. (1999): Complete nucleotide sequence of the prophage VT2-Sakai carrying the verotoxin 2 genes of the enterohemorrhagic *Escherichia coli* O157:H7 derived from the Sakai outbreak. Genes Genet. Syst., 74: 227-239.

26. Mazariego-Espinosa, K., Cruz, A., Ledesma, M.A., Ochoa, S.A. and Xicohtencatl-Cortes, J. (2010): Longus, a type IV pilus of enterotoxigenic *Escherichia coli*, is involved in adherence to intestinal epithelial cells. J. Bacteriol., 192: 2791-2800.

27. Navarro, A., Eslava, C., Perea, L., Inzunza., Delgado, G., Morales-Espinosa, R., Cheasty, T. and Cravioto, A. (2010): New enterovirulent *Escherichia coli* serogroup 64474 showing antigenic and genotypic relationships to *Shigellaboydii* 16. J. Med. Microbiol., 59: 453-461.

28. Nowrouzian, F.L., Wold, A.E. and Adlerberth, I. (2005): *Escherichia coli* strains belonging to phylogenetic group B2 have superior capacity to persist in the intestinal microflora of infants. J. Infect. Dis., 191: 1078-1083.

29. Ohnishi, M., Murata, T., Nakayama, K., Kuhara, S., Hattori, M., Kurokawa, K., Yasunaga, T., Yokoyama, K., Makino, K., Shinagawa, H. and Hayashi, T. (2000): Comparative analysis of the whole set of rRNA operons between an enterohemorrhagic *Escherichia coli* O157:H7 Sakai strain and an *Escherichia coli* K-12 strain MG1655. Syst. Appl. Microbiol., 23: 315-324.

30. Orskov F, and Orskov I. Serotyping of *Escherichia coli*. In T. Bergan, (Ed.). (1984): Methods in Microbiology. Academic Press Ltd, London. 14: 43-112.

31. Pavlovic, M., Luze, A., Konrad, R., Berger, A., Sing, A., Busch, U. and Huber, I. (2011): Development of a duplex real-time PCR for differentiation between *E. coli* and *Shigella* spp. J. Appl. Microbiol. ,110: 1245-1251.

32. Peng, J., Yang, J. and Jin, Q. (2009): The molecular evolutionary history of *Shigella* spp. and enteroinvasive *Escherichia coli*. Infect. Genet. Evol., 9: 147-152.

33. Peng, J., Zhang, X., Yang, J., Wang, J., Yang, E., Bin, W., Wei, C., Sun, M. and Jin, Q. (2006): The use of comparative genomic hybridization to characterize genome dynamics and diversity among the serotypes of *Shigella*. BMC Genomics, 7: 218.
34. Pupo, G.M., Lan, R. and Reeves, P.R. (2000): Multiple independent origins of Shigella clones of Escherichiacoli and convergent evolution of many of their characteristics. Proc. Natl. Acad. Sci. USA., 97: 10567–10572.
35. Reid, S.D., Herbelin, C.J., Bumbaugh, A.C., Selander, R.K. and Whittam, T.S. (2000): Parallel evolution of virulence in pathogenic Escherichiacoli. Nature, 406: 64–67.
36. Rodas, C., Iniguez, V., Qadri, F., Wiklund, G., Svennerholm, A.M. and Sjöling, A. (2009): Development of multiplex PCR assays for detection of enterotoxigenic Escherichiacoli colonization factors and toxins. J. Clin. Microbiol., 47: 1218-1220.
37. Yang, J., Nie, H., Chen, L., Zhang, X., Yang, F., Xu, X., Zhu, Y., Yu, J. and Jin, Q. (2007): Revisiting the molecular evolutionary history of Shigella spp. J. Mol. Evol., 64: 71–79.
38. Ziebell, K., Konczy, P., Yong, I., Frost, S., Mascarenhas, M., Kropinski, A. M., Whittam, T.S., Read, S.C. and Karmali, M.A. (2008): Applicability of phylogenetic methods for characterizing the public health significance of verocytotoxin-producing Escherichiacoli strains. Appl. Environ. Microbiol.,74: 1671-1675.