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RESEARCH ARTICLE

Escherichiacoli DERIVED FROM DIFFERENT SOURCES SHARE ANTIGENIC CHARACTERISTICS WITH *Shigellaboydii* 18AND VIRULENCE FACTORS WITH ENTEROTOXIGENIC *E. coli*

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Abstract

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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,sc*3 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 44037antisera 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*, *uid* 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 *sc*3 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.

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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

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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 similarto 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 $X10^8$ 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 6000*g* 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 13000*g* 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 13000*g* 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 *lac*Z,*uid*A and *cyd* 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 denaturationat 94°C for 30 sec, annealingat 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 *ltA*, *sth*, *stp*, *cfaI*, *cs1*, *cs3* y *cs21* genes from ETEC employing the previously described conditions (Bekal, 2003; Rodas, 2009; Mazariego-Espinosa, 2010; Chattopadhyay, 2012).

The same PCR technique was used to determine the presence of the *wzx* (flippase) and *wxy* (polymerase) genes, which relate to the biosynthesis of the somatic antigen of *S. boydii* 18. The nucleotide sequences of the *wzx* 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) 30μg, 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. Inorder 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 anti-serum against *E. coli* O152 that reacted against the *S. boydii* 18 antigen at a low titer level (1:100). However, anti-sera 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*

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 *lac*Z, *uidA*, *cyd,lac*Y, 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 *ltA* 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 *wxz* 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 *wzx* and *wzy* genes in the *E. coli* strains confirm the existence of common epitopes similarto 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 *ltA* 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 *uid*A, *cyd*, *lac*Y, *lac*Z and *ipaH* genes in order to establish whether the strains contained *Shigella* genes. The results showed that all the strains were positive for *uid*A, *cyd*, *lac*Y and *lac*Z 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* y *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 *Enterobactercloacae* and *Citrobacterfreundii*. 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 themajority 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* 16 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.

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Table 1:- PCR Primers.

Genes	CODE	Nucleotide Sequence 5´-3´	Molecular Weight (pb)	Reference
*ipa*H	EM_Sh ipaH_F	ATCTAATAACTTTGGATGTGTCCGA	187	Current study
	EM_Sh_ipaH_R	TTAAGATTAGAAGGCAGAGATGGAA		
*lac*Y	EM LacY_F	CAATAATCAGTTCGTTTTCTGGC	241	
	EM LacY_R	AGCAAACTGTTGGTCAAAAACAT		
*lac*Z	LacZ-F	ATGAAAGCTGGCTACAGGAAGGCC	264	Horáková(2006)
	LacZ-R	GGTTTATGCAGCAACGAGACGTCA		
*uid*A	UidA-F	ATCGGCGAAATTCCATACCTG	319	
	UidA-R	GTTCTGCGACGCTCACACC		
cyd	Cyd-F	CCGTATCATGGTGGCGTGTGG	393	
	Cyd-R	GCCGGCTGAGTAGTCGTGGAAG		
*yja*A	yjaA-F	CAAACGTGAAGTGTCAGGAG	211	Clermont (2013)
	yjaA-R	AATGCGTTCCTCAACCTGTG		
TspE4	TspE4-F	CACTATTCGTAAGGTCATCC	152	
	TspE4-R	AGTTTATCGCTGCGGGTCGC		
arpA	arpA-F	AACGCTATTCGCCAGCTTGC	400	
	arpA-R	TCTCCCCATACCGTACGCTA		
chuA	chuA-F	ATGGTACCGGACGAACCAAC	288	
	chuA_R	TGCCGCCAGTACCAAAGACA		
wzx	EM-WZX-L60	GCAGGGCACAAACTATCTGT	340	Current study
	EM WZX-R399	GAGCATATTCCCAATTACAGCGA		
wzy	EM-WZY-L70	GGCTTTATGGGTATGCCAAA	537	
	EM-WZY-R606	CGCTTTCGCGTAATCTTTTT		
cfaI	CFAI-F	GGTGCAATGGCTCTGACCACA	479	Bekal (2003)
	CFAI-R	GTCATTACAAGAGATACTACT		
cs1	Cs1-F	GCTCACACCATCAACACCGTT	321	
	Cs1-R	CGTTGACTTAGTCAGGATAAT		
cs3	CS3-F	GGGCCCACTCTAACCAAAGAA	401	
	CS3-R	CGGTAATTACCTGAAACTAAA		
cs21	CS21-F	ATGAGCCTGCTGGAAGTTATCATTG	608	Mazariego-Espinosa (2010)
	CS21-R	TTAACGGCTACCTAAAGTAATTGAGTT		
ltA	LtA-F	ACGGCGTTACTATCCTCTC	273	Rodas (2009)
	LtA-R	TGGTCTCGGTCAGATATGTG		
sth	Sth-F	CTTTCTGTATTATCTTTTTCACCTTT	181	Chattopadhyay (2012)
	Sth-R	CACCCGGTACAAGCAGGATTAC		
stp	Stp-F	TCTTTCCCCTCTTTTAGTCAG	166	Rodas (2009)
	Stp-R	ACAGGCAGGATTACAACAAAG		

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 ofboiled *E. coli* and*Shigella*strains					
				S. boydii 18	44037	O152	*S. boydii* 18	O152	44037
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 (%)
			(O:H)	*ltA*	*stp*	*cfaI*	*cs3*	*wzx*	*wzy*	ipaH ‡	*lacZ*	*lacY* ‡	*uidA*	*cyd*		
Reference Strains		1	O152:H-	−	−	−	−	−	−	+	+	−	+	+	A	
		1	*Sboydii* 18	−	−	−	−	+	+	+	−	−	+	+	A	
				N (%)												
Mexico	1986	3	44037:H-	3	−	3	2	3	3	−	3	3	3	3	A	3 (13)
		3	44037:H-	2	−	3	2	3	3	−	3	3	3	3	B1	3 (13)
		1	44037:H2	−	−	1	−	1	1	−	1	1	1	1	A	1 (4)
		2	44037:H2	2	−	2	2	2	2	−	2	2	2	2	B1	2(9)
	1999	1	44037:H2	−	−	1	1	1	1	−	1	1	1	1	I	2 (9)
	2000	1	44037:H16	1	1	1	−	1	1	−	1	1	1	1		
Egypt	1999	2	44037:H2	1	−	2	1	2	2	−	2	2	2		I	2 (9)
		1		−	−	1	−	1	1	−	1	1	1	1	D	1 (4)
	2005	1		−	−	1	−	1	1	−	1	1	1	1	I	1 (4)
Dairy cattle herd, Mexico	2007	1	44037:H3	1	1	1	1	1	1	−	1	1	1	1	A	1 (4)
		2		−	1	1	1	2	2	−	2	2	2	2	B2	3 (13)
		1	44037:H9	1	1	−	−	1	1	−	1	1	1	1		
		2	44037:H3	1	1	2	2	2	2	−	2	2	2	2	I	2 (9)
		1		1	1	1	−	1	1	−	1	1	1	1	ND	1 (4)
		1	44037:H48	1	−	1	1	1	1	−	1	1	1	1	I	1 (4)
Total		23		14 (61)	6 (26)	21 (91)	13 (57)	23 (100)	23 (100)		23 (100)	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
				N (%)				
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				R	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.

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