Relationship among *Shigella* spp. and enteroinvasive *Escherichia coli* (EIEC) and their differentiation

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Submitted: May 28, 2013; Approved: December 13, 2013.

Abstract

Shigellosis produces inflammatory reactions and ulceration on the intestinal epithelium followed by bloody or mucoid diarrhea. It is caused by enteroinvasive *E. coli* (EIEC) as well as any species of the genus *Shigella*, namely, *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. This current species designation of *Shigella* does not specify genetic similarity. *Shigella* spp. could be easily differentiated from *E. coli*, but difficulties observed for the EIEC-*Shigella* differentiation as both show similar biochemical traits and can cause dysentery using the same mode of invasion. Sequencing of multiple housekeeping genes indicates that *Shigella* has derived on several different occasions via acquisition of the transferable forms of ancestral virulence plasmids within commensal *E. coli* and form a *Shigella*-EIEC pathovar. EIEC showed lower expression of virulence genes compared to *Shigella*, hence EIEC produce less severe disease than *Shigella* spp. Conventional microbiological techniques often lead to confusing results concerning the discrimination between EIEC and *Shigella* spp. The lactose permease gene (*lacY*) is present in all *E. coli* strains but absent in *Shigella* spp., whereas β-glucuronidase gene (*uidA*) is present in both *E. coli* and *Shigella* spp. Thus *uidA* gene and *lacY* gene based duplex real-time PCR assay could be used for easy identification and differentiation of *Shigella* spp. from *E. coli* and in particular EIEC.

Key words: diarrhea, *E. coli*, *Shigella*, real-time PCR

Introduction

Bacillary dysentery like shigellosis, endemic throughout the world, is one of the major causes of morbidity and mortality, especially among children > 5 years of age in low and middle income countries including Bangladesh (Bardhan et al., 2010; Wen et al., 2012). The disease is caused by enteroinvasive *Escherichia coli* (EIEC) or any of the four species or groups of *Shigella*: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. In the nineties, there are about 165 million cases of *Shigella* infection annually worldwide and 1.1 million *Shigella*-related deaths, with 98% of those cases in low income countries (Kotloff et al., 1999). According to a recent review on Asian countries (Bardhan et al., 2010), about 125 million cases of endemic shigellosis occur per year. The study showed that the incidence of shigellosis is similar to an earlier report (Kotloff et al., 1999), however, the overall mortality rate due to shigellosis has come down to ~ 0.01% and ~0.89% among the youngest age group. Although EIEC is one of the etiologic agents of diarrhea, very few epidemiologic studies have been done globally to estimate the actual disease burden due to EIEC, individual risk factors for infection or prospective reservoirs of EIEC. Adequate attention was not given to the epidemiology of EIEC, since it is often found to be rare etiological agent of diarrhea compared to other diarrhea-causing enteropathogens (Vieira et al., 2007; Moreno et al., 2010). Identification and differentiation of *Shigella* spp. and EIEC from environmental and clinical specimens by conventional culture and biochemical assays...
are complex and time consuming. This review focuses on phenotypic and genotypic relationship among EIEC and *Shigella* spp., and recent progress of clinical and practical research, and utility of currently available molecular methods for differentiation between *Shigella* spp. and *E. coli*, in particular emphasis on EIEC based on literature study.

**Identification and Classification**

The Japanese scientist Kioshi Shiga first identified *Shigella* in the 1898s. He termed it as *Bacillus* because it seemed to be related to *Bacillus coli*, which is now referred to as *E. coli* (Hale, 1991). In taxonomy, *Shigella* strains were classified into a different genus from *E. coli* in the 1940s due to their pathological significance. The genus is divided into four species and at least 54 serotypes based on their biochemical and/or the structure of the O-antigen component of LPS present on the cell wall outer membrane: *S. dysenteriae* (subgroup A, 16 serotypes), *S. flexneri* (subgroup B, 17 serotypes and sub-serotypes), *S. boydii* (subgroup C, 20 serotypes), and *S. sonnei* (subgroup D, 1 serotype) (Simmons and Romanowska, 1987; Talukder and Azmi, 2012).

On the other hand, EIEC was first reported as ‘paracolon bacillus’ in 1944, but it was later designated as *E. coli* O124. EIEC is associated with specific *E. coli* O-serotypes: O28ac:NM, O29:NM, O112ac:NM, O121:NM, O124:NM, O124:H30, O136:NM, O136:NM, O143:NM, O144:NM, O152:NM, O159:H2, O159:NM, O164:NM, O167:H4, O167:H5, O167:NM and O173:NM (Orskov et al., 1991; Matsushita et al., 1993; Nataro and Kaper, 1998; Martinez et al., 1999; Gibotti et al., 2004). All of the serotypes are nonmotile except few biotypes of O28ac, O29, O124, O136 and O143 (Silva et al., 1980; Martinez et al., 1999). Some of these EIEC-associated O antigens are identical or similar to O antigens present in *Shigella* spp., namely: O112ac, O124, O136, O143, O152, and O164 with *Shigella* O antigens of *S. dysenteriae* 2/ *S. boydii* 15/ *S. boydii* 1, *S. dysenteriae* 3/ provisional *Shigella* serovar 3615.53, *S. dysenteriae* 3/ *S. boydii* 1, *S. boydii* 8, provisional *Shigella* serovar 3341.55, and *S. dysenteriae* 3, respectively (Cheasty and Rowe, 1983; Landersjo et al., 1996; Linnerborg et al., 1999). In fact, differentiation between *Shigella* and EIEC strains with the same serotype is often difficult due to their nearly identical physio-biochemical traits. EIEC strains can be differentiated from typical members of commensal *E. coli* strains by the Sereny test and/or the identification of bacterial invasion-associated proteins or genes via specific tests. However, these methods are not routinely used for laboratory diagnosis and EIEC strains are only provisionally identified by O serotyping with commercially available antisera in diagnostic laboratories (Beutin et al., 1997). Where a typable isolate additionally carries a serotype occurring in both EIEC and *Shigella* spp., a taxonomic classification could be at best possible on the basis of genes responsible for the higher metabolic activity of EIEC (Ewing, 1986; Kibbee et al., 2013).

**Phenotypic and Genotypic Relationship**

Although *Shigella* and *E. coli* are closely related, *E. coli* always show some different physio-biochemical properties than *Shigella*. More than 80% of *E. coli* are proteotropic, motile, able to decarboxylate lysine and ferment many sugars, produce gas from D-glucose and indole positive, whereas *Shigella* are auxotrophic, obligate pathogens, non-motile, unable to decarboxylate lysine, ferment few sugars and never produce gas from D-glucose, except *Shigella flexneri* 6, *S. boydii* 13 and *S. dysenteriae* 3 (Clayton and Warner, 1929; Downie et al., 1933; Stypulkowska, 1964; Rowe et al., 1975; Silva et al., 1980; Toledo and Trabulsi, 1983; Baumann and Schubert, 1984; Ewing, 1986; Scheutz F, 2005). *Shigella* spp. are unable to ferment salicine and hydrolyze esculine (Bopp et al., 2003). Of four *Shigella* spp., only *S. sonnei* is able to ferment lactose slowly and can be mucate positive (Goodman and Pickett, 1966). However, some EIEC strains have remarkable phenotypic and genotypic similarity with *Shigella* species (Farmer et al., 1985; Lan et al., 2004). They are usually nonmotile, lactose negative and lysine-decarboxylase negative except for a few serotypes, which are the Sereny test negative and motile (Farmer et al., 1985). The few biochemical properties that enable differentiation of *E. coli* and *Shigella* spp. are mucate fermentation and acetate utilization. EIEC may be positive for one or both of the properties, in contrast *Shigella* strains are negative for both and more than 90% of other *E. coli* strains are positive for both with very few exceptions (Bopp et al., 2003; Lan et al., 2004).

It was reported that *Shigella* and *E. coli* show 80-90% similarity at the nucleotide level, while other *Escherichia* spp. have a much lower degree of similarity and are genetically distant (Brenner et al., 1972). Multilocus enzyme electrophoresis and ribotyping analyses revealed that *Shigella* genotypes are interspersed within *E. coli* genotypes, irrespective of their nomen-species (Rolland et al., 1998). Likewise, a high degree of relatedness between *Shigella* and *E. coli* is found based on 16S rDNA analysis (Christensen et al., 1998). DNA-DNA re-association studies, sequencing of numerous other housekeeping genes, virulence genes and complete genome sequencing showed that EIEC and *Shigella* spp. formed a distinctive *E. coli* pathovar (Brenner et al., 1972; Brenner, 1973; Lan et al., 2004). However, the discrimination between EIEC and *Shigella* spp. is necessary as both exhibit a number of clinical differences. For example, *Shigella* spp. can cause haemolytic gastric syndrome (HUS), a clinical syndrome characterized by progressive renal failure associated with microangiopathic (nonimmune, Coombs-negative) haemolytic anaemia and thrombocytopenia, whereas EIEC is not known to cause HUS (Johnson, 2000).
Genetic Structure of Shigella

Each of the Shigella genomes includes a single circular chromosome and/or a circular virulence plasmid (VP) that harbors conserved primary virulence determinants (Sasakawa et al., 1992). The whole-genome sequencing of all four Shigella spp. and E. coli revealed that they share a fundamental core genome of approximately 3 Mb (Lukjancenko et al., 2010). The Shigella chromosome has more than 200 pseudogenes, 300–700 copies IS-elements, numerous deletions, insertions, translocations and inversions. Although, Shigella chromosomes share most of their genes with E. coli K12 strain MG1655, bacteriophage-mediated horizontal gene transfer is the main contributor for the massive diversity of putative virulence genes (Venkatesan et al., 2001). Shigella spp. became highly virulent pathogens with distinct clinical and epidemiological features via gaining and loss of gene functions in adaptation and convergent evolution, by means of bacteriophage integration, IS-mediated transposition and formation of pseudogenes (Yang et al., 2005; Peng et al., 2006).

Phylogenetic Relationship

The sequencing of multiple housekeeping genes indicates that Shigella has risen on several different occasions from several independent ancestors by acquisition of the transferrable forms of ancestral VPs within the group of nonpathogenic E. coli. Based on the analysis of sequence variation in eight housekeeping genes of Shigella, Pupo et al. proposed that Shigella strains fall into three main clusters and four outliers (Pupo et al., 2000). In 2007, Yang et al. showed a similar phylogenetic tree using the DNA sequences of 23 housekeeping genes, which corroborated the credibility of the previous conclusions (Yang et al., 2007). The most striking features are that each cluster includes strains from different Shigella species. The phylogenetic tree shows that most of the Shigella strains belong to three clusters (C1, C2 and C3) leaving S. sonnei (SS), S. dysenteriae (D) serotype 1, 8, 10 and S. boydii serotype 13 (B13) as outliers. Cluster 1 can be sub-clustered to SC1, SC2, SC3, and a minor branch consisting of only S. dysenteriae 7. Sub-cluster 1 contains only D strains (D3-4, D6, D9, D11-13), SC2 contains mostly B strains (B1, B3, B6, B8, B10, and B18) but also D5, and SC3 contains three B strains (B2, B4, and B14) and F6. Cluster 2 is mainly composed of B strains (B5, B7, B9, B11, and B15-17) and D2. Cluster 3 consists of mostly S. flexneri (F) strains (F1a, F1b, F2a, F2b, F3, F4a, F4b, F5, Fx, and Fy) and B12. The most profound observation is that each of the five clusters/sub clusters contains strains mostly from only one serogroup indicates that serological classification is highly correlated with the genotypes and continues to be useful in epidemiologic and diagnostic investigations. In the phylogenetic tree, B13 is distant from all the E. coli/Shigella strains, which is consistent with a recent report that B13 and E. albertii strains form a distinct lineage of enteric pathogens that had separated from an E. coli-like ancestor about 28 million years ago (Hyma et al., 2005). MLST analysis revealed that EIEC strains grouped into four clusters with one outlier strain (which was found in Shigella cluster 2), indicating the independent derivation of EIEC several times (Lan et al., 2004). In comparison of EIEC with Shigella clusters, the authors showed that EIEC clusters have diverged less than Shigella clusters, although Shigella-EIEC forms one single pathovar of E. coli (Lan et al., 2004).

Pupo et al. proposed that the three main clusters of Shigella had independently evolved from multiple E. coli ancestors with diverse genetic backgrounds 35,000-270,000 years ago (Pupo et al., 2000). This indicated that dysentery existed long before civilization and was one of the early infectious diseases of human. S. sonnei had developed as a human-pathogenic clone of E. coli approximately 10,000 years ago (Shepherd et al., 2000). The descent times are relatively recent when one takes in account that a major nonpathogenic E. coli cluster diverged from other bacteria 8–22 million years ago. These data are probably no coincidence because pathogenesis of Shigella is based on surviving in the intestinal epithelial cells of humans only-a perfect host-adaptation (Pupo et al., 2000). Sequence variations in the clusters of Shigella and EIEC indicated that EIEC might have arisen from E. coli ancestors after Shigella developed. Based on this derivation of EIEC, two hypotheses about EIEC in relation to Shigella were stated. First, EIEC strains are in an intermediate stage and are a potential precursor of ‘full-blown’ Shigella strains. Second, like Shigella, EIEC is a distinct group of organisms that is adapted to human hosts, but is better equipped to survive outside the host (Lan et al., 2004).

The critical step for Shigella creation is the acquisition of the antecedent forms of the VP, which is a non self-transferable large single-copy plasmid of 180-230 kb (Hale et al., 1983). This VP is essential for invasiveness, cell survival and apoptosis of Macrophages (Harris et al., 1982; Sansonetti et al., 1982b; Sansonetti et al., 1983). The virulence associated genes on the pINV are probably acquired horizontally from another unrelated genus, because the A+T content of the nucleotides of these genes is 75%, while the A+T content of all Shigella and E. coli genomes is 50% (Adler et al., 1989; Hale, 1991). Based on the analysis of three virulence genes (ipgD, mxiA, and mxiC) that are located on the invasion region of VP in Shigella and EIEC strains, two forms of VPs (pINV A and pINV B) were found (Lan et al., 2001; Yang et al., 2007). Lan et al. extensively studied 32 EIEC strains and found that all but two EIEC strains have the pINV A form (Lan et al., 2004). S. sonnei has only pINV B and other serotypes have mixed form. The acquisition of the VP in an ancestral E. coli strain preceded the diversification by radiation of all Shigella and EIEC groups. The DNA sequence indicated that a 31-kb entry region of VP encodes components of the Mxi [membrane
excretion of Ipa]-Spa[surface presentation of invasion plasmid antigens] TTSS [type three secretion system] apparatus, substrates of this apparatus (IpaA-D [invasion plasmid gene]), their dedicated chaperones (IpgA, IpgC, IpgE and Spa15) and two transcriptional activators (VirB and MxiE) (Buhrissier et al., 2000). Outside of the entry region, there are i) virG gene, encoding outer membrane protein (VirG), responsible for bacterial movement within the cytoplasm of infected cells, ii) virF gene, encoding a transcriptional activator (VirF), controls expression of icsA and virB, and iii) the sepA gene encodes a secreted serine protease of the autotransporter family. Moreover, the virulence plasmid also contains two copies of the shet2 gene encoding a putative enterotoxin, and genes encoding several secreted proteins (VirA, IpaH4.5, IpaH7.8, IpaH9.8) and six uncharacterized protein designated (gutur Shigella proteins): OspB, OspC1, OspD1, OspE1, OspF, and OspG (Harris et al., 1982; Sansonetti et al., 1982a; Sansonetti et al., 1983). The plasmid encoded proteins are directly involved in the entry into host epithelial cells. With the acquisition of the pINV, Shigella and EIEC were able to live in the human intestinal epithelial cells. For the invasion and maintaining in the host, Shigella and EIEC need a combined expression of genes located on the pINV and chromosome (Sansonetti et al., 1982b; Maurelli et al., 1998). The Shigella genome has adapted to the acquisition of invasion plasmid by multiple different events, such as: (i) controlling at promoter level, (ii) mutations within genes, (iii) the suppression or over expression of certain genes, or (iv) deletion of anti-virulence genes which is called “black hole” to evolve toward a pathogenic lifestyle (Maurelli et al., 1998). For example, the loss of cadA gene is a black hole in EIEC and Shigella. CadA encodes for lysine decarboxylase activity (LDC), which is present in almost all non-entero invasive E. coli. Cadaverine produced by lysine decarboxylase has been shown to attenuate the bacteria’s ability to induce polymorphonuclear leucocytes transepithelial migration. Because of the inhibiting influence of cadaverin on the virulence of Shigella, LDC activity was lost by genome deletion (Maurelli et al., 1998). Consequently, LDC is a biochemical trait which can be used to differentiate between other E. coli vs. Shigella and EIEC, but not between EIEC and Shigella.

Pathogenesis Process

The illness caused by Shigella or EIEC is characterized by the destruction of the colonic epithelium caused by the inflammatory response induced upon invasion of the mucosa by bacteria (Parsot, 2005). It is well established that the disease induced by EIEC is generally less severe than Shigella does (DuPont et al., 1989; Moreno et al., 2009; Bando et al., 2010; Moreno et al., 2012). Recombination techniques and the sequencing of the invasion plasmid and chromosomal genes associated with virulence, gave insight of the precise mechanism of infection by Shigella. First, the bacteria in the intestinal lumen invade the colon by transcytosis through microfold cells (M-cells) of the Follicle-Associated Epithelium (FAE) to reach the underlying submucosa (Croxen and Finlay, 2010). The disruption of tight junctions and the damage that is caused by inflammation also give Shigella entry to the submucosa. Although Shigella phagocytosed by resident macrophages, it can escape from the phagosome, and caspase-1-dependent inflammation activation resulting ultimate release from macrophages. After cell death, the bacteria, released in the submucosa, invade epithelial cells by endocytosis. During the invasion of the epithelial cells, ipaBCD and mxiAB genes of the ipa-mxi-spa island on the VP are brought to expression (Sansonetti et al., 1981; Sansonetti et al., 1982b; Buyssse et al., 1987; Venkatesan et al., 2001; Moreno et al., 2009; Croxen and Finlay, 2010). IpaD is believed to play a role in attaching to host cell membranes, and subsequently IpaB plays a role in the endocytic uptake of the bacteria. The roles of the other known virulence genes associated with invasion of the cell have yet to be discovered. Once internalized, the phagocytic vacuole is quickly lysed by the invading bacterium, thereby allowing its escape into the host cell cytoplasm, where it nucleates and assembles an F-actin comet at one of its poles (Bernardini et al., 1989). This result in the bacterium moving inside epithelial cells and passing from cell to cell, thereby causing a very efficient process of intracellular colonization. Shigella actin based motility is mediated by a single outer membrane protein, IcsA (VirG) (Lett et al., 1989). Glycine-rich repeats in the amino terminal end of IcsA (VirG) bind neuronal Wiskoff-Aldrich Syndrome Protein (N-WASP) (Suzuki et al., 1998), a member of the WASP family of Cdc42-dependent mediators of actin nucleation via the Arp 2/3 complex. Formation of a complex between IcsA, N-WASP, and Arp 2/3 at the bacterial surface is sufficient to cause actin nucleation/polymerization in the presence of actin monomers (Egile et al., 1999). Motile intracellular Shigella then involve components of the cell intermediate junction to form a protrusion that is internalized by the adjacent cell, thus causing cell-to-cell spread (Sansonetti et al., 1994). Invasion of epithelial cells by Shigella stimulates the release of proinflammatory cytokines and chemokines, such as IL-8 attacks polymorphonuclear leukocytes (PMN) to the infection site and their transmigration through the epithelium, which results in major tissue destruction and inflammation.

Difference in Pathogenicity and Virulence Genes Expression

EIEC produce less severe disease than S. flexneri (Moreno et al., 2009; Bando et al., 2010; Moreno et al., 2012). An inoculum of 10^6 EIEC cells is sufficient for infection, whereas as low as 10^5 Shigella cells can cause successful infection (DuPont et al., 1971). S. flexneri induced
keratoconjunctivitis quickly and more severely than the EIEC strains. One of the hypotheses for this phenomenon is the increased efficiency of Shigella in spreading through epithelial cells compared to EIEC. (Moreno et al., 2009). Moreover, it is noteworthy that Shigella and EIEC showed significant differences in the expression of regulatory and pathogenic genes (icsA, icsB, ipaA-D, virF and virB). It was found that S. flexneri expresses pathogenic genes at significantly higher levels than that of EIEC (Moreno et al., 2009; Bardhan et al., 2010). The virF and virB genes act in a regulatory cascade to trigger virulence genes transcription following the receipt of specific environmental signals by the bacterium (Adler et al., 1989; Prosseda et al., 1998). The VirF protein initiates the transcription of the virB regulatory gene, and the product of this gene (VirB) in turn activates the promoters of the structural virulence genes (Porter and Dorman, 2002). Among the studied genes, only the virF gene was more expressed by EIEC than by S. flexneri. All the other genes were less expressed in EIEC. The lower expression of these genes might lead to significant differences in virulence between EIEC and Shigella, leading to a weaken dissemination capacity of EIEC. These data also corroborated the differences in the mechanism by which EIEC and S. flexneri manipulate the host intestinal cells, and suggest that their genes respond specifically to the environment of the host cell milieu, resulting in different disease outcomes (Moreno et al., 2009). Regarding the immune response related to dendritic cells (DCs), the innate immune response upon EIEC infection are preserved although DCs fail to activate naïve T lymphocytes (Moreno et al., 2012). Moreover, EIEC showed a late killing effect in 374 macrophage cultures in compare to S. flexneri (Bando et al., 2010). This data could explain why EIEC takes longer time than Shigella species to cause diarrhea.

Current Approaches to Differentiate Shigella spp. and EIEC

The above described characteristics suggest that Shigella/EIEC could be differentiated genetically from typical E. coli by targeting marker genes. But designing a rapid, sensitive and reliable molecular technique for differentiation and differentiation between EIEC and Shigella spp. is very difficult due to their close biochemical similarity. Till now few molecular methods have been described for identification of the members of Shigella-EIEC pathovar from other typical E. coli. For example, apyrase-based colorimetric test (Sankaran et al., 2009), loop-mediated isothermal amplification method targeting the ipaH gene (Song et al., 2005), PCR-ELISA (Sethabutr et al., 2000), IpaC and IpaH gene -specific ELISA (Oberhelman et al., 1993; Pal et al., 1997), large invasive plasmid (120-140 Mda) analysis based method (Ud-Din et al., 2010), colony blotting using 2.5 kb HindIII fragment of invasion plasmid (Small and Falkow, 1988). Moreover, PCR based assay targeting- IS630-probes (Houng et al., 1997), virF gene (Wang and Chen, 2012), ipaH gene (Thiem et al., 2004) and IS1 region (Hsu et al., 2007), multiplex PCR (Antikainen et al., 2009; Fujioka et al., 2013) and singleplex real-time PCR (Liu et al., 2013) have been reported previously to detect the presence or absence of Shigella/EIEC. Most recently, Ojha et al. developed a pentaplex PCR which is able to detect and differentiate among Shigella spp. (Ojha et al., 2013). Unfortunately, this method is also unable to differentiate EIEC from Shigella spp.

Lactose fermentation is the biochemical hallmark of E. coli which is exploited extensively for its detection by conventional culture methods (Ito et al., 1991; Stoebel, 2005). The lacY gene, a gene encoding lactose permease, is present in different members of the family Enterobacteriaceae like E. coli, Enterobacter cloacae, Citrobacter freundii or Kluvyser ascorbata, while the b-glucuronidase enzyme (uidA), which encodes the beta-glucuronidase enzyme is present in E. coli and Shigella spp. (Horakova et al., 2008), Horakova et al. reported that the lacY gene is a putative genetic marker for differentiation of Shigella spp. from E. coli (Horakova et al., 2008). They developed a conventional multiplex PCR, which seemed to work well to differentiate Shigella from E. coli but not for EIEC-Shigella differentiation. Additionally, this conventional PCR failed to differentiate EIEC from Shigella due to presence of similar sized non-specific amplicons for Shigella spp. In consequence, Pavlovic M et al. developed a simple, rapid, reliable and specific probe-based duplex real-time PCR assay specific for the genes uidA and lacY to minimize the risk of detection of nonspecific targets (Pavlovic et al., 2011). They successfully differentiated ninety-six isolates including 11 EIEC isolates of different serotypes and at least three representatives of each Shigella species correctly. All the tested Shigella and E. coli including EIEC isolates were positive for the uidA gene. Additionally, all E. coli isolates were positive for the lacY gene, whereas none of the tested Shigella isolate harbored the lacY gene. Even cross reacting serotypes of EIEC (O112ac, O124 and O152) were clearly differentiated from Shigella as EIEC by the duplex real-time PCR. The selectivity of the lacY-uidA duplex real-time PCR was 100%.

Conclusion

In summary, Shigella and EIEC can be differentiated from commensal E. coli by testing for presence of the ipaH-gene. Since Shigella and EIEC have similar physio-biochemical characteristics, conventional identification systems will identify members of the Shigella-EIEC pathovar as either E. coli or Shigella. Conventional cultural techniques often lead to confusing results concerning the discrimination of EIEC and Shigella spp. The duplex real-time PCR assay, which is simple, rapid, reliable and specific, can be used for differentiation of Shigella spp. from E. coli and in particular EIEC.
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