Published in final edited form as:

Pediatr Infect Dis J. 2013 October ; 32(10): 1122–1126. doi:10.1097/INF.0b013e318293306c.

Detection of Secretory Immunoglobulin A in Human Colostrum as Mucosal Immune Response against Proteins of the Type Three Secretion System of *Salmonella*, *Shigella* and Enteropathogenic *Escherichia Coli*

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Abstract

Background—Some enteropathogens use the type three secretion system (T3SS) to secrete proteins that allows them to interact with enterocytes and promote bacterial attachment or intracellular survival. These proteins are *Salmonella* invasion proteins (Sip), invasion plasmid antigens (Ipa) of *Shigella* and *E. coli* secreted proteins (Esp) of enteropathogenic *E. coli* (EPEC). There are no previous studies defining the presence of colostral sIgA against all these three major enteric pathogens.

Objective—To evaluate the presence of sIgA in colostrum against proteins of the T3SS of *Salmonella*, *Shigella* and EPEC.

Methods—We collected 76 colostrum samples from puerperal women in Lima, Peru. These samples were reacted with T3SS proteins extracted from bacterial culture supernatants and evaluated by Western Blot.

Results—Antibodies were detected against *Salmonella* antigens SipA in 75 samples (99%), SipC in 62 (82%) and SipB in 31 (41%); against *Shigella* antigens IpaC in 70 (92%), IpaB in 68 (89%), IpaA in 66 (87%) and IpaD in 41 (54%); and against EPEC EspC in 70 (92%), EspB-D in 65 (86%) and EspA in 41 (54%). 10% of samples had antibodies against all proteins evaluated; and 42% against all except one protein. There was no sample negative to all these proteins.

Conclusions—The extraordinarily high frequency of antibodies in colostrum of puerperal women detected in this study against these multiple enteric pathogens, shows evidence of

immunological memory and prior exposure to these pathogens, in addition to its possible protective role against infection.

Keywords

Colostrum; sIgA; T3SS; *Salmonella*; *Shigella*; EPEC

INTRODUCTION

Diarrhea is a major cause of illness and death among children in developing countries, especially in infants younger than 1 year of age.¹ Among the causative agents there is a group of bacteria that have developed a special mechanism for infection, the type III secretion system (T3SS). The T3SS forms a needle-complex/injectisome through which secretes a set of effector proteins that are translocate into the host cell cytoplasm allowing the colonization or invasion of the small intestine.^{2,3} T3SS effector proteins required for enterocytes invasion in *Salmonella* and *Shigella* are called Sip (*Salmonella* invasion proteins) and Ipa (Invasion plasmid antigens), respectively.⁴ In enteropathogenic *Escherichia coli* (EPEC), the T3SS effector proteins, denominated Esp (*E. coli* secreted proteins), are responsible for bacterial adherence/colonization to enterocytes resulting in the characteristics attachment and effacement (A/E) lesion.⁵ Cellular functions for the effector proteins of these three enteropathogenic bacteria have been previously described and are summarized on table 1.

Breastfeeding reduces the risk of diarrhea and other infections in children living in developing countries. Ingestion of colostrum loads the infant gut with antibodies that protect against potentially lethal infant diarrhea.⁶ Secretory immunoglobulin A (sIgA) is the main immunoglobulin isotype in colostrum; it represents over 90% of the immunoglobulin present in milk.⁷ The protective effect of sIgA against various enteropathogens relates to its ability to inhibit cell adhesion,⁸ as well as its action against lipopolysaccharide (LPS).^{9,10} Antibodies against T3SS-secreted proteins appear to be key in this inhibition of bacteria-enterocyte adhesion.^{11,12}

The aim of this study was to evaluate colostrum from puerperal women living in Lima for the presence of sIgA against the major proteins secreted by the T3SS of *Salmonella*, *Shigella* and EPEC. Such antibodies reflect previous exposure and immunological memory on the mother and provide insight into the range of antibodies consumed by infants in a developing country setting.

MATERIAL AND METHODS

Patients and human colostrums samples collection

A total of 76 human colostrum samples were collected during the first five days after birth from mothers with non-premature newborns at Cayetano Heredia National Hospital in Lima, Peru. This is a referral hospital for urban and peri-urban communities of low and middle socio-economic class serving the northern district of Lima. The mean age of women was 25 ± 6.1 years; the mean birth weight was 3434.1 ± 477.6 g; and the median newborn age at the time of colostrum collection was 72 hours (range: 48–120). The colostrum samples (5 ± 2 mL) were obtained by hand pumping using gentle pressure to move secretions toward the nipple into sterile flasks. Colostrum samples were centrifuged at 12 000 g for 15 minutes and the top layer of fat was removed in order to obtain the liquid fraction.⁹ The samples were aliquoted and stored at -20°C until analysis. Participating mothers signed an informed consent prior to data and sample collection. The study and procedures were approved by the

Ethics Research Institutional Committees at Cayetano Heredia National Hospital and Universidad Peruana Cayetano Heredia.

Bacterial strains and induction of virulence protein expression

Strains used in this study included: *Shigella flexneri* M90T, *Salmonella enterica* ser. Typhimurium SL1344 and EPEC E2348/69. Additionally, *Salmonella* ser. Typhimurium SL1344 *hliA* mutant, lacking the *hliA* gene, which is necessary for the expression of the genes encoding the T3SS and the effector proteins required for invasion. *Salmonella* and *Shigella* strains were grown in Luria broth (LB) at 37 °C, whereas EPEC was grown in Dulbecco's minimal Eagle medium (DMEM) at 37 °C. Each strain was incubated overnight in LB at 37 °C and at 350 rpm. The next day, these cultures were then diluted 1:5 in LB and 1:100 in DMEM, respectively, and grown for an additional 4.5–6 hrs at 37 °C to late-log/early-stationary phase ($OD_{600nm} = 1.1–1.3$).

Collection of protein components secreted in media

Supernatant protein extraction was carried out according to Komoriya et al. (1999)¹³ with small modifications. Briefly, after desired OD was reached for each strain, 6mL of bacterial culture were centrifuged at 12500 g for 15 minutes, then 4 mL of supernatant were removed and 706 µL of trichloroacetic acid (TCA 25%, Merck) was added (final concentration 15%). This suspension was incubated at 4°C between 6-hours and overnight and then centrifuged at 12 5000 g for 15 minutes. The pellet was resuspended in Laemmli sample buffer, aliquoted and stored at –20 °C until use.

Analysis of protein secretion profiles of bacterial strains

Through a vertical denaturing electrophoresis (SDS-PAGE at 12.5%) secretion profiles of all 3 bacteria were determined with Coomassie blue. The presence of each specific protein was preliminary determined based on the molecular weight previously reported for each protein (Table 1) and confirmed by Western Blot using monoclonal and polyclonal antibodies against EspB and EspD, respectively,¹⁴ monoclonal antibodies against IpaB and IpaC;¹⁵ and monoclonal antibodies against SipB and SipC (provided by Dr. Jorge E. Galan, Yale University). We did not have antibodies to the other proteins, therefore the diagnosis was presumptive. We were not able to adequately separate EspB and EspD because their molecular weights are too close; therefore the band detected by antibodies to EspB or D was reported as EspB-D.

Western Blot and quantification

The protein pellets resuspended in Laemmli sample buffer were separated and analyzed in a 12.5% SDS-PAGE and then electrophoretically transferred to 0.45µm pore size nitrocellulose membranes. These membranes were incubated with blocking buffer (3% skim milk in tris buffer saline [TBS] combined with 0.05% Tween 20) at room temperature for 2 hours and placed on a support: Mini-PROTEAN II Multiscreen Apparatus (Bio-Rad, Hercules, CA, USA). The dilution used for the primary antibody (IgA present in human colostrum) was 1/10 for membranes with *Salmonella* and *Shigella* secreted proteins; and 1/40 for membranes with EPEC secreted proteins. The membranes were incubated overnight with 300 µl of primary antibody at room temperature and then 3 times washed with milk buffer. The membranes were then incubated with 300 µl of secondary antibody (Anti-human IgA peroxidase conjugated) in a dilution of 1/200 for *Salmonella* and *Shigella* strains; and in a dilution of 1/800 for EPEC. After 2 h incubation at room temperature, membranes were washed three times with TBS 0.05%-Tween 20 and then another 3 times only with TBS. For developing, Horseradish peroxidase (HRP) Conjugate Substrate Kit (Bio-Rad, Hercules, CA, USA) was employed; the reaction was stopped when the bands became visible.

In order to estimate the amount of anti-TTSS IgA present in each colostrum sample, we have quantified the intensity of each Western Blot band using the Quantity One v4.6.3 software (Bio-Rad, Hercules, CA, USA), which measures the band intensity by OD (peaks density). Since the amount of the TTSS proteins loaded in each SDS-PAGE was the same (one large comb was used for each gel), and all colostrum samples had the same dilution, we have used the band intensity as a proxy for the amount of antibody present against each TTSS protein.

RESULTS AND DISCUSSION

Antibodies were detected against *Salmonella* antigens SipA 75/76(99%), SipC 62/76(82%) and SipB 31/76(41%); against *Shigella* antigens IpaC 70/76(92%), IpaB 68/76 (89%), IpaA 66/76(87%) and IpaD 41/76(54%); and against EPEC EspC 70/76(92%), EspB-D 65/76(86%) and EspA 41/76(54%) (Table 2). Thirty different patterns were found among 76 profiles analyzed. The most common pattern (n=32, 42%) was the presence of antibodies to all except one of the major secreted proteins of *Shigella*, *Salmonella* and EPEC; 13 samples lacked antibodies to SipB, 6 lacked antibodies to EspA, 3 lacked antibodies to IpaD, and 2 lacked antibodies to SipC. When combinations of proteins were evaluated, presence of sIgA against all Ipas was the most common combination, present in 54% (41 samples), followed by reactivity against all Esps in 53% (40 samples), and reactivity against SipA- SipC in 47% (36 samples). There were only six colostrum samples that lacked antibodies to all shigella T3SS proteins, four that lacked antibodies to EPEC T3SS proteins, and one that lacked antibody to the *Salmonella* T3SS proteins.

Antibodies to *Salmonella* T3SS effector proteins SipA and SipC were the most prevalent. These proteins are among the first to be released by the T3SS targeting host cells.² On the other hand, responses to other components of the same translocon varied markedly in the same samples (e.g. SipC was recognised in 82% but SipB in 41%). The difference in the expression of components of translocon SipB and SipC, may be due to the fact that compared to SipC, SipB is secreted for a short period of time at the initial stage of the invasion.¹⁶ Therefore, SipB has a lower probability of interacting with the host immune system antibodies to *Shigella* T3SS effector proteins (IpaC and IpaB) were also very prevalent. Previous studies have shown that antibodies against these antigens are present in milk from women living in both high and low-endemic areas of Shigellosis,^{11,17} suggesting that milk mucosal immunity reflects the mother's lifetime experience with these organisms.

An EPEC effector protein, EspC was the most prevalent followed by EspB-D. Although the EspC is an autotransporter protein secreted by a type five secretion system, this can also be secreted through the T3SS, which improves its efficiency of translocation into the epithelial cell.¹⁸ The presence of sIgA against EspA has been described with large variability. Parissi-Crivelli *et al.* (2000)¹⁹ found EspA antibodies in 16 of 21 (76%) of colostrum samples from Mexican women. On the other hand, Noguera-Obenza *et al.* (2003)¹² found a higher frequency in both Mexican women 68/73 (93%) and American women 45/50 (90%).

When we quantified the Western Blot band intensity for each TTSS protein and each colostrum sample we found large variability on these measurements, but with a normal distribution in general (Table 3). This suggests that the amount of sIgA against each protein varies in each milk sample, probably reflecting the frequency, degree and time of mothers' previous exposure to these pathogens.

Previous studies in developing countries have evaluated the presence of sIgA in human colostrum against the most frequent LPS serogroups of *Salmonella*, *Shigella* and EPEC, showing evidence of immunological memory and prior exposure to these pathogens, in addition to its possible protective role against infection.^{9,10} However, antibodies to specific

LPS by definition do not protect all serotypes. Hence such antibodies represent a poor candidate for investigating milk protective antibodies against all *Salmonella*, all *Shigella* and all EPEC. Thus, the proteins of the shared virulence mechanism represent a more plausible set of antigens to study milk-related infant protection. The study of these T3SS antibodies in milk may aid in the understanding of the host immune response and the development of enteric vaccines.

Previously, Sip and Ipa proteins were reported as homologous proteins,^{20,21} however analysis of amino acid sequences have determined them to be orthologous proteins with a low number of sequences conserved between them.^{22,23} Even though the structure of the proteins is different, their homologous functions could thus confer cross-reactivity. It is important to point out that antibodies against the T3SS effector proteins of EPEC are likely to also be relevant for protection from enterohemorrhagic *E. coli* or Shiga-toxin producing *E. coli* (EHEC/STEC) infection, since the effector proteins are highly conserved between these bacteria. For example, based on BLAST search data through NCBI the degree of amino acid identity between E2348/69 and *E. coli* O157:H7 EDL933 for EspA is 81%, for EspB 61%, for EspC 53%, and for EspD 74%. Thus, despite the fact that these proteins are not identical, it is likely that if antibodies protect against EPEC infection, they are also likely to protect against EHEC/STEC infection.

This study has several limitations. First, we used a qualitative method (presence or absence of an antibody) instead of quantitative method. Since amount may matter, we have tried to indirectly estimate the amount of antibody in each sample. Although this method provides some information, future studies are needed using specific quantitative methods (i.e. ELISA) or performing serial dilutions of all milk samples for the Western Blot analysis. Second, this study did not use purified antigens. Although this is an approach used in many studies, we were not able to discriminate between antibodies detecting EspB and EspD. Third, colostrum samples were collected from puerperal women at one hospital which may not represent the immunological memory of women from different socioeconomic levels in Lima. Finally, we have not determined the functional protective effects of these antibodies, which is beyond the scope of this study. However, some previous studies have demonstrated the protective effect of specific colostrum and milk antibodies *in vitro*.^{8,32,33} Nevertheless, this is one of the few studies that have searched simultaneously for the T3SS effector protein antibodies in colostrum against these important enteric pathogens. Indeed the extraordinarily high frequency of antibodies detected in this study against these multiple pathogens also suggest that milk antibodies reflect the mothers' long term immunologic experience.

Acknowledgments

Dr. Jorge E. Galan, Yale University for kindly providing monoclonal antibodies against SipB and SipC proteins. This study was funded by Public Health Service award 1K01TW007405 (T.J.O) from the National Institutes of Health.

References

1. Fischer Walker CL, Perin J, Aryee MJ, Boschi-Pinto C, Black RE. Diarrhea incidence in low- and middle-income countries in 1990 and 2010: a systematic review. *BMC Public Health*. 2012; 12:220. [PubMed: 22436130]
2. Haraga A, Ohlson MB, Miller SI. Salmonellae interplay with host cells. *Nature Reviews Microbiology*. 2008; 6:53–66.
3. Schroeder GN, Hilbi H. Molecular pathogenesis of *Shigella spp.*: controlling host cell signaling, invasion, and death by type III secretion. *Clin Microbiol Rev*. 2008; 21:134–156. [PubMed: 18202440]

4. Cossart P, Sansonetti PJ. Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science*. 2004; 304:242–248. [PubMed: 15073367]
5. Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. *Curr Opin Infect Dis*. 2011; 24:478–483. [PubMed: 21857511]
6. Clemens J, Elyazee RA, Rao M, et al. Early Initiation of Breastfeeding and the Risk of Infant Diarrhea in Rural Egypt. *Pediatrics*. 1999; 104:1–5. [PubMed: 10390252]
7. Morrow AL, Rangel JM. Human milk protection against infectious diarrhea: implication for prevention and clinical care. *Semin Pediatr Infect Dis*. 2004; 15:221–228. [PubMed: 15494945]
8. Carbone CB, Carbone SB, Carneiro-Sampaio MMS. Secretory immunoglobulin A obtained from pooled human colostrum and milk for oral passive immunization. *Pediatr Allergy Immunol*. 2005; 16:574–581. [PubMed: 16238582]
9. Hayani KC, Guerrero ML, Ruiz-Palacios GM, Gómez HF, Cleary TG. Evidence for Long-Term Memory of the Mucosal Immune System: Milk Secretory Immunoglobulin A against *Shigella* Lipopolysaccharides. *J Clin Microbiol*. 1991; 29:2599–2603. [PubMed: 1774268]
10. Nathavitharana KA, Catty D, McNeish AS. IgA antibodies in human milk: epidemiological markers of previous infections? *Arch Dis Child Fetal Neonatal*. 1994; 71:F192–F197.
11. Hayani KC, Guerrero ML, Morrow AL, et al. Concentration of milk secretory immunoglobulin A against *Shigella* virulence plasmid-associated antigens as a predictor of symptom status in *Shigella*-infected breast-fed infants. *J Pediatr*. 1992; 121:852–856. [PubMed: 1447644]
12. Noguera-Obenza M, Ochoa TJ, Gomez HF, et al. Human Milk Secretory Antibodies against Attaching and Effacing *Escherichia coli* Antigens. *Emerg Infect Disease*. 2003; 9:545–551. [PubMed: 12737737]
13. Komoriya K, Shibano N, Higano T, Azuma N, Yamaguchi S, Aizawa SI. Flagellar proteins and type III-exported virulence factors are predominant proteins secreted into the culture media of *Salmonella typhimurium*. *Molec Microbiol*. 1999; 34:767–779.
14. Ochoa TJ, Noguera-Obenza M, Ebel F, Guzman CA, Gomez HF, Cleary TG. Lactoferrin Impairs Type III Secretory System Function in Enteropathogenic *Escherichia coli*. *Infect Immun*. 2003; 71:5149–5155. [PubMed: 12933858]
15. Gomez HF, Ochoa TJ, Carlin LG, Cleary TG. Human Lactoferrin Impairs Virulence of *Shigella flexneri*. *The Journal of Infectious Diseases*. 2003; 187:87–95. [PubMed: 12508150]
16. Gong H, Su J, Bai Y, et al. Characterization of the expression of *Salmonella* Type III secretion system factor PrgI, SipA, SipB, SopE2, SpaO, and SptP in cultures and in mice. *BMC Microbiol*. 2009; 9:73. [PubMed: 19371445]
17. Cleary TG, West MR, Ruiz-Palacios G, et al. Human milk secretory immunoglobulin A to *Shigella* virulence plasmid-coded antigens. *J Pediatr*. 1991; 118:34–38. [PubMed: 1986095]
18. Vidal JE, Navarro-García F. EspC translocation into epithelial cells by enteropathogenic *Escherichia coli* requires a concerted participation of type V and III secretion systems. *Cell Microbiol*. 2008; 10:1975–1986. [PubMed: 18547338]
19. Parissi-Crivelli A, Parissi-Crivelli JM, Girón JA. Recognition of Enteropathogenic *Escherichia coli* virulence determinants by human colostrums and serum antibodies. *J Clin Microbiol*. 2000; 38:2696–2700. [PubMed: 10878066]
20. Kaniga K, Tucker S, Trollinger D, Galán JE. Homologs of the *Shigella* IpaB and IpaC invasins are required for *Salmonella typhimurium* entry into cultured epithelial cells. *J Bacteriol*. 1995a; 177:3965–3971. [PubMed: 7608068]
21. Kaniga K, Tucker S, Trollinger D, Galán JE. Identification of two targets of Type III protein secretion system encoded by the *inv* and *spa* loci of *Salmonella typhimurium* that have homology to the *Shigella* IpaD and IpaA proteins. *J Bacteriol*. 1995b; 177:7078–7085. [PubMed: 8522512]
22. Mattei PJ, Faudry E, Job V, Izoré T, Attree I, Dessen A. Membrane targeting and pore formation by the type III secretion system translocon. *FEBS J*. 2011; 278:414–426. [PubMed: 21182592]
23. Worrall LJ, Lameignere E, Strynadka NC. Structural overview of the bacterial injectisome. *Curr Opin Microbiol*. 2011; 14:3–8. [PubMed: 21112241]
24. Lara-Tejero M, Galán JE. *Salmonella enterica* Serovar Typhimurium Pathogenicity Island 1-Encoded Type III Secretion System Translocases Mediate Intimate Attachment to Nonphagocytic Cells. *Infection and Immunity*. 2009; 77:2635–2642. [PubMed: 19364837]

25. DeMali KA, Jue AL, Burrige K. IpaA targets beta 1 integrins and rho to promote actin cytoskeleton rearrangements necessary for *Shigella* entry. *J Biol Chem*. 2006; 281:39534–39541. [PubMed: 17060328]
26. Hale TL. Genetic basis of virulence in *shigella* species. *Microbiol Rev*. 1991; 55:206–224. [PubMed: 1886518]
27. Moreira CG, Palmer K, Whiteley M, et al. Bundle-forming pili and EspA are involved in biofilm formation by enteropathogenic *Escherichia coli*. *J Bacteriol*. 2006; 188:3952–3961. [PubMed: 16707687]
28. Abe A, Heczko U, Hegele RG, Finlay BB. Two Enteropathogenic *Escherichia coli* Type III Secreted Proteins, EspA and EspB, Are Virulence Factors. *J Exp Med*. 1998; 188:1907–1915. [PubMed: 9815268]
29. Lizumi Y, Sagara H, Kabe Y, et al. The enteropathogenic *E. coli* effector EspB facilitates microvillus effacing and antiphagocytosis by inhibiting myosin function. *Cell Host Microbe*. 2007; 2:383–392. [PubMed: 18078690]
30. Daniell SJ, Delahay RM, Shaw RK, et al. Coiled-coil domain of enteropathogenic *Escherichia coli* type III secreted protein EspD is involved in EspA filament-mediated cell attachment and hemolysis. *Infect Immun*. 2001; 69:4055–4064. [PubMed: 11349076]
31. Mellies JL, Navarro-Garcia F, Okeke I, Frederickson J, Nataro JP, Kaper JB. espC pathogenicity island of enteropathogenic *Escherichia coli* encodes an enterotoxin. *Infect Immun*. 2001; 69:315–3124. [PubMed: 11119520]
32. Fernandez RM, Carbonare SB, Carneiro-Sampaio MMS, Trabulsi LR. Inhibition of enteroaggregative *Escherichia coli* adhesion to HEp-2 cells by secretory immunoglobulin A from human colostrum. *Pediatr Infect Dis J*. 2001; 20:672–678. [PubMed: 11465839]
33. Palmeira P, Carbonare SB, Amaral JA, Tino-De-Franco M, Carneiro-Sampaio MMS. Colostrum from healthy Brazilian women inhibits adhesion and contains IgA antibodies reactive with Shiga toxin-producing *Escherichia coli*. *Eur J Pediatr*. 2005; 164:37–43. [PubMed: 15549380]

Table 1

T3SS effector proteins of *Salmonella*, *Shigella* and EPEC.

Protein	Cellular function	Molecular weight	Source
<i>Salmonella</i>			
SipA	Polimerizes actin. Potentiates SipC activity. Induces polymorphonuclear cell transepithelial migration	80 KDa	Haraga et al. ² Kaniga et al. ²¹
SipB	Translocon component. Activates caspase-1 triggering apoptosis. Induces autophagy in macrophages.	68 KDa	Haraga et al. ² Kaniga et al. ²⁰
SipC	Translocon component. Presents nucleation and bundling actin activity.	41 KDa	Haraga et al. ² Kaniga et al. ²⁰
SipD	Associated with translocon component. Present at the needle-component tip prior to contact with the host cell.	37 KDa	Kaniga et al. ²¹ Lara-Tejero M, Galán JE. ²⁴
<i>Shigella</i>			
IpaA	Induces actin reorganization in bacterial entry site with or without binding to vinculin.	78 KDa	Schroeder GN, Hilbi H, ³ DeMali et al., ²⁵ Hale TL. ²⁶
IpaB	Translocon component. Activates caspase-1 in macrophages triggering apoptosis and induces cell death. Interacts with CD44 receptor stimulating basolateral invasion.	62 KDa	Schroeder GN, Hilbi H, ³ DeMali et al., ²⁵ Hale TL. ²⁶
IpaC	Translocon component. Induces polymerization of actin and filopodia formation during invasion. Associated with pathogen escape of the phagosome.	43 KDa	Schroeder GN, Hilbi H, ³ Hale TL. ²⁶
IpaD	Present at the tip of the needle-component prior to host cell contact. Regulates IpaB/IpaC proteins secretion.	38 KDa	Schroeder GN, Hilbi H, ³ Hale TL. ²⁶
EPEC			
EspA	Conforms the needle-complex extension. Related with adherence and associated with biofilm formation.	25 KDa	Moreira et al. ²⁷ Abe et al. ²⁸
EspB	Translocon component. Inhibits the interaction between myosin and actin triggering microvillus effacing and suppressing phagocytosis.	37 KDa	Abe et al., ²⁸ Lizumi et al. ²⁹
EspD	Translocon component. Associated with the regulation of EspA filament formation and hemolysis.	40 KDa	Abe et al. ²⁸ Daniell et al. ³⁰
EspC	Enterotoxin of the Serine Protease Autotransporters of the Enterobacteriaceae (SPATE) family. Secreted by a Type Five Secretion System.	110 KDa	Vidal JE, Navarro-García F, ¹⁸ Mellies et al. ³¹

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<i>Salmonella</i>				<i>Shigella</i>				EPEC			n
SipA	SipB	SipC	IpaA	IpaB	IpaC	IpaD	EspA	EspB-D	EspC		
+	-	+	+	+	+	-	-	-	-	1	
+	-	+	+	+	+	+	-	-	-	1	

Table 3

Quantification of Western Blot bands' intensity of T3SS proteins of *Salmonella*, *Shigella* and EPEC.

Individual protein		Number of positive bands	Band intensity (OD)	
			mean $\pm$ SD	Median (Range)
<i>Salmonella</i>	SipA	75	160 $\pm$ 68	175 (21 – 255)
	SipB	31	97 $\pm$ 51	80 (30 – 204)
	SipC	62	126 $\pm$ 66	122 (23 – 254)
<i>Shigella</i>	IpaA	66	119 $\pm$ 36	127 (37 – 180)
	IpaB	68	125 $\pm$ 35	127 (41 – 191)
	IpaC	70	129 $\pm$ 32	132 (46 – 193)
	IpaD	41	103 $\pm$ 37	99 (32 – 178)
EPEC	EspA	41	54 $\pm$ 38	42 (9 – 174)
	EspB-D	65	115 $\pm$ 62	105 (19 – 243)
	EspC	70	105 $\pm$ 56	106 (20 – 210)