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# Modulation of the enterohemorrhagic *E. coli* virulence program through the human gastrointestinal tract

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Enteric pathogens must not only survive passage through the gastrointestinal tract but must also coordinate expression of virulence determinants in response to localized microenvironments with the host. Enterohemorrhagic *Escherichia coli* (EHEC), a serious food and waterborne human pathogen, is well equipped with an arsenal of molecular factors that allows it to survive passage through the gastrointestinal tract and successfully colonize the large intestine. This review will explore how EHEC responds to various environmental cues associated with particular microenvironments within the host and how it employs these cues to modulate virulence factor expression, with a view to developing a conceptual framework for understanding modulation of EHEC's virulence program in response to the host. In vitro studies offer significant insights into the role of individual environmental cues but in vivo studies using animal models as well as data from natural infections will ultimately provide a more comprehensive picture of the highly regulated virulence program of this pathogen.

## Introduction

Infection with enterohemorrhagic *Escherichia coli* (EHEC) is a leading cause of bloody diarrhea and hemorrhagic colitis, occasionally resulting in life-threatening systemic complications including hemolytic uremic syndrome (HUS).[1,2] This food and waterborne zoonotic agent has been associated with numerous outbreaks worldwide and constitutes a serious public health threat. Of over 380 EHEC serotypes, the O157:H7 serotype is the one most highly associated with outbreaks and severe disease in North America.[3,4] However, the non-O157 serotypes also represent a significant public health concern, and are frequently associated with HUS, particularly in Latin America, Europe, and Australia.[3,5]

EHEC infection typically begins with watery diarrhea, vomiting and abdominal cramping that then progresses to bloody diarrhea. In the majority of infected individuals, the infection resolves within a week to 10 d. However, in 5–7% of infected individuals, the infection leads to a systemic, sometimes life-threatening complication known as hemolytic uremic syndrome (HUS). HUS is characterized by thrombocytopenia, hemolytic anemia, and acute renal failure. Currently, treatment consists primarily of supportive therapy including rehydration.[6-11] Conventional antibiotic treatment is generally not recommended although there is no clear consensus on this matter. In vitro studies have shown that, at least for EHEC O157, sublethal doses of antibiotics, particularly trimethoprim, the quinolones or furazolidone, promote the production and release of Shiga toxins, a development that constitutes a risk factor for progression to HUS.[12-18] A number of clinical studies have also shown that patients on antibiotic therapy for hemorrhagic colitis have a higher risk of developing HUS.[8,13,18-21] However, it should be noted that these studies are often limited by small sample size as well as the advanced stage of illness of the patients and the findings may be more relevant for certain EHEC seropathotypes. There is evidence that antibiotic treatment of EHEC O104:H4 does not promote Shiga toxin release[22] and two clinical studies found that antibiotic treatment of EHEC O104:H4 infection did not enhance the risk of HUS.[23,24] Other agents often used to treat bacterial infection including antimotility agents, narcotics and non-steroidal anti-inflammatory medication are also not recommended for EHEC infection. Given the increasing number of EHEC outbreaks and HUS complications and the lack of available therapeutic strategies, there is a significant, critical need for new approaches to the prevention and treatment of EHEC infection. Recent research on toxin antibodies, novel peptides and small molecule drugs as well as zinc-based salts have shown some promise in the quest for effective preventative and/or very early treatment strategies.[25-29]

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## EHEC Virulence Factors

EHEC was first identified as the pathogen responsible for colitis-mediated HUS by Karmali et al. who found that patients presenting with diarrhea and HUS were positive for a toxin capable of inducing irreversible cytotoxicity in cultured Vero cells.[30] The toxin, originally referred to as Verotoxin, was later shown to be structurally and antigenically similar to the toxin produced by *Shigella dysenteriaie* type 1, a finding which resulted in the name, Shiga toxin (Stx).[31] Stx contains two major structural subunits, A and B, the former which has RNA N-glycosidase activity against 28S rRNA, resulting in protein synthesis inhibition and the latter

which binds to globotriaosylceramide-3 (Gb3) on the surface on endothelial cells, permitting toxin dissemination and toxin-mediated tissue damage.[32-36] Human renal glomerular endothelial tissue express high levels of surface Gb3 which may explain why Stx production results in acute renal failure.[30,37]

EHEC pathogenesis is not however limited to toxin-mediated effects. Hemorrhagic colitis, an earlier event in the infection, is thought to be promoted by a number of virulence factors that include fimbrial and nonfimbrial adhesins, flagella, Stx, and the Type III secretion system. The primary adhesin, intimin, a bacterial outer membrane protein encoded in the chromosomal pathogenicity island, LEE (locus for enterocyte effacement), promotes bacterial adhesion to the columnar epithelial cells lining the terminal ileum and transverse colon through binding to its own injected receptor, Tir (translocated intimin receptor), as well as binding to host cell proteins, integrin and nucleolin.[38-42] However, intimin mutants still bind to host epithelial cells, providing persuasive evidence of the involvement of other adhesins.[43,44] A number of other non-fimbrial EHEC adhesins have been implicated in adhesion including the plasmid-encoded *toxB,* the chromosomal genetic locus, *efa1* (EHEC factor for adherence), and the chromosomally-encoded adhesins, Iha (*Vibrio cholerae* IrgA homolog), Cah (calcium-binding antigen 43 homolog), and OmpA (outer membrane protein A).[44-46] Fimbrial structures that have been implicated in host adhesion include two long polar fimbriae, F9 (a type 1 pilus homolog), two type IV pili (HCP in EHEC O157 and TFP in a non-O157 EHEC), the sorbitol-fermenting EHEC O157:H-plasmid-encoded fimbriae, SFP and ECP (*E. coli* common pilus), a pilus structure produced by both pathogenic and nonpathogenic *E. coli*.[47] The long polar fimbriae of EHEC O157:H7 appear to play a role in sheep and pig colonization although some studies have suggested they may also play complementary roles in adhesion to human cells.[48] By contrast, F9 fimbriae may actually prevent or disrupt adhesion since F9 mutants show increased adhesion to cultured epithelial cells.[49] HCP are recognized by the sera of HUS patients and may function in a synergistic manner with the adhesion-receptor pair, intimin-Tir.[50,51] ECP may play a role in colonization of pathogenic and commensal *E. coli* strains, promoting interbacterial interactions in biofilm communities.[52,53] Flagella have also been reported to function as adhesins, mediating bacterial adherence to mucins, the primary component of the mucous coat in the gastrointestinal tract.[54] Finally, Stx has also been shown to promote EHEC adhesion to host epithelial cells by upregulating surface expression of two receptor candidates, phosphatidylethanolamine and nucleolin.[55,56]

Other virulence factors include the LEE-encoded type III secretion system translocation (TTSS) and effector proteins, as well as a variety of non-LEE encoded effector proteins,[57] all of which contribute to the modulation of host cell signaling to support bacterial replication and survival, host colonization and the development of disease. Host cell changes include marked cytoskeletal rearrangements, disruption of intestinal barrier function, downregulation of the host inflammatory response, and induction of host cell apoptosis.[58] Although intimin and Tir are the primary mediators of human intestinal adhesion, other LEE-encoded proteins, including EspA, EspH, Map, EspF, and EspG, also promote EHEC colonization.[59,60]

## Expression of Virulence Factors

It is well established that EHEC virulence factor expression is influenced by numerous experimental conditions including temperature, culture media, pH, bile salts, and even host cell factors.[61-71] Clearly, the pathogen successfully survives passage through the human gastrointestinal tract (GIT), but recent research suggests that it may also use exposure to different GIT environments to regulate expression and function of virulence factors. The idea that environmental conditions may cue temporal-spatial expression of virulence factors in a pathogen has been garnering significant attention recently.[68,72] In this review, we will examine key host environmental cues that influence EHEC virulence factor expression with a view to understanding how they may play a role in the temporal-spatial regulation of EHEC virulence during passage through the human GIT.

## Acid Stress

Depending on intestinal motility and the location within the food bolus, bacteria must be able to survive up to 2.5 h at pH values ranging from 2–6, in order to successfully transit from the human stomach. Both nonpathogenic and pathogenic *E. coli* encode four different acid resistance systems that provide protection against exposure to pH as low as 2–2.5.[73,74] Since the infectious dose of EHEC is typically very low (50–100 organisms), acid tolerance and resistance are critical virulence traits. The acid resistance systems are dependent on culture conditions and growth phase and are employed differentially by EHEC for survival in foods vs. the intestinal tract.[73,75] The glutamate-dependent acid-resistance system (Gad; AR3) is one of three amino acid decarboxylase systems (Ar2–Ar4) and is thought to offer the best protection below pH 3. The AR1 system which employs the stationary phase alternative sigma factor, RpoS, and the global regulatory protein, cAMP receptor protein (CRP), provides an acid adaptation or tolerance response (ATR) that permits *E. coli* exposed to sublethal pH values (pH 5) to survive subsequent exposure to lower pH values (pH 2.5). Since the pH of the human stomach can increase to pH 6 after a large meal before dropping to pH 2, the acid adaptation response may be physiologically relevant in the survival of ingested EHEC. However, the acid resistance/adaptation response can also be triggered prior to ingestion, either in the bovine intestinal tract or within acidic foods.[73] Studies have further revealed that EHEC O157:H7 that are already expressing acid resistance remain acid resistant for at least a month during refrigeration and that no further induction upon encountering pH 2.5 is required.[74] These findings suggest that EHEC in contaminated foods are well prepared to survive the acid stress of gastric passage.

In addition to the expression of acid resistance systems, exposure to acid can trigger induction or repression of specific virulence genes and sets of genes in EHEC. In a DNA microarray study, investigators examined the gene expression profiles of

EHEC O157 that had been acid stressed and then neutralized relative to the same unstressed strain.[66] There were significant expression changes in virulence factors associated with adhesion, motility and type III secretion including genes encoding known and putative adhesins, fimbria, flagella, and curli as well as many of the LEE-encoded type III translocation and effector proteins. These changes correlated with changes in virulence properties including enhanced motility and host cell adhesion following acid stress and neutralization. The TTSS genes whose products mediate colonization and infection in the large intestine were downregulated following acid stress and this is consistent with their negative regulation by two regulators, GadE and H-NS, under acid conditions.[76,77] Adhesin expression profiles were more variable, depending on the nature and duration of the acid stress with several known adhesins including intimin showing little change and a few novel adhesins showing significant upregulation, suggesting that acid stress alters the adhesin profile. Interestingly, adhesion of acid-stressed EHEC to human epithelial cells is increased and at least one of the novel adhesins appears to play a role in the acid-induced adhesion.[78] Flagellar synthesis genes were also upregulated under acute acid stress along with a modest increase in motility, a response which may offer a defense strategy against acute acid. Interestingly, there was no change in *stx* gene expression and no increase in Stx-mediated cytotoxicity after acid stress which is consistent with the fact that Stx-induced cytotoxicity is generally associated with large intestine. Collectively, these findings suggest that acid stress serves to arm EHEC with defensive strategies including acid resistance and enhanced motility for escape as well as downregulation of genes whose proteins are typically involved in colonization and subsequent infection of the large intestine.

Recent studies have revealed that EHEC also employs the transcriptional regulator SdiA to coordinate the transcription of the LEE genes needed for A/E lesion formation and the *gad* genes required acid resistance, at least in cattle.[79-81] SdiA is a member of the LuxR family of transcriptional regulators and it senses acyl-homoserine lactones (AHL) produced by other bacteria. Based on these studies, it has been proposed that SdiA senses AHL, upregulates *gad* genes required for acid resistance, and downregulates LEE genes required for colonization. These findings support a model for modulation of the EHEC virulence program in the bovine intestinal tract, where EHEC resides as a commensal organism.

## Bile

Bile resistance, a critical virulence property of gastric pathogens, is generally achieved through active efflux using a variety of resistance nodulation division (RND) efflux systems and altered outer membrane permeability often achieved through modifications of lipopolysaccharide layer.[82-86] Studies have shown that bile also serves an environmental cue for a number of enteric bacteria including *Salmonella*, enteropathogenic *E. coli*, and EHEC by modulating the expression of specific virulence factors.[69,83,87-98] DNA microarray analysis of EHEC O157:H7 treated with bile salts showed upregulation of genes encoding the AcrAB efflux pump, a two component signal transduction system (*basRS/pmrAB*) and a lipid A modification pathway (*arnBCADTEF* and *ugd*).[69] Interestingly, this correlated with bile salt-induced resistance to the antimicrobial agent, polymyxin B, which was *basS*- and *arnT*-dependent. *ArnT* encodes the enzyme that transfers L-Ara4N to lipid A, a modification that decreases the negative charge of the lipopolysaccharide and has been shown to enhance resistance to several cationic antimicrobial peptides.[89,99,100] The authors also reported that bile salt treatment did not enhance Shiga toxin-mediated cytotoxicity, a finding that was consistent with the downregulation of *stx2* genes after bile salt treatment. Finally, expression of several other well established virulence factors including those encoded on the LEE pathogenicity island, was not altered by bile salt treatment. These findings suggest that bile secreted into the small intestine serves an environmental cue for EHEC, signaling changes that result in protective modifications of the bacterial outer membrane, thereby enhancing successful migration of the pathogen through the small intestine while at the same time suppressing the expression of virulence factors required for subsequent colonization and infection of the large intestine.

## Ethanolamine

The constant turnover of intestinal epithelial cells and commensal flora in the human gastrointestinal tract generates a large number of membrane lipid metabolites including the breakdown product of phosphatidylethanolamine, ethanolamine (EA).[101] Through degradation to ammonia and acetaldehyde, EA can serve as a source of nitrogen and occasionally carbon for some bacteria. Recent studies indicate that several GI pathogens including *Clostridium*, *Listeria*, *Enterococcus*, EHEC, enteropathogenic *E. coli* (EPEC), and *Salmonella* possess genes necessary to catabolize EA and that EA utilization (Eut) may be a possible virulence determinant.[101] In *Salmonella enterica*, the global virulence regulators, Fis and CsrA, have been found to regulate *eut* genes and mutations in those genes triggered a loss of virulence in a mouse model of infection.[102,103] Recent studies have also shown that EA serves as a source of nitrogen but not carbon for EHEC grown under conditions that mimic the intestinal environment.

Interestingly, the source of host-generated EA, phosphatidylethanolamine, has also been found to play a role in EHEC pathogenesis. EHEC preferentially binds to phosphatidylethanolamine (PE) in the host epithelial cell membrane and induces apoptosis in the host cell, resulting in the upregulation of outer leaflet PE levels and increased adhesion to the apoptotic cells.[56,104] Studies with the related attaching and effacing pathogen, EPEC, showed that PE binding by EPEC modulated host phospholipid metabolism, leading to increased outer leaflet PE and similar to EHEC, increased adhesion to the host cell.[105-107] These data suggest that the elevated host outer-leaflet PE levels triggered by pathogen binding to epithelial cells in the large intestine may be providing a critical source of EA for the pathogen.[101]

Recent studies by Kendall et al. now indicate that EA may also serve as an environmental cue to EHEC to modulate virulence factor expression.[70] EHEC cultured in minimal media

containing EA showed increased expression of genes encoding virulence regulators (Ler, QseE, and QseC) as well as Shiga toxin (*Stx2a*) and also showed increased characteristic attaching and effacing (A/E) lesions on host epithelial cells. However, the nature of that regulation is still not fully understood. While the *eut* genes are upregulated by culture in EA, the increased expression of virulence genes appears to be independent of the Eut catabolic enzymes. A positive transcriptional regulator of the *eut* locus, EutR, appears to partially regulate expression of the virulence genes under certain growth conditions in EA but the data also suggest the involvement of a second as yet unidentified regulator. Nevertheless these data provide evidence that EA in the microenvironment of the intestinal lumen may be acting as an environmental cue for virulence modulation in EHEC to assist in colonization of the large intestine.

## Microbial Flora Metabolites

During passage through and colonization of the human GIT, EHEC encounters the highly complex microflora and the metabolites that they produce. These metabolities can include simple metabolic byproducts such as short chain fatty acids (SCFA) as well as metabolites that allow the microflora populations to modulate their metabolism according to the size of their populations. The three principal SCFAs present in the intestine are acetate, propionate and butyrate and the concentrations of these acids vary through the ileum and the colon.[108-111] High concentrations of SCFAs (above 50 mM) more typical of that found in the proximal and distal colon have been shown to inhibit the growth of EHEC while low concentrations, particularly butyrate, (from 6.25 to 25 mM) more typical of the distal ileum enhance expression of virulence genes involved in motility, adhesion and induction of A/E lesion formation,[112,113] suggesting that SCFAs may be cueing EHEC migration and adhesion to the distal ileum. However, another study reported that high concentrations of SCFAs (172 mM) more typical of the distal colon were associated with increased expression of the gene encoding the adhesin Iha[114] suggesting that at least this adhesin is promoting host adherence in the colon. Since Iha can also function as an iron siderophore and, in this study, was upregulated along with TonB, the outer membrane ferrichrome transport protein, it is also possible that SCFAs may be cueing the pathogen to increase its iron-scavenging capacity in the colonic environment. EHEC is also able to sense the quorum signaling molecule AI-3, secreted by commensal flora, using the histidine kinase-response regulator two component signaling system, QseCB.[115-117] EHEC responds to AI-3 with increased flagellar synthesis and motility and it is thought that increased motility permits the pathogen to more closely approach the mucosal epithelium at the site of colonization. Collectively, these studies suggest that EHEC employs certain molecular cues secreted by commensal flora to upregulate virulence factors that enhance motility, adhesion and iron-scavenging, all of which promote the establishment of infection.

However there are other molecular structures secreted by the normal gut microbiota that may protect the host against infection. De Sablet et al. showed that one or more factors secreted by a complex human gut microbiota including the predominant species, *Bacteroides thetaiotaomicron*, repressed *stx2* mRNA expression in a manner independent of SdiA, QseA, QseC, or AI-3.[118] This is consistent with other studies that show that pure cultures of several probiotic strains inhibit *stx2* transcription in laboratory media.[119] It is also well established that certain commensal bacteria exert generalized antibacterial effects against enteric pathogens through the production of antimicrobial proteins such as colicins, lantibiotics, and microcins, which typically result in inhibited pathogen growth.[120] These data point to the positive benefit of normal gut microbiota in protecting against EHEC infection, leading to the speculation that disruptions in gut microbiota may enhance risk of EHEC infection.

In the final analysis, the impact of normal gut microbiota on EHEC infection must be contextualized within our enhanced understanding of mucosal microbial populations based on recent, in-depth investigations.[121] As we begin to fully appreciate the extensive variation in the microbial community structure along the entire length of the human gastrointestinal tract, we recognize the need for further research to better understand the roles of the gut microbiota within specific microenvironments in the modulation of the EHEC virulence program.

## Host Hormones, Epinephrine, and Norepineprine

EHEC, along with a number of other disease-causing organisms including ETEC, *Salmonella enterica*, *Vibrio parahemolyticus*, and *Edwardsiella tarda*, have been shown to use host-generated hormones epinephrine and norepinephrine as signals for differential regulation of virulence factors mediating invasion, motility, and in the case of EHEC and EPEC, A/E lesion formation.[115,117,122-125] EHEC uses the histidine sensor kinases QseC and QseE as sensors of the two hormones.[126] QseC, via its cognate response regulator QseB, regulates flagellar and motility genes and through QseF, QseC is able to activate production of Stx.[79,117] Through another response regulator, KdpE, QseC also upregulates LEE genes.[117] Not surprisingly, deletion of *qseC* attenuates EHEC virulence. The second sensor, QseE, responds to epinephrine as well as to phosphate and sulfate and now appears to regulate the LEE genes and *nleA* (nonLEE-encoded effector A) negatively.[79] However, this negative regulation is mediated indirectly through transcriptional inhibition of the response regulator, RcsB, which is a positive regulator of the LEE. Regulation of EHEC virulence by epinephrine and norepinephrine appears to be quite complex and is still not fully resolved. Nevertheless, that data collectively suggest that EHEC coordinates a temporal response to the microbial flora-produced AI-3 and the two host-derived hormones epinephrine and norepinephrine to assist in cueing the site of colonization and to enhance approach to the epithelial layer through increased motility and increased A/E lesion formation.[79,117]

## Low Oxygen

The environment of the intestinal tract is characterized by variable oxygen levels and a number of studies on other

**Table 1.** Modulation of EHEC virulence program by microenvironmental cues in the human gastrointestinal tract

| Local GIT Environment | Cue | Regulons Involved | Virulence factors: expression changes | Virulence modulation | References |
|---|---|---|---|---|---|
| Stomach | Low pH | RpoS, CRP, H-NS, GadE | ↑ AR1–4, ↑ Flagella and motility genes, ↑ novel adhesins ↓ LEE genes | ↑ acid resistance, ↑ motility ↑ adhesion | 66, 73 and 77 |
| Duodenum | Bile | BasRS, PhoP? | ↑ *arnBCADTEF* ↑ acrAB ↓ *stx2* | LPS modification, ↑ Bile and CAMP resistance | 69 and 89 |
| Ileum | AI-3(quorum sensing) | QseCB, SdiA | ↑ *gad* genes, flagella | ↑ motility, ↑ acid resistance (to SCFAs?) | 81 and 115 |
| | SCFA (< 25 mM) | | ↑ LEE, flagella | ↑ adhesion, ↑ motility | 112, 113 |
| Colon | EA | EutR, Ler, QseE, QseC | ↑ Stx2a | ↑ cytotoxicity | 70 |
| | SCFA (> 50 mM) | | ↑ Iha | ↑ adhesion, ↑ iron scavenging | 114 |
| | Low oxygen | Fnr, AcrA | ↑ EspA, ↑ TTSS effectors (at microaerobic oxygen levels) | ↑ adhesion, A/E lesion | 132 and 133 |
| | Epinephrine, norepinephrine | QseCB, QseCF, QseC/KdpE | ↑ flagella ↑ LEE genes ↑ Stx | ↑ motility, ↑ A/E lesion ↑ cytotoxicity | 117 |
| | Epinephrine, phosphate, sulfate | QseE | Inhibits RcsB, ↓ LEE? | ↓ A/E lesion? | 79 |

Cues encountered at various locations within the GIT are provided along with associated changes in the expression of specific virulence factors and properties (increased, ↑, or decreased, ↓). Regulons reported to be involved in the responses are also provided.

pathogens report that oxygen levels do modulate pathogen virulence.[72,127,128] While the lumen of the intestinal tract is relatively anaerobic, there is a zone of relative oxygenation adjacent to the mucosal surface that is generated by diffusion from the microvilli capillary network.[128,129] *E. coli* can sense changes in oxygen availability and switch from aerobiosis to either anerobiosis or microaerobiosis, a process which is governed by two global regulators, Fnr (anaerobiosis) and ArcA (microaerobiosis).[130-132] Studies reveal that *E. coli* are alternatively dependent on microaerobic and anaerobic respiration and that this respiratory flexibility is a key determinant in their ability to successfully colonize the human intestine. Despite our understanding of this respiratory flexibility in *E. coli*, there is very little known about how varying oxygen concentrations modulate EHEC virulence. A recent study examined a model of EHEC infection of the apical side of polarized epithelial cells under oxygen concentrations of 1–2% atmospheric pressure (considered microaerobic) and found that EHEC-host adhesion was increased and that expression and translocation of EHEC TTSS effector proteins were also increased.[133] The increased adhesion appeared to be mediated primarily by the TTSS translocon, EspA, while other potential adherence factors including flagella and the *E. coli* common pilus were only minimally expressed. These results suggest that the microaerobic environment adjacent to the intestinal microvilli may upregulate expression of EHEC virulence factors that promote successful colonization of the large intestine. They also point to the need for further study on the role of oxygen availability in modulating EHEC virulence.

## Temporal-Spatial Cueing

We have yet to put together the picture of how EHEC adapts to each of the successive environments of the human GIT and responds to these various cues in a temporal-spatial fashion. By assembling a sequential list of environmental cues encountered by the pathogen along with data on the modulation of virulence factors and properties, one can begin to envision a model for the temporal-spatial regulation of the EHEC virulence program (**Table 1**). Passage through each of the local GIT environments appears to differentially arm EHEC with specific protective mechanisms appropriate to the local environments including enhanced resistance to acid, bile, and cationic antimicrobial proteins along with increased motility which could mediate escape from stressful environments. Exposure to different microenvironmental cues also alters expression of virulence factors and properties that may provide EHEC with selective advantages in current or upcoming local environments including AI-3-induced acid tolerance possibly to upcoming SCFA stress, and flagella-mediated motility toward the gut epithelium. As EHEC approaches the site of colonization and infection, exposure to environmental cues in the ileum and colon including short chain fatty acids, quorum signals, ethanolamine, host hormones, and changes in oxygen levels is accompanied by the upregulation of virulence factors that promote adhesion, A/E lesion formation, and cytotoxicity, all of which promote colonization and the establishment of infection. While the picture is beginning to emerge, there are still many gaps and some inconsistencies. What is still missing is the integration of signals delivered in a

sequential but not necessarily spatially isolated fashion. Large scale studies using molecular genomics, genetics, and proteomic approaches have generated huge amounts of information but determining the physiological relevance of these data remains a challenge. Natural infections can provide us with retrospective information but again the data are difficult to evaluate in the absence of appropriate controls. Animal models will likely provide us with the most useful insight but it is still difficult to appreciate the value of each cue or sets of cues or sequence of cues in a definitive manner. Expression of EHEC virulence factors, both the timing and the level of expression, is highly regulated by the environment and to more fully understand this regulation will involve well-designed animal infection models and more data from infection outbreaks.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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### References


1. Karmali MA. Infection by Shiga toxin-producing Escherichia coli: an overview. Mol Biotechnol 2004; 26:117-22; PMID:14764937; http://dx.doi.org/10.1385/MB:26:2:117
2. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev 1998; 11:142-201; PMID:9457432
3. Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, et al. Association of genomic O island 122 of Escherichia coli EDL 933 with verocytotoxin-producing Escherichia coli seropathotypes that are linked to epidemic and/or serious disease. J Clin Microbiol 2003; 41:4930-40; PMID:14605120; http://dx.doi.org/10.1128/JCM.41.11.4930-4940.2003
4. Banatvala N, Griffin PM, Greene KD, Barrett TJ, Bibb WF, Green JH, et al.; Hemolytic Uremic Syndrome Study Collaborators. The United States National Prospective Hemolytic Uremic Syndrome Study: microbiologic, serologic, clinical, and epidemiologic findings. J Infect Dis 2001; 183:1063-70; PMID:11237831; http://dx.doi.org/10.1086/319269
5. Coombes BK, Wickham ME, Mascarenhas M, Gruenheid S, Finlay BB, Karmali MA. Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing Escherichia coli strains. Appl Environ Microbiol 2008; 74:2153-60; PMID:18245257; http://dx.doi.org/10.1128/AEM.02566-07
6. Ake JA, Jelacic S, Ciol MA, Watkins SL, Murray KF, Christie DL, et al. Relative nephroprotection during Escherichia coli O157:H7 infections: association with intravenous volume expansion. Pediatrics 2005; 115:e673-80; PMID:15930195; http://dx.doi.org/10.1542/peds.2004-2236
7. Palermo MS, Exeni RA, Fernández GC. Hemolytic uremic syndrome: pathogenesis and update of interventions. Expert Rev Anti Infect Ther 2009; 7:697-707; PMID:19681698; http://dx.doi.org/10.1586/eri.09.49
8. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet 2005; 365:1073-86; PMID:15781103
9. Bavaro MF. Escherichia coli O157: what every internist and gastroenterologist should know. Curr Gastroenterol Rep 2009; 11:301-6; PMID:19615306; http://dx.doi.org/10.1007/s11894-009-0044-0
10. Goldwater PN, Bettelheim KA. Treatment of enterohemorrhagic Escherichia coli (EHEC) infection and hemolytic uremic syndrome (HUS). BMC Med 2012; 10:12; PMID:22300510; http://dx.doi.org/10.1186/1741-7015-10-12
11. Serna A 4th, Boedeker EC. Pathogenesis and treatment of Shiga toxin-producing Escherichia coli infections. Curr Opin Gastroenterol 2008; 24:38-47; PMID:18043231; http://dx.doi.org/10.1097/MOG.0b013e3282f2dfb8
12. Scheiring J, Andreoli SP, Zimmerhackl LB. Treatment and outcome of Shiga-toxin-associated hemolytic uremic syndrome (HUS). Pediatr Nephrol 2008; 23:1749-60; PMID:18704506; http://dx.doi.org/10.1007/s00467-008-0935-6f
13. Zimmerhackl LB. E. coli, antibiotics, and the hemolytic-uremic syndrome. N Engl J Med 2000; 342:1990-1; PMID:10874069; http://dx.doi.org/10.1056/NEJM200006293422611
14. Karch H, Schmidt H, Janetzki-Mittmann C, Scheef J, Kröger M. Shiga toxins even when different are encoded at identical positions in the genomes of related temperate bacteriophages. Mol Gen Genet 1999; 262:600-7; PMID:10628842; http://dx.doi.org/10.1007/s004380051122
15. Matsushiro A, Sato K, Miyamoto H, Yamamura T, Honda T. Induction of prophages of enterohemorrhagic Escherichia coli O157:H7 with norfloxacin. J Bacteriol 1999; 181:2257-60; PMID:10094706
16. Wagner PL, Livny J, Neely MN, Acheson DW, Friedman DI, Waldor MK. Bacteriophage control of Shiga toxin 1 production and release by Escherichia coli. Mol Microbiol 2002; 44:957-70; PMID:12010491; http://dx.doi.org/10.1046/j.1365-2958.2002.02950.x
17. Rahal EA, Kazzi N, Nassar FJ, Matar GM. Escherichia coli O157:H7-Clinical aspects and novel treatment approaches. Front Cell Infect Microbiol 2012; 2:138; PMID:23162800; http://dx.doi.org/10.3389/fcimb.2012.00138
18. Safdar N, Said A, Gangnon RE, Maki DG. Risk of hemolytic uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 enteritis: a meta-analysis. JAMA 2002; 288:996-1001; PMID:12190370; http://dx.doi.org/10.1001/jama.288.8.996
19. Slutsker L, Altekruse SF, Swerdlow DL. Foodborne diseases. Emerging pathogens and trends. Infect Dis Clin North Am 1998; 12:199-216; PMID:9494839; http://dx.doi.org/10.1016/S0891-5520(05)70418-9
20. Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic-uremic syndrome after antibiotic treatment of Escherichia coli O157:H7 infections. N Engl J Med 2000; 342:1930-6; PMID:10874060; http://dx.doi.org/10.1056/NEJM200006293422601
21. Wong CS, Mooney JC, Brandt JR, Staples AO, Jelacic S, Boster DR, et al. Risk factors for the hemolytic uremic syndrome in children infected with Escherichia coli O157:H7: a multivariable analysis. Clin Infect Dis 2012; 55:33-41; PMID:22431799; http://dx.doi.org/10.1093/cid/cis299
22. Corogeanu D, Willmes R, Wolke M, Plum G, Utermöhlen O, Krönke M. Therapeutic concentrations of antibiotics inhibit Shiga toxin release from enterohemorrhagic E. coli O104:H4 from the 2011 German outbreak. BMC Microbiol 2012; 12:160; PMID:22853739; http://dx.doi.org/10.1186/1471-2180-12-160
23. Geerdes-Fenge HF, Löbermann M, Nürnberg M, Fritzsche C, Koball S, Henschel J, et al. Ciprofloxacin reduces the risk of hemolytic uremic syndrome in patients with Escherichia coli O104:H4-associated diarrhea. Infection 2013; In press; PMID:23292662; http://dx.doi.org/10.1007/s15010-012-0387-6
24. Menne J, Nitschke M, Stingele R, Abu-Tair M, Beneke J, Bramstedt J, et al.; EHEC-HUS consortium. Validation of treatment strategies for enterohaemorrhagic Escherichia coli O104:H4 induced haemolytic uraemic syndrome: case-control study. BMJ 2012; 345:e4565; PMID:22815429; http://dx.doi.org/10.1136/bmj.e4565
25. Dolgin E. As E. coli continues to claim lives, new approaches offer hope. Nat Med 2011; 17:755; PMID:21738132; http://dx.doi.org/10.1038/nm0711-755
26. Lino M, Kus JV, Tran SL, Naqvi Z, Binnington B, Goodman SD, et al. A novel antimicrobial peptide significantly enhances acid-induced killing of Shiga toxin-producing Escherichia coli O157 and non-O157 serotypes. Microbiology 2011; 157:1768-75; PMID:21454368; http://dx.doi.org/10.1099/mic.0.047365-0
27. Stearns-Kurosawa DJ, Collins V, Freeman S, Debord D, Nishikawa K, Oh SY, et al. Rescue from lethal Shiga toxin 2-induced renal failure with a cell-permeable peptide. Pediatr Nephrol 2011; 26:2031-9; PMID:21603905; http://dx.doi.org/10.1007/s00467-011-1913-y
28. Silberstein C, Lucero MS, Zotta E, Copeland DP, Lingyun L, Repetto HA, et al. A glucosylceramide synthase inhibitor protects rats against the cytotoxic effects of shiga toxin 2. Pediatr Res 2011; 69:390-4; PMID:21270676; http://dx.doi.org/10.1203/PDR.0b013e318211dd57
29. Crane JK, Byrd IW, Boedeker EC. Virulence inhibition by zinc in shiga-toxigenic Escherichia coli. Infect Immun 2011; 79:1696-705; PMID:21245267; http://dx.doi.org/10.1128/IAI.01099-10
30. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing Escherichia coli. J Infect Dis 1985; 151:775-82; PMID:3886804; http://dx.doi.org/10.1093/infdis/151.5.775
31. O'Brien AD, Lively TA, Chen ME, Rothman SW, Formal SB. Escherichia coli O157:H7 strains associated with hemorrhagic colitis in the United States produce a shigella dysenteriae 1 (shiga)-like cytotoxin. Lancet 1983; 321:702; http://dx.doi.org/10.1016/S0140-6736(83)91987-6
32. O'Brien AD, Tesh VL, Donohue-Rolfe A, Jackson MP, Olsnes S, Sandvig K, et al. Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis. Curr Top Microbiol Immunol 1992; 180:65-94; PMID:1324134; http://dx.doi.org/10.1007/978-3-642-77238-2_4
33. Lingwood CA, Law H, Richardson S, Petric M, Brunton JL, De Grandis S, et al. Glycolipid binding of purified and recombinant Escherichia coli produced verotoxin in vitro. J Biol Chem 1987; 262:8834-9; PMID:3298243
34. Waddell T, Head S, Petric M, Cohen A, Lingwood CA. Globotriosyl ceramide is specifically recognized by the Escherichia coli verocytotoxin 2. Biochem Biophys Res Commun 1988; 152:674-9; PMID:3284526; http://dx.doi.org/10.1016/S0006-291X(88)80091-3

35. Bergan J, Dyve Lingelem AB, Simm R, Skotland T, Sandvig K. Shiga toxins. Toxicon 2012; 60:1085-107; PMID:22960449; http://dx.doi.org/10.1016/j.toxicon.2012.07.016
36. Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara T, Igarashi K. Site of action of a Vero toxin (VT2) from Escherichia coli O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. Eur J Biochem 1988; 171:45-50; PMID:3276522; http://dx.doi.org/10.1111/j.1432-1033.1988.tb13756.x
37. Williams JM, Boyd B, Nutikka A, Lingwood CA, Barnett Foster DE, Milford DV, et al. A comparison of the effects of verocytotoxin-1 on primary human renal cell cultures. Toxicol Lett 1999; 105:47-57; PMID:10092056; http://dx.doi.org/10.1016/S0378-4274(98)00383-X
38. Frankel G, Lider O, Hershkoviz R, Mould AP, Kachalsky SG, Candy DCA, et al. The cell-binding domain of intimin from enteropathogenic Escherichia coli binds to beta1 integrins. J Biol Chem 1996; 271:20359-64; PMID:8702771; http://dx.doi.org/10.1074/jbc.271.34.20359
39. McKee ML, Melton-Celsa AR, Moxley RA, Francis DH, O'Brien AD. Enterohemorrhagic Escherichia coli O157:H7 requires intimin to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells. Infect Immun 1995; 63:3739-44; PMID:7642319
40. Sinclair JF, O'Brien AD. Cell surface-localized nucleolin is a eukaryotic receptor for the adhesin intimin-gamma of enterohemorrhagic Escherichia coli O157:H7. J Biol Chem 2002; 277:2876-85; PMID:11704679; http://dx.doi.org/10.1074/jbc.M110230200
41. Jerse AE, Yu J, Tall BD, Kaper JB. A genetic locus of enteropathogenic Escherichia coli necessary for the production of attaching and effacing lesions on tissue culture cells. Proc Natl Acad Sci U S A 1990; 87:7839-43; PMID:2172966; http://dx.doi.org/10.1073/pnas.87.20.7839
42. Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB. Enteropathogenic E. coli (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell 1997; 91:511-20; PMID:9390560; http://dx.doi.org/10.1016/S0092-8674(00)80437-7
43. Dytoc MT, Ismaili A, Philpott DJ, Soni R, Brunton JL, Sherman PM. Distinct binding properties of eaeA-negative verocytotoxin-producing Escherichia coli of serotype O113:H21. Infect Immun 1994; 62:3494-505; PMID:7518809
44. Torres AG, Zhou X, Kaper JB. Adherence of diarrheagenic Escherichia coli strains to epithelial cells. Infect Immun 2005; 73:18-29; PMID:15618137; http://dx.doi.org/10.1128/IAI.73.1.18-29.2005
45. Johnson JR, Jelacic S, Schoening LM, Clabots C, Shaikh N, Mobley HLT, et al. The IrgA homologue adhesin Iha is an Escherichia coli virulence factor in murine urinary tract infection. Infect Immun 2005; 73:965-71; PMID:15664939; http://dx.doi.org/10.1128/IAI.73.2.965-971.2005
46. Tatsuno I, Horie M, Abe H, Miki T, Makino K, Shinagawa H, et al. toxB gene on pO157 of enterohemorrhagic Escherichia coli O157:H7 is required for full epithelial cell adherence phenotype. Infect Immun 2001; 69:6660-9; PMID:11598035; http://dx.doi.org/10.1128/IAI.69.11.6660-6669.2001
47. Brunder W, Khan AS, Hacker J, Karch H. Novel type of fimbriae encoded by the large plasmid of sorbitol-fermenting enterohemorrhagic Escherichia coli O157:H(-). Infect Immun 2001; 69:4447-57; PMID:11401985; http://dx.doi.org/10.1128/IAI.69.7.4447-4457.2001
48. Low AS, Holden N, Rosser T, Roe AJ, Constantinidou C, Hobman JL, et al. Analysis of fimbrial gene clusters and their expression in enterohaemorrhagic Escherichia coli O157:H7. Environ Microbiol 2006; 8:1033-47; PMID:16689724; http://dx.doi.org/10.1111/j.1462-2920.2006.00995.x
49. Low AS, Dziva F, Torres AG, Martinez JL, Rosser T, Naylor S, et al. Cloning, expression, and characterization of fimbrial operon F9 from enterohemorrhagic Escherichia coli O157:H7. Infect Immun 2006; 74:2233-44; PMID:16552054; http://dx.doi.org/10.1128/IAI.74.4.2233-2244.2006
50. Xicohtencatl-Cortes J, Monteiro-Neto V, Ledesma MA, Jordan DM, Francetic O, Kaper JB, et al. Intestinal adherence associated with type IV pili of enterohemorrhagic Escherichia coli O157:H7. J Clin Invest 2007; 117:3519-29; PMID:17948128; http://dx.doi.org/10.1172/JCI30727
51. Xicohtencatl-Cortes J, Monteiro-Neto V, Saldaña Z, Ledesma MA, Puente JL, Girón JA. The type 4 pili of enterohemorrhagic Escherichia coli O157:H7 are multipurpose structures with pathogenic attributes. J Bacteriol 2009; 191:411-21; PMID:18952791; http://dx.doi.org/10.1128/JB.01306-08
52. Rendón MA, Saldaña Z, Erdem AL, Monteiro-Neto V, Vázquez A, Kaper JB, et al. Commensal and pathogenic Escherichia coli use a common pilus adherence factor for epithelial cell colonization. Proc Natl Acad Sci U S A 2007; 104:10637-42; PMID:17563352; http://dx.doi.org/10.1073/pnas.0704104104
53. Martínez-Santos VI, Medrano-López A, Saldaña Z, Girón JA, Puente JL. Transcriptional regulation of the ecp operon by EcpR, IHF, and H-NS in attaching and effacing Escherichia coli. J Bacteriol 2012; 194:5020-33; PMID:22797761; http://dx.doi.org/10.1128/JB.00915-12
54. Erdem AL, Avelino F, Xicohtencatl-Cortes J, Girón JA. Host protein binding and adhesive properties of H6 and H7 flagella of attaching and effacing Escherichia coli. J Bacteriol 2007; 189:7426-35; PMID:17693516; http://dx.doi.org/10.1128/JB.00464-07
55. Robinson CM, Sinclair JF, Smith MJ, O'Brien AD. Shiga toxin of enterohemorrhagic Escherichia coli type O157:H7 promotes intestinal colonization. Proc Natl Acad Sci U S A 2006; 103:9667-72; PMID:16766659; http://dx.doi.org/10.1073/pnas.0602359103
56. Barnett Foster D, Abul-Milh M, Huesca M, Lingwood CA. Enterohemorrhagic Escherichia coli induces apoptosis which augments bacterial binding and phosphatidylethanolamine exposure on the plasma membrane outer leaflet. Infect Immun 2000; 68:3108-15; PMID:10816451; http://dx.doi.org/10.1128/IAI.68.6.3108-3115.2000
57. Tobe T, Beatson SA, Taniguchi H, Abe H, Bailey CM, Fivian A, et al. An extensive repertoire of type III secretion effectors in Escherichia coli O157 and the role of lambdoid phages in their dissemination. Proc Natl Acad Sci U S A 2006; 103:14941-6; PMID:16990433; http://dx.doi.org/10.1073/pnas.0604891103
58. Frankel G, Phillips AD. Attaching effacing Escherichia coli and paradigms of Tir-triggered actin polymerization: getting off the pedestal. Cell Microbiol 2008; 10:549-56; PMID:18053003; http://dx.doi.org/10.1111/j.1462-5822.2007.01103.x
59. Campellone KG, Roe AJ, Løbner-Olesen A, Murphy KC, Magoun L, Brady MJ, et al. Increased adherence and actin pedestal formation by dam-deficient enterohaemorrhagic Escherichia coli O157:H7. Mol Microbiol 2007; 63:1468-81; PMID:17302821; http://dx.doi.org/10.1111/j.1365-2958.2007.05602.x
60. Ritchie JM, Waldor MK. The locus of enterocyte effacement-encoded effector proteins all promote enterohemorrhagic Escherichia coli pathogenicity in infant rabbits. Infect Immun 2005; 73:1466-74; PMID:15731044; http://dx.doi.org/10.1128/IAI.73.3.1466-1474.2005
61. Abe H, Tatsuno I, Tobe T, Okutani A, Sasakawa C. Bicarbonate ion stimulates the expression of locus of enterocyte effacement-encoded genes in enterohemorrhagic Escherichia coli O157:H7. Infect Immun 2002; 70:3500-9; PMID:12065489; http://dx.doi.org/10.1128/IAI.70.7.3500-3509.2002
62. Nakanishi N, Abe H, Ogura Y, Hayashi T, Tashiro K, Kuhara S, et al. ppGpp with DksA controls gene expression in the locus of enterocyte effacement (LEE) pathogenicity island of enterohaemorrhagic Escherichia coli through activation of two virulence regulatory genes. Mol Microbiol 2006; 61:194-205; PMID:16824105; http://dx.doi.org/10.1111/j.1365-2958.2006.05217.x
63. Sperandio V, Li CC, Kaper JB. Quorum-sensing Escherichia coli regulator A: a regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic E. coli. Infect Immun 2002; 70:3085-93; PMID:12011002; http://dx.doi.org/10.1128/IAI.70.6.3085-3093.2002
64. Rosenshine I, Ruschkowski S, Finlay BB. Expression of attaching/effacing activity by enteropathogenic Escherichia coli depends on growth phase, temperature, and protein synthesis upon contact with epithelial cells. Infect Immun 1996; 64:966-73; PMID:8641808
65. Jandu N, Ho NK, Donato KA, Karmali MA, Mascarenhas M, Duffy SP, et al. Enterohemorrhagic Escherichia coli O157:H7 gene expression profiling in response to growth in the presence of host epithelia. PLoS One 2009; 4:e4889; PMID:19293938; http://dx.doi.org/10.1371/journal.pone.0004889
66. House B, Kus JV, Prayitno N, Mair R, Que L, Chingcuanco F, et al. Acid-stress-induced changes in enterohaemorrhagic Escherichia coli O157 : H7 virulence. Microbiology 2009; 155:2907-18; PMID:19497950; http://dx.doi.org/10.1099/mic.0.025171-0
67. Carruthers MD, Minion C. Transcriptome analysis of Escherichia coli O157:H7 EDL933 during heat shock. FEMS Microbiol Lett 2009; 295:96-102; PMID:19473256; http://dx.doi.org/10.1111/j.1574-6968.2009.01587.x
68. Gyles CL. Relevance in pathogenesis research. Vet Microbiol 2011; 153:2-12; PMID:21592684; http://dx.doi.org/10.1016/j.vetmic.2011.04.020
69. Kus JV, Gebremedhin A, Dang V, Tran SL, Serbanescu A, Barnett Foster D. Bile salts induce resistance to polymyxin in enterohemorrhagic Escherichia coli O157:H7. J Bacteriol 2011; 193:4509-15; PMID:21725004; http://dx.doi.org/10.1128/JB.00200-11
70. Kendall MM, Gruber CC, Parker CT, Sperandio V. Ethanolamine controls expression of genes encoding components involved in interkingdom signaling and virulence in enterohemorrhagic Escherichia coli O157:H7. MBio 2012; 3:e00050-12; PMID:22589288; http://dx.doi.org/10.1128/mBio.00050-12
71. Pacheco AR, Sperandio V. Shiga toxin in enterohemorrhagic E.coli: regulation and novel anti-virulence strategies. Front Cell Infect Microbiol 2012; 2:81; PMID:22919672; http://dx.doi.org/10.3389/fcimb.2012.00081
72. Marteyn B, Gazi A, Sansonetti P. Shigella: a model of virulence regulation in vivo. Gut Microbes 2012; 3:104-20; PMID:22356862; http://dx.doi.org/10.4161/gmic.19325
73. Foster JW. Escherichia coli acid resistance: tales of an amateur acidophile. Nat Rev Microbiol 2004; 2:898-907; PMID:15494746; http://dx.doi.org/10.1038/nrmicro1021
74. Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW. Mechanisms of acid resistance in enterohemorrhagic Escherichia coli. Appl Environ Microbiol 1996; 62:3094-100; PMID:8795195
75. Price SB, Wright JC, DeGraves FJ, Castanie-Cornet MP, Foster JW. Acid resistance systems required for survival of Escherichia coli O157:H7 in the bovine gastrointestinal tract and in apple cider are different. Appl Environ Microbiol 2004; 70:4792-9; PMID:15294816; http://dx.doi.org/10.1128/AEM.70.8.4792-4799.2004

76. Kailasan Vanaja S, Bergholz TM, Whittam TS. Characterization of the Escherichia coli O157:H7 Sakai GadE regulon. J Bacteriol 2009; 191:1868-77; PMID:19114477; http://dx.doi.org/10.1128/JB.01481-08
77. Laaberki MH, Janabi N, Oswald E, Repoila F. Concert of regulators to switch on LEE expression in enterohemorrhagic Escherichia coli O157:H7: interplay between Ler, GrlA, HNS and RpoS. Int J Med Microbiol 2006; 296:197-210; PMID:16618552; http://dx.doi.org/10.1016/j.ijmm.2006.02.017
78. Chingcuanco F, Yu Y, Kus JV, Que L, Lackraj T, Lévesque CM, et al. Identification of a novel adhesin involved in acid-induced adhesion of enterohaemorrhagic Escherichia coli O157 : H7. Microbiology 2012; 158:2399-407; PMID:22767547; http://dx.doi.org/10.1099/mic.0.056374-0
79. Nguyen Y, Sperandio V. Enterohemorrhagic E. coli (EHEC) pathogenesis. Front Cell Infect Microbiol 2012; 2:90; PMID:22919681; http://dx.doi.org/10.3389/fcimb.2012.00090
80. Hughes DT, Terekhova DA, Liou L, Hovde CJ, Sahl JW, Patankar AV, et al. Chemical sensing in mammalian host-bacterial commensal associations. Proc Natl Acad Sci U S A 2010; 107:9831-6; PMID:20457895; http://dx.doi.org/10.1073/pnas.1002551107
81. Kanamaru K, Kanamaru K, Tatsuno I, Tobe T, Sasakawa C. SdiA, an Escherichia coli homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic Escherichia coli O157:H7. Mol Microbiol 2000; 38:805-16; PMID:11115115; http://dx.doi.org/10.1046/j.1365-2958.2000.02171.x
82. Lacroix FJ, Cloeckaert A, Grépinet O, Pinault C, Popoff MY, Waxin H, et al. Salmonella typhimurium acrB-like gene: identification and role in resistance to biliary salts and detergents and in murine infection. FEMS Microbiol Lett 1996; 135:161-7; PMID:8595853; http://dx.doi.org/10.1111/j.1574-6968.1996.tb07983.x
83. Prouty AM, Brodsky IE, Manos J, Belas R, Falkow S, Gunn JS. Transcriptional regulation of Salmonella enterica serovar Typhimurium genes by bile. FEMS Immunol Med Microbiol 2004; 41:177-85; PMID:15145463; http://dx.doi.org/10.1016/j.femsim.2004.03.002
84. Thanassi DG, Cheng LW, Nikaido H. Active efflux of bile salts by Escherichia coli. J Bacteriol 1997; 179:2512-8; PMID:9098046
85. Cerda-Maira FA, Ringelberg CS, Taylor RK. The bile response repressor BreR regulates expression of the Vibrio cholerae breAB efflux system operon. J Bacteriol 2008; 190:7441-52; PMID:18776020; http://dx.doi.org/10.1128/JB.00584-08
86. Wibbenmeyer JA, Provenzano D, Landry CF, Klose KE, Delcour AH. Vibrio cholerae OmpU and OmpT porins are differentially affected by bile. Infect Immun 2002; 70:121-6; PMID:11748172; http://dx.doi.org/10.1128/IAI.70.1.121-126.2002
87. de Jesus MC, Urban AA, Marasigan ME, Barnett Foster DE. Acid and bile-salt stress of enteropathogenic Escherichia coli enhances adhesion to epithelial cells and alters glycolipid receptor binding specificity. J Infect Dis 2005; 192:1430-40; PMID:16170762; http://dx.doi.org/10.1086/462422
88. Torres AG, Tutt CB, Duval L, Popov V, Nasr AB, Michalski J, et al. Bile salts induce expression of the afimbrial LDA adhesin of atypical enteropathogenic Escherichia coli. Cell Microbiol 2007; 9:1039-49; PMID:17381433; http://dx.doi.org/10.1111/j.1462-5822.2006.00850.x
89. Gunn JS. Mechanisms of bacterial resistance and response to bile. Microbes Infect 2000; 2:907-13; PMID:10962274; http://dx.doi.org/10.1016/S1286-4579(00)00392-0
90. Prouty AM, Gunn JS. Salmonella enterica serovar typhimurium invasion is repressed in the presence of bile. Infect Immun 2000; 68:6763-9; PMID:11083793; http://dx.doi.org/10.1128/IAI.68.12.6763-6769.2000
91. Hung DT, Zhu J, Sturtevant D, Mekalanos JJ. Bile acids stimulate biofilm formation in Vibrio cholerae. Mol Microbiol 2006; 59:193-201; PMID:16359328; http://dx.doi.org/10.1111/j.1365-2958.2005.04846.x
92. Kristoffersen SM, Ravnum S, Tourasse NJ, Økstad OA, Kolstø AB, Davies W. Low concentrations of bile salts induce stress responses and reduce motility in Bacillus cereus ATCC 14579 [corrected]. J Bacteriol 2007; 189:5302-13; PMID:17496091; http://dx.doi.org/10.1128/JB.00239-07
93. Malik-Kale P, Parker CT, Konkel ME. Culture of Campylobacter jejuni with sodium deoxycholate induces virulence gene expression. J Bacteriol 2008; 190:2286-97; PMID:18223090; http://dx.doi.org/10.1128/JB.01736-07
94. Prouty AM, Brodsky IE, Falkow S, Gunn JS. Bile-salt-mediated induction of antimicrobial and bile resistance in Salmonella typhimurium. Microbiology 2004; 150:775-83; PMID:15073288; http://dx.doi.org/10.1099/mic.0.26769-0
95. Pumbwe L, Skilbeck CA, Nakano V, Avila-Campos MJ, Piazza RM, Wexler HM. Bile salts enhance bacterial co-aggregation, bacterial-intestinal epithelial cell adhesion, biofilm formation and antimicrobial resistance of Bacteroides fragilis. Microb Pathog 2007; 43:78-87; PMID:17524609; http://dx.doi.org/10.1016/j.micpath.2007.04.002
96. Rychlik I, Barrow PA. Salmonella stress management and its relevance to behaviour during intestinal colonisation and infection. FEMS Microbiol Rev 2005; 29:1021-40; PMID:16023758; http://dx.doi.org/10.1016/j.femsre.2005.03.005
97. van Velkinburgh JC, Gunn JS. PhoP-PhoQ-regulated loci are required for enhanced bile resistance in Salmonella spp. Infect Immun 1999; 67:1614-22; PMID:10084994
98. Nikaido E, Yamaguchi A, Nishino K. AcrAB multidrug efflux pump regulation in Salmonella enterica serovar Typhimurium by RamA in response to environmental signals. J Biol Chem 2008; 283:24245-53; PMID:18577510; http://dx.doi.org/10.1074/jbc.M804544200
99. Moskowitz SM, Ernst RK, Miller SI. PmrAB, a two-component regulatory system of Pseudomonas aeruginosa that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. J Bacteriol 2004; 186:575-9; PMID:14702327; http://dx.doi.org/10.1128/JB.186.2.575-579.2004
100. McPhee JB, Lewenza S, Hancock RE. Cationic antimicrobial peptides activate a two-component regulatory system, PmrA-PmrB, that regulates resistance to polymyxin B and cationic antimicrobial peptides in Pseudomonas aeruginosa. Mol Microbiol 2003; 50:205-17; PMID:14507375; http://dx.doi.org/10.1046/j.1365-2958.2003.03673.x
101. Garsin DA. Ethanolamine: a signal to commence a host-associated lifestyle? MBio 2012; 3:e00172-12; PMID:22761393; http://dx.doi.org/10.1128/mBio.00172-12
102. Lawhon SD, Frye JG, Suyemoto M, Porwollik S, McClelland M, Altier C. Global regulation by CsrA in Salmonella typhimurium. Mol Microbiol 2003; 48:1633-45; PMID:12791144; http://dx.doi.org/10.1046/j.1365-2958.2003.03535.x
103. Kelly A, Goldberg MD, Carroll RK, Danino V, Hinton JC, Dorman CJ. A global role for Fis in the transcriptional control of metabolism and type III secretion in Salmonella enterica serovar Typhimurium. Microbiology 2004; 150:2037-53; PMID:15256548; http://dx.doi.org/10.1099/mic.0.27209-0
104. Barnett Foster DE, Philpott D, Abul-Milh M, Huesca M, Sherman PM, Lingwood CA. Phosphatidylethanolamine recognition promotes enteropathogenic E. coli and enterohemorrhagic E. coli host cell attachment. Microb Pathog 1999; 27:289-301; PMID:10545256; http://dx.doi.org/10.1006/mpat.1999.0305
105. Wu Y, Lau B, Smith S, Troyan K, Barnett Foster DE. Enteropathogenic Escherichia coli infection triggers host phospholipid metabolism perturbations. Infect Immun 2004; 72:6764-72; PMID:15557596; http://dx.doi.org/10.1128/IAI.72.12.6764-6772.2004
106. Abul-Milh M, Wu Y, Lau B, Lingwood CA, Barnett Foster DE. Induction of epithelial cell death including apoptosis by enteropathogenic Escherichia coli expressing bundle-forming pili. Infect Immun 2001; 69:7356-64; PMID:11705908; http://dx.doi.org/10.1128/IAI.69.12.7356-7364.2001
107. Khursigara C, Abul-Milh M, Lau B, Giron J, Lingwood CA, Barnett Foster DE. Enteropathogenic Escherichia coli virulence factor bundle-forming pilus has a binding specificity for phosphatidylethanolamine. Infect Immun 2001; 69:6573-9; PMID:11598024; http://dx.doi.org/10.1128/IAI.69.11.6573-6579.2001
108. Macfarlane GT, Gibson GR, Cummings JH. Comparison of fermentation reactions in different regions of the human colon. J Appl Bacteriol 1992; 72:57-64; PMID:1541601
109. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987; 28:1221-7; PMID:3678950; http://dx.doi.org/10.1136/gut.28.10.1221
110. Argenzio RA, Southworth M, Stevens CE. Sites of organic acid production and absorption in the equine gastrointestinal tract. Am J Physiol 1974; 226:1043-50; PMID:4824856
111. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. J Clin Gastroenterol 2006; 40:235-43; PMID:16633129; http://dx.doi.org/10.1097/00004836-200603000-00015
112. Nakanishi N, Tashiro K, Kuhara S, Hayashi T, Sugimoto N, Tobe T. Regulation of virulence by butyrate sensing in enterohaemorrhagic Escherichia coli. Microbiology 2009; 155:521-30; PMID:19202100; http://dx.doi.org/10.1099/mic.0.023499-0
113. Tobe T, Nakanishi N, Sugimoto N. Activation of motility by sensing short-chain fatty acids via two steps in a flagellar gene regulatory cascade in enterohemorrhagic Escherichia coli. Infect Immun 2011; 79:1016-24; PMID:21149585; http://dx.doi.org/10.1128/IAI.00927-10
114. Herold S, Paton JC, Srimanote P, Paton AW. Differential effects of short-chain fatty acids and iron on expression of iha in Shiga-toxigenic Escherichia coli. Microbiology 2009; 155:3554-63; PMID:19684070; http://dx.doi.org/10.1099/mic.0.029454-0
115. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. The QseC sensor kinase: a bacterial adrenergic receptor. Proc Natl Acad Sci U S A 2006; 103:10420-5; PMID:16803956; http://dx.doi.org/10.1073/pnas.0604343103
116. Reading NC, Torres AG, Kendall MM, Hughes DT, Yamamoto K, Sperandio V. A novel two-component signaling system that activates transcription of an enterohemorrhagic Escherichia coli effector involved in remodeling of host actin. J Bacteriol 2007; 189:2468-76; PMID:17220220; http://dx.doi.org/10.1128/JB.01848-06
117. Njoroge J, Sperandio V. Enterohemorrhagic Escherichia coli virulence regulation by two bacterial adrenergic kinases, QseC and QseE. Infect Immun 2012; 80:688-703; PMID:22144490; http://dx.doi.org/10.1128/IAI.05921-11
118. de Sablet T, Chassard C, Bernalier-Donadille A, Vareille M, Gobert AP, Martin C. Human microbiota-secreted factors inhibit shiga toxin synthesis by enterohemorrhagic Escherichia coli O157:H7. Infect Immun 2009; 77:783-90; PMID:19064636; http://dx.doi.org/10.1128/IAI.01048-08

119. Carey CM, Kostrzynska M, Ojha S, Thompson S. The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic Escherichia coli O157:H7. J Microbiol Methods 2008; 73:125-32; PMID:18328583; http://dx.doi.org/10.1016/j.mimet.2008.01.014
120. Liévin-Le Moal V, Servin AL. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. Clin Microbiol Rev 2006; 19:315-37; PMID:16614252; http://dx.doi.org/10.1128/CMR.19.2.315-337.2006
121. Macfarlane S, Bahrami B, Macfarlane GT. Mucosal biofilm communities in the human intestinal tract. Adv Appl Microbiol 2011; 75:111-43; PMID:21807247; http://dx.doi.org/10.1016/B978-0-12-387046-9.00005-0
122. Bearson BL, Bearson SM. The role of the QseC quorum-sensing sensor kinase in colonization and norepinephrine-enhanced motility of Salmonella enterica serovar Typhimurium. Microb Pathog 2008; 44:271-8; PMID:17997077; http://dx.doi.org/10.1016/j.micpath.2007.10.001
123. Nakano M, Takahashi A, Sakai Y, Nakaya Y. Modulation of pathogenicity with norepinephrine related to the type III secretion system of Vibrio parahaemolyticus. J Infect Dis 2007; 195:1353-60; PMID:17397007; http://dx.doi.org/10.1086/513275
124. Lyte M, Arulanandam B, Nguyen K, Frank C, Erickson A, Francis D. Norepinephrine induced growth and expression of virulence associated factors in enterotoxigenic and enterohemorrhagic strains of Escherichia coli. Adv Exp Med Biol 1997; 412:331-9; PMID:9192038
125. Wang X, Wang Q, Yang M, Xiao J, Liu Q, Wu H, et al. QseBC controls flagellar motility, fimbrial hemagglutination and intracellular virulence in fish pathogen Edwardsiella tarda. Fish Shellfish Immunol 2011; 30:944-53; PMID:21288493; http://dx.doi.org/10.1016/j.fsi.2011.01.019
126. Hughes DT, Clarke MB, Yamamoto K, Rasko DA, Sperandio V. The QseC adrenergic signaling cascade in Enterohemorrhagic E. coli (EHEC). PLoS Pathog 2009; 5:e1000553; PMID:19696934; http://dx.doi.org/10.1371/journal.ppat.1000553
127. Baxt LA, Goldberg MB. Anaerobic environment of the intestine primes pathogenic Shigella for infection. Expert Rev Anti Infect Ther 2010; 8:1225-9; PMID:21073287; http://dx.doi.org/10.1586/eri.10.123
128. Marteyn B, West NP, Browning DF, Cole JA, Shaw JG, Palm F, et al. Modulation of Shigella virulence in response to available oxygen in vivo. Nature 2010; 465:355-8; PMID:20436458; http://dx.doi.org/10.1038/nature08970
129. Saldeña TA, Saraví FD, Hwang HJ, Cincunegui LM, Carra GE. Oxygen diffusive barriers of rat distal colon: role of subepithelial tissue, mucosa, and mucus gel layer. Dig Dis Sci 2000; 45:2108-14; PMID:11215723; http://dx.doi.org/10.1023/A:1026411118033
130. Dibden DP, Green J. In vivo cycling of the Escherichia coli transcription factor FNR between active and inactive states. Microbiology 2005; 151:4063-70; PMID:16339951; http://dx.doi.org/10.1099/mic.0.28253-0
131. Jones SA, Chowdhury FZ, Fabich AJ, Anderson A, Schreiner DM, House AL, et al. Respiration of Escherichia coli in the mouse intestine. Infect Immun 2007; 75:4891-9; http://dx.doi.org/10.1128/IAI.00484-07; PMID:17698572
132. Alexeeva S, Hellingwerf KJ, Teixeira de Mattos MJ. Requirement of ArcA for redox regulation in Escherichia coli under microaerobic but not anaerobic or aerobic conditions. J Bacteriol 2003; 185:204-9; PMID:12486057; http://dx.doi.org/10.1128/JB.185.1.204-209.2003
133. Schüller S, Phillips AD. Microaerobic conditions enhance type III secretion and adherence of enterohaemorrhagic Escherichia coli to polarized human intestinal epithelial cells. Environ Microbiol 2010; 12:2426-35; PMID:20406285; http://dx.doi.org/10.1111/j.1462-2920.2010.02216.x