Animal and Veterinary Sciences
2019; 7(3): 83-93
http://www.sciencepublishinggroup.com/j/avs
doi: 10.11648/j.avs.20190703.13
ISSN: 2328-5842 (Print); ISSN: 2328-5850 (Online)

A Review on Virulence Factors of *Escherichia Coli*

Eshetu Shumi Gebisa[1, *], Minda Asfaw Gerasu[2], Diriba Taddese Leggese[1]

[1]College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia
[2]College of Agriculture and Environmental Science, Arsi University, Asela, Ethiopia

Email address:

eshetu.shumi@yahoo.com (E. S. Gebisa)
[*]Corresponding author

To cite this article:

Eshetu Shumi Gebisa, Minda Asfaw Gerasu, Diriba Taddese Leggese. A Review on Virulence Factors of Escherichia Coli. *Animal and Veterinary Sciences*. Vol. 7, No. 3, 2019, pp. 83-93. doi: 10.11648/j.avs.20190703.13

Received: March 12, 2019; **Accepted**: April 17, 2019; **Published**: July 10, 2019

Abstract: Most *Escherichia coli (E. coli)* strains are normal commensals found in the intestinal tract of both humans and animals, while others are pathogenic to animals and humans. Pathogenic *E. coli* distinguished from normal flora by their possession of virulence factors. Hence, the main objective of this review is to appraise different virulence factors associated with occurrence of pathogenic *E. coli* infections. Some pathogenic strains cause diarrhoeal disease and are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O: H serogroups. In this review, the most important virulence factors of *E. coli* including acid resistance, different adhesion proteins like fimbriae, fibrillae, curli and outer membrane protein A, the use of type III secretion systems by the bacteria to subvert eukaryotic signaling pathways by injecting virulence proteins into the host cell cytoplasm, the alkaline phosphatase encoded by PhoA gene in *E. coli*, the repeatsin toxin pore-forming toxins, oligopeptide toxin of *E. coli*, heat-labile enterotoxins, Vero/Shiga toxins and different pathogenicity islands which encode a variety of different virulence factors like adhesins, toxins, invasins, protein secretion systems, iron uptake systems and others were critically conferred. Thus, this review paper call for pioneering research on different virulence factors of *E. coli* in order to apply a well-coordinated control interventions.

Keywords: Acid Resistance, Adhesion Proteins, Escherichia Coli, Pathogenecity Islands, Toxins, Virulence Factor

1. Introduction

Escherichia coli were first isolated by a German paediatrician, Theodore Esherich, in 1884 from faeces of human neonates (Khan and Steiner, 2002). For the genus *E. coli,* there are hundreds of serotypes of *E. coli* which are classified on the bases of various surface antigens referred to as Somatic (O), Capsular (K), Flagellar (H) and Fimbrial (F). The first confirmed isolation of *E. coli* O157:H7 in the United States of America was in 1975 from a Californian woman with bloody diarrhoea, while the first reported isolation of the pathogen from cattle was in Argentina in 1977, while the bacterium was first identified as a human pathogen in 1982 [1].

Most *E. coli* strains are harmless commensals; however, some strains are pathogenic and cause diarrhoeal disease. *E. coli* strains that cause diarrhoeal illness are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O: H serogroups. These categories include enteropathogenic *E. coli* strains (EPEC), enteroinvasive *E. coli* strains (EIEC), diffuse adhering *E. coli* strains (DAEC), enteroaggregative *E. coli* strains (EAggEC) and enterohaemorrhagic *E. coli* strains (EHEC) [1].

According to Eisenstein *et al.* (2000), *E. coli* are the most prevalent infecting organism in the family of gram negative bacteria known as enterobacteriaceae. Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora. However, there are some strains that have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands. This pathogenic *E. coli* can be categorized based on serogroups, pathogenicity mechanisms, clinical symptoms, or virulence factors [2].

Escherchia coli are today divided into several pathogenic strain causing different intestinal, urinary tract or internal infection and pathologies, in all animal species and humans. Those pathogenic E. coli serotype where therefore named by

the clinical syndrome they can cause, diarrhoeagenic *E.coli* (DEC), urophathogenic *E. coli* (UPEC), septicemic *E.coli* (SePEC), neonatal meningitis associated *E. coli* (NMEC), etc. or sometimes according to the target host; avian phatogenic *E. coli* (APEC) [3]

The question soon changed to how identifying *in vitro* the pathogenic strains of *E. coli*. The first answer came from the serotyping system developed by Kauffmann in the 1940s, *i.e.* the identification of somatic (O antigen), capsular (K antigen) and flagellar (H antigen) surface antigens. Some serotypes are indeed more frequently than others associated with specific clinical syndromes [4].

Fortunately, from the late 1960s on wards, several specific properties that differentiate the pathogenic strains from each other and from non-pathogenic strains were unraveled. It therefore became possible to name the different classes of pathogenic *E. coli* on the basis of their specific properties instead of the clinical syndrome they can produce. Focusing on diarrhoeagenic *E. coli*, three classes were so defined in humans in the 1970s enterotoxigenic *E. coli* producing enterotoxins causing hypersecretion of electrolytes and water by enterocytes of domestic animals and humans, EIEC invading the enterocytes of humans and primates, and enteropathogenic *E. coli* belonging to specific serotypes, pathogenic for humans, but whose virulence properties were still unknown at the time. [3].

Therefore, the objective of this review paper is to appraise the most important virulence factors associated with the properties of O157:H7 and other shiga toxin-producing *E. coli* including acid resistance factor, different classes of adhesion proteins, different proteinous toxinsandas well as to present different type 3 secretion system which bacteria can utilize to interfere with eukaryotic signaling pathways by injecting virulence proteins into the host cell and different pathogenecity islands which encode a variety of virulence factors.

2. Characteristics of Escherichia Coli

Escherichia coli can be characterized by sereotyping, a method based on differences in antigenic structure on the bacterial surface. The serotype is defined by the bacterium's O-antigen refers non spreading growth on agar (Ohne), a polysaccharide domain in the bacterium's lip polysaccharide (LPS) in the outer membrane, and the H-antigen (Hauch) consisting of flagella protein. Serotyping may also include the K antigen (Kapsel) and the F-antigen (Fimbriae). There are many known O, H, K and F antigens and the existing number of different serotypes is known to be very high. Serotyping is an important tool which can be used in combination with other methods to distinguish pathogenic *E. coli* strains as specific pathogenicity attributes are often linked to certain serotypes [5].

Among *E. coli* isolates, there is considerable variation and many combinations of somatic (O and K) and flagellar (H) antigens. *E. coli* are routinely characterized by serological identification of somatic O, flagellar H and capsular K antigens. However, while some serotypes correlate closely with certain clinical syndromes, differentiation of pathogenic strains from the normal flora depends on the identification of virulence characteristics. Among pathogenic strains, there are few patterns of these antigens and few phylogenetic groupings. For *E. coli*, there are over 150 antigenically unique O-antigens. K type capsular material occurs in two or four forms on the basis of physical, biochemical and genetic criteria [6].

Over 80 serologically and chemically distinct capsular polysaccharides have been recognized. In addition, a slime layer, colonic acid extracellular polysaccharide, is common to many *E. coli* isolates and can be co-expressed with some K-type capsules. There are 53 H-antigen specificities among *E. coli* [7].

2.1. Overview of Escherichia Coli as a Pathogen

Escherichia coli are Gram-negative, facultative anaerobic, rod-shaped and highly motile bacteria. They are often classified under enterobacteriacea known to be normal inhabitants of the gastrointestinal tract of both animals and human beings but only some strains of *E. coli* have become highly adapted to cause diarrhoea and a range of extra-intestinal diseases [8].

It was since 1982 that EHEC have been recognized as an important aetiological agent of diarrhoeal diseases in man and animals. *E coli* O157 was described as a rare serotype. Studies conducted between 1983 and 1985 in the United States and Canada, have linked EHEC infection to haemorrhagic colitis (HC) and it had a close relation with the classical form of haemolytic uraemic syndrome (HUS) (Karmali *et al*, 1985). As a result of these and other studies re-examined isolates of *E coli* belonging to the O157 serogroup that had been submitted to the International *Escherichia* and *Klebsiella* Centre. Three isolates were found that had the H7 antigen. These three isolates were from the faeces of one animal out of a batch of 39 calves with colibacillosis in Argentina [9].

Pathogenesis of Escherichia Coli O157:H7

Escherchia coli O157:H7 can with stand the acidic environment of the human stomach and begins the arduous and complex process of infection. From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days. During this time, the bacteria progress through several phases of infection including adherence, colonization and the production and release of Stxs. First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells. The association between the bacterial and host cells consequently induces the expression of the Type three secretion system (TTSS) genes located on the locus of enterocyte effacement (LEE). Following their synthesis, the TTSS proteins are systematically assembled [2].

The membrane-bound proteins first associate and form the foundation of the TTSS followed then by the proteins that form the extracellular channel and by the protein that create

the pore in the host cell membrane. Once assembled, a multitude of effectors proteins are shuttled through the TTSS channel and into the cytoplasm of the host cell. After the effectors proteins invade the cytoplasm, they alter the host cell's normal patterns of signal transduction in order to accommodate bacterial adherence. The alterations in signal transduction are accomplished through the activities of the bacterial effector proteins, and, by selective phosphorylation, the effector proteins force actin topolymerize and the cytoskeleton to reorganize [10].

Reorganization results in the effacement of host cell microvilli. At this point, the bacteria may superficially adhere to the host cell while simultaneously preparing for intimate adherence. Intimate adherence of the bacterial cell to the host cell requires the orchestrated activities of LEE-encoded intimin and the specialized translocated intimin receptor (Tir) [11]. Intimin is an outer membrane protein whose presence and proper activity are critical to intimate adherence and the formation of the attaching and effacing lesion. A proposed auto transport system is believed to be responsible for shuttling and inserting intimin into the outer membrane of the bacterial cell where intimin then adopts a β–barrel conformation [12].

Although the N-terminus of intimin is highly conserved, the C-terminus is slightly more variable in comparison. As a consequence, approximately 14 subtypes of intimin are now recognized and each variant has a slightly different tissue tropism for the assorted gastrointestinal cells. On the other hand, Tir is more unique, because it is a protein receptor manufactured by the bacterial cell itself, which appears to be a distinctive trait of attaching-effacing bacteria. During infection, Tir is synthesized in the bacterial cell and then translocates through the TTSS to the host epithelial cell. After translocation, Tir inserts spontaneously into the plasma membrane and adopts a hairpin loocon formation as it is presented on the surface of the epithelial cell [13].

Once inserted, intimin, located on the surface of the bacterial cell, associates with Tir and causes a more exaggerated rearrangement of the host cytoskeleton. This rearrangement forms the characteristic pedestal of the A/E lesion and enables the bacterial cell to intimately adhere to the host cell. As more *E. coli* O157:H7 intimately adhere to host epithelial cells and form the A/E lesions, the bacteria begin to overwhelm the normal microflora of the intestinal tract and successfully colonize the host [14].

However, the exact means by which *E. coli* O157:H7 establishes and sustains colonization in the host remains elusive. Once it has successfully colonized and established itself within the host, *E. coli* O157:H7 produces and releases it Stxs in the intestinal lumen [3]. The B subunits of the Stxs then bind to their corresponding Gb3 receptors on the surface of epithelial and, unlike the LEE-encoded effector proteins, to endothelial cells as well. The A subunit of the Stxs is then internalized into the host cell through the process of receptor-mediated endocytosis and transported to the Golgi apparatus where furin, a serine protease, cleaves the A subunit. Next, the A subunit is shuttled to the endoplasmic reticulum where a critical disulfide bond is reduced, releasing the active fragment he active fragment functions as an RNA N-glycosidase that selectively depurinates an adenine residue in the 28S rRNA of the large ribosomal subunit. This prohibits elongation factors 1 and 2 from binding to the ribosome and inhibits host cell protein synthesis, ultimately forcing the host cell to undergo apoptosis [15].

In addition, it is believed that apoptosis results in the local symptoms of disease. Furthermore, the Stxs can translocate from intestinal epithelial cells into the bloodstream. Here, the Stxs bind to the Gb3 receptors on glomerular endothelial cells. The Stxs injure the glomerular cells and cause platelets and fibrin to deposit within the glomeruli. Eventually, the deposits decrease renal filtration and lead to the acute kidney damage characteristic of HUS [16].

2.2. Acid Resistance

Multiple acid resistance (AR) mechanisms have been described for E. coli. Four of the five primary systems utilize a pyridoxal-5'-phosphate (PLP)-dependent amino acid decarboxylase with an externally derived amino acid to consume a proton and generate a byproduct and CO_2. A corresponding anti porter exchanges the amino acid and byproduct across the membrane. The glutamate dependent system named as AR2 or GDAR is the most robust system, allowing up to 80% survival after 2 hr in extremely low pH and producing GABA as byproduct [17].

Other amino acid dependent systems are the arginine dependent system (AR3 or ADAR) the lysine-dependent system (AR4 or LDAR), and the more recently discovered ornithine-dependent system (ODAR). The first described AR system, AR1, is an oxidative AR system repressed by glucose that is σ^S-dependent and does not require an externally-derived amino acid. Despite the fact that AR1 was the first discovered AR system, its mechanism has still not been determined [18].

The main transcriptional regulatory elements of amino acid dependent AR have been characterized. Glutamate decarboxylase GadE is the primary regulator of AR2 and serves as a transcriptional activator for genes encoding two glutamate decarboxylase isoforms (gadA and gadB) and the glutamate/GABA antiporter (gadC). Transcriptional activation of gadA/B requires heterodimerization of GadE with RcsB. Regulation of gadE, in turn, is complex and involves the activities of multiple circuits whose effects are integrated by binding to the large intergenic region upstream of gadE (Sayed and Foster, 2009). AdiY is the primary regulator of AR3 that, together with CysB, coordinately regulates the corresponding arginine decarboxylase gene (adiA). CadC is the primary regulator of AR4, regulating both the lysine decarboxylase (cadA) and antiporter (cadB) genes [19].

The regulatory network for ODAR is not well-defined. With the exception of the binding of GadE-RcsB to the cadBA promoter, it is not known whether or how the regulation different system AR systems and adaptations are coordinated. Elements of AR2 can be induced by non-acid

stimuli including treatment with acetate and entry into stationary phase. In addition, acid stress leads to adaptations beyond the amino acid-dependent AR machinery including expression of the electron transport chain, the envelope stress response and alterations in membrane permeability to protons, a for mate hydrogen lyase system that reduces protons to hydrogen gas, and reversals in the cell potential that may drive a chloride/proton antiporter, and numerous metabolic processes. The regulatory mechanisms underlying these expression changes have not been established, and the coordination of these and other acid responses with broader cellular metabolism has not been fully explored [20].

2.3. Adhesion

Following passage through the stomach, viable organisms must adhere to the bowel mucosa to prevent their removal by the peristaltic flow. Other pathogenic types of *E. coli,* such as EPEC and ETEC, have well characterized adhesion structures that allow them to adhere to the bowel. It is likely that EHEC are also capable of adhering to the bowel mucosa as one of the first stages of the infection process. At least three different general classes of *E. coli* adhesion can be defined; fimbriae, fibrillae and curli, the afimbrial family (Afa), and specific outer membrane protein like the intimin [2, 3].

2.3.1. The Appendices

Escherichia coli fimbriae and fibrillae adhesins were at first described on ETEC from pigs, calves and then humans. They were there after also described on UPEC, SePEC, APEC and NTEC *E. coli* [5].

They received different names at the time of their description, but today most are named by the letter F followed by numbers. F2 (Colonization Factor Antigen (CFA) 1), F3 (CFA2), F4 (K88 antigen), F5 (K99 antigen), F6 (987 strain pili), F18 (F107, 88 18,2134pili), F41 (B41 strain fimbriae) of ETEC; F7–F16 (Pap/ Prs fimbriae) of UPEC, SePEC, APEC NTEC; F1 (type1) causing mannose sensitive haemagglutination produced by almost all strains of *E. coli* and probably involved in the early steps of the colonization of the air sacs by APEC; F17 (FY, Att25, Att111, Vir adhesin) produced by different ETEC, SePEC, UPEC, APEC, and NTEC. The genes coding for the different fimbriae and fibrillae can be plasmid located, especially in ETEC or chromosome located sometimes on specific pathogenicity islands, like in UPEC [21].

The *E. coli* best known type 4 fimbriae, or bundle forming pili (BFP) are produced by typical enteropathogenic *E. coli* (tEPEC) a sub class of human EPEC. Non BFP producing EPEC from humans and animals are called atypical (aEPEC) (Hernandes*et al*.,2009). The BFP, that are encoded by plasmid located genes, were at first thought to be involved in adherence to the human enterocytes, but are today believed to help bacteria to attach together forming micro colonies at the surface of the host cells, also called "Localized Adherence" (LA) pattern. Other type 4-like fimbriae, the Longus Pilus, are produced by some human ETEC strains that, in addition to promoting intestinal colonization, may also play a role in the protection of the bacteria from antimicrobial agents [22]

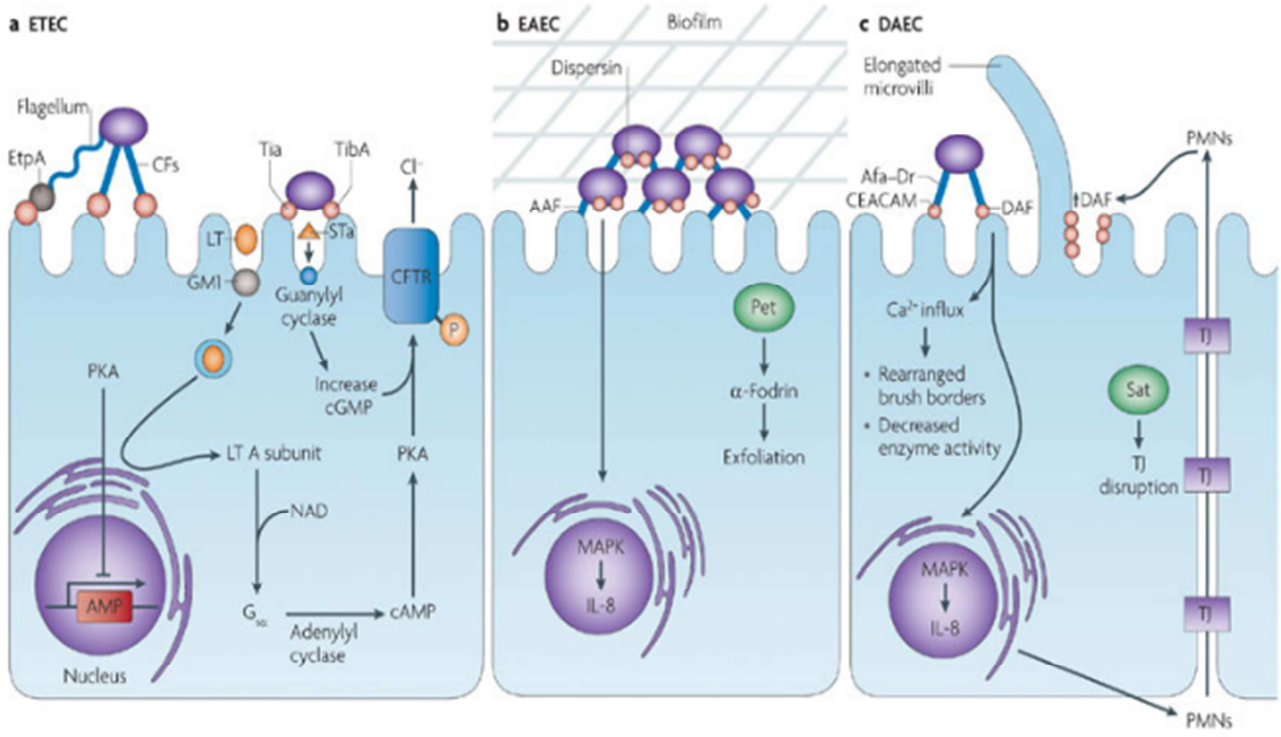
Curli that are encoded by chromosome located genes, are produced by different pathogenic *E. coli* strains, more especially SePEC and some EHEC. Curli may be involved in the adherence of SePEC to the mucosal extracellular matrix after fragilization of the epithelium and in the formation of biofilm on organic (not only animals, but also plants) and non-organic surfaces by O157:H7 EHEC and other pathogenic strains. But more studies are needed, especially *in vivo* to more precisely understand their role in *E. coli* pathogenicity [23].

All fimbriae are good immunogens since the major subunit is a 15–25kD a protein and is present in several hundreds of copies. Vaccination of the pregnant dams to protect newborn calves, lambs and piglets against infections by ETEC *via* colostral antibodies is actually based on vaccines containing semi purified fimbriae (F4, F5, F6, F17 and/or F41) [24]

2.3.2. Virulence Mechanisms of Enterotoxigenic, Enteroaggregative and Diffusely Adherent Escherichia Coli

Enterotoxigenic *E. coli*becomes anchored to enterocytes of the small bowel through colonization factors (cfs) and an adhesinthat is found at the tip of the flagella. Tighter adherence is mediated through tia and tiba. Two toxins, heat-labile enterotoxin (st2) and heat-stable enterotoxin (st2), are secreted and cause diarrhoea through cyclic amp (camp) and cyclic gmp (cgmp)-mediated activation of cystic fibrosis transmembrane conductance regulator. Enteroagreggative *E. coli* attaches to enterocytes in both the small and large bowels through aggregative adherence fimbriae (aaf) that stimulate a strong interleukin-8 (IL-8) response, allowing biofilms to form on the surface of cells. Plasmid-encoded toxin is a serine protease autotransporter of the enterobacteriaceae (spate) that targets α-fodrin (also known as sptan1), which disrupts the acting cytoskeleton and induces exfoliation [25].

Diffusely adherent *E. coli* (DAEC) forms a diffuse attaching pattern on enterocytes of the small bowel, which is mediated through afimbrial (afa) and fimbrial adhesins, which are collectively known as afa-dr fimbriae. Most afa-dr fimbriae bind to complement decay-accelerating factor (daf); a subset of afa-dr fimbriae bind to receptors in the carcino embryonic antigen related cell adhesion molecule (ceacam) family. The autotransported toxin sat has been implicated in lesions of tight junctions (tjs) in afa-dr expressing daec, as well as in increased permeability. Polymorphonuclear leukocyte infiltration increases surface localization of daf. Antimicrobial peptides; $g_{s\alpha}$, stimulatory guanylyl-nucleotide-binding (g) protein α-subunit; mapk, mitogen-activated protein kinase; pka, protein kinase A [25].



Source: Croxen and Finlay, (2010)

Figure 1. *Virulence mechanisms of ETEC, EAEC and DAEC.*

2.3.3. Role of Outer Membrane Proteins in Adhesion (OMP)

Outer membrane protein A (OmpA) is one of the major proteins in the membrane of *E. coli* and is highly conserved among gram negative bacteria. It plays a multifunctional role in the biology of the bacteria, acting as a phage and colicin receptor, serving as a mediator in F-factor dependent conjugation (Delcour and A. H. 2002,) and maintaining structural integrity of membranes and generation of normal cell shape. In *E. coli* K1 strains, OmpA is also known to be associated with the pathogenesis of neonatal meningitis because it mediates invasion of *E. coli* K1 to brain microvascular endothelial cells and plays a key role during the initial processes of bacterial adhesion and invasion [26].

More recently, the role of OmpA in adherence was established during the screening of an EHEC O157:H7 transposon insertion mutant library for hyper adherent mutants. A mutation of the tcdA gene, encoding a transcriptional activator associated with L-threonine transport and degradation, resulted in elevated expression of OmpA and hyperadherence of EHEC O157:H7 to HeLa and Caco-2 cells. Inactivation of OmpA in the tdcA mutant abolished the hyper adherent phenotype, and mutation of OmpA alone in the wild-type EHEC O157:H7 strain reduced adherence by 13.5%. Furthermore, OmpA specific antiserum inhibited the adherence of other EHEC O157:H7 strains to HeLa cells [27].

It has been shown that antigen presenting cells (APCs) can recognize Klebsiella pneumonia OmpA and are activated by this interaction. K. pneumonia OmpA induces the expression of co stimulatory molecules and CD83 on human dendritic cells (DCs) and triggers cytokine production by murine and human macrophages [28]. OmpA appears to possess a new type of pathogen associated molecular pattern (PAMP) because it is conserved among the Enterobacteriaceae family, is essential for bacterial survival and virulence, and can activate APCs. DCs are potent APCs with the unique ability to activate native T cells. DCs are widely distributed in the skin and the mucosal tissues in the intestinal tract and airways. Upon recognizing PAMPs via specialized receptors such as the C-type lectin, Fc receptors, and Toll-like receptors, Dendritic cells detect the presence of bacteria in the host tissues, capturing their products, migrate to the T-cell areas in lymph nodes, and activate native T cells. During these processes, DCs become mature and are activated to produce diverse cytokines. Over the past few years, much has been learned about the biology of EHEC O157:H7 strains, but it is not known whether these bacteria and their products interact with DCs. It is possible that OmpA of EHEC O157:H7 can function as a PAMP to stimulate dendritic cells cytokine production and migration [29].

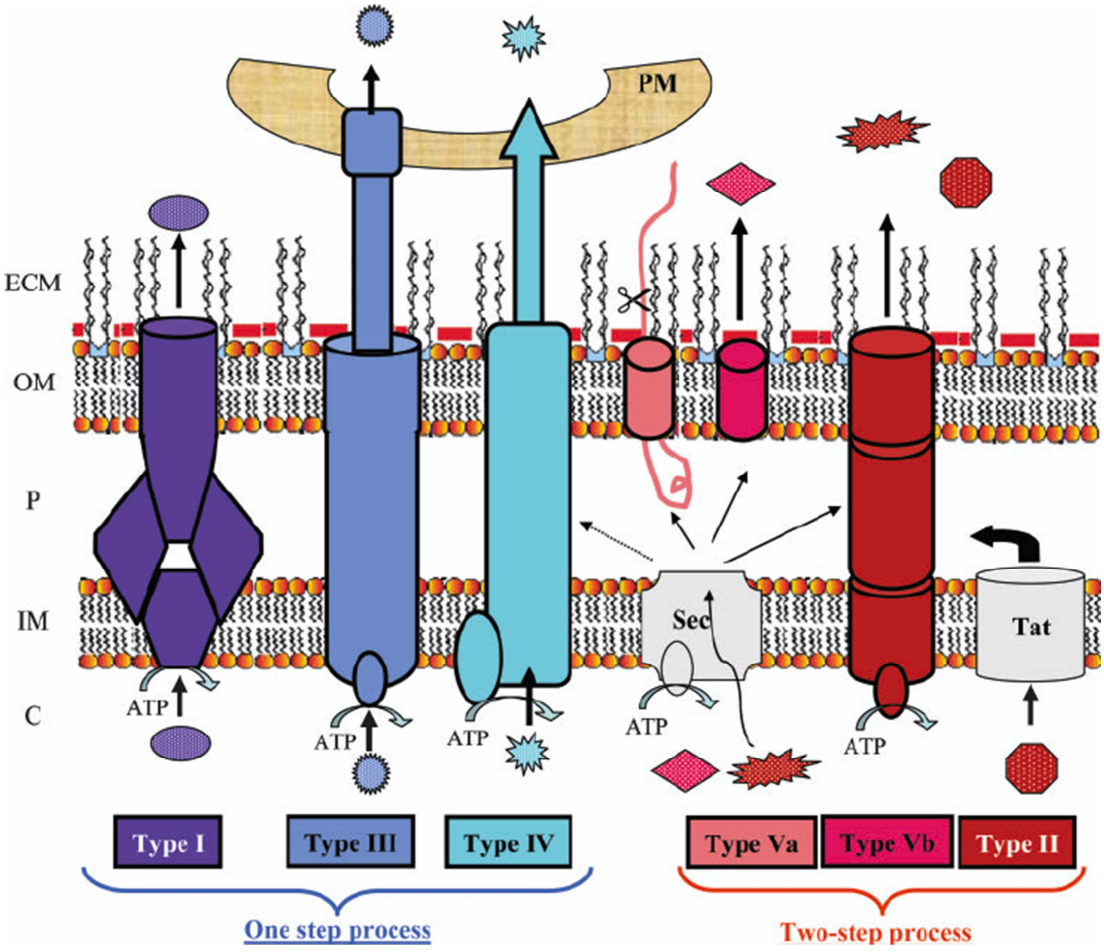
2.4. The Type 3 Secretion System (T3SS) and Other

Many pathogenic Gram-negative bacteria utilize type III secretion systems (T3SSs) to subvert eukaryotic signaling pathways by injecting virulence proteins into the host cell cytoplasm [30]. Enteropathogenic *E. coli* (EPEC) and

enterohemorrhagic *E. coli* (EHEC) use the T3SS to deliver effector proteins that result in the creation of the attaching and effacing (A/E) lesions. The complete genome sequencing of two, EHEC O157: H7 strains, EDL933 and Sakai, revealed the presence of a gene cluster predicted to encode an additional T3SS. This T3SS was designated as the *E. coli* type III secretion system 2 (ETT2), to distinguish it from the locus of enterocyte effacement locus for enterocyte effacement encoded T3SS, which is now called ETT1 [31].

The genes required for ETT1 function, including the T3SS apparatus and the secreted proteins, are encoded on the locus of enterocyte effacement (LEE) in EPEC and EHEC. This virulence locus is about 35.4-kb in size and contains 41 open reading frames (ORFs). Up to 32 other non-LEE-encoded effectors proteins can also be delivered into host cells by the ETT1 to subvert eukaryotic cell biology [32].

A large bacterial chromosome pathogenicity island, the locus of enterocyte effacement (LEE) carries all according to the seropathotypes gene necessary for the formation of the AE lesion. They are grouped into several functional gene clusters, the most important being the espA, B, D genes coding for the type 3 secretion system (T3SS) structural proteins (or injectisome) gene coding for the anchor structure of the injectisome within the outer and inner bacterial membranes; the tir and several other genes coding for the type 3-secrated effectors into the eukaryotic cells; and the experimental autoimmune encephalomyelitis (eae) gene coding for the intimin adhesin located within the bacterial outer membrane. The injectisome is composed of polymerized EspA protein with the EspB and EspD proteins at the top forming a pore within the eukaryotic cytoplasmicmembrane. To some extent the injectisome functions as one adhesion connecting the bacteria to the eukaryotic cell, through which the type 3-secrated effectors translocate into the enterocytes [30].



Source: Merrell and Falkow, (2004).

Figure 2. *Secretion type systems in Gram negative bacteria.*

Type I, type III and type IV SSs (left) are believed to transport proteins in one step from the bacterial cytosol to the bacterial cell surface and external medium. In the case of type III and type IV SSs, the proteins are transported from the bacterial cytoplasm to the target cell cytosol. One exception for type IV is the pertussis toxin, which is secreted in two steps and released into the extracellular medium. This exception is represented by the dotted arrow, which connects Sec and the type IV SS. Type II and type V SSs transport proteins in two steps. In that case, proteins are first transported to the periplasm via the Sec or Tat system before reaching the cell surface. Type Va is a putative

autotransporter, indicating that the C-terminus of the protein forms the outer-membrane channel (cylinder) whereas the N-terminus (pink line) is exposed to the surface or released by proteolytic cleavage (scissors). C, bacterial cytoplasm; IM, bacterial inner membrane; P, bacterial periplasm; OM, bacterial outer membrane; ECM, extracellular milieu. PM (brown zone), host cell plasma membrane. When appropriate, coupling of ATP hydrolysis to transport is highlighted. Arrows indicate the route followed by transported proteins [33].

2.5. Alkaline Phosphatase

Alkaline phosphatase is encoded by the alkaline phosphatase A (phoA) gene in *E. coli.* The wild type PhoA protein has a signal-sequence that allows the export of alkaline phosphatase into the periplasm where it is located and active [34].

Escherichia Coli Disulphide Bond System

Disulphide bonds are covalent, single, non-polar, σ-bonds that are formed between two sulfur atoms of the thiol groups of cysteines, and they are important features for the three-dimensional structure of many proteins. The disulfide-bond (Dsb) system is responsible for the formation of disulfide bonds in bacteria during the process of protein folding. In *E. coli* K12 the DsbA and DsbB proteins are involved in the oxidative pathway where they introduce disulfide bonds into newly synthesized proteins translocated into the periplasm. The other pathway is the isomerase pathway where the DsbC, DsbG and DsbD catalyze the disulphide bond reshuffling/isomerisation, when incorrect disulphide bonds are introduced either by DsbA or under the conditions of copper oxidative stress [35].

2.6. Exotoxins

2.6.1. Repeatsin Toxin Pore-Forming Toxins

Protein toxins are prominent virulence factors in many pathogenic bacteria. While toxins of Gram-positive bacteria do not generally require activation, many toxins of the Gram negatives are translated into an inactive form and require a processing step. The most common such step involves a proteolytic cleavage to generate the active form, especially in those toxins with enzymatic activity. Toxins are activated by proteolysis in a variety of ways: As examples, the anthrax toxin is proteolyzed after its interaction with the receptor on the target cell to promote the formation of a prepore.

The pore-forming α-hemolysin (HlyA) of *E. coli*, a member of the RTX toxins, represents a unique class of bacterial toxins that require for activation a posttranslational modification involving a covalent amide linkage of fatty acids to two internal lysine residues [36].

Pore-forming α-hemolysin (HlyA) belongs to one class of a wide range of host cell specific toxins. HlyA acts on a variety of cell types from several species. E.g, red blood cells, embryo and adult fibroblasts, granulocytes, lymphocytes, and macrophages, also binds to and disrupts protein-free liposomes. The host environments encountered by the ExPEC are extremely nutrient poor; and the function of HlyA has generally been thought to be primarily the destruction of host cells, thereby facilitating the release of nutrients and other factors, such as iron, that are critical for bacterial growth. The lytic mechanism of HlyA is a complex process. Three stages seem to be involved that ultimately lead to cell lysis: binding, insertion, and oligomerization of the toxin within the membrane. Studies that have explored the binding of HlyA to membranes and the characterization of a putative toxin specific receptor have produced contradictory results. First, the lymphocyte function associated antigen (LFA-1) (CD11a/CD18; 12 integrin), was reported to serve as the receptor for HlyA on polymorphonuclear neutrophils and HlyA was found to recognize and bind the N linked oligosaccharides to their !2-integrin receptors [33].

This finding raises the possibility that the initial binding of the toxin to various cells might occur through the recognition of glycosylated membrane components, such as glycoproteins and gangliosides. Recently, found that HlyA binds to the glycophorin of horse erythrocytes and that this binding was abolished by atrypsinization of the membranes. In addition, these authors found that the glycophorinpurified from erythrocyte ghosts and reconstituted in liposomes significantly increased liposomal sensitivity to HlyA. Amino acids 914-936 of HlyA were subsequently hypothesized to be responsible for binding to the ghost receptor [37].

Other studies, however, indicated that the binding of HlyA to cells occurred in a nonsaturable manner and that the toxin did not interact with a specific protein receptor either on granulocytes or erythrocytes. Nevertheless, HlyA produces protein-free liposome disruption [38].

These results demonstrated that the presence of a receptor was not necessary for hemolysis to occur. These contradictory findings regarding the presence or absence of a toxin-specific receptor might be related to the different amounts of toxin and/or the different types and animal species of target cells used in the various studies. At all events, the interaction of HlyA with a target cell membrane devoid of any specific proteinaceous receptor appears to occur in two steps: an initial reversible adsorption of the toxin that is sensitive to electrostatic forces followed by an irreversible membrane insertion. Studies with the isolated calcium binding domain of HlyA revealed that that part of the protein may be adsorbed onto the membrane during the early stages of HlyA membrane interaction [39].

The next step in the hemolytic process is the insertion of the toxin into the membrane. The major region of HlyA that inserts into the membrane is located between residues 177 and 411. The insertion is furthermore independent of membrane lysis since HlyA protein mutants that are completely nonlyticcan insert into lipid monolayers. In addition, a binding of calcium to the toxin was shown to induce a protein conformational change that made the insertion process irreversible. Once the toxin is inserted, an oligomerization process occurs. We previously found that the fatty acids covalently bound to the toxin induce

conformational changes that expose intrinsically disordered regions so as to promote protein-protein interactions Thus; the oligomerization process of the toxin is facilitated by micro domains within the membrane [40]. The HlyA pore that is formed is highly dynamic because the size depends on both the interaction time and the concentration of the toxin [16].

2.6.2. The Oligopeptide Toxin of Escherichia Coli

Several bacterial species produce oligopeptide toxins, like *E.coli*, *Vibriocholerae, Yersinia enterocolitica*, *Klebsiella pneumoniae*, and Enterobacteraero genes. *E. coli* even produces different oligopeptide toxins, like the heat-stable enterotoxins of enterotoxigenic strains (STaP, STaH and STb) and the heat-stable enterotoxin of enteroaggregative strains (EAST1). STaP and STaH are 18 and 19 amino-acid peptides respectively of a molecular weight of less than 2 kDa, whereas STb is a 48 amino-acid peptide of ca. 5 kDa and EAST1 is a 38 amino-acid toxin of ca. 4kDa. Because of their low molecular weight, no oligopeptide toxin is a good immunogen.

STaP is produced by animal (bovine, ovine, porcine and canine) and human ETEC strains, while STaH is produced only by human ETEC strains, STb by porcine ETEC strains, and EAST1 by human EAggEC and by bovine, human and porcine ETEC, EPEC, VTEC strains. All STaP STaH, STb and EAST1 encoding genes are plasmid located. STaH STaP and probably EAST1 (based on structural similarity, exert their toxicity via activation of an intra cellular cascade. While STb seems is to act as a non-specific pore forming toxin [41].

Both STaP and STaH are synthesized as a preprotoxin of 72 amino acids that is secreted by a type 2 secretion system (general or Sec-dependent secretion pathway) into the periplasmic space of the bacteria after removal of the 18 amino-acid long signal sequence. Further processing occurs in the periplasmic space or during the crossing of the outer membrane to produce the active 18 (STaP) or 19 (STaH) amino-acid toxins with three disulfide bonds. The receptor of both STa is a transmembrane glycoprotein with guanylate cyclase activity (guanylyl cyclase C) of the intracytoplasmic domain, that is present at the height of the microvilli of the enterocytes (Gyles and Fairbrother, 2010). The intracellular levels of cyclic guanosine monophosphate (cGMP) regulate several cellular processes including the activity of ion pumps, like the main ion channel of epithelial cells (the cystic fibrosis transmembrane conductance regulator: CTFR). The action of either STa results in the stimulation of chloride and water secretion and the inhibition of sodium absorption, leading to watery diarrhoea [2].

The AB toxins some bacterial AB toxins are actually composed of two separate subunits, with one copy of the A subunit and one to several copies of the B subunit. Other AB toxins are actually single proteins composed of two domains corresponding to the A and B subunits. The A subunit/ domain is the toxic component and the B subunit /domain binds the whole toxins to the receptors on the eukaryotic cell membrane. After activation by enzymatic cleavage, the A subunit/ domain can exert its toxic (enzymatic) activity: interference with the cytoskeleton integrity, the protein synthesis, the DNA metabolism, or different secretion pathways. As examples, two types of AB *E. coli* toxins whose interactions with eukaryotic cells are well understood and whose role in vivo has been proved are going to be described: the heat-labile enterotoxin (LT) of human, porcine and ruminant ETEC and the Vero/Shiga toxins (VTx/Stx) of human, porcine and bovine EHEC and VTEC [3].

2.6.3. The Heat-Labile Enterotoxins

Besides the STa and STb oligopeptide enterotoxins, porcine and human ETEC strains can also produce an A1B5 heat-labile enterotoxin or LT Porcine and human LT (LTp and LTh) are both related to the cholera toxin (CT) of Vibriocholerae but slightly differ anti genetically from each other. Genes coding for LTp and LTh are located on plasmids [2].

Heat labile enterotoxin is secreted by the type 2 secretion system, involving the removal of the signal sequences. After binding of the B subunits to their specific receptors (the main receptor of LT and E. coli T is ganglioside GM1) on the host cells, whole LT are internalized by receptor mediated endocytosis and begin a retrograde transport in the endocytic vesicles to the endoplasmic reticulum via the Golgi apparatus. There the A subunit is enzymatically cleaved into a large A1 and a small A2 fragments and the A1 fragment translocate into the cytoplasm. The enzymatic activity of the A1 fragment is NAD dependent ADP ribosylation of the stimulatory regulator of the enzyme adenyl cyclase, causing its permanent activation and high levels of cyclic adenosine monophosphate (cAMP) in the target cells. The action of LT also results in the opening of the cystic fibrosis transmembrane conductance regulator with increased secretion of chloride and carbonate ions and of water and in the inhibition of sodium absorption, leading to watery cholera-like diarrhoea. Newborn and weaned piglets and humans in developing countries are especially sensitive to the action of LT that can also be involved in traveler's diarrhoea cases [42].

Two other LT enterotoxins (LT-IIA and LT-IIb) that differ from LTp/h and CT antigenically and by their main receptor specificity (GD1b for LT-IIA and GD1a for LT-IIb), but that possess similar enzymatic activity, can be produced by E. coli isolated from humans, bovines and water buffaloes suffering diarrhoea. The genes coding for LT- IIa and LT-IIb are chromosome located and all LT enterotoxins are very good immunogens and LTpis present in vaccines against neonatal diarrhoea in piglets [43].

2.6.4. The Vero/Shiga Toxins

In 1977, culture supernatants of some E. coli isolates produced a profound cytotoxic effect on Vero cells. Two distinct Verotoxin (VTx) families have been described, VTx1 andVTx2. VTx1 is nearly identical to the Shiga toxin of Shigella dysenteriae serotype 1 and the term Shiga toxin (Stx) was also proposed to design this group of cytotoxins. In addition, three variant forms of VTx1 and seven variant

forms of VTx2 have been described, called VTx1a, VTx1c, VTx1d and VTx2a through VTx2g, respectively. VTx are produced by human, porcine and bovine VTEC and EHEC. Strains producingVTx2a, VTx2c and/or VTx2d are more often associated with the haemolytic uremic syndrome (HUS) in humans while VTx2e is associated with oedema disease in swine. The majority of VTx are encoded by phage located genes that can be lost or acquired by other E. coli not only in vitro, but also in vivo [44].

Verotoxin (VTx) are also A1B5 toxins using Gb3, or Gb4 for VTx2e, as cell surface receptors, but their target cells in vivo are not the enterocytes. VTx can indeed cross the human and porcine intestinal epithelium by transcytosis through the enterocytes, i.e. endocytosis followed by exocytosis at the basal pole. Still, the actual mechanism of crossing is not fully understood and may differ between humans and piglets since the human enterocytes donot express the Gb3 receptor, while porcine enterocytes do express the Gb4 receptor [6].

In humans, and also probably in piglets, VTx subsequently travel in the blood stream in association with leucocytes and attach to the receptors on the endothelial cells, more especially of the renal glomerulus vessels in humans, but of vessels all over the body in pigs. After internalization, the whole toxin begins a retrograde transport in the endocytic vesicles to the endoplasmic reticulum via the Golgi apparatus where the A subunit is enzymatically cleaved. There after the activated A1 fragment translocates into the cytoplasm and cleaves the 28S rRNA via N-glycosylation of a specific adenine residue. The protein synthesis is inhibited in the endothelial cells and the blood vessel walls are damaged [45].

Verotoxin (VTx)-producing E. coli strains can also colonize the intestines of ruminants, though VTx cause no vascular lesion and no disease in ruminants (healthy carriers) for at least two reasons. At first VTx do not cross the intestinal epithelium though bovine crypt enterocytes express the Gb3 receptor. In deed after endocytosis the intracellular trafficking localizes VTx in lysosomes leading to abrogation of transcytosis. Moreover the Gb3 receptor is not expressed on endothelial cells of cattle. VTx are good immunogens and active immunization with a VTx2e toxoid can protect piglets against oedema disease [46].

2.7. Pathogenecity Islands of Escherichia Coli

Pathogenicity islands (PAIs) are mobile genetic elements in the chromosome of bacteria which carry virulence genes. They range in size from 10 to 200Kb and are more susceptible to incursion by foreign DNA than the pan genome (or supra-genome). The insertion of PAIs to a strain is not a permanent event. PAIs are present only in the pathogenic variants of E. coli and rarely or not in the non-pathogenic variants. PAIs are also present in animals and plants genomes. The presence of PAIs in pathogenic strains of E. coli makes the difference between commensal K-12 strains and the pathogenic variants [47].

There are four different PAIs in the E. coli ExPEC strain (UPEC 536 (O6:K15:H31)) and they encode a variety of different virulence factors: adhesins, toxins, invasins, protein secretion systems, iron uptake systems, and others. PAI I and PAI II are inserted into two genes that encode for Leuand Sec (Selenocysteine) tRNAs. PAIs are not unique just to E. coli species but they are also found in a variety of both Gram-negative bacteria and also Gram-positive bacteria. The PAIs of Gram positive bacteria are more stable and do not carry mobility genes as compared to the PAIs of Gram negative bacteria [9].

Some pathogenicity islands are more stable than others in terms of deletion frequencies due to environmental stimuli such as temperature, nutrient availability and cell density. The recombination processes that lead to the integration of PAIs are not specific, since both UPEC and EPEC code for different genes but are located at identical base pairs in selCtRNA genes of the PAIs. Point mutations, genome rearrangement and the acquisition of new genes by horizontal gene transfer is the current basis of understanding the evolution of microbial pathogens from related non-pathogenic bacteria, as well as for the generation of new variants of pathogens. Following the acquisition of new genetic information, the stabilization and optimization of the expression of the new genetic elements becomes important. Loss-of-gene function by point mutation in the genome may enhance bacterial virulence without horizontal gene transfer of a DNA fragment, carrying a specific virulence factor [33].

3. Conclusion and Recommendations

There are several highly adapted E. coli clones that have acquired specific virulence attributes, which confers an increased ability to adapt to new niches and allows them to cause a broad spectrum of disease. Virulence factor in E. coli include the ability to resist phagocytosis, to tolerate an extremely low pH (highly acidic environment) by using multiple acid resistance mechanisms, utilization of highly efficient iron acquisition systems, expression of different adhesion proteins to prevent their removal by the peristaltic flow following passage through the stomach, the type 3 secretion systems, production of toxins (heat stable toxin, heat labile toxins and Vero/Shiga toxins) and acquisition of different pathogenecity islands that encode a variety of different virulence factors including adhesins, toxins, invasins, protein secretion systems, iron uptake systems, and others.

In order to effectively control the pathogenic E. coli infections, in-depth investigation on virulence factors of E. coli strains circulating in the country should be undertaken. Since the routine identification and differentiation of different pathogenic E. coli strains virulence factors based on the molecular techniques, and genotypic characterization requires the availability of different kit, establishment of functional molecular laboratory with respected kit at both national and regional laboratories has to be encouraged.

More proactive measures should be taken to protect animals and human populations from pathogenic E. coli infection to reduce its economic impact to different food, dairy and meat

industry and the risk of infection in exposed human population.

Acknowledgements

First and foremost, I would like to thank God for making all this possible. I would then like to express my deepest gratitude to my adviser Dr. Minda Asfaw for his constant follow up, guidance, provision of material and encouragement throughout the review of my seminar paper. And also, I would like to extend my sincere appreciation to my family for their fruit full apprise and moral support without them this would have not been possible. Last but not least I would like to thank my friends.

References

[1] Fernandez, T. F, *E. coli* O157: H7.. *Vet. World,* 2008. 1 (3): p. 83-87.

[2] Kaper, J. B, Nataro, J. P, Mobley, H. L, Pathogenic *Escherichia coli. Nat. Rev. Microbiol,* 2004 2: p. 123-140.

[3] Mainil, J, Molecular and cellular pathogenesis of bacterial infections Colonisation of the mucosae; adherence factors and their interaction with host cells. *Ann. Vet. Supp*, 2005 12,: p. 5-14.

[4] Lior, H, Classification of Escherichia coli. In: Gyles, C. L. (Ed.), *Escherichia coli* in Domestic Animals and Humans. *CabInternational, Wallingford, Oxon, U. K,* 1994.: p. 31-72.

[5] Gyles, C. L, Prescott J. F, Songer, F, Airbrother, J. M, J. G. and Thoen, C, *Escherichia coli. I*n: Pathogenesis of bacterial infections in animals. *Wiley-Blackwell, Ames, IA, U. S. A,* 2010. 12: p. 267-308.

[6] Waddell, T. E, Ling wood, C. A, Gyles, C. L, Interaction of Verotoxin 2e with pig intestine. *Infect. Immun,* 1996. 64: p. 1714-1719.

[7] Schreiber, W, Donnenberg, M. S. and Donnenberg, M. S, *Escherichia coli* Virulence Mechanisms of a Versatile Pathogen. *Academic Press New York, NY,..* 2002 4: p. 417.

[8] Khan, M. A. a. S, T. S, Mechanism of emerging diarrheagenic *Escherichia Coli i*nfections. *Current Inf. Dis. Rep,* 2002. 4: p. 112-117.

[9] Sabate M, M. E, Perez T, Andreu A, Prats G, Pathogenicity island markers in commensal and uropathogenic Escherichia coli isolates. *ClinMicrobiol Infect* 2006. 12: p. 880-886.

[10] Jores, J, Rumer, L, and Wieler, L, Impact of the locus of enterocyte effacement pathogenicity island on the evolution of pathogenic *Escherichia coli*. *Int. J. Med. Microbio,* 2004. 294: p. 103-113.

[11] Ceponis. P, R, J. and Sherman, P, Epithelial cell signaling responses to entero hemorrhagic Escherichia coli infection. *Memriasdo Instituto Oswaldo Cruz,* 2005. 100: p. 199- 203.

[12] Garmendia, J, Frankel, G. and Crepin, V, Enterpathogenic and enterohemorrhagic *Escherichia coli infections:* translocation, translocation, translocation. *Infection and Immunity,* 2005. 73: p. 2573-2585.

[13] Lim, J, Sheng, H, Seo, K, Park, Y. and Hovde. C, Characterization of an *Escherichia coli* O157:H7 plasmid O157 deletion mutant andits survival and persistence in cattle. *Applied and Environmental Microbiology,* 2007. 73: p. 2037-2047.

[14] LeBlanc, J, Implication of virulence factors of *Escherichia coli* O157: H7 pathogenesis. *Clinical Microbiology Review,* 2003. 29: p. 277-296.

[15] Robins-Browne, *T*he relentless evolution of pathogenic *Escherichia coli. Clinical Infecious Diseases,* 2005. 41: p. 793-794.

[16] Welinder-Olsson, C. a. K, B, Enterohemorrhagic *Escherichia coli* (EHEC). *Scandanavian Journal of Infectious Diseases,* 2005 37: p. 405-416.

[17] Lu P, M. D, Chen Y, Guo Y, Chen GQ, Deng H, and Shi Y, L-glutamine provides acid resistance for Escherichia coli through enzymatic release of ammonia. *Cell Res,* 2013 23 (5): p. 635-44.

[18] Foster, J. W, *Escherichia coli* acid resistance: tales of an amateur acidophile. *Nat. Rev. Microbiol,* 2004. 2 (11): p. 898-907.

[19] Kuper C, J. K. C, mediated activation of the cadBA promoter in *Escherichia coli. J Mol Microbiol Biotechnol,* 2005. 10 (1): p. 26-39.

[20] Kanjee U, H. W, *Mechanisms of acid resistance in Escherichia coli.. Annu RevMicrobiol,* 2013. 67: p. 65-81.

[21] Rasko, D. A, Rosovitz, M. J, Myers, G. S, Mongodin, E. F, Fricke, W. F, Gajer, P, Crabtree, J, Sebaihia, M, Thomson, N. R, Chaudhuri, R, Hender son, I. R, Sperandio, V, Ravel, J, The pan genome structure of *Escherichia coli:* comparative genomic analysis of E. coli commensal andpathogenicisolates. *J. Bacteriol,* 2008. 190: p. 6881-6893.

[22] Clavijo, A. P, Bai, J. and Gomez-Duarte, O. G, The longus type IV pilus of Enterotoxigenic *Escherichia coli (ETEC)* mediate bacterial self-aggregation and protection from*antimicrobial* agents. *Microbiol. Pathog,* 2010. 48: p. 230-238.

[23] Saldana, Z, Sanchez, E, Xicohtencatl-Cortes, J, Puente, J. L, Giron, J. A, Surface Structures involved in plants to mata and leaf colonization by Shiga toxigenic *Escherichia coli* O157:H7. *Frontiers Microbiol,* 2011. 2,: p. 1-9.

[24] Nagy, B, Fekete, P. Z, Enterotoxigenic*Escherichiacoli (*ETEC) infarm animals. *Vet. Res,* 1999. 30 (259-284.).

[25] Croxen, M. A. a. F, B. B, Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiol,* 2010. 8 (1): p. 26-38.

[26] Shin, S, G. Lu, M. Cai, and K. S. Kim, *Escherichia coli* outer membrane protein A adheres to human brain microvascular endothelial cells. *Biochem. Biophys.. Res. Commun,* 2005. 330: p. 1199-1204.

[27] Cells. *Infect. Immun,* 2003. 71: p. 4985-4995.

[28] Jeannin, P, B. Bottazzi, M. Sironi, A. Doni, M. Rusnati, M. Presta, V. Maina, G. Magistrelli, J. F. Haeuw, G. Hoeffel, N. Thieblemont, N. Corvaia, C. Garlanda, Y. Delneste, and A. Mantovani, Complexity and complementarity of outer membrane protein A recognition by cellular and humoralinnate immunity receptors. *Immunity* 2005. 22: p. 551-560.

[29] Moll, H, Dendritic cells and host resistance to infection. *cell. micro-biol,* 2003. 5: p. 493-500.

[30] Bolton, D. J, Vero cytotoxigenic (Shiga toxin producing) *Escherichia coli:* Virulence factors and pathogenicity in the farm to fork paradigm. *Food borne Pathog. Dis,* 2011. 8: (357-365.).

[31] Hayashi T, M. K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han CG, Ohtsubo E, Nakayama K, Murata T, Tanaka M, Tobe T, Iida T, Takami H, Honda T, Sasakawa C, Ogasawara N, Yasunaga T, Kuhara S, Shiba T, Hattori M, Shinagawa H:, Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 *and genomic comparison with a laboratory strain K-12. DNA Res,* 2001. 8: p. 11 - 22.

[32] Tobe T, B. S, Taniguchi H, Abe H, Bailey CM, Fivian A, Younis R, Matthews S, Marches O, Frankel G, Hayashi T, Pallen MJ:, An extensiverepertoire of type III secretion effectors in Escherichia coli O157 and therole of lambdoid phages in their dissemination. *Proc Natl AcadSci U S A,* 2006. 103: p. 14941 - 14946.

[33] Morova, J, R. Osicka, J. Masin and P. Sebo RTX cytotoxins recognize beta2 integrin receptors through N-linked oligosaccharides.*"P Natl AcadSci USA* 2008. 105: p. 5355-5360.

[34] Calamia. J. and Manoil, C, Lac permease of Escherichia coli: Topology and sequence elements promoting membrane insertion. *Pro. of the Natio. Acad. Scie. Un. Stat. America,* 1990. 87: p. 4937-4941.

[35] Kim Jy, D. A, Chen Dj, Cremona gh, shuler ml, putnam d, delisamp, Engineered bacterial outer membrane vesicles with enhanced functionality. *j. mol. bio,* 2008 380: p. 51-66.

[36] van der Goot, G. a. J. A. Y, Receptors of anthrax toxin and cell entry. *Mol Aspects Med,* 2009 30 (6): p. 406-412.

[37] Cortajarena, A. L, F. M. Goñiand H. Ostolaza A receptor binding region in *Escherichia coli* haemolysin. *J. Biol. Chem,* 2003 278: (21): p. 19159-19163.

[38] Valeva, A, I. Walev, H. Kemmer, S. Weis, I. Siegel, F. Boukhallouk, T. Wassenaar, T. Chavakis and S. Bhakdi Binding of *Escherichia coli* Hemolysin and Activation of the Target Cells is not Receptor-dependent.." *J BiolChem*, 2005 280: p. 36657-36663.

[39] Sanchez-Magraner, L, A. R. Viguera, M. Garcia-Pacios, M. P. Garcillan, J. L. Arrondo, F. de la Cruz, F. M. Goniand H. Ostolaza The calcium-binding C-terminal domain of Escherichia coli alpha-hemolysin is a major determinant in the surface-active properties of the protein. *"J BiolChem* 2007 282 (16): p. 11827-11835.

[40] Herlax, V, S. Mate, O. Rimoldi and L. Bakas Relevance of fatty acid covalently bound to *Escherichia coli* alpha-hemolysin and membrane microdomains in the oligomerization process. *"J Biol Chem* 2009. 284 (37): p. 199-210.

[41] Weintraub, A, Enteroaggregative *Escherichia coli:* epidemiology, virulence and detection. *J. Med. Microbiol,* 2007 56: p. 4-8.

[42] Flores, J. a. O, P. C, Entero aggregative *Escherichia coli* infection. *Curr. Opin. Gastro Enterol,* 2009. 25: p. 8-11.

[43] Radostits, O, Gay, C. C, Hinchcliff, K, Constable, P. D, Veterinary Medicine A Textbook of the Diseases of Cattle, Horses, Sheep, *Pigs.* 2007.

[44] Scheutz, F, Teel, L. D, Beutin, L, Piérard, D, Buvens, G, Karch, H, Mellmann, A, Caprioli, A, Tozzoli, R, Morabito, S, Strockbine, N. A, Melton-Celsa, A. R, Sanchez, M, Persson, S, O'Brien, A. D, Multicenter evaluation of a sequence based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *J. Clin. Microbiol,* 2012 50, (2951-2963.).

[45] Johannes, L, R omer, W, Shigatoxins from cell biology to Biomedical applications. *Nat. Rev. Microbiol,* 2010). 8: p. 105-116.

[46] Hoey, D. E, Sharp, L, Currie, C, Lingwood, C. A, Gally, D. L, Smith, D. G, Verotoxin1 binding to intestinal crypt epithelial cells results in localization to lysosomes and abrogation of toxicity. *cell. microbiol,* 2003. 5: p. 85-97.

[47] Hacker J, K. J, Pathogenicity islands and the evolution of microbes. *Annu Rev Microbiol*, 2000. 54: p. 641-679.