

Review

Toxigenic *Escherichia coli* and *Vibrio cholerae*: Classification, pathogenesis and virulence determinants

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***Escherichia coli* and *Vibrio cholerae* are pathogenic bacteria commonly found in various contaminated sources and pose a major health risk, causing a range of human enteric infections and pandemics, especially among infants in Africa. Virulence and pathogenesis of these organisms is specifically based on the expression of certain virulence determinants, distinctive mucosal interactions as well as the production of enterotoxins or cytotoxins. The *E. coli* strains that cause human disease are generally grouped into six pathotypes based on their pathogenic mechanisms of which the enterohemorrhagic and enterotoxigenic groups have been shown to be the most severe. Of the *V. cholerae* pathogens, the 01 and 0139 serotypes have been identified as being toxigenic due to the CTX genetic element and *V. cholerae* pathogenicity Island, possessed by the respective serotype. This article thus provides an overview of both the enterohaemorrhagic and enterotoxigenic *E. coli* as well as toxigenic *V. cholerae*, and their respective virulence genes determinants involved in pathogenicity.**

Key words: Diarrhoea, *Escherichia coli*, pathogenicity, toxigenicity, *Vibrio cholerae*, virulence determinants.

INTRODUCTION

Diarrhoeal diseases caused by enteric infections remain a leading global health problem. Two to four billion episodes of infectious diarrhoea have been estimated to occur annually in developing countries, resulting in 3 to 5 million deaths (Sánchez and Holmgren, 2005). Almost half of all cases of diarrhoea are due to bacteria that cause disease by producing one or more enterotoxins (Sánchez and Holmgren, 2005). The virulence of a pathogen is dependent on a discrete set of genetic determinants and their well-regulated expression (Chakraborty et al., 2001). During evolution, bacterial species have become capable of transferring virulence genes not only between members of a particular species but also between different bacterial species, creating new pathotypes with new combinations of different virulence

genes (Schubert et al., 1998; Kaper et al., 2004). The detection of specific virulence attributes of a given strain allows for the determination of potential reservoirs of virulence genes (Feng and Monday, 2000; Kuhnert et al., 2000; Ram et al., 2009). These are expected to play a key role as the origin of emerging diseases caused by pathogens and is a useful tool in the analysis and detection of new strains (Feng and Monday, 2000; Kuhnert et al., 2000; Sharma, 2002; Sharma and Dean-Nystrom, 2003; Chassagne et al., 2009; Hidaka et al., 2009; Ram et al., 2009).

Virulent strains of *Escherichia coli* cause a series of human diseases, such as gastroenteritis, urinary tract infection and neonatal meningitis as well as haemolytic uremic syndrome (HUS), peritonitis, mastitis, and septicemia in rare cases (Todar, 2007). Also, *Vibrio cholerae*, a motile Gram negative curved-rod shaped bacterium with a polar flagellum causes cholerae in humans (Faruque and Nair, 2008), which is a common pandemic disease, especially in Africa. This review provides an overview of

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the enterohaemorrhagic and enterotoxigenic *E. coli* as well as toxigenic *V. cholerae*, their virulence determinants and associated malaises.

Escherichia coli

Certain strains of *E. coli*, the widely used 'indicator' of the microbiological quality of surface waters, have virulence properties that may account for life-threatening infections. The pathogenicity of a particular *E. coli* strain is primarily determined by specific virulence factors which include adhesins, invasins, haemolysins, toxins, effacement factors, cytotoxic necrotic factors and capsules (Kuhnert et al., 2000; Galane and Le Roux, 2001). Additional genes detected in pathogenic *E. coli* encode various virulence factors which directly indicate their virulence and pathotype (Kuhnert et al., 2000). Three general clinical syndromes resulting from infection with natural pathogenic *E. coli* strains include: Urinary tract infections, sepsis/meningitis and enteric/diarrhoeal diseases (Nataro and Kaper, 1998).

Most *E. coli* serotypes are non-pathogenic; however those that do cause disease are classified and grouped according to their pathogenic mechanisms (Oregon Health Services, 2002). Currently, six *E. coli* pathotypes recognized to cause diarrhoea in humans are: Enteropathogenic *E. coli*, enteroinvasive *E. coli*, enterohaemorrhagic *E. coli* (EHEC) or shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli*, enterotoxigenic *E. coli* (ETEC) and diffusely adhering *E. coli* (Turner et al., 2006). However, certain pathotypes of *E. coli* including STEC and ETEC are potent pathogens associated with waterborne disease outbreaks and mortality in humans (Ram et al., 2007). Although ETEC and STEC are known to be associated with food and waterborne diseases, relatively few studies have been performed to determine their distribution in environmental surface waters (Obi et al., 2004; Begum et al., 2005; Higgins et al., 2005; Hamelin et al., 2006; Shelton et al., 2006; Ram et al., 2008).

Enterohaemorrhagic *E. coli* (EHEC)

Enterohaemorrhagic *E. coli* or shiga toxin-producing *E. coli* has been shown to be the most important group among the six characterized groups of diarrheagenic *E. coli* in developed countries and numerous outbreaks of patients developing life-threatening complications such as hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC) have been reported (Leelaporn et al., 2003; Matsell and White, 2009). *E. coli* O157:H7 is a dominant STEC serotype in many parts of the world and historically has been the type most commonly associated with large outbreaks (Paton and Paton, 1998). The STEC have been classified into pathogroups A to E based on the

severity of diseases they cause and their association with outbreaks (Karmali et al., 2003). The ingestion of as few as 1 to 10 cells may cause illness in humans (Chart, 2000; Kuhnert et al., 2000). Because STEC/EHEC is asymptomatic in animals, cattle, sheep and goats represent the main reservoir of STEC as the organism is shed in their faeces (Shelton et al., 2006; Williams et al., 2006). This in turn may serve as a means of maintenance and spread of these pathogens among them (Franz et al., 2007). The organism has also sporadically been detected in chickens, pigs, horses and dogs (Beutin et al., 1993; Karch et al., 1999; Fratamico et al., 2008).

The pathogenicity of STEC for humans is related to the production of Stx (shiga toxins) and additional virulence factors such as enterohaemorrhagic *E. coli* (EHEC) hemolysin encoded by *hlyA* gene which acts as pore forming cytotoxin on eukaryotic cells and intimin, an outer membrane protein responsible for the attaching and effacing phenotype, encoded by a chromosomal *eaeA* gene (Leelaporn et al., 2003; Ram et al., 2009). The *chuA* gene is part of heme transport locus that encodes for a 69-kDa outer membrane protein responsible for heme transport, the bifunctional catalase peroxidase KatP enzyme and the secreted serine protease which can cleave human coagulation factor V (Torres and Payne, 1997; Leelaporn et al., 2003; Ram et al., 2009). STEC may also possess genes that encode fimbrial and nonfimbrial adhesins, proteases, and other toxins, including *astA* (enteroaggregative *E. coli* heat-stable enterotoxin, EAST1) and *cdt* (cytotoxic distending toxin) (Gyles, 2007).

The Stx family contains two major groups called Stx1 and Stx2. A EHEC strain may express Stx1 only, Stx2 only, or both toxins or even multiple forms of Stx2. Stx1 from EHEC is identical to the Shiga toxin from *Shigella dysenteriae* I (O' Brien et al., 1992; Takeda, 1995). Members of the Stx family have compound, the holotoxin (approximately 70 kDa), comprising a single 32-kDa A subunit and a multimeric B subunit (7.7-kDa monomers) which binds the toxin to a specific glycolipid receptor, globotriaosylceramide or Gb₃, which is present on the surface of eukaryotic cells (Sandvig and van Deurs, 1994). During internalization, A clathrin-coated pit formed in the cell membrane, which subsequently pinches off to form a sealed coated vesicle with toxin bound to the internal surface (Paton and Paton, 1998). The A subunit is translocated to the cytoplasm and proteolytically nicked by a membrane-bound protease furin to yield A₁ (27-kDa N-terminal) and A₂ (4-kDa C-terminal) peptides, which are linked by a disulfide bond. The A₁ peptide contains the enzymatic activity, and the A₂ peptide serves to bind the A subunit to a pentamer of five identical 7.7-kDa B subunits (O' Brien et al., 1992; Paton and Paton, 1998). The disulfide bond is subsequently reduced, thereby releasing the active A1 component which enzymatically inactivates the 60S ribosomal subunit by depurination of specific residues of the host cell ribosomes, thus

inhibiting the peptide chain elongation step of protein synthesis. This leads to the death of renal endothelial cells, intestinal epithelial cells or any cells, which possess the Gb₃ receptor (Sandvig and van Deurs, 1994).

The STEC isolates also produce a hemolysin, encoded by the *hlyA* gene which is located on a large virulence 60-MDa plasmid (O' Brien et al., 1992). The hemolysin antigen has been detected in the serum of 95% of patients with HUS (Schmidt et al., 1995). The role of the hemolysin in the pathogenesis of infection is unclear although it has been shown to increase the secretion of interleukin (IL) 1-beta which enhances the toxicity of the shiga toxin toward human vascular epithelial cells through up-regulation of the cell surface receptor for the toxin (Law and Kelly, 1995; Mills and Payne, 1995). *E. coli* O157 contains a specialized iron transport system which allows this organism to use heme or haemoglobin as an iron source and the lysis of erythrocytes by one or more of the hemolysins could release these sources of iron, thereby aiding infection (Law and Kelly, 1995; Mills and Payne, 1995).

The final virulence factor is the ability of *E. coli* O157 to adhere to and colonize intestinal surfaces. Attachment to mucosal surfaces prevents the loss of the bacteria via peristalsis and promotes delivery of the toxin to the cell surface in a concentrated manner. The *eae* gene of O157 encodes the intimin protein which facilitates actin polymerization and cytoskeletal rearrangement of the intestinal cells, causing the development of characteristic attaching and effacing (A/E) lesions (Donnenberg et al., 1993; Caprioli et al., 2005). The complex mechanism of A/E adhesion is genetically governed by a large pathogenicity island defined as the locus of enterocyte effacement (LEE), consisting of three functionally different modules; the first encodes a type III secretion system (TTSS) that exports effector molecules, the second encodes the secreted proteins EspA, B, and D, which function as part of the type III secretion apparatus, while the third encodes the intimin and the translocated intimin receptor (Tir), which is translocated into the host cell plasma membrane by the TTSS (Caprioli et al., 2005). The mechanisms by which *E. coli* O157 causes HC and HUS are not fully understood. The organism is believed to adhere closely to mucosal cells of the large bowel, disrupting the brush border (Mead and Griffin, 1998). This process alone may be sufficient to produce non-bloody diarrhoea. Shiga toxins have effects on the intestine and are probably critical to the development of bloody diarrhoea. Damage of the endothelial cells, mediated by Shiga toxins, may trigger platelet and fibrin deposition, leading to injury of passing erythrocytes (haemolysis) and occlusion of renal microvasculature (renal failure) (Mead and Griffin, 1998). Thrombocytopenia is believed to reflect trapping of platelets in involved organs and removal by the liver and spleen. Although the kidneys are preferentially involved, other organs including the brain may be affected, resulting in a

wide range of complications (Mead and Griffin, 1998).

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* refers to those which specifically adhere to the microvilli of the small intestinal epithelial cells and which produce site-specific enterotoxins (Nagy and Fekete, 1999). These bacteria cause secretory diarrhoea in humans throughout the world (Albert et al., 1995), especially in children below 5 years due to the consumption of contaminated water and food (Qadri et al., 2005). ETEC is also an important pathogen in the farming industry and causes diseases in cattle, neo-natal and post-weaning pigs (Turner et al., 2006). These pathogenic ETEC strains are characterized by the production of either large molecular weight heat labile enterotoxins (LT) or small molecular weight heat-stable peptide toxins (ST) which serve as the main virulence determinants (Nagy and Fekete, 1999). Enterotoxins are defined as extracellular proteins or peptides which affect the respective intestinal epithelial cells. ETEC causes travelers' diarrhoea by producing different combinations of heat labile (LT) and heat stable (ST) enterotoxins along with one or more of the 22 colonizing factors (Turner et al., 2006). The LTs of *E. coli* are oligomeric toxins that are closely related to the cholerae enterotoxin (CT) expressed by *V. cholerae* (Sixma et al., 1993). There are two major serogroups of LT, LT-I and LT-II, which do not cross-react immunologically. LT-I is expressed by *E. coli* strains that are pathogenic for both humans and animals. LT-II is found primarily in animal *E. coli* isolates and rarely in human isolates, but in neither animals nor humans has it been associated with disease (Prescott et al., 2005). In contrast to the large, oligomeric LTs, the STs are small, monomeric toxins that contain multiple cysteine residues, whose disulfide bonds account for the heat stability of these toxins (Prescott et al., 2005). The genes for the LT and ST enterotoxins could be encoded together or separately on large variable plasmids called Ent plasmids (Prescott et al., 2005; Turner et al., 2006).

The universal trait of all ETEC infections involves the adherence by fimbriae or pilli, to the small intestinal epithelial cells thus allowing them to colonize the gut and transfer the respective enterotoxins more effectively (Nagy and Fekete, 1999). This pathogenic process is initiated via colonization of the ETEC bacteria, followed by multiplication within the small intestine in order to aid production of the abovementioned toxins. Colonization is usually associated with the presence of surface adhesins known as the colonization factors (CFs) that allow attachment to the small intestine (Ansaruzzaman et al., 2007). After binding to the host cell membranes, the toxin is endocytosed and translocated through the cell in a process involving trans-Golgi vesicular transport (Lencer et al., 1995). ST binds to a glycoprotein receptor that is

coupled to guanylate cyclase on the surface of intestinal epithelial cells. Activation of guanylate cyclase stimulates the production of cyclic guanosine monophosphate (cGMP), which leads to the secretion of electrolytes and water into the lumen of the small intestine, manifested as watery diarrhoea characteristic of an ETEC infection (Crane et al., 1992; Mezzoff et al., 1992). LT binds to specific gangliosides on the epithelial cells and activates membrane-bound adenylate cyclase, which leads to an increased production of cyclic adenosine monophosphate (cAMP), resulting in the hypersecretion of electrolytes and water into the intestinal lumen (Prescott et al., 2005). The epidemiologic pattern of ETEC disease is determined by a number of factors: (a) Mucosal immunity to ETEC infection develops in exposed individuals; (b) Even immune asymptomatic individuals may shed large numbers of virulent ETEC organisms in the stool; and (c) The infection requires a relatively high infectious dose (DuPont et al., 1971). The illness is typically abrupt in onset with a short incubation period (14 to 50 h) (DuPont et al., 1971; Nalin et al., 1975). The general mode of action of the ST and LT enterotoxins is the functional changes which are brought about, such as the increased secretion of H₂O, Na⁺ and Cl⁻ and resultant decrease of fluid absorption, leading to excessive dehydration or acidosis (Nagy and Fekete, 1999). The diarrhoea is watery, usually without blood, mucus, or pus; fever and vomiting are present in a minority of patients (Levine et al., 1987). Administration of antibiotics to which ETEC strains are susceptible has been shown to decrease both the duration of diarrhoea and the intensity of ETEC excretion (Black et al., 1982).

Vibrio cholerae

Cholera is an epidemic disease of major global and public health significance and is caused by the organism, *V. cholerae* which is an autochthonous inhabitant of riverine, brackish and estuarine ecosystems. It is widespread in Southern Asia, parts of Africa and America where seasonal outbreaks are common and predominantly associated with both poverty and poor sanitation and hygiene (Faruque et al., 1998). Outbreaks of cholerae cause approximately 120,000 deaths worldwide annually with the majority of these deaths occurring in children (Cooke, 2010). The seventh cholerae pandemic started in Indonesia in 1961, reached Africa in 1970 and arrived in Latin America in 1991 with Peru being the first country to be hit with cholerae. In 1999, a small outbreak occurred in Paranaguá Bay, Paraná State (South of Brazil) where cholerae incidents had never been reported (Rivera et al., 2003). In Argentina, there were seven epidemics since 1992, with these outbreaks occurring mainly during the summer months and were consistent with reports from other geographic regions of the world (Binsztein et al., 2004).

In August 2000, South Africa experienced one of the worst cholerae epidemics in the country's history. Initial reports of the cholerae outbreak came from the largely rural and impoverished communities on the outskirts of the Ngwelezane Township, near the Empangeni town. The source of the epidemic was traced to the Mhlathuze River, also in the Northern part of the KwaZulu-Natal province (Jenkins, 2000). However, by the end of the year 2000, the Northern KwaZulu-Natal cholerae outbreak had replicated itself in eight of South Africa's nine provinces and registered over 114,000 cases and 260 reported deaths by the end of January 2002, nearly all from KwaZulu-Natal (KZN Department of Health media release, 7 February 2002). The disease also claimed at least 5000 lives in Angola in 2006 and neighbouring countries (Thompson et al., 2008).

V. cholerae is a heterogeneous species with 206 serotypes identified to date based on the heat-stable somatic O antigen. Among these only two serotypes, O1 and O139, have been characterized as toxigenic and have caused epidemics of cholerae (Rivera et al., 2003). *V. cholerae* non O1 or non O139 is isolated in abundance from aquatic environments whereas *V. cholerae* O1 is seldom recovered from these ecosystems in the inter-epidemic periods of the disease or it may be found in the non-toxigenic form, "viable but non-culturable" form or in the form of biofilms (Leal et al., 2008). Also, interaction with plankton appears to play an important role in the ecology of the microorganism and to facilitate persistence, mainly in response to low temperatures and reduced nutrient concentrations (Binsztein et al., 2004). *V. cholerae* O1 is divided into two biotypes, classical and El Tor, which are distinguished by a variety of phenotypic markers (Kaper et al., 1995). However, three variants of the El Tor biotype were recently described in Bangladesh, Mozambique and other regions of Asia and Africa (Taneja et al., 2009).

The ability of *V. cholerae* to cause disease is dependant on multiple factors that allow the pathogen to colonize the epithelium of the small intestine and produce the respective enterotoxins that disrupts ion transport. In addition to this, the expression of two virulence factors, the cholerae toxin (CT) which is a potent enterotoxin and a pilus-colonization factor know as the toxin-coregulated pilus (TCP) are also important for pathogenicity (Faruque and Mekalanos, 2003). Both virulence factors are encoded by genes that form part of larger genetic elements namely the *ctxAB* gene which encodes for CT and the TCP-ACF element encoding for TCP, alternatively referred to as the *Vibrio* pathogenicity island (Faruque and Mekalanos, 2003). CT is a heterodimeric protein exotoxin which consists of two parts that are encoded by two separate but overlapping open reading frames: (1) An enzymatically active A subunit which acts as an ADP-ribosyl transferase and elevates intracellular cyclic AMP levels, known as CTA, and (2) A pentamer of B subunits (CTB) which bind the holotoxin to its receptor

which is the ganglioside GM₁ located on eukaryotic cells (Murley et al., 2000). CT initiates its toxic action on cells by binding with high affinity and exquisite specificity to cell membrane receptors (GM₁), which appears to be localized mainly in lipid rafts on the cell surface, and is endocytosed by the cell. In order for cell intoxication to occur, the A subunit needs to be transported to the cytosol to induce the activity of adenylate cyclase (AC). After endocytosis, CT travels to the endoplasmic reticulum (ER) via a retrograde transport pathway after which CTA dissociates from CTB (Feng et al., 2004). The arrival of CTA in the cytosol is the crucial step for intoxication because this peptide catalyzes the ADP ribosylation of a specific component of AC. This causes AC to remain in its GTP-bound state, resulting in enhanced AC activity and an increased intracellular cAMP concentration (Sánchez and Holmgren, 2005). Higher levels of cAMP produce an imbalance in electrolyte movement in the epithelial cell. The decrease in sodium uptake reduces water intake by the enterocyte and simultaneously increased anion extrusion to cause sodium outflow, which consequently results in water secretion and abundant net fluid loss from the intestine (Sánchez and Holmgren, 2005).

Apart from CT and TCP production, pathogenesis further relies on the coordinated interaction of a range of other virulence genes which occur in clusters. One such is the TCP pathogenicity island which shares several similar characteristics with other pathogenic bacteria such as a virulence gene regulator, a transposase gene, attachment sites on either side of the pathogenicity island and an integrase which exhibits homology to that of a particular phage integrase (Faruque et al., 1998). More than 95% of *V. cholerae* strains belonging to the O1 and O139 serogroups produce CT which is central to the disease process (Chakraborty et al., 2001). TCP is an adhesin that is coordinately regulated with CT production (Taylor et al., 1987) and is the only *V. cholerae* pilus that plays a role in colonization of the gut mucosa of humans (Chakraborty et al., 2001). Both CT and TCP are presumed to be exclusively associated with clinical strains of *V. cholerae* as reports on the incidence of CT and TCP among environmental strains are rare, suggesting these virulence factors are associated only with virulent *V. cholerae* O1 or O139 (Chakraborty et al., 2001).

Both the O1 and O139 pathogenic serogroups contain two main regions related to pathogenicity: CTX genetic element and VC pathogenicity island (VPI) (Karaolis and Kaper, 1999; Schmidt and Hensel, 2004). Under certain environmental conditions, non-toxigenic *V. cholerae* can transform into the toxigenic form with epidemic potential by acquisition of the CTX Φ (lysogenic filamentous bacteriophage) and VPI Φ phages which carry the genes for the CT as an operon (*ctxAB*) and the gene for the major subunit of the TCP (*tcpA*) (Faruque and Nair, 2002; Faruque et al., 2006). Transcription of the *tcpA* gene

together with an operon of 12 genes is involved in processing and assembly of TCP on the surface of *V. cholerae* (Murley et al., 2000). Regulation of expression of these virulence genes are dependent on ToxR (transmembrane DNA-binding protein) and ToxS (second regulatory protein) and these are both encoded in an operon (Miller and Mekalanos, 1984). These proteins are essential for the transcription of a two genes encoding the outer membrane protein of *V. cholerae* known as *ompU* (Crawford et al., 1998) and a second regulatory protein known as ToxT (Higgins and DiRita, 1994). In turn, ToxT activates the transcription of the *ctxAB* and *tcpA* operons (Higgins and DiRita, 1994). Although CT and TCP are undoubtedly the main virulence factors of *V. cholerae*, several accessory factors have also been described. These range from additional pilus structures, such as the fucose-binding and mannose-binding hemagglutinins which might or might not contribute to colonization of classical and El Tor biotypes, respectively, to various soluble factors. In addition to mucinase and neuraminidase, the latter group includes a variety of 'minor toxins' that might contribute to cholera diarrhea (Sánchez and Holmgren, 2005).

CONCLUSION

E. coli and *V. cholerae* are two of the major etiological agents causing many life-threatening diseases and has had serious implications in the public health system for many years (Rivera et al., 2003; Binsztein et al., 2004). Important preventative measures should be implemented in order to curb the spread of future infections and prevent future pandemics (Faruque and Mekalanos, 2003). These measures should include proper education of respective individuals in order to avoid possible future exposure, preventing cross-contamination, implementing proper hygiene control mechanisms, proper isolation and restrictions with regards to hospitalized patients and food handlers as well as the implementation of proper environmental measures (Oregon Health Services, 2002). Both *E. coli* and *V. cholerae* offer natural systems that allow for study of both the co-evolution and virulence-associated genetic factors which can aid in obtaining knowledge about possible emerging pathogens. Irrespective of current attempts, there remain many unknown virulence genes that are yet to be identified; therefore, continuous surveillance and characterization of virulence signature of these organisms is necessary to provide further insight into the potential health hazard associated with these pathogens and the development of possible prevention strategies and treatments.

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