

# **Persistence of foodborne Diarrheogenic *Escherichia coli* in the agricultural and food production environment: implications for food safety and public health**

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## **Abstract**

Diarrheogenic *Escherichia coli* (DEC) is a leading cause of foodborne illness associated with intestinal disease. While known over the years that contamination of food sources occurs via the oral faecal-route, the mechanisms underlying its persistence within the open environments including the food chain remains virtually known. Therefore, in this mini-review we will shed light on bacterial processes such as initial attachment, biofilm formation, horizontal gene transfer and response to environmental stresses. These factors may enable persistence of DEC as well as the emergence of potentially more virulent strains within the agricultural and food production environment. Mechanistic studies in clinical microbiology and immunology have elucidated infection pathways in the human and other animal bodies leading to diagnostic and treatment solutions. Therefore, understanding DEC behaviour in the agricultural and food production environment is crucial for ensuring food safety and public health by reducing the burden of foodborne illnesses.

Key words: Diarrheogenic *Escherichia coli*, Lateral gene transfer, environmental response, biofilm formation, foodborne illness, bacterial adherence

## Highlights

- Diarrheagenic *Escherichia coli* (DEC) is a leading cause of foodborne outbreaks worldwide
- DEC's ability to survive within the open environment has been scarcely explored.
- DEC characteristics may aid survival in open the environment subsequently contaminating food sources.
- Bacterial adherence, lateral gene transfer and environmental response may persistence of DEC persistence.
- These factors elucidate how foodborne pathogens spread along the food chain.

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### 1. *Escherichia coli*

*Escherichia coli* is a facultative Gram-negative facultative anaerobe of the phylum Proteobacteria and Family *Enterobacteriaceae* which forms a large part of the normal microflora of the human gut (Shin et al., 2015). The high numbers in the gut suggest a commensal or symbiotic relationship with other microbial constituents of the normal human microbiota. While many strains from this species present no viable health risk, certain strains do have the ability to acquire virulence factors enabling them to attack specific parts of the human body and other animals causing diseases (Leimbach et al., 2013). Alternatively, *E. coli* has also been referred to as a laboratory ‘work-horse’, a term used to refer to its ease of culture at different growth conditions facilitating its convenient easy use for elucidating bacterial processes in laboratories around the world (Blount, 2015). For example, over the last century the commensal strain *E. coli* K-12 has been used to unravel many fundamental processes underlying biological phenomena, many of which have gone on to be utilized in various industries from biotechnology to health and other applications (Blount, 2015).

### 1.1. Categorization of pathogenic *E. coli*

Pathogenic strains of *E. coli* include those that cause disease in the human gastrointestinal tract (DEC) and extra-intestinal *E. coli* (ExPEC) which cause infections in the urinary tract (uropathogenic *E. coli*, UPEC), septicemia associated *E. coli* (SEPEC) and meningitis-associated *E. coli* (MNEC). Infections caused in other parts of the body include those causing blood infections (bacteremia). Pathogenic *E. coli* has also been shown to cause diarrheal disease in domestic farm animals such piglets and poultry (Avian Pathogenic *E. coli*, APEC) depicting its wide distribution amongst humans and animals and thereby potential circulation within the human agricultural and food production environment. For example, the global dissemination of the ExPEC strain *E. coli* sequence type (ST131) has been associated with chronic infections and antibiotic resistance affecting many people (Nicolas-Chanoine et al., 2014).

DEC have acquired virulence genes through horizontal genetic transfer (HGT) and to a lesser extent random mutation enabling the organisms to attach to the human gut surfaces leading to diseases among which diarrhea is a symptom (Croxen et al., 2013; Kaper et al., 2004). The virulence factors enable attachment to the gut mucosal lining leading to persistent infections and inflammation. DEC are categorized into pathotypes with each description based on which virulence factors it possesses as well as the mechanism of pathogenesis.

Common DEC pathotypes commonly associated with human disease include; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), shigatoxin producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), diffusely adherent (DAEC), enteroinvasive *E. coli* (EIEC) and adherently invasive *E. coli* (AIEC) (Croxen et al., 2013). The common virulence factors associated with many of these pathotypes include attachment proteins such as intimin (*eae*) and bundle forming pili (*bfp*) for EPEC, cytotoxin production as noticed with STEC (shigatoxins 1, *stx1* and 2, *stx2* as well as the locus of enterocyte effacement, LEE) as well as heat labile (*LT*) and heat stable (*ST*) toxins for ETEC and epithelial cell adherence patterns for EAEC (stacked-brick pattern) and DAEC (random attachment along the whole cell surface) (Croxen et al., 2013). It has been suggested that driven environmental and genetic factors have led to the rise of multiple clones of these pathogens leading to the great heterogeneity observed among groups such as EAEC, ETEC and AIEC (Leimbach et al., 2013).

## **1.2. DEC a foodborne pathogen**

Diarrheagenic *E. coli* (DEC) is a leading cause of foodborne disease around the world affecting all age groups and demographics with symptoms including but not limited to diarrhoea, gastroenteritis, inflammation and nutrient malabsorption (Croxen et al., 2013; Leimbach et al., 2013). At highest risk of illness and death are new-borns, infants, the elderly as well as individuals with compromised immunity such as those living with Human Immuno-Deficiency Virus (HIV). While specific numbers relating to different geographical areas differ, higher rates of infection and disease have been observed in developing and least developed nations of the world (Acosta et al., 2016; Kotloff et al., 2013). However, recurrent outbreaks are also frequently reported in the developed world (Kaur et al., 2010; Rasko et al., 2011). Interestingly, the causes of illness in both these areas differ with poor sanitation being blamed for endemic DEC prevalence in less developed nations (Ahmed et al., 2013; Baker et al., 2016). In contrast, within developed countries, cross-contamination of food products along the heavily internationalized logistics food chain involving heavy mechanization at large food processing and distribution centres is a significant risk factor attributed to outbreaks.

The main pathotypes which are defined by how they illicit disease and having significant association with foodborne illness include; enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), shigatoxin producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC) and adherent and invasive *E. coli* (AIEC). However, EPEC, ETEC, EHEC and EAEC are noted to constitute the highest burden to human disease. It has been suggested as mainly transferred through the oral-faecal route hence making them foodborne pathogens (Kirk et al., 2017). Proper sanitation within domestic and food processing environments is an effective prevention strategy.

## **2. The agricultural and food production environment as a hotbed for emerging Diarrheagenic *Escherichia coli***

The emergence of highly virulent strains causing large-scale outbreaks is a big concern. STEC O157:H7 as well as other shigatoxin (STEC) producing *E. coli* strains (non-O157 STEC) still cause many outbreaks in developed countries (Havelaar et al., 2010; Kirk et al., 2017; Tauxe et al., 2010). This is in spite of developed countries having better tracking systems for foodborne pathogens and illness than developing nations (Nhampossa et al., 2015; Oundo et al., 2008). In 2011, a highly virulent EAEC strain O104:H4 code named the 'German outbreak strain' producing Shiga toxins was clinically described as

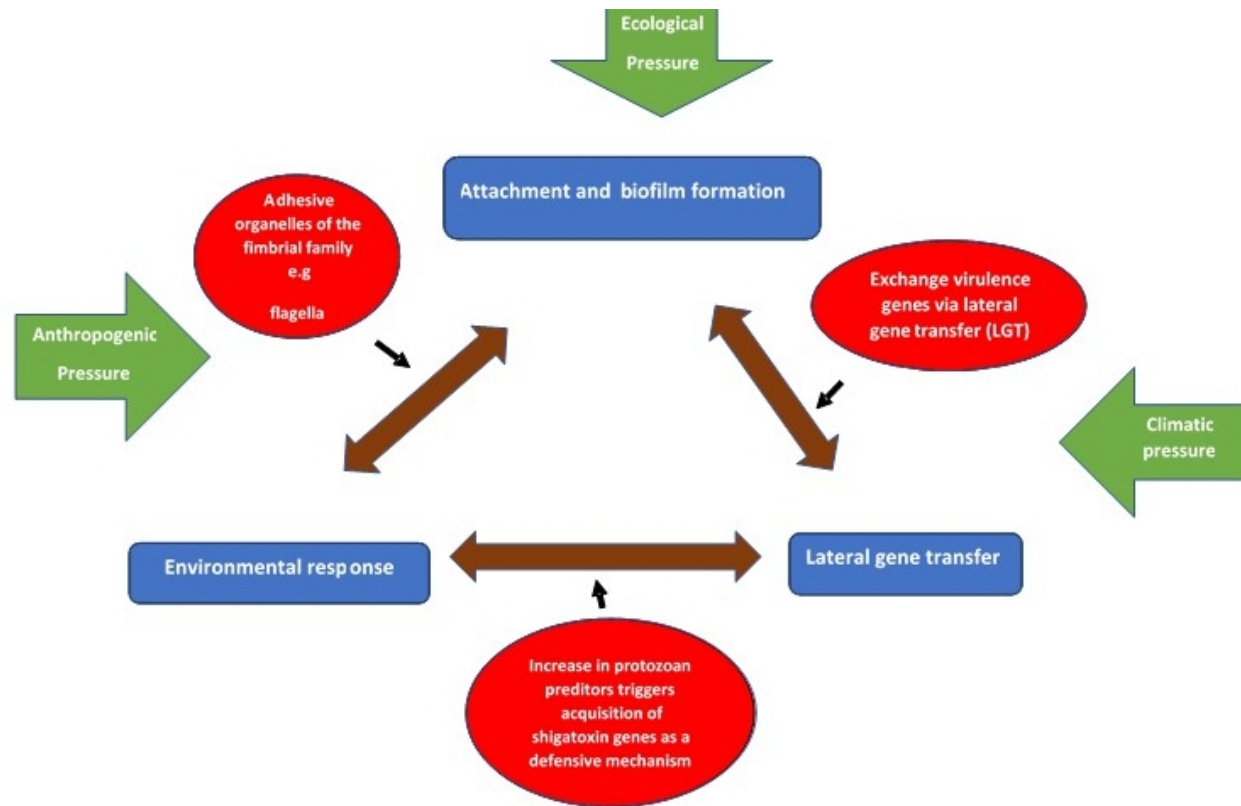
enteroaggregative haemorrhagic *E. coli* (EAHEC) (Beutin and Martin, 2012; Bielaszewska et al., 2011; Rasko et al., 2011). This strain caused the deadliest foodborne outbreak associated with *E. coli* in Europe. This outbreak affected many sectors of European society such as health, trade and politics (Commission of The European Communities, 2011). This outbreak leads to speculation that genetic mechanisms in the DEC group may have favoured the emergence of highly virulent foodborne within the agricultural and food production ecosystem.

### **2.1. Linking Diarrheagenic *Escherichia coli* persistence in the agricultural and food production environment to inherent bacterial attributes**

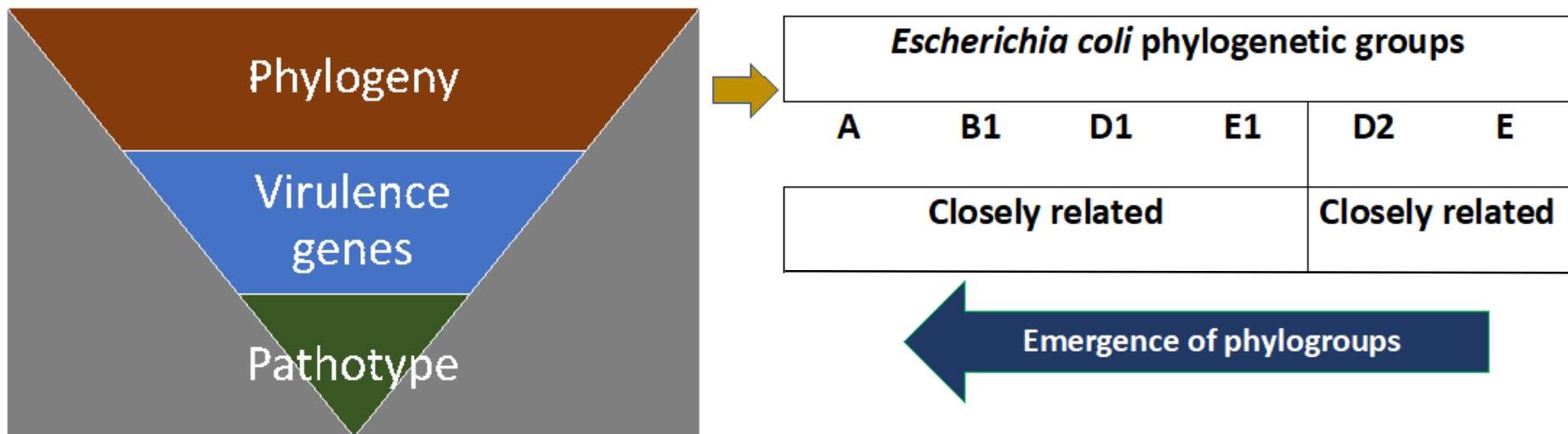
A great body of work has elucidated the pathogenicity of most DEC pathotypes within the human body (Blount, 2015; Croxen et al., 2013; Leimbach et al., 2013; Nataro and Kaper, 1998). However, food and environmental microbiologists lack a comparable mechanistic understanding of phenotypic or genotypic factors enabling environmental prevalence (Berg et al., 2014; Dini-Andreote et al., 2012; Martínez, 2013; van Elsas et al., 2011) and subsequent survival of DEC.

Fortunately, new research has begun to shed more light on how these pathogens may survive within hostile environments including water, soil and plants (Chandran and Mazumder, 2015; Franz et al., 2011; Macarisin et al., 2012; Nagy et al., 2015; Saldaña et al., 2011). Reperant et al., 2012 suggest that gut microbes including DEC have co-evolved with humans over the past several thousand years specifically targeting and responding accordingly to lifestyle changes within their human hosts which enables seamless transfer into the host. Additionally, some of the DEC virulence genes may provide an evolutionary advantage helping to sustain life of the strains in the open environment (Martínez, 2013). As environmental changes driven by climatic and anthropogenic activities escalate, understanding how these processes affect the prevalence and persistence of foodborne pathogens such as DEC is crucial. This is because DEC are still a menace to food safety, food security and human health.

In this mini-review, we therefore aim to shed light on some of the work that has sought to understand the relationship of DEC in the open environment that provides a direct link with persistence along the human food chain (Fig 1). Most of the reported work is understandably focused on outbreak pathogens such as *E. coli* O157:H7 and *E. coli* O104:H4 which may obscure the risks presented by other strains because of the high heterogeneity within many DEC pathotypes. However, because the former strains have previously been implicated in



**Fig. 1.** Interplay of interdependent bacterial processes that may facilitate the persistence of Diarrheagenic *Escherichia coli* (DEC) in the open environment. The processes can be bi-directional depending on the prevailing environmental conditions influenced by anthropogenic, climatic and ecological factors



**Fig. 2.** The phylogeny of *E. coli* influences the acquisition of virulence genes and subsequently the diarrheagenic pathotype DEC which may inadvertently lead to emergence of new pathotypes in varying environmental niches such as the agricultural and food production setting. The phylogenetic neighbourhood of geographically remote *E. coli* supports the notion of a rapid worldwide spread of an evolutionary common ancestor maybe with the advent of mammals and selection in specific habitats. Chaudhuri and Anderson, . phylogroups A mostly commensals and non shigato in producing TEC strains form the newest clades. phylogroups D uropathogenic EC and enteroaggregative EAEC strains and E separate clade of TEC and enteropathogenic E EC strains . phylogroups D and E EC strain E and e traintestinal E EC strains form the oldest clades.



large-scale outbreaks, they can be used as standard models for studying DEC behaviour as seen in the clinical setting with the use of prototypes.

The validation of these outbreak strains with a larger set of strains will shed more information regarding their persistence within the food and agricultural open environment and thereby risk posed to food safety and public health. Additionally, understanding the genotypic and phenotypic characteristics enabling DEC environmental persistence shall provide insight into how new pathotypes emerge (Fig 2). In our review, we shall focus on three broad categories that may work in tandem to enable DEC environmental persistence. They include; attachment to biotic and abiotic surfaces, evolution and adaptation as driven by lateral gene transfer and response to stressful conditions in the environment.

### **2.1.1. Diarrheagenic *Escherichia coli* attachment to biotic and abiotic surfaces**

To survive within the external environment, DEC needs to establish itself onto a biotic host such as a plant or an environmental surface. Bacterial attachment subsequently leading to colonization and persistence is a complex interaction between planktonic bacteria, surface associated bacteria and the surface (Carter et al., 2016). The attachment process is dynamic and governed by bacterial cellular appendages (Wong et al., 2017). Additionally, it is sequential and bi-directional and depends on the prevailing environmental conditions (Beloin et al., 2008). The control of attachment occurs through different regulatory systems such as the *Rcs* two-way component regulatory pathway that aids remodelling of the bacterial surface. On the other hand, the *Enz/OmpR* two-component pathway helps sense changes in osmolarity (Beloin et al., 2008). For example the ompR234 protein promotes biofilm formation by binding the *csgD* promoter region and stimulating its transcription (Prigent-combaret et al., 2001).

Additionally, the *csgD* gene encodes the transcription regulator csgD which in turn activates the transcription of the *csgBA* operon encoding curli which are extracellular structures involved in bacterial attachment (Prigent-combaret et al., 2001). *E. coli* attachment to surfaces is either reversible or irreversible (Beloin et al., 2008). Reversible attachment involves physicochemical and electrostatic interactions between the bacterial envelope and environmental substrates while, irreversible attachment uses adhesive organelles of the fimbrial family such as; type I fimbriae, curli and conjugative pili (Beloin et al., 2008).

Additionally, irreversible attachment leads to progressive inter-bacterial attachment and build-up of the resulting biomass forming a biofilm, which describes a group of single or

multispecies bacteria living together as a multifunctional unit of existence for the benefit of all organisms (Wood, 2008). Cellular appendages and bacterial secretion systems (SS) such as the Type 3SS (Saldaña et al., 2009), Type 6SS (Bernard et al., 2010; Boyer et al., 2009) and Type 7SS (Hufnagel et al., 2015) are associated with attachment and colonization of abiotic surfaces through biofilm formation in a complex interdependent way. This process follows different regulatory pathways and may differ from strain to strain (Niba et al., 2007).

#### **2.1.1.1. Initial attachment as an adaptive strategy to initiate colonization of environmental surfaces and habitats by DEC**

For DEC to persist within the open environment, the persistence usually begins with planktonic bacteria multiplying to form a whole community leading to colonization. The factors enabling this persistence may include cell surface appendages such as curli, type I fimbria, type IV pili and flagella (Beloin et al., 2008). Additionally, bacterial secretion systems aid in this process by facilitating the functioning of bacterial appendages through the type 2SS (Beloin et al., 2008), type 3SS (Saldaña et al., 2009), type 6SS (Silverman et al., 2012) and type 7SS (Hufnagel et al., 2015). Additionally, the initial attachment can be facilitated and reinforced by extracellular polysaccharide substances such as cellulose (Borgersen et al., 2018; Macarisin et al., 2012).

Different *E. coli* appendages were shown to play a crucial role in its initial attachment and stability to abiotic surfaces such as silica (Wong et al., 2017). Total internal reflection fluorescence (TIRF) microscopy was used to investigate the attachment and stability of *E. coli* deletion mutants of curli ( $\Delta csgA$ ), flagella ( $\Delta flhA\Delta c$ ) and type I fimbriae on silica surfaces (Wong et al., 2017). It was found that the absence of curli and flagella diminished ability to attach strongly in comparison to type I fimbriae deficient strains that were comparable to the wild-type strains thereby indicating that removal of fimbriae did not affect initial attachment to abiotic surfaces (Wong et al., 2017).

Due to many foodborne outbreaks associated with fresh green produce, studies have sought to elucidate the mechanisms through which DEC attaches and persists on these ready-to-eat minimally processed food products. For example, Macarisin et al., (2012) used two *E. coli* O157:H7 outbreak strains, to investigate the role of pili and cellulose expression in adherence to spinach leaves and noticed that curli strains had a stronger association with leaf surfaces than curli mutants. Additionally, attachment increased with incubation time (0, 24, 48 h). Cellulose mutants had strong attachment nonetheless and curli expressing strains had an

extracellular matrix (Macarisin et al., 2012). Similarly, (Saldaña et al., 2009) had previously reported that curli and cellulose were co-expressed in the presence of the transcriptional activator *csgD* in attaching and effacing *E. coli* strains such as EHEC and EPEC. Additionally, they noticed that *Fis* (factor for inversion stimulation) was a negative transcriptional activator for curli expression (Saldaña et al., 2009).

The persistence of DEC strains in the open environment has been suggested to follow the same mechanism or at least share similarities with observations in the human host (Berg et al., 2014; Dini-Andreote et al., 2012). For instance, the locus of enterocyte effacement (LEE) pathogenicity island found in EPEC and STEC facilitated attachment of *E. coli* O157:H7 strains to baby spinach leaves, entry into the stomata, intracellular and vascular tissue (xylem and phloem) (Saldaña et al., 2011). This process occurred through the type III secretion system and effector genes that coordinated production of curli, the *E. coli* common pilus, haemorrhagic coli type 4 pilus and flagella (Saldaña et al., 2011). These bacterial surface structures have also been shown to attach equally strongly to abiotic surfaces such as glass and stainless steel which are common food contact surfaces. For example, filament deficient O157:H7 mutants were shown to bind less strongly to glass (Nagy et al., 2015) and steel (Carter et al., 2016) surfaces compared to the wild strains. However, curli based attachment was shown to vary largely depending on the strain, growth medium and abiotic surface (Carter et al., 2016).

Like STEC and EPEC attachment and persistence mechanisms within the open environment, EAEC strains have been shown to use virulence factors in human infections. The attachment of EAEC to human epithelial cells is facilitated by fimbrial and afimbrial (outer membrane proteins) adhesins that help in formation of the aggregative adherence (AA) phenotype in both typical and atypical EAEC strains (Estrada-Garcia and Navarro-Garcia, 2012). Aggregative adherence fimbriae (*AAF I to III*) are associated with attachment and the AA phenotype in typical EAEC while strains lacking these structures have been shown to use a type IV pilus (atypical strain C1096) encoded by genes located in an *Inc11* plasmid (Dudley et al., 2006a) while the *Hda* adhesin is responsible for attachment in strains lacking the *AAF* (Boisen et al., 2008).

To investigate the adhesive mechanism of the German outbreak *E. coli* O104:H4 strain on fresh produce and abiotic surfaces, Nagy et al., (2016) noticed that deletion of the *aggA* gene which codes for the *AAF/I* fimbriae subunit (*AggA*) greatly reduced colonization. This

observation suggests that it plays a role in the adherence and subsequent colonization of spinach leaves. In contrast to the attachment mechanism of typical EAEC foodborne pathogen such as *E. coli* 104:H4 possessing the transcriptional activator *AggR*, that regulates virulence mechanisms involved in attachment (Estrada-Garcia and Navarro-Garcia et al. 2012), atypical EAEC (lacking *AggR*) were noted to mediate AA and attachment to abiotic surfaces such as glass through a type IV pilus carried on the Incompatibility class 1 (*Incl1*) plasmid of the EAEC strain C1096 (Dudley et al., 2006a).

This observation shows the various differing mechanisms that facilitate the persistence of DEC foodborne pathogens in the open environment thereby complicating potential intervention and prevention measures.

#### **2.1.1.2. Biofilm formation as an adaptation utilized by DEC to enhance environmental persistence in the open environment**

Biofilms describe sessile communities of single or multiple species that are characterized by long-term bacteria to bacteria and subsequently bacteria to surface adherence that favour attachment to surfaces in comparison to a planktonic state (Niba et al., 2007). Biofilm formation occurs through the Type 6 secretion system in and depending on the availability of favourable conditions occurs in a contact-dependent mechanism of effector delivery (Silverman et al. 2012). The ability of DEC to form biofilms has been shown to support persistence within the food production environment (Giaouris et al., 2015) and domestic setting (Ahmed et al., 2013) supporting its ability to cause human infections through cross-contamination. For example, the formation of biofilms by ETEC on drinking water contact surfaces is linked to the warm and humid months in Bangladesh, a period when high rates of diarrheal disease (epidemic levels) are noted among poor households (Ahmed et al., 2013).

Similarly, biofilm formation by potentially pathogenic *E. coli* was noticed to occur under conditions relevant to the food production chain (Nesse et al., 2014). In this work, intimin positive (*eae+*) strains had higher biofilm formation capacity at lower temperatures (12° and 20°C) than those without intimin (*eae-*) (Nesse et al., 2014). Intimin negative (*eae-*) strains instead had higher biofilm capacity at a high temperature (37°C) suggesting that the intimin attachment protein played a role in environmental attachment albeit at lower temperatures (Nesse et al., 2014). The observations from this work suggest that DEC may use different mechanisms to promote biofilm formation and thereby persistence based on the prevailing conditions. Secondly, the same attachment structures such as intimin that facilitate persistence of EPEC and STEC in the human body may also be applicable in the open

external environment. These scenarios further emphasize DEC versatility and the risk posed to food contamination and subsequent illness. Aside from temperature, environmental factors such as nutrient medium and the attachment surface affect the ability of DEC to form biofilms and consequently further persistence within a food production environment. On using two *E. coli* O157:H7 outbreak strains possessing curli fimbriae to assess biofilm formation in differential media and two different abiotic surfaces, (Carter et al., 2016) noticed higher biofilm formation on stainless steel (Luria broth without salt) and polypropylene (spinach lysates). The change in physiological state of the strains when grown in spinach lysates preventing curli production coupled with the presence of other uncharacterized adherence factors was suggested as the cause of reduced biofilm production observed on stainless steel surfaces in the respective samples (Carter et al., 2016).

### **2.1.2. Lateral gene transfer as a tool utilized by DEC for adaptation to the agricultural and food production environment**

The diversity of DEC is primarily associated with the acquisition of mobile elements such as plasmids, bacteriophages, genomic islands and chromosomal DNA through mechanisms such as conjugation, transduction and natural transformation (Blount, 2015; Leimbach et al., 2013). The foreign genetic material can be acquired through exchange with closely and distantly related strains within a specified host (human gut) or within the open environment by the acquisition of naked free flowing DNA packaged in a plasmid or bacteriophage.

Through the acquisition of foreign DNA, its survival of cellular defence mechanisms, establishment via Lateral gene transfer (LGT) into the main chromosome or as an extrachromosomal element and clonal multiplication in the new host, the newly acquired genetic information may become abundant in the host cell population. Additionally, via mutations, expression of the obtained DNA may be further regulated and tuned with the encoded proteins for better integration into the bacterial cellular networks (Skippington and Ragan, 2011). The core bacterial chromosomal genes that initiate cellular processes such as replication, transcription and translation are tightly regulated. Consequently, acquiring foreign DNA is crucial in helping adjust to new environmental pressures (Leimbach et al., 2013).

#### **2.1.2.1. Mobile genetic elements assisting in the adaptation to a hostile open environment**

It has suggested that only genes necessary for critical bacterial processes will be absent in the open environment (Touchon et al., 2009). This is because apart from the genes required for vital metabolic and nutritional bacterial processes necessary for survival, the rest are

accessory genes that can be acquired from or dispelled into the environment (Soborg et al., 2013). Additionally, based on the prevailing environmental conditions, these accessory genes facilitate adaptation to a given environmental niche (Soborg et al., 2013; Touchon et al., 2009). This suggestion has been corroborated by (Sidhu et al. 2013) and Soborg et al. (2013) who found many bacterial virulence genes associated with DEC including toxins, adhesins, secretion systems and regulators of virulence factors within the soil and freshwater environments. The acquisition of these genes correlated to several factors such as genome size, genome G-C composition, carbon utilization and oxygen tolerance (Jain et al., 2003; Skippington and Ragan, 2011) with the process favouring more closely related strains.

The family *Enterobacteriaceae*, in which DEC resorts, forms part of a large group of bacterial species that have commensal, symbiotic or pathogenic relationships within the open environment (Van Overbeek et al., 2014). Such as scenario can potentially aid persistence and fitness of DEC through the exchange of mobile genetic elements (Fletcher et al., 2013; Van Overbeek et al., 2014). Foodborne DEC have larger genomes than commensals suggesting the ability to tolerate variable conditions facilitated by a more extensive repertoire of adaptive genes (Van Overbeek et al., 2014). This evolutionary adaptation has been suggested as a means by which DEC increase environmental persistence to heightening the chance of entering the human body through the faecal-oral route (Xicohtencatl-Cortes et al., 2009) because these pathogens have evolved to closely match changes in human diets (Reperant et al., 2012).

These findings suggest that DEC faces pressures within the open environment that select for survival traits, many of which are virulence genes (Soborg et al., 2013).

#### **2.1.2.2. Lateral gene transfer and its role in the emergence of Diarrheagenic *Escherichia coli* pathotypes and subsequent environmental persistence**

DEC pathotypes have evolved independently through multiple acquisitions of virulence factors by Lateral gene transfer (LGT) resulting in diverse and dynamic genomic structures that may represent overlapping ecological niches as they have a different distribution in humans, domesticated animals and wild animals (Tenailon et al., 2010). However, *E. coli* classification based on DEC pathotypes mostly provides a clinical/public health categorization of a small group of strains within the more abundant species with a usually vague description of environmental distribution. Therefore, this categorization unintentionally neglects crucial factors such as phylogeny that may influence the ecology and

distribution of this group of pathogens within the open environment outside the human host (Robins-Browne et al., 2016).

#### **2.1.2.2.1. DEC phylogeny and the emergence of new pathotypes**

DEC pathotypes fall within all phylogroups of *E. coli* phylogeny including phylogroups A, B1, B2, D1, D2 and E (Chaudhuri and Henderson, 2012). A close relationship exists among phylogroups A, B1, D1 and E of which (A and B1) (Figure 2.3) form the most recently emerged phylogroups of strains (Chaudhuri and Henderson, 2012).

On the other hand, phylogroups B2 and D2 form the backbone of the ancient strains from which the other groups have emerged making them the earliest members on the ancestry tree (Leimbach et al., 2013). Group A is mostly composed of commensals, B1 has non-O157 EHEC, D1 has EAEC and EHEC O157 while ETEC does not fall in any phylogroup but rather occurs across the whole spectrum. This broad spectrum of characterization can complicate the ability to study the ecology of DEC strains (Leimbach et al., 2013). The close relationship of phylogroups A, B1, D1 and E1 which consist of common foodborne pathogens, as well as commensals, may facilitate the exchange of virulence factors within the open environment and hence enhance the emergence of new strains (commensal and pathogen) or more lethal pathogens (pathogen to pathogen). This could result from the high gene flux in *E. coli* which causes closely related strains to share a significant amount of accessory genes (Touchon et al., 2009). Additionally, at broader taxonomic scales lateral gene transfer is more frequent within than between taxonomic groups because such strains usually share a similar life style (Skippington and Ragan, 2012). For example, intra-group edges linking phylogenetic groups A, B1 and B2 with D are more frequent as most are commensal compared to extra-intestinal pathogens (B2 and D) which raises the possibility of preferential transfer (Skippington and Ragan, 2012). Additionally, phylogeny has been suggested as more important than ecology in influencing genetic exchange because genetic material exchanged among closely related individuals can be integrated by homologous recombination and has greater compatibility with the native host (Jain et al., 1999). Furthermore, the shared evolutionary history of closely related individuals biases the uptake of genetic material including phage host infection biases, DNA uptake specificity and quorum sensing (Thomas and Nielsen, 2005). The bacterial life style, environmental niche and phylogeny do not substantially hinder lateral gene transfer although transfer is biased towards closely related species (Skippington and Ragan, 2012).

Consequently, phylogeny may help food safety and public health practitioners to better understand the ecology as well as metabolic and phenotypic characteristics associated with the different DEC pathotypes within the open environment.

This information can be used to infer DEC ecological distribution over geographic and temporal scales which is vital in coming up with appropriate interventions for preventing the contamination and subsequent proliferation of these pathogens on food and within environmental sources, thereby reducing the risk of foodborne illness. Additionally, by using phylogeny which describes the genetic ancestry of DEC through the broader spectrum of the whole *E. coli* species, we can potentially assess the risk of emerging foodborne pathogens through the acquisition of virulence factors by Lateral gene transfer.

### **2.1.3. Increased environmental persistence and virulence of DEC through the acquisition of mobile genetic elements**

By acquiring new virulence genes, DEC pathotypes may be able to survive longer in the open environment through increased virulence which usually correlates with resistance to increasing environmental pressure (Soborg et al., 2013). Additionally, new pathotypes may emerge that could increase the risk of causing human illness through the faecal-oral route. Phylogenetically related DEC such as non-O157 STEC (B1), O157 STEC (E) and EAEC (B1) may easily exchange genes leading to the emergence of new pathotypes (Chaudhuri and Henderson, 2012). This scenario is not unprecedented and has been reported in recent times such as during the 2011 German *E. coli* outbreak associated with *E. coli* O104:H4 strain that had characteristics of both EAEC and STEC (Rasko et al., 2011). This emerging foodborne pathogen was suggested to have acquired a plasmid containing the *AAF/II* locus but having lost the *AAF/II* locus while gaining a plasmid with the gene encoding *CTX-M-15* extended-spectrum beta-lactamase (ESBL) producing antibiotics (Van Overbeek et al., 2014). Additionally, this pathotype was noticed to have recently acquired the *Stx2* encoding phage leading to its notably increased virulence compared to prototypical EAEC strains (Rasko et al., 2011) further suggesting that emergence of new pathogenic subtypes is ongoing and presents a viable risk to food safety and public health.

Similarly, *E. coli* O157:H7 is suggested to have diverged from an O55:H7 EPEC precursor by the acquisition of virulence factors such as phage-encoded Shiga toxin (Leimbach et al., 2013). The ability to acquire this toxin has been suggested as a defensive mechanism against predation by protozoa in the open environment (Martínez, 2013) which indirectly ended up as



a negative consequence to human health by causing haemorrhagic colitis (HC) and haemorrhagic uremic syndrome (HUS). Additionally, STEC strains such as O157:H7 strain EDL 933 use the locus of enterocyte effacement (LEE), a genomic island whose actions are facilitated through the T3SS to facilitate the persistence on green leafy vegetables as well as internalization into the stomata and plant vascular tissues (Xicohtencatl-Cortes et al., 2009). The LEE is a virulence factor in EPEC and EHEC intestinal infections that is associated with the attaching and effacing phenotype (Kaper et al., 2004).

In the food industry, heat resistance within bacterial pathogens is a primary concern that can complicate the provision of safe food since heat treatment is a conventional processing technique that helps kill pathogens such as DEC. However, foodborne *E. coli* strains have shown high heat resistance (Mercer et al., 2015). In this study, it was reported that strains were found to possess a  $D_{60}$ -value of greater than 10 minutes with subsequent genomic analysis of the strains revealing possession of a pathogenic island, the locus of heat resistance (LHT), with high homology to those found in *Cronobacter sakazakii* and *Klebsiella pneumoniae* (Mercer et al., 2015). The transfer of the LHT into commensal strains led to the acquisition of the heat resistant phenotype (Mercer et al., 2015) further exemplifying how these elements can lead to the emergence of highly resilient strains.

#### **2.1.3.1. *Escherichia coli* response to the changing environmental conditions with emphasis on Diarrheogenic *Escherichia coli***

To survive the harsh conditions of the open environment, DEC have evolved a complex interplay of bacterial response systems that facilitate adjustment to the prevailing conditions. Principal among these interconnected response systems includes the alternate sigma factor, *RpoS* that is a global response mechanism to stressful conditions (Battesti et al., 2011). Additionally, bacterial secretion systems including the type 3 secretion systems, type 4 secretion systems and type 6 secretion systems form apparatuses that aid in translocation of proteins and DNA through and across cellular membranes enabling response to conditions such as changes in temperature, osmotic pressure, oxygen levels and moisture (Green and Mecsas, 2015).

#### 2.1.3.1.1. *Escherichia coli* response to environmental stresses with emphasis on Diarrheagenic *Escherichia coli* in the open environment outside the human host

*E. coli* when encountering stressful conditions such as temperature, pH, osmotic pressure and nutrient starvation can trigger global stress response mechanisms dependant on the alternate sigma factor *RpoS* (Battesti et al., 2011). The *RpoS* through interaction with the core RNA polymerase (RNAP) and in the process controlling approximately 500 genes allows bacteria to withstand stressful conditions and treatments (Ng and Bassler, 2009). For example, in the common foodborne pathogen *E. coli* O157:H7 this factor enables increased tolerance to low pH conditions (Chauret, 2011), a hurdle commonly used to improve the shelf life of food. Additionally, the *RpoS* gene has been noted to control the expression of the heat shock (*dnak*) and cold shock (*CspA*, *yfiA*) proteins which have been shown to have high expression in *E. coli* O157: H7 during stress conditions (Chauret, 2011).

In a bid to study the proteome of *E. coli* O157:H7 and *E. coli* O104:H4 using tandem mass spectrometry under minimal nutrient conditions, Islam et al., (2016), noticed in both pathogens high prevalence of the virulence factors commonly associated with human disease. For example, proteins from O157:H7 include LEE proteins such as intimin and *Tir* involved in iron scavenging (Islam et al., 2016). Similarly, proteins from O104:H4 included *AAFI*, serine protease autotransporters, beta-lactamases and Shiga toxin 2 subunit B (Islam et al., 2016). These results suggest that to survive outside the human intestines, DEC must be able to respond to stresses of poor nutrient environments probably by facilitating expression of virulence genes (Islam et al., 2016). Increases in expression of virulence and antibiotic resistance genes maybe a response mechanism to stresses such as limited nutrient conditions as a means of facilitating adaption to environmental conditions outside the human host (Islam et al., 2016).

The ability to metabolize different environmental substrates may also provide an evolutionary advantage for pathogens such as *E. coli* O157:H7 to survive in the open environment (Franz et al., 2011). For example, the ability to grow on propionic, alpha-ketobutyric and alpha-hydroxybutyric acid correlated with an increased survival time of *E. coli* O157:H7 in manure-amended soils (Franz et al., 2011).

Additionally, the *E. coli* pentabolome which describes all its metabolic reactions is composed of mostly (57%) core reactions that are common to all strains (Leimbach et al.,

2013). The majority of the pentabolome reactions are anabolic (molecule building) while most catabolic (molecule breakdown) reactions make up the dispensable metabolome (Leimbach et al., 2013). Therefore, *E. coli* and notably DEC have evolved mechanisms that can acquire or dispense genes to respond to the prevailing environmental conditions facilitating growth and colonization of a given environment.

#### **2.1.3.1.2. *Escherichia coli* bacterial secretion systems and their role in environmental persistence**

The type 3 secretion system spans three cellular membranes (inner, outer and eukaryotic host cell membrane) enabling bacteria to deliver effector proteins into host cells allowing bacterial survival and colonization (Deng et al., 2017). In STEC O157:H7 strain EDL933, the Type 3 secretion system gene cluster was noted to enable persistence on leafy green vegetables (Xicohtencatl-Cortes et al., 2009). Similarly, the Type 4 secretion system clusters are large protein complexes traversing the cell envelopes of many bacteria and contain a channel through which protein-protein or protein DNA complexes can be translocated enabling transfer of virulence genes and hence facilitation of environmental adaptation (Skippington and Ragan, 2011). For example, most type 4SS clusters in Gram-negative bacteria encode a small protein that resembles the *TraA* pilin (encoded by the *E. coli* F plasmid) which might help establish contact between donor and target cells (Skippington and Ragan, 2011).

Furthermore, the Type 6 secretion system also negotiates interactions with eukaryotic and prokaryotic competitors in Gram-negative bacteria by encoding cytoplasmic, periplasmic and membrane proteins to form a trans envelop apparatus (Silverman et al., 2012). The type 6 secretion system is tightly regulated together with other virulence gene determinants such as quorum sensing and flagella synthesis to help the bacteria respond to changes in the different environments such as water, soil or specific host tissues (Leung et al., 2011).

This coordinated response occurs through the activation of bacterial secretion systems and flagella synthesis at the proper time (Leung et al., 2011). For example, in EAEC, expression of the *sci-2* gene cluster (a T6SS gene cluster), is positively regulated by *AggR*, and *Arac*-like transcriptional factors also inducing expression of plasmid carried genes mediating aggregative adherence (Dudley et al., 2006b).

### **3. Conclusion and future perspectives**

In this mini-review, we have sought to explore some of the inherent bacterial properties responsible for the persistence of DEC in the open environment. Our specific focus on outbreak pathogens suggests they employ the same virulence genes at least in some part to navigate the harsh environmental conditions. This scenario implicates virulence genes in the facilitation of DEC persistence within the human body and open environment.

Elucidating these mechanisms of persistence will enable the use of effective food safety interventions that shall prevent contamination and reduce the persistence of DEC within the food chain thereby reducing the risk of foodborne illness. For instance, understanding how DEC attaches to different food contact surfaces coupled with the identification of processing conditions favouring expression of attachment extracellular proteins (biofilm formation) shall enable the design of resilient food contact surfaces and optimized of processing conditions.

While a decent body of literature is emerging regarding the circulation of DEC within the open environment, there is still a lot of information lacking regarding the distribution of these pathogens in different geographical and temporal regions. Most large-scale studies have focused on characterization of DEC from clinical specimen although these pathogens spread via the oral-faecal route. Therefore, more studies tracking the prevalence of these pathogens within food and environmental sources is warranted since these pathotypes have emerged independently worldwide within different ecological niches (Chaudhuri and Henderson, 2012; Leimbach et al., 2013).

The rise of microbiome research (human, soil, water, built environment) should help shed more light on the ecology of DEC within the open environment. The total number of microbiota and genes within a given environment (microbiota) has been shown to influence not only the overall ecological health but also the health of humans and animals within it (Alivisatos et al., 2015; Mariadassou et al., 2015; Martiny et al., 2015; Ramirez et al., 2018). Additionally, the role of higher taxonomic groups in predicting the overall population of lower groups (species) has been suggested as a means of tracking prevalence of pathogenic and non-pathogenic microbial groups over geographical and temporal sites leading to what ecologists would describe as biogeography (Philippot et al., 2010).

Some questions that may require answers in the future include the following: What type of microbiome favours the emergence and persistence of DEC within an agricultural food

processing and environment? What environmental pressures either anthropogenic through factors such as antibiotic resistance or climatic through warmer temperatures drive the emergence of new DEC pathotypes in previously unaffected foods and ecological habitats? How can we track such heterogeneous pathogens using quick diagnostics especially in the changing landscape of microbiology that is encouraging targeting of microbial communities rather than single strains?

Answers to many of these questions will be obtained through the collaborative effort of agricultural, clinical, environmental, food and veterinary practitioners seeking to understand the underlying mechanisms enabling proliferation of such pathogens (Fletcher et al., 2013). For instance, global initiatives such as the ‘one health’ approach that seeks to bring together environmental and health professional and make their research collaborative (Atlas, 2012), is a step in the right direction.

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