

Review of *Escherichia coli* O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective

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Abstract: *Escherichia coli* O157:H7 is an important food-borne and water-borne pathogen that causes hemorrhagic colitis and the hemolytic-uremic syndrome in humans and may cause serious morbidity and large outbreaks worldwide. People with bloody diarrhea have an increased risk of developing serious complications such as acute renal failure and neurological damage. The hemolytic-uremic syndrome (HUS) is a serious condition, and up to 50% of HUS patients can develop long-term renal dysfunction or blood pressure-related complications. Children aged two to six years have an increased risk of developing HUS. Clinical enteropathogenic *Escherichia coli* (EPEC) infections show fever, vomiting, and diarrhea. The EPEC reservoir is unknown but is suggested to be an asymptomatic or symptomatic child or an asymptomatic adult carrier. Spreading is often through the fecal-oral route. The prevalence of EPEC in infants is low, and EPEC is highly contagious in children. EPEC disease in children tends to be clinically more severe than other diarrheal infections. Some children experience persistent diarrhea that lasts for more than 14 days. Enterotoxigenic *Escherichia coli* (ETEC) strains are a compelling cause of the problem of diarrheal disease. ETEC strains are a global concern as the bacteria are the leading cause of acute watery diarrhea in children and the leading cause of traveler's diarrhea. It is contagious to children and can cause chronic diarrhea that can affect the development and well-being of children. Infections with diarrheagenic *E. coli* are more common in African countries. Antimicrobial agents should be avoided in the acute phase of the disease since studies showed that antimicrobial agents may increase the risk of HUS in children. The South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs has reported increased antimicrobial resistance in *E. coli*. Pathogenic bacterial strains have developed resistance to a variety of antimicrobial agents due to antimicrobial misuse. The induced heavy metal tolerance may also enhance antimicrobial resistance. The prevalence of antimicrobial resistance depends on the type of the antimicrobial agent, bacterial strain, dose, time, and mode of administration. Developing countries are severely affected by increased resistance to antimicrobial agents due to poverty, lack of proper hygiene, and clean water, which can lead to bacterial infections with limited treatment options due to resistance.

Keywords: *Escherichia coli*, O157:H, Shiga-toxins, hemolysin, LEE, metagenomic, PhiG17, verotoxin, zoonosis, One health, antimicrobial resistance, acid resistance

Introduction

Escherichia coli (*E. coli*) was first described in 1885 by Theodor Escherich.¹ It is a Gram-negative, bar cylindrical shape-like structure, with flagella, non-sporulating, and facultative anaerobic bacteria,^{1,2} classified as one of the most genetically versatile bacterial species,² belonging to the *Enterobacteriaceae* family.^{2,3} It is generally motile by peritrichous flagella, regularly found within the gastrointestinal tract (GIT) of humans and vertebrate animals,^{4,5} and spread by fecal contamination.⁶ It can also be found due to contamination during food animal slaughter, but in most cases, it is found in soil, water^{6,7} and food⁷ due to fecal contamination,⁷ and those environments are referred to as secondary habitats² which are becoming an ecological issue of

significance,⁷ while the gut of vertebrates (mammals and birds)⁵ is referred to as its primary habitats where the bacterium lives as a commensal.² Therefore, there are comprehensively two types of *E. coli*: commensal and pathogenic *E. coli*.⁴

Commensal *E. coli* bacteria are essential as a major aspect of the typical gut flora and contribute to the innate and adaptive immunity, and additionally play a significant role in the GIT.^{1,2} Extrapathogenic *E. coli* can be categorized into different principle genetic groupings which include one of the phylogroups A, B1, B2, C, D, E, F, and clade I.² Group A usually represents isolates from the duodenum, ileum, and colon.²

The pathogenic *E. coli* is classified into serotypes due to distinctive exterior differences of the antigens, as they can be speculated by distinguishing genes involved in antigen synthesis or identified by agglutination assays.⁴ However, different studies that have been conducted over the past years for the identification and analyses of *E. coli* using various methodologies were not able to distinguish any meaningful differences regarding virulence profiles and genetics in various *E. coli* pathotypes irrespective of the host origin. Therefore, *E. coli* has been recognized to potentially play a role in zoonotic infections³ as illustrated in Figure 1.

It is difficult to characterize an *E. coli* infection based solely on clinical symptoms, as there are several different diseases with similar symptoms. It is a well-known fact that various infections can occur within similar groups, but in addition, they have similar symptoms.^{3,4} In Africa, the evidence of Shiga toxin-producing *E. coli* (STEC) O157:H7 infection among the environment, animals, and humans, in general, is not conclusive. In addition, the South African National Veterinary Surveillance and Monitoring Programme for Resistance to Antimicrobial Drugs have shown increased antimicrobial resistance in *E. coli*, and these results were similar to results in European countries.⁴ The high prevalence of pathogenicity and antimicrobial resistance in *E. coli* is a worrying issue as it increases pathogenicity. However, such challenges cannot be addressed without sufficient research. Therefore, more studies are needed to be conducted, especially in developing countries such as South Africa.⁴ *E. coli* is one of the components of the natural microflora of the GIT of animals and humans, but pathogenic strains such as O157:H7 can cause a variety of diseases through different virulence determinants.⁵ According to Figure 1, *E. coli* released from human and animal feces can survive in environments such as water and soil.⁵ *E. coli* O157:H7 infection in humans has been well documented to be transmitted from animal food sources.⁶

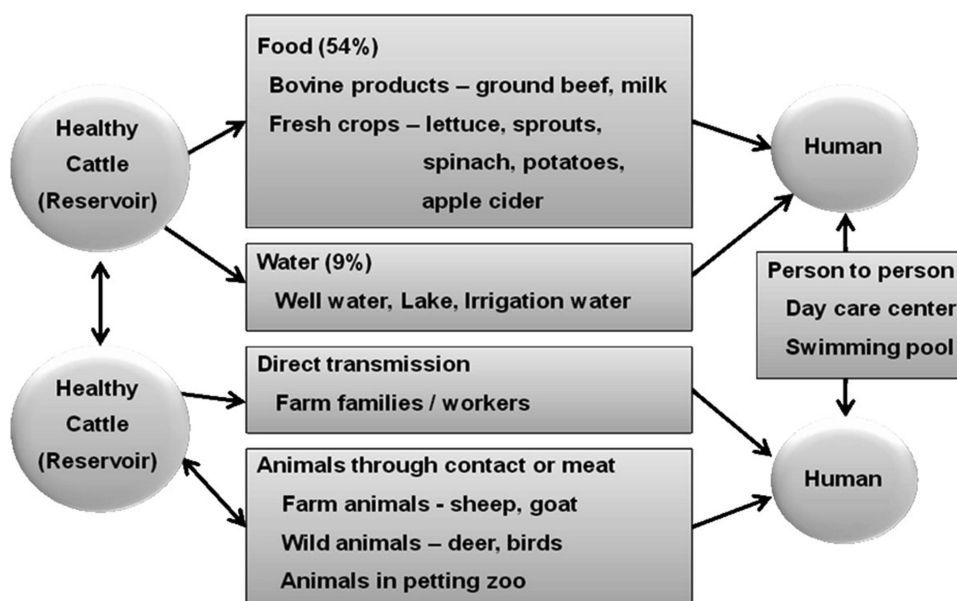


Figure 1 *E. coli* O157:H7 infection. Healthy cattle are the main reservoir of *E. coli* O157:H7 and temporarily carry this microorganism without any visible symptoms. Contaminated cattle products and culture are the main causes of human infection. Reproduced with permission from Lim JY, Yoon JW, Hovide CJ. A Brief Overview of *Escherichia coli* O157:H7 and Its Plasmid O157. *J Microbial Biotechnol*, 2010;20(1):5–14. Copyright © The Korean Society for Microbiology and Biotechnology. Creative Commons Attribution 4.0 license (CC BY).¹

Diarrheagenic *Escherichia coli* (DEC)

ETEC bacteria are recognized as a compelling cause of the overall problem of diarrheal disease worldwide.^{8,9} ETEC strains are of global importance as they are the leading cause of acute watery diarrhea (sometimes fatal) in children in developing countries and the leading cause of traveler's diarrhea.^{9,10} Verotoxin-producing *Escherichia coli* (VTEC) is of particular concern as a food poisoning pathogen, especially in the United States. There have been many improvements in food safety, promoted by the outbreak of *E. coli* O157:H7 from ground beef.¹⁰ Infections with diarrheagenic *E. coli* are likewise more frequent in African countries, mostly in Ethiopia, Nigeria, and South Africa.¹⁰

Enterotoxigenic *Escherichia coli* (ETEC)

ETEC refers to strains of *E. coli* that produce one or more of the two enterotoxins, a heat-labile toxin (LT) and a heat-stable toxin (ST).¹¹ Various pathogenic ETEC strains can excrete one or both toxins, but the diseases caused by each toxin are comparable.^{12,13} It is characterized by containing pathogenic *E. coli* strains that secrete multiple toxins from two characteristic groups of enterotoxins: ST and LT. ETEC strain was first identified as the cause of diarrheal disease in piglets, and the infection continues to cause fatal infections in young animals.¹³ A study conducted with ETEC in piglets first elucidated the mechanism of the disease using both plasmid-encoded enterotoxins.^{8,13} The most important region that can serve as a host for parasite interactions is the proximal small gastrointestinal tract where ETEC colonizes, and LT or ST proliferates.¹³ When the small intestine becomes infected with LT or ST, the lining of the gastrointestinal tract is irritated, secreting extreme water, and causing diarrhea.¹¹

Enteropathogenic *Escherichia coli* (EPEC)

EPEC is characterized by the diarrheal agent *E. coli* releasing the attaching and effacing (A/E) lesions from enterocytes and belonging to one of the different serotypes.¹⁴ It attaches to the small intestinal cells via bundle-forming pilus and penetrates the microvilli structure by inducing typical A/E lesions.¹⁴ Cytoskeleton imbalances are complemented by an inflammatory response, active ion secretion, increased intestinal permeability, and loss of absorbable surface areas due to the disappearance of microvilli, thus causing diarrhea.¹⁴

Clinical EPEC disorders are characterized by fever, malaise, vomiting, and diarrhea, with a significant amount of fluid, but no blood.¹⁵ The EPEC reservoir is unknown but is believed to be an asymptomatic or symptomatic child and an asymptomatic adult carrier. Spreading is often through the fecal-oral route.¹⁵ The prevalence of EPEC in infants is rather low, and EPEC is highly contagious in children. EPEC disease in children tends to be clinically more severe than many other diarrheal infections in this group. Some children experience persistent symptoms of diarrhea that last for more than 14 days.^{11,13}

Some verocytotoxin-producing *E. coli* induce adherent and decongestive lesions, EPEC induces lesions in the small gastrointestinal tract, while VTEC induces lesions in the colon.¹⁵ A distinguishing feature of VTEC is the expression of verocytotoxin, which is systematically absorbed and can cause life-threatening complications. VTEC is classified as *E. coli* with the presence of the *vtx* gene or the potential production of verocytotoxin (VT).^{15,16}

Clinically, VTEC disease is described by acute gastrointestinal infections with diarrhea, abdominal pain, vomiting, and fever. One in three people suffering from bloody diarrhea appears to be associated with an increased risk of developing serious complications such as acute renal failure and neurological damage such as paralysis.^{11,15–17} Hemolytic-uremic syndrome (HUS) is a serious condition, and up to 50% of HUS patients can develop long-term renal impairment or blood pressure-related complications. Children aged two to six years are at increased risk of developing HUS.^{16,17} The risk of VTEC infection progressing to HUS depends on the characteristics of the infected strain, which is a subtype of the *vtx* gene. Some strains have been designated as HUS-induced or high-risk strains.^{10,15,18} VTEC reservoirs are usually found in the intestines of cultured ruminants, such as cattle, sheep, and goats.^{16,19}

Figure 1 shows that the source of infection is beef, contaminated fruits, vegetables, or raw milk. VTEC can be transmitted via water or food and has a low infection rate (less than 100 VTEC bacteria), so human-to-human transmission is possible.^{16,17} Farmers who produce food or work with vulnerable groups (nursery children, patients, seniors) are quarantined if found to be infected with VTEC. Infected workers cannot go to work or access the facility until two negative samples are collected.^{19,20} The

incubation period is 1-8 days, and the illness period is usually 5-20 days.¹ Treatment of VTEC diseases is limited to supportive measures. The use of antimicrobial agents and antidiarrheal treatments have been shown to increase the risk of HUS in children^{16,20} and are therefore these agents are recommended to be avoided in the acute phase.^{16,18,20}

Verotoxin-Producing *Escherichia coli* (VTEC)

VTEC is called Shiga toxin-producing *E. coli* (STEC), but because it is a subset of STEC, it is also classified as enterohemorrhagic *E. coli* (EHEC). These types can cause the most extreme consequences, such as renal failure and death. The most widely recognized O group for this pathotype is the O157, even though the other O groups are observed in outbreaks.^{10,11,15,21} VTEC is frequently detected in food poisoning outbreaks worldwide.^{10,11,15,21,22} Infections can have harmful effects and are expected to cause serious cases in the medical field. However, in outbreak situations, it is essential to identify the source of infection and limit further infections.^{10,15,21} ETEC infection is known as traveler's diarrhea. This is because infectious diseases are common when traveling to developing countries, which have serious implications for tourism and thus the economy.^{10,11,15,16,22,23} EPEC is an important cause of diarrhea in infants. This, along with Shigella, was a major cause of diarrhea in children in hospitals.^{10,11,16,22,24} It is highly contagious to children and can cause long-term or chronic diarrhea that can affect the development and well-being of infected children.^{10,15,23,25}

Classification and Pathogenicity of *Escherichia coli*

The classification of the three serotypes of *E. coli* is identified by considering the presence of the antigen O (lipopolysaccharide (LPS)), H (flagellar protein), and K (polysaccharide).⁴ The serotype is defined as a mixture of O and H antigens that is regularly used as an indicator to detect the potential for pathogenic genes. The exterior portion of the polysaccharide or lipopolysaccharide is classified as antigen, and it is stored in the Gram-negative bacterial cell wall, such as *E. coli*. However, *E. coli* may additionally carry F (fimbria) and K (capsule) antigens.⁴ *E. coli* bacteria have optimal conditions for becoming pathogenic such as ideal intestinal conditions, receptors, temperature, and genetic synthesis (the ability to transform or maintain pathogenic genes and maintain resistance genes).^{1,26} There are some important diarrheagenic pathogenic types (pathotypes) of *E. coli* such as *E. coli* that produce Shiga toxins (STEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC), Shigella/enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC) and adherent-invasive *E. coli* (AIEC).²⁶ Diarrhoea due to *E. coli* is classified particularly as a leading cause of mortality and desolation in nations that are still developing associated with bacterial infections among other pathogens. Infectious diarrhoea is associated with moderate mortality and is generally less severe in industrialized countries but affects many people and represents a significant disease burden.²

A particular combination of virulence genes gives *E. coli* the potential to be pathogenic and cause certain diseases in humans and animals. *E. coli* with virulence genes belong within the "pathogenic form" defined by the disease they cause,⁴ but individual strains can have virulence attributes in excess of one pathotype. There are six commonly recognized pathogenic *E. coli* types. Types of *E. coli* that cause diarrheal disease: enterohemorrhagic (EHEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), and enteroinvasive (EIEC). Additionally, infections within the extraintestinal occur due to by ExPECs (extraintestinal pathogenic *E. coli*) such as urinary tract pathogenic meningitis/sepsis-related *E. coli* (MNEC) and uropathogenic *E. coli* (UPEC).⁴

Various strains of *E. coli* infections cause a decline in animal production, especially in the poultry industry, due to diseases such as abdominal sepsis, urinary tract infections, blood poisoning diarrhoea, haemolytic uremic syndrome, and haemorrhagic colitis.⁷ This is a major global public health concern for humans and livestock. There is a great concern about the proper implementation of hygiene protocol in livestock production systems.^{4,28} It is estimated that approximately 70% to 95% of cases reported worldwide are caused by the pathogenic form of *E. coli*. In addition, these bacterial strains cause more infections in the chicken and poultry industries around the world and are greatly affected by the great financial burden.^{6,7} In this regard, poultry products are an important source of food-derived and antibacterial resistant *E. coli* strains in humans which may cause infectious diseases.^{6,7,29} Blanco et al reported that STEC was detected using a variety of techniques, including serotypes widely used to classify *E. coli* strains. The H antigen from the flagella protein and the O antigen detected by the polysaccharide portion of the cell wall lipopolysaccharide (LPS) are used to classify the serotypes of *E. coli* isolates.^{30,31}

Approximately there are about 174 O-antigens,^{29,31} numbered 1 to 181, excluding numbers 31, 47, 67, 72, 93, 94 and 122. There are also 53 H antigens in the serotyping^{17,26} also reported that there are *E. coli* isolates containing various mixtures of H and O antigens. However, the majority of STEC serotypes are non-motile (NM), or H-antigen-free mutants.^{29,30} In addition, the method can detect the serotypes to which these NM strains belong and the differences between what are considered non-NM serotypes.²⁹ Common O157:H7-STECS may contain mutant O157:NM-STECS strains.³² O157:H7 STEC cannot ferment sorbitol, while O157:NM STEC can ferment sorbitol and was detected in some parts of Europe.^{29,30} The importance of the O157:H7 serotype helps detect human infections caused by *E. coli*. It is common to classify STEC serotypes into two basic categories, non-O157 and O157. STEC seropathotypes A, B, C, D, and E cause regular infections in humans of varying degrees of severity.^{26,30} Serotype A contains highly toxic O157:H7 and highly toxic O157:NM. Serotype B includes serotypes O145:NM, O121:H19, O26:H11, O103:H2, and O111:NM, which, like O157-STECS, can cause severe outbreaks and illness. However, there are only a limited number of cases associated with these serotypes.^{27,30} Serotype C contains serotypes that may be associated with normal HUS, but these serotypes are not associated with outbreaks and are composed of O113:H21 and O91:H21.^{30–32} Serotype D includes many serotypes associated with normal cases of diarrhea.³² Serotype E includes several STEC serotypes that are not associated with human infections.^{28,30} Presumably toxic genetic determinants are considered indicators of serotyping, and such indicators have proven to be efficient. However, the samples used were assigned to one of the serotypes without being separated into restricted groups.^{17,31} The idea of serum pathology is useful for studying the genetic determinants of bacteria that support infection and infectivity, and can be refined to help diagnosis. Serotyping helps reveal some of the diversity between STECS.^{27,30,32}

Shiga-Toxin producing *Escherichia coli* (STEC)

E. coli that produce Shiga toxins (STEC) has been classified as a significant food-borne zoonosis accompanied by continuous outbreaks and irregular cases of the haemolytic uraemic syndrome, diarrhoea, and haemorrhagic colitis in people.^{8,33,34} Various STEC serotypes exist related to a particular disease. However, serotypes with the ability to trigger deadly diseases in infected humans comprise O111: NM, O145: NM, O104:H4, O26:H11, O121:H19, and O103:H2.³¹ Serotype O157:H7 will be the major focus of this review, which is the most widespread source of enterohaemorrhagic *E. coli*, haemolytic uraemic syndrome, and haemorrhagic colitis (HC) cases and outbreaks in various nations.^{28,34}

Shiga toxin (Stx), is known as a strong cytotoxin and encrypt bacteriophages (as illustrated in Figure 2). Stx is single and extended. It damages transcriptional units and numerous cell types.³³ Stx can be divided into two groups called Stx1 and Stx2, but they do not produce cross-reactive antibodies with 56% homology in amino acid sequence.^{42,43} Stx1 is the same as Stx2 of *Shigella dysenteriae* because of the difference in sole amino acid. *E. coli* O157:H7 toxic isolate can contain either only Stx1 or Stx2, or sometimes both toxins. Shiga toxin, which is more toxic and more common, is Stx2 compared to the Stx1 strain, and it is

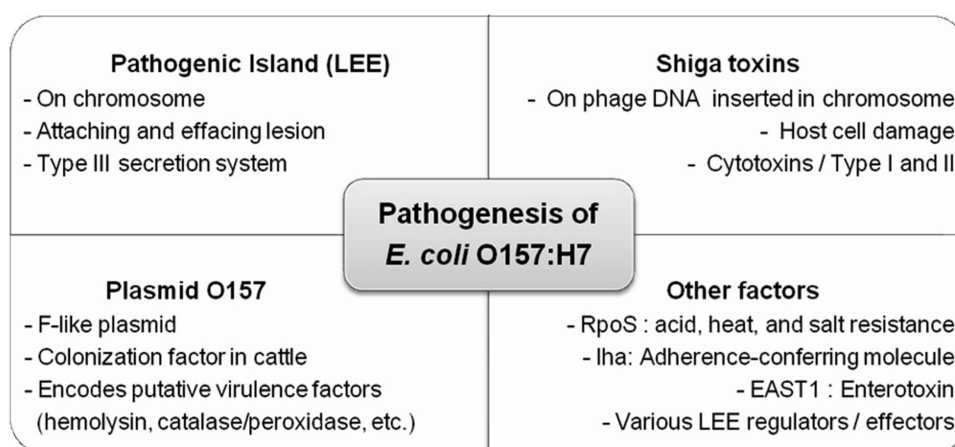


Figure 2 Different virulence factors produced by pathogenic *E. coli* O157:H7 which causes infections. Reproduced with permission from Lim JY, Yoon JW, Hovide CJ. A Brief Overview of *Escherichia coli* O157:H7 and Its Plasmid O157. *J Microbiol Biotechnol*, 2010;20(1):5–14. Copyright © The Korean Society for Microbiology and Biotechnology. Creative Commons Attribution 4.0 license (CC BY).¹

highly linked to HUS or HC infections in humans.^{9,34,35} Studies have shown that Shiga toxin has a conserved structure consisting of one enzymatically active A subunit (A1) which noncovalently joins to a pentamer of 5 identical B subunits (B5).^{36,43} The subunit (B5) attaches to a particular host either as an alternative globotriaosylceramide (Gb4) or as a receptor globotriaosylceramide (Gb3).³⁶ Following the joining of Shiga toxin (A1B5) through a host cell within the cytoplasm, subunit A is then incorporated. Then, A1 suppresses the synthesis of protein through detaching of a sole adenine excess from 28S rRNA 60S ribosome subunit.^{34,35} (Figure 2) The detailed mechanism of Stx translocation to the various organizations is not fully understood.^{36–38}

Intestinal Colonization by STEC

For intestinal *E. coli* infections, the infection process is thought to be accompanied by toxin damage and intestinal colonization due to invasion.³⁹ Colonization is the process by which STEC overcomes the host's defense mechanisms and becomes part of the bacterial fauna in the intestine. Gastric acid is an important host defense mechanism in the gut, but acid resistance is a well-known property of *E. coli*, a well-developed property of O157 pathogens and various STEC serotypes.³⁹ Serotype O157:H7 STEC is generally reported to survive on acidic foods such as salami and apple juice. Therefore, even low bacterial infection rates can cause infections in humans.^{39,40} However, various studies have reported significant differences between strains within this serotype. Exposure to a weakly acidic environment allows the strain to respond to acid resistance and tolerate higher acidic pH³⁹ as illustrated in Figure 2.

Adhesion to intestinal epithelial cells is one of the first advances in STEC evolution that affects disease and is usually intensively studied in vivo using cultured cell lines of different origins. Interactions and binding patterns between STEC strains and epithelial cells are unique for *eae*-positive and *eae*-negative STEC strains.^{36,40} However, *eae*-positive STEC strains form the A/E lesions typical of gastrointestinal epithelial cells.^{36,39} A/E lesions are not important for HUS or bloody diarrhea in humans, but most strains associated with these syndromes are positive. Therefore, many EHEC strains are *eae*-positive, and *eae* gene has been identified as a risk factor for HUS.⁴⁰ The *eae*-positive STEC has a pathogenic islet called the locus of enterocyte effacement (LEE) which encodes a bacterial protein, intimin, and is important for the development of the A/E lesions on the intestinal epithelia.^{36,39} Using the development of A/E lesions of enterohemorrhagic *E. coli* (EPEC) in response to EPEC infection and the similarity of LEE to understand equivalent events within STEC.³ LEEs are classified into five basic polycistronic operons named LEE1, LEE2, LEE3, LEE4, and LEE5.^{36,38,40} LEE results in a type III secretory system (LEE1, LEE2, and LEE3) (refer to Figure 2). Protein translocation system, LEE4, intimin or *eae* gene (corrosion-attached and absorptive proteins) and their receptors, the translocated intimin receptor (TIR). Both are coded by LEE5. Effector protein that can be moved by the secretory system.⁴¹ The secretory system is a hollow molecular structure that begins in the cytoplasm of the bacterium, extends through the intima and adventitia, and travels across the host cell membrane.⁴⁰ The secreted protein is then transported from the bacterial cytoplasm to the host cell through this structure.^{3,37} The secretory protein encoded via LEE is composed of EspG, EspF (*E. coli* secreted protein F), EspZ, mitochondria-related protein, Tir, and EspH.^{3,39,41,42}

The LEE secretory system is used to transfer many proteins that are not LEE-encoded.³⁹ The TIR protein is then introduced into the host cell membrane and functions as a receptor for the intimin gene outside the bacterium, while other host cell compounds also bind to the intimin gene.^{35,36} TIR and other secreted proteins activate many signaling cascades that lead to reorganization of intestinal epithelial cell structure and changes in cell physiology.^{3,42} The non-LEE-encoded secretory protein EspJ has been identified as a genetic antitoxic determinant.^{36,44} The deletion has been suggested to prolong the survival of lambs and mice, and promote the transmission and survival of host pathogens.^{3,39,43} At least about 17 intimin genes^{39,40} are associated with heterogeneity within the C-terminal of molecules involved in binding to TIR.^{3,45} These close differences are divided between STEC and EPEC of animal and human origin. γ -Intimin is associated with pathogenic STEC serotypes, such as O145:H and O157:H7.^{7,43} Alternating use of *eae* O157 and EPEC strain *eae* resulted in colonization by the modified O157:H7 STEC strain in the small intestines of experimentally infected pigs.^{39,45,46} By comparison, wild-type O157:H7 STEC colonized the colon. Various studies have shown that TIR also binds to several host cell structures. However, studies have found that intimin from EPEC binds to a subset of β 1-integrins.^{3,36,46} Other research studies have reported that Tir from EHEC O157:H7 attaches to the outer nucleoli of Hep2 cells and there is competition between Tir and nucleoli. Recent studies have also noted that β 1-integrin and nucleoli are closely associated

with adherent calf and pig adherence to EHEC O157:H7. A/E lesions are associated with close attachment of bacteria outside the host cell and structural changes to epithelial cells.^{39,45,47} The structural modifications include accumulation of cytoskeletal proteins beneath the adherent bacteria, loss of pedestal formation, and microvilli. Less information is available regarding adherence of *eae* negative STEC.^{3,36,39} A study investigated the attachment of *eae* negative STEC of serotype O113:H21 to cultured epithelial cells (HEp2) and rabbit gastrointestinal tract in vivo.^{39,45,47} In a study conducted by Dallman et al⁴⁸, it was confirmed that there had been a reason for microvilli use effacement beneath the bacteria but that the cytoskeletal reorganization trait of the A/E lesion no longer developed.⁴⁹ Even though STEC strains have a few abilities for invasion of enterocytes, the infection seems to be localized with no indication of septicemia.⁵⁰ Effects on the whole body are mainly detectable by the movement of toxins absorbed from the intestines. A previous study provided information that supports or consolidates the notion that the degree of in vitro compliance with STEC may be related to its ability to cause disease.^{43,49}

The Natural Reservoir of *E. coli* O157:H7

Among livestock, cattle being a natural reservoir of *E. coli* O157:H7 is always carried with faeces when excreted,^{13,50} as shown in Figure 1, and can infect about 1% to 50% of healthy cows.^{38,50} Contaminated minced meat is the most common medium of *E. coli* infections. Beef products are more frequently contaminated by pathogenic *E. coli* O157:H7 during animal slaughter; beef milling can also transmit pathogens to the inside of the meat from its surface.³⁸ Therefore, if the beef that is minced through milling is not fully cooked, then the pathogen can thrive. Additionally, to ground beef, various foods are linked to the company of *E. coli*, particularly O157:H7 contamination, including fresh foods such as radish sprouts, lettuce, unsterilized milk, drinking water, fresh spinach, cider, and salami. The major outbreak occurred due to contamination of radish sprouts in Osaka, Japan in 1996 where about 7966 people were infected. The population was diagnosed with a confirmed *E. coli* O157:H7 infection.²⁴ It was an epidemiological investigation where food seems to be contaminated with cow dung. For this reason, mitigation, prevention, and control of bovine *E. coli* O157:H7 has been highlighted as a vital management methodology to mitigate, control, and prevent the frequent occurrence of *E. coli* O157:H7 within the production systems and smallholder farms. Various other treatment and prevention measures have been proposed including the use of probiotic cultures, identification of inhibitory feeds, advanced practices for cattle management, the use of feeding additives, and immunization.^{24,51}

E. coli O157:H7 is also observed as an animal, water, and soil reservoir (as illustrated in Figure 1). Studies have shown that the *E. coli* O157:H7 can survive about 365 days with fertilized soil, and about 730 days (almost 2 years) with uncomposed raw fertilizer.⁵² Composite fertilizers are more efficient in eliminating *E. coli* O157:H7 when heat increases and kept at 50°C or higher during the first 6 days. *E. coli* O157:H7 strain was reported to persist in water for long periods and more ideally at low temperatures. The deposits in the pail contaminated with cow dung serve as durable (>8 months) reservoir for the O157:H7 strain and persisting on contaminated bacterial pathogen can be a major source of infection.¹⁷ It was reported⁵³ that *E. coli* O157:H7 can replicate and survive in *Acanthamoeba polyphaga*. *Acanthamoeba polyphaga* is a familiar free-living environmental protozoon more commonly disseminated in water, faeces, and soil. Therefore, in these kinds of environments, it may be an efficient means of transporting and transmitting *E. coli* O157:H7 strain. *E. coli* O157:H7 was reported to thrive in different environmental fluctuations or extreme changes in temperature, pH, and osmotic conditions which are common conditions it comes across in nature. As an example, *E. coli* production of exopolysaccharide (EPS) *E. coli* O157:H7 is linked to acid and heat resistance, and changes in lipid composition membrane changes are caused by thermal stress.⁵⁴ Therefore, environmental adaptations of the O157:H7 strain increase the sustainability and spread of this microorganism on the farm and its transmission from animal-to-animal (horizontal transfer). It also enhances its capability to persist when outside the reservoir of the host. There is also a risk of O157:H7 strain of pathogenic bacteria *E. coli* contaminating crops that are produced through the use of contaminated water for irrigation purposes, proximity to infected animals, or cow dung contamination.⁵⁴

Epidemiology

E. coli O157:H7 infections pose a major risk and great public health concern globally. The total number of cases due to infections by O157:H7 are beneath other intestinal pathogenic bacteria, such as *Campylobacter* and *Salmonella*

infections.⁵⁵ However, infections posed by *E. coli* O157:H7 have recorded more hospitalization, including mortality in severe cases.⁵⁵ Infections in humans posed by the O157:H7 strain may have a comprehensive clinical range originating in patients that do not show any clinical symptoms of mortality. Oftentimes, it starts with non-bloody diarrhoea and is self-solving in the absence of any additional complexity. In some cases, patients develop bleeding diarrhoea or HC in a few days, about one to three days. Furthermore, in about 5–10% of patients with HC, the illness can advance to the life-threatening effect of thrombocytopenic purpura (TTP) or HUS.⁵⁶ *E. coli* O157:H7 strain was reported as the leading source of haemolytic uremic disease in the United States, as the elderly and children have a higher probability of severe clinical symptoms such as bloody diarrhoea, TTP, and HUS. Several treatment strategies are being investigated, including the use of antimicrobials and vaccination.⁵⁵ However, direct treatment has a specific action for people infected with the O157:H7 strain. Prescription utilization of antimicrobial agent has a possibility to be controversial. However, the remedy primarily helps limit or prevent periods of symptoms and systemic complications. Therefore, it is recommended to implement proper measures to effectively prevent and control *E. coli* O157:H7 infections.⁵⁵

The US Centers for Disease Control and Prevention (CDC) have approximated that O157:H7 strain infectious diseases cause illnesses (73,000), hospitalizations (2200), and deaths (60) in the United States annually.⁵⁵ The US CDC occurrence monitoring data is *E. coli* O157:H7 reported after an outbreak in 1999, and since then infections have declined. However, large-scale sporadic outbreak cases will carry on happening. *E. coli* O157:H7 infections have an estimated annual cost of illness of about \$405 million, which includes medical care, reduced livestock productivity, and premature death.⁵⁵ Due to the expensive cost of the disease, more direct and effective efforts are needed to combat this pathogenic bacteria.⁵⁵

E. coli O157:H7 strain exhibits somatic (O) antigen 157 and flagella (H) antigen 7. O157:H7 strain has a delayed sorbitol fermentation (>24 hours) production of β -glucuronidase capable of hydrolysing the synthetic molecule 4-methylumbelliferyl-D-glucuronide (MUG).⁵⁷ Sorbitol-MacConkey (SMAC) agar medium complemented with MUG is utilized to detect O157:H7. Furthermore, to strengthen the selectivity of *E. coli*, tellurite potassium, cefixime and vancomycin were included in the SMAC agar plate to suppress other bacteria that have the same Gram-negative characteristics. Latex agglutination assay that is commercially available can be used to further confirm serotypes O157 and H7.⁵⁸

E. coli O157:H7

The chromosome size of hemolysin (H7) is 5.5 Mb. The genome contains 4.1 Mb. With the backbone sequence preserved completely in all strains of *E. coli*. The rest is unique to *E. coli* O157:H7.²⁴ In addition, the genome comparison of pathogenic *E. coli* strain O157:H7 and non-pathogenic *E. coli* K12, *E. coli* O157:H7 DNA is missing 0.53 Mb. This was observed in genomics analyses of O157:H7 strain. Reduction furthermore took part in the *E. coli* O157:H7 strain evolution.^{59,60} Most *E. coli* O157:H7 distinct DNA sequence (1.4 Mb) is horizontally transmitted foreign DNA by elements like prophage. *E. coli* O157:H7 carries about 463 phage-related genes, while *E. coli* K12 contains only 29.²⁶ Changes in G + C content are one of the signs that genomic regions have been gained using horizontal gene transfer. It was determined that not less than 53 individual species were found to have imparted onto the distinctive sequences of the O157:H7 strain. Pathogenicity-related genes connecting the two sequenced O157:H7 strains are almost 99% similar. However, both loss and addition of DNA had a key capacity role in the evolution of the etiology of *E. coli* O157:H7.^{59–61}

Some epidemiological investigations and comparisons have indicated that *E. coli* O157:H7 is derived from non-toxic and less toxic *E. coli* O55:H7.⁶¹ *E. coli* O157:H7 arose from 4 consecutive occurrences. The addition of *stx*₂ includes (i) addition of pO157 and *rfb* regions, (ii) addition of *stx*₁ inability to ferment sorbitol and loss of β -glucuronidase activity, (iii) bacteriophage (iv) including bacteriophage.⁶¹

Clonal origin of all O157:H7 prevented the preliminary aim to identify groupings in the serotype, considering the diversity of housekeeping genes.⁶² Insertion and deletion, for example, as a migratory genetic element, not a point mutation.⁶³ Genomic analysis of existing strains encodes VTEC O157:H7 from O55:H7 ancestors such as EPEC, pO157 pathogenic plasmids, prophage encoding verotoxin, and other phage containing several T3SS effector genes. It shows that it has evolved through the gradual acquisition of gene regions^{61,64,65} and an antigenic shift from O55 to O157 was caused by the gaining of another *rfb* region.⁷⁹ mutations also resulted in a loss of the capability to ferment sorbitol, including a loss of β -glucuronidase activity in many O157:H7.⁶⁵ The EPEC O55:H7 strain, which may be closely

associated with O157:H7, is responsible for neonatal diarrhoea in many countries.⁶⁷ A subset of O157:H7 could ferment sorbitol but forfeited motility and formed the O157:H lineage. It gives rise to severe EHEC infections in humans.⁶⁸

Genomic analyses of extant lines show particularly VTEC O157:H7 that is developed from an EPEC-like O55:H7 ancestor through stepwise acquisition of the pO157 virulence plasmid, other phage-encoded genetic regions, as well as a few T3SS effector genes and verotoxin-encoding prophages,^{61,64,65} and an antigenic shift from O55 to O157 given rise by the gaining of another *rfb* region.^{61,66} Mutations, on the other hand, additionally caused the loss of the capability to ferment sorbitol and in addition a forfeit of β -glucuronidase activity in most O157:H7.⁶⁵ Strains of EPEC O55:H7 which might be firmly associated with O157:H7 continue to be a causative agent of diarrhoea in newborn children in various nations.⁶⁷ However, a subset of O157:H7 held the potential to ferment sorbitol lost its motility, forming an O157:H-lineage, which likewise gives rise to serious EHEC infections in humans.⁶⁸

Considering an octamer-based genome scan of a restricted portion of the genome of an isolate that was collected from the United States, Kim et al⁶⁹ proposed the existence of two distinct lines of O157:H7, Line I (LI) and Line II (LII).³³ The two lines are thought to be ruggedly disseminated between human and livestock resources, and LII tends to be a “livestock bias”. Direct and simple assays utilizing six fragment length polymorphisms (lineage-specific polymorphisms, LSPA6) prove to be a more useful alternative to genomic scans in distinguishing between these genealogies or lineages. Recent studies using different methods were done,⁶⁹ including microarray analysis,^{68,69} which is used to distinguish intermediate genealogy, LI/II.^{68,69} The presence of three similar genes is a tremendous network analysis based entirely on data from multiple multi-loci typing methods,⁷⁰ and next-generation sequencing to investigate SNP variability throughout the genome.^{70–72}

A clade is classified based on a sequence of 96 single nucleotide polymorphisms (SNP) between clinical isolates of the strain O157:H7.⁷³ It was then proposed to divide O157:H7 into nine branches.⁷³ Clade 8 is associated with more extreme illnesses, as shown by analysis of outbreak data.⁷³ Clade typing is largely consistent with the strains described above, but isolates with multiple strains are found in the exact clade.^{74,75} This may be suitable for homoplasmy or recombination apparent within the networked idea of the phylogenetic network of Manning’s primary study,⁷³ but in addition, to strains by different authors. This may be due to an inconsistent interpretation of the LSPA6 profile.^{73,75} Some drawbacks of the clade framework are that it is dependent on clinical isolates and therefore does not fully characterize significant SNP fluctuations between O157:H7 strains that cause little or no symptoms.⁷³

Many infections in people are linked to clades 6 and 8 of *E. coli* strains.^{73,76,77} Like the lineage system, it is additionally due to a restrained arrangement of isolates as far as topographic inception. Moreover, as genome data from pathogenic O157:H7 bacterial strains of *E. coli* from around the world are regularly being made accessible, several of those predicaments could be relied upon being solved soon⁷⁶ so that there can be a solid classification of *E. coli* clades.^{76,77}

The pO157 plasmid conveys a gene encoding the type of EHEC haemolysin that could bring about lysis and probably play a part in the etiology of the O157:H7 and apoptosis of human microvascular endothelial cells.⁷⁸ This plasmid contains genes for presumed virulence genes like catalase/peroxidase (*katP*), cytotoxin B (*toxB*)^{79,80} serine proteases (*espP*), and some of the type II excretion.⁸¹ *E. coli* O157:H7 which is associated with serious human disease is required to colonize the intestinal lining, and carrying pO157 which is also correlated with the pathogenicity where the strains of O157:H7 cured from the pO157 plasmid has a low capability to infect animals.^{79,80,81}

Hemolysin

E. coli hemolysin, also termed α -hemolysin was the first virulence factor described for pO157.^{82,83} Haemolysin operon (*EhxCABD*) may be of foreign origin due to its different G + C content and codon usage than the surrounding genetic contents.⁷⁹ The 3.4 kb fragment encodes the gene that encodes haemolysin.^{83,84} This is required in transport and synthesis, and this is useful as a diagnostic probe for *E. coli*. It is already being used in O157:H7 strains and more often on isolates of EHEC. Some experiments have shown particularly haemolysin to be very high, and it is preserved among various EHEC serotypes such as O8:H19, O111:H8, and O157:H7. However, it is unclear whether they have the same biological activity.^{82,84}

The gene for catalase-peroxidase activity (*katP*) has been detected and observed from pO157.⁸⁵ The *katP* gene has a size of about 2.2 kb, and it is distinctly homologous to the bacterial bifunctional catalase-peroxidase.^{41,46} *E. coli* O157:H7 KatP enzyme activity was demonstrated in both periplasm and cytoplasm fractions. Now, within the cytoplasmic membrane, there is the N-terminal signal sequence that is based on this enzyme.⁴¹ Furthermore, the *katP* gene has been detected on the whole spectrum of *E. coli* O157:H7 strains, it was not detected in EAggEC, ETEC, EPEC, and EIEC, strains.^{41,46} *katP* enzyme enables or assists O157:H7 strains to colonize the host intestine by minimizing oxidative stress and utilizing by-products of oxygen when the host intestines are depleted or depleted of oxygen.⁸⁵

Plasmid pO157 encodes 13 ORFs named *etpO* from *etpC*, showing high similarity to T2SS of Gram-negative bacteria.⁸⁵ However, these genes are found next to the haemolysin locus. Therefore, an insertion element such as IS911 has been detected to be distant from the *ehx* and *etp* genes. The *etp* gene was also detected in all *E. coli* O157:H7 strains and resembles in addition to the *katP* gene, and a portion of some was detected in EHEC strains other than O157 and was not detected in EAggEC, ETEC, EPEC, and EIEC strains. However, the T2SS is comparable to *Klebsiella oxytoca* pullulans secretory pathway (pulO), but with its function was not identified.^{41,46,85}

Pepsin A and human coagulation factor V are known to be produced by EspP which is a pO157-encoded V-secreted serine protease.⁸⁶ The EspP extracellular enzyme resembles some secretory enzymes or surface-bound proteins containing PssA from IgA1 Neisseria protease, EPEC EspC, and EHEC O26:H.⁸⁷ Not long ago, it was reported that EspP affects calf intestinal colonization and attachment to bovine primary intestinal epithelium cells. In addition, EspP-mediated degradation of human coagulation factor V may play a role in it and mucosal bleeding is distinguished in HC patients.^{86,87}

pO157 has been reported to encode the metalloprotease StcE and specifically cleaves C1 esterase inhibitor.⁸⁸ C1 esterase inhibitors are host regulators based on several proteolytic cascades of inflammatory pathways to the same degree as a classical complement, endogenous coagulation, together with contact activation. StcE is secreted by T2SS encoded in pO157, LEE-encoded regulator (Ler).^{88,89} StcE contributes to intimate adherence to the O157:H7 strain. *StcE* gene and Hep2 cells in vitro, all were found in the O157:H7 strain, and a few of them were found in the O55:H7 EPEC serotype that causes diarrhea.⁸⁸

The *toxB* gene is encoded in a 9.5 kb sequence, the expected product of which is 20%. Similarities of *Clostridium difficile* to toxin B.⁹⁰ In a recent study, ToxB in *E. coli* O157 contributes to the attachment of H7 onto Caco2 cells and secretion of TTSS.⁹¹ In addition, sequence comparisons showed a ToxB of 28%. Amino acid identity and 47% comparability onto the predicted product of *lifA/efa-1*, another pathogenic gene that is commonly detected on the chromosomes of non-O157 EHEC and EPEC isolates.⁹² The existence of the *lifA/efa-1* gene is present in humans and gastrointestinal lymphocytes in mice, and therefore ToxB may be involved in the inhibition of host lymphocytes.⁹⁰ However, mutations in the *toxB* and *efa1* genes did not affect the colonization of calves or sheep colons.⁹¹

It was recently reported that pO157 encodes the temperature *ecf* operon (*ecf1-4*). It is coordinated by essentially curved DNA.⁹³ Both *ecf1* and *ecf2* encode estimates polysaccharide deacetylase and LPS- α 1,7N-acetylglucosamine transferase, each and both of which are distinctive to pO157.⁹³ *ecf3* and *ecf4* exhibit comparability to estimate *E. coli* K1 outer membrane protein associated with bacterial invasion.⁹⁴ *msbB2* encodes a second copy of lipid A myristoyltransferase.^{79,93} The lipid A double mutant with a deletion in *ecf4* and its chromosomal copy from *E. coli* lpxM O157:H7 alters the structure belonging to lipid A together with the fatty acid composition of the membrane, in addition to minimized persistence within the gastrointestinal tract of cattle.^{79,93} A single variant of *ecf4* showed no significant difference in contrast to wild-type *E. coli* O157:H7.^{79,93}

Acid Resistance

Acid resistance is described as the capacity of pathogens to shield themselves in distinction to very low pH (pH 3.0).⁹⁴ The low pH of the stomach (pH 1.5–3.0) is described as one of the host's first defence mechanisms. However, *E. coli* can survive gastric acidity and volatile fatty acids produced as a result of fermentation in the intestine.^{94,96} The potential to survive in the acidic conditions of the stomach increases the probability that a pathogen will settle in the intestines and give rise to infections. Acid Resistance is closely correlated with reduced infection with enteric pathogens.⁹⁴ The low pH resistant infection is one of the *E. coli* O157:H7 most known features and makes it a highly infectious bacteria (see illustration in Figure 2). Numerous research demonstrated reports that there is a mechanism of acid resistance for *E. coli*

O157:H7 strain.^{95,96} These investigations have identified three efficient acid resistance systems. One of the acid resistance systems needs an alternative sigma factor RpoS and glucose suppression. *E. coli* O157:H7 RpoS mutant eliminated the number based on experimentally infected calves and mice are small. Another acid resistance system is the addition of arginine during exposure to acidic conditions.^{94–96} Arginine decarboxylase (*adiA*) and the regulator (*cysB*) are described within this second acid resistance system. The third acid resistance system prerequisites a glutamic acid for its defence mechanism at low pH conditions. The required component of this acid resistance system combines two isozymes (*gadA* or *gadB*) of suspected glutamic acid and glutamate decarboxylase, aminobutyric acid antiporter (*gadC*). This ensures that at least either one or both glutamate decarboxylase is required; therefore, glutamic acid decarboxylase is required for preservation at pH 2.5.⁹⁶

Previous findings showed that acid resistance relies on glutamate as a considerably more valuable protection at pH 2.0 on complex media. In the O157:H7 strain, there are three overlapping acid resistance systems; however, there are controls and requirements for acid resistance activity in any acid resistance system. In addition to the already mentioned three acid resistance systems, some proteins also take part in acid resistance of *E. coli*.⁹⁵ These proteins have been identified in *E. coli* O157:H7 which include RNA polymerase-related chaperones, DNA-binding protein Dps, SspA, and HdeA Protein. Also, the production of colonic acid or cell wall membrane is linked with the success of acid resistance in the O157:H7 strain. Therefore, the O157:H7 strain uses various acid resistance systems based on the variety of acidic environments it encounters in nature.^{95,96}

Routes of Transmission of O157:H7

E. coli O157:H7 can be transmitted through contaminated foods and it is classified as a food-borne pathogen. Various contaminated foods can transmit this pathogen including vegetables, different meat products, ground beef (Figure 1), and unpasteurized dairy products are the main origin of significant outbreaks around the world.⁹⁷ In the United States, the link between *E. coli* O157 with outbreaks of haemorrhagic colitis in humans was observed for the first time in 1982.⁹⁸ Afterward, outbreaks of diseases linked with *E. coli* O157 have been observed and documented all around the globe.⁹⁹ *E. coli* O157:H7 infections may lead to HUS, HC,¹⁰⁰ or TTP.^{100,101} *E. coli* O157:H7 strain is found in faeces secreted by sheep, birds, cattle, bats, pigs, goats, and dogs. Both cattle and sheep are the main reservoirs of *E. coli* O157:H7 serotype and are also major sources of human O157:H7 disease. The faecal release of *E. coli* O157 can bring about illnesses in people through contact with water and soil contaminated with animal excrements such as butchers and brushes¹⁰⁰ or with animals.^{101,102}

O157:H7 strain is known to be potentially serious worldwide zoonotic pathogen.¹⁰³ O157:H7 strain can be distinguished in faeces produced by cattle, sheep, goats, pigs, dogs, bats, and avian species. Both cattle and sheep are the fundamental reservoirs of the O157:H7 serotype strain of *E. coli*.^{103,104} and are also the primary etiology of O157:H7 infection in human.¹⁰³ The faecal release of *E. coli* O157 has a potential to cause disease in humans because of contact with animals, or with soil, and water contaminated with animal excreta such as butcher or brushing.¹⁰⁴

E. coli, which can be pathogenic to humans, has several genetic pathogenicity determinants harbored on plasmids wherever possible.¹⁰⁵ The largest VTEC serotype is associated with at least one large plasmid that encodes several virulence factors such as haemolysin, each with a different genetic makeup from the plasmid backbone, suggesting a complex evolutionary history.⁴¹ The large O157:H7 pathogenic plasmid pO157 is 92 kbp.¹⁰⁵ The pO157 plasmid exhibits a gene encoding a type of EHEC haemolysin that could bring about lysis and apoptosis of human microvascular endothelial cells and can presumably play a part in the pathogenesis of O157:H7.⁸⁸ The plasmid additionally includes the serine protease (*espP*), putative virulence factors cytotoxin B (*toxB*), components of type II secretory system,⁸¹ and catalase/peroxidase (*katP*).^{41,106}

Evolution of *Escherichia coli*

The driving force behind the evolution of *E. coli* is the acquisition and loss of mobile genetic elements. It has the potential to encode a heterogeneity of virulence factors and survival, as well as genes associated with the elemental transfer. These elements can be transferred horizontally, which means that they can maneuver among multiple strains of bacteria or even between bacterial species.¹⁰⁵ However, the most important types of chromosomal elements in *E. coli*

include pathogenic islands and prophage. Elements that have been assimilated into bacterial chromosomes for generations often become increasingly immobile because of the assemblage of mutations that result in immobile parts of the host genome. *E. coli* virulence determinants can be located exterior of the chromosome encoded by the plasmid and can be horizontally transmitted.¹⁰⁵ Most human pathogenic *E. coli* strains rely on at least this type of pathogenic plasmid to elicit an infectious phenotype.¹⁰⁵ The gaining of the mobile elements of an *E. coli* strain is an ongoing activity as old elements deteriorate and are lost, no useful benefit is obtained from them and additional elements are incorporated.¹⁰⁵

Nonetheless, the acquisition of virulence determinants by horizontal gene transfer is the powerful force in the evolution of *E. coli*. Insertion sequences (IS) are small transposable elements commonly found in the bacterial genome.^{106,107}

Metagenomic Analysis

To directly detect microorganisms and their proportions within the microbial community, the pyrosequencing of the 16S rRNA gene can be used.¹⁰⁸ There is no need for laborious procedures for conventional culturing methods of individual bacterial species for identification. This methodology has been developed for determining the admixture of the bacterial community and was tested and applied to the surface of ready-made vegetables containing spinach.¹⁰⁸ The use of this kind of method to discover the presence of foodborne pathogenicity in bacterial communities linked with bagged fresh produce such as leafy vegetables could offer the possibility that the number of pathogens is currently available and can recognize the pathogenicity faster and allow proper measures to be taken much quicker. Nonetheless, high throughput metagenomic shotgun sequencing have been utilized in studies that detect the taxonomic composition of microbial communities and can yield higher resolution taxonomic data. The 16S rRNA sequence uses a clade-specific marker gene^{108,109} or matches an entry-specific database.^{108,110,111} The identifiable kmer method of metagenomic sequence data classification has increased the speed of data processing, and it is a key aspect of pathogen detection methods. The 16S rRNA sequence reveals evidence of potentially contaminated *E. coli* strains and shotgun metagenome sequencing enable higher resolution classification.^{108,109} Only for *E. coli* phylogroups and shotgun metagenome sequencing, if the appropriate sequence depth is once achieved, it will provide data for the characterization of pathogenic genes required to estimate the possibility of pathogenicity of the strain. Nonetheless, the purpose of the study was to use a metagenomic shotgun sequencing method to detect contaminated STEC of packaged spinach.^{108,109} The issue of the amount of contamination required to detect spinach samples without concentration and the time required for the concentration process to detect very low STEC levels was addressed, and the contamination detection method according to the established culture protocol of the US FDA bacteriological analytical manual.^{108,112} In addition, this method was able to identify bacterial communities associated with sack spinach, so we focused on changes in bacterial communities in terms of changes in enrichment time and enrichment process.¹⁰⁸

Whole-Genome Sequencing of Phage PhiG17 (*Escherichia coli* O157:H7)

The whole-genome sequence of phage G17 resulted in a genome of about 68,270 bp assembled into a single contig with an N50 value of 68,270 bp with an average GC content of 43.5%.⁶⁴ Coding sequences and genes in the genome were estimated and annotated Quick annotation using Phage Search Tool (PHAST)⁶⁴ together with subsystem technology (RAST) v2.0 server¹¹³ and an online analysis tool. Estimated proteins in the genome are more advanced NCBI's non-redundant GenBank database is annotated by BLAST.¹¹⁴ The PhiG17 genome has been detected to carry a sum of 78 coding sequences that differ in length from 114 bp to 10,353 bp which also carries one isoleucine tRNA, as estimated by ARAGORN^{113,115} together with tRNAscanSE.^{113,116} As determined by ResFinder v3.0,¹¹⁷ there are no known genes encoding antimicrobial resistance in the genome. The gene encoding the toxin and the gene involved in transduction was missing within the genome of phage PhiG17. Genome nucleotide BLAST searches revealed nucleotide similarity of 94% with APEC7 (GenBank accession number KF562340), also 95% with APEC5 (GenBank accession number KF192075). They are both members of the newly assigned G7C virus. Another Core Genes analysis of the genome^{113,117} showed significant comparability to the same recently assigned G7c virus, especially the *Escherichia* phage vB EcoP PhAPEC7 (GenBank accession number KF562340). However, members within the new category are classified as members of the

genus *G7C virus* and the family Podoviridae and are regarded as a safe biocontrol group.^{118,119} Therefore, it is suggested that the *E. coli* phage G17 is a member of the *G7C virus*.^{113,120}

***Escherichia coli* in Africa**

Isolation of the O157:H7 strain that produces Shiga toxin in humans, animals, and food together with the environment has been documented all around the African continent. The first human infection was detected and documented as early as 1990 in the city of Johannesburg, South Africa.^{121,122} However, in Central Africa during 1996, pathogenic bacteria were isolated from people with haemorrhagic colitis in the Central African Republic, resulting in mortality. STEC O157:H7 isolation in people was documented in 1998 after the development of bloody diarrhoea in Cameroon.¹²³ In East Africa, pathogen isolation was reported in Ethiopia, Kenya, and Tanzania. Ethiopia ranks second only to Nigeria in the outbreak of zoonotic diseases on the African continent.¹²¹ Ethiopia is in sub-Saharan Africa and is facing the world's largest outbreak of food poisoning. In Ethiopia, *E. coli* O157 is the leading cause of food poisoning that threatens to prolong lifespan.^{121,122} In Ethiopia, diarrhoea has shortened life expectancy, with approximately 2.6 million registered in 2010, and lower respiratory tract infections are the second leading cause of sudden death after lower respiratory tract disease.⁸⁵ Data on *E. coli* O157 infection in humans have not been studied much in Ethiopia and most African countries.¹²³

In 2006, Morogoro, Tanzania, reported a prevalence of over 7% STEC O157:H7 in patients with diarrhoea.¹²⁴ During a study that was done in Kenya in 2012, pathogenic bacteria were detected and isolated from cattle within the same region and had a prevalence of 0.9%, STEC O157:H7 was detected in a 2-year-old boy with symptoms of haemorrhagic colitis.^{123,125} Later, in the same country, bovine faeces and milk were detected to carry the same pathogenic bacteria. Beef, mutton,^{123,124} sheep, and goat droppings (4.7%), swabs taken from the skin (8.7%), water samples (4.2%), pre-washed carcasses (8.1%), and post-washed carcasses (8.7%) were included in the reports on outbreaks of STEC O157:H7 and are made accessible and available from North African nations, such as Algeria, Egypt, Morocco, and Tunisia. The investigation that was done in Algeria found a 7% prevalence of carcasses of cattle.¹²³ However, in Morocco, there was a prevalence of 11.1% for meat sold in Rabat, and 9.1% for dairy products were reported. On the Mediterranean coast of Morocco, a 1.9% prevalence of shellfish was reported in 2011. In Tunisia, Shiga toxin-producing *E. coli* O157:H7 isolates from human stool samples had a prevalence of 3.4%. Detection and isolation of bacterial pathogens from various sources have also been reported in Egypt. For example, a study that was conducted in Egypt discovered that slaughterhouses, supermarkets, and farmers had a prevalence of 4% from chicken, 6% from beef, 6% from milk, and 4% from lamb samples. However, in West Africa, the majority of STEC O157:H7 is majorly reported in studies from Nigeria. About 6% prevalence of diarrhoea were recorded in Lagos.¹²³ Whereas within the area of Ibadan, O157:H7 STEC was found in cattle, sheep, goats, pig faeces, and beef chevon (goat). The prevalence of goats is 5%. In Zarya, this strain was isolated from diarrheal stools in children younger than 4 and 5 years with a prevalence of 2.2% and 5.4% from surface water. However, STEC O157:H7 isolated in Nigeria indicated the presence of pathogens in the environment (water), animals, meat, and humans.¹²³

Studies in Ghana, which is in the coastal savanna, have not reported the isolation of the *E. coli* O157:H7 strain in dairy products and freshly produced milk,^{123,125} however, this cannot be a guarantee that the bacterial pathogens are not there. During the data collection of *E. coli* O157:H7 recovery, a few West African nations were not part of this recovery program study, including Benin, Burkina Faso, Cape Verde, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Mali, Mauritania, Niger, Sierra Leone, and Togo.¹²³ However, even though there are similarities when observing the environments of the West African countries, this pathogenic *E. coli* strain is likely to be present in West African countries that were not included in the study. The insufficient pathogenic bacterial research and insufficient reports of *E. coli* O157:H7 isolation in some African nations may be due to poor diagnostic ability, and more so in underdeveloped and rural areas where infections may not be diagnosed.⁸⁷ The Southern African region includes Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Zambia, and Zimbabwe.^{123,126} Within the region of South Africa (Eastern Cape), vegetable samples recorded a prevalence of 10.3% of STEC O157:H7.^{123,126,127} Moreover, within the same location in the Eastern Cape region, meat and meat products had a prevalence of 2.8% of the bacterial

pathogen. Botswana, on the other hand, which is adjacent to South Africa, STEC O157:H7 was detected in Gaborone fresh sausages, minced meat, and meat cubes which had a prevalence of 2.26%, 3.76%, and 5.22%, respectively.¹²⁷

The consequences of these beef products pose a risk of infection to consumers. In Lungwena, Malawi, homemade food samples (beans, vegetables, corn porridge, and fish) were tested for pathogens and it was reported that a percentage of 8% was contaminated with STEC O157:H7. *E. coli* O157:H7 in most areas of the continent of Africa (East, West, Central, North, South) indicates that pathogenic bacteria are occurring all over the African continent. About 15 countries reported recovery from pathogenic *E. coli* O157:H7 from the environment, food, animals, or human beings. Of the 30 cases surveyed, 10 (33.3%) came from patients; moreover, the remaining 20 isolates (66.7%) were food, cows, water, and others such as sheep and goats, one vegetable, and one crustacean.^{123,128,129}

Contact Transmission in Developing Countries

Pathogenic bacteria can be transmitted via blood products from living animals and domestic slaughterhouses, but the actual route of transmission is often unknown. In developing countries, livestock breeding is a common practice for many families, and animals are often stored in the backyard of the home for egg, milk, or meat production.^{130–133} Backyard poultry farming is often associated with avian influenza transmission. China has a wide variety of livestock farming, from poultry farming with people at all stages of the production cycle¹³⁴ to large herds of industrial cattle.¹³⁵ Both reported zoonotic diseases from livestock to humans, avian influenza, and *T. verrucosum*.¹³⁵ In summary, it can be said that the literature so far does not state, which patterns of contact between livestock and humans in developing countries lead to the transmission of zoonotic diseases.^{133,135}

Resistance to Antimicrobial Agents

The high resistance of O157:H7 strain is known as one of the major factors that give the potential to increase the pathogenicity of the bacteria. Animal-derived food samples, especially meat, contain *E. coli* O157:H7, which is highly resistant to a group of commonly utilized antimicrobial agents such as quinolones, aminoglycosides, macrolides, cephalosporins, sulfonamides, fluoroquinolones, and tetracyclines. Several antimicrobial-resistance genes enable resistance including resistance genes of ampicillin (CITM), cephalothin (*bla_{SHV}*), chloramphenicol (*catI* and *cmlA*), fluoroquinolone (*qnr*), gentamicin (*aac(3)-IV*), sulphonamide (*sulI*), tetracycline (*tetA* and *tetB*), and trimethoprim (*dfrA1*). However, aminoglycosides resistance genes (*aadA1*) were reported to be responsible for the antimicrobial resistance of STEC strains.^{136–138} Given the uncertainty in the spread of *E. coli* O157:H7 in samples of raw meat, it is important to investigate the distribution and the prevalence of antimicrobial-resistance factors and antimicrobial resistance patterns of O157:H7 strains obtained from raw beef. The investigation should include sheep, goat meat, camel, chicken, turkey, and meat samples.^{138–140}

Various investigations in the African continent have detected and reported resistance to various antimicrobial agents of STEC O157:H7. For example, in Egypt, multidrug resistance STEC O157:H7 has been detected and documented and it was isolated from the environment, animals, and humans.¹²³ In South Africa, a multidrug-resistant STEC O157:H7 was detected and reported to be isolated from bovine. Similar results have been reported in Nigeria. One of the major concerns is that antimicrobial agents are sorely not only used to treat STEC O157:H7 infections, and so this gives rise to multidrug resistance, and it does not seem to be taken with high importance, but it helps in the selection of resistance genes.¹²³

Guidelines for Antimicrobial Agents Usage

The World Health Organization (WHO) has developed global antimicrobial guidelines but that does not seem to be followed or applied in many countries. Therefore, many countries supplement livestock with antimicrobial agents that are used for human health, which is another major concern. It has been reported that developing countries such as South Africa and Thailand have made efforts to reduce antimicrobial resistance.^{141,142} This was done through the implementation of a national framework for antimicrobial resistance from 2014 to 2024, and by implementing an antimicrobial resistance containment program from 2012 to 2016.¹⁴¹ Multidrug resistance is one of the major challenges of the antimicrobial resistance crisis.^{141,142}

Multidrug resistance is defined as an isolate that lacks susceptibility to at least three different classes of antimicrobial agents¹⁴² and has few antimicrobials available for effective treatment. Therefore, more frequently consumed antimicrobials develop resistant bacterial populations that arise in symbiotic bacteria from more and more animals within pathogens and exposed bacterial populations, including practices that treat non-bacterial animals. Antimicrobial agents may induce multidrug-resistant isolates.^{136,142} Interestingly,¹⁰⁸ the global average of livestock antimicrobial spending per kilogram of animals produced is projected to increase to 67% by 2030 to meet basic animal protein requirements. However, this is not ecological in the long run, as increased resistance and reduced efficacy of antimicrobials affect the health of humans, animals, and the environment. Resistance can also result from improper use of antimicrobials in viral infections, antimicrobials given at lower concentrations than necessary, or failure to use the correct antimicrobial selected to combat the infection.¹⁴³ Elevation and scope of antimicrobial-resistant genes in bacteria are a complex process, primarily driven by transposons, inclusion groups, integrins, and plasmids, some of which are homologous in both livestock and human isolation.¹⁴² Therefore, studying mobile genetic elements, especially plasmids, is an important factor needed to better understand the distribution of ESBL genes.¹⁴² Different classes of antimicrobial agents and their relevant modes of action and possible resistance mechanisms are shown in Table 1.

Antimicrobial Resistance (Genetic and Non-Genetic)

Non-genetic resistance can be described as a state whereby bacteria are resistant to antimicrobials in the absence of genetic change, which could be plasticity resistance, as seen with biofilms and persistent drug ineffective. Non-genetic resistance has other mechanisms that are demonstrated by changes in the phenotype as a response to bacterial metabolic status.¹⁴⁴ Studies have shown that many genes play a role in phenotypic resistance, and a few of those genes are also associated with the metabolism of a bacterial cell. Therefore, because the resistant phenotype is within the process of metabolic control, alterations in the bacterial metabolism due to internal and external factors may change the susceptibility to antimicrobials.¹⁴⁴

During the steady phase of bacterial growth, this is usually where a state of bacterial drug indifference to antimicrobials is usually found. This is because immobile bacteria show resistant patterns to some antimicrobials and are moderately sensitive to other antimicrobials. Antimicrobials experience higher levels of activity against the bacteria than if the cells were actively proliferating and will be lower.¹⁴⁴ A subpopulation of bacterial cells, classified as persistent cells, are dormant during culturing and are consequently unaffected by antimicrobial treatment even if the remaining population dies.¹⁰⁷ Pathogens of resistant strains can resume growth without antimicrobials but become more susceptible and do not survive when antimicrobials are reintroduced.¹⁴⁴ The resistance mechanism involved in persistent cells depends on the toxin-antitoxin element.^{145,146}

Table 1 Selected Different Classes of Antimicrobial Agents and Their Modes of Action and Resistance Mechanisms*

Antibiotic Class	Examples	Mode of Action	Resistance Mechanisms
β-Lactams	Ampicillin, Cefotaxime, Aztreonam	Inhibition of cell wall biosynthesis	Enzymatic inactivation of the antibiotic (β-lactamases), and mutation of penicillin-binding proteins; porins
Fluoroquinolones	Norfloxacin, Ciprofloxacin	Inhibition of DNA gyrase during DNA replication	Mutation of DNA gyrase and drug efflux
Aminoglycosides	Gentamicin, Tobramycin	Impairment of codon-anticodon interaction causing accumulation of defect proteins	Enzymatic inactivation/ modification of the antibiotic
Tetracycline/ Glycylcyclines	Tigecycline	Inhibition of protein synthesis (affect t-RNA binding to 30S ribosome)	Efflux of drug and enzymatic inactivation of the antibiotic

Note: *Table was compiled accordingly to reference.¹²⁵

Biofilms are bacteria and coherent that grow at a reduced rate within an encapsulated matrix attached to the surface.¹⁴⁷ Under these conditions, gradients are developed due to differences in the depths of the nutrients, oxygen, and biofilm, which then alters the metabolic state of the bacteria at different depths, creating the bacteria indirectly showing resistance to several antimicrobials.¹⁴⁴ Diffusion of certain antimicrobials through biofilms is impaired by compounds in the matrix,¹⁴⁵ reducing free antimicrobial concentrations.¹⁴⁴ Another mechanism studied as quorum sensing may reorient the sensitivity of antimicrobials depending on the depth of the biofilm.¹⁴⁴ Persistent cells are involved in biofilm maintenance and survival.¹⁴⁵

The availability of metabolic conditions, temperature changes, inducers, and reactive oxygen species can transfigure antimicrobial sensitivity by altering its permeability to bacterial cells. Bacteria can alter the lipopolysaccharide layer and trigger a series of processes.¹⁴⁴ Such processes incorporate changing the number or type of porin to prevent antimicrobials from invading the cell, reducing antimicrobial binding, expanding the surface area to minimize the effect of antimicrobials per cell, and expressing an excretion pump to remove antimicrobials, if present, entering the cytoplasm of the cell.¹⁴⁴ One study interpreted the capacity of chemical signalling from metabolic by-products in antimicrobial resistance. In *E. coli*, indole is produced in large concentrations when under stress and is used as a cell signalling semi-chemical that induces cell protection against specific antimicrobials in the population.¹⁴⁸ Polyamines are also generated by bacterial cells, including *E. coli*, and the compound has also been shown to induce antimicrobial resistance at different concentrations in different bacterial species.¹⁴⁸ Ammonia is a volatile compound that increases the resistance of *E. coli* to certain antimicrobials.¹⁴⁸

Bacteria exhibit three types of resistance mechanisms, including endogenous, adaptive, and acquired.¹⁴⁹ Intrinsic resistance is inherent to microbial cells and is species-specific. Alternatively, the acquired resistance is the result of the microorganism acquiring new genetic material, such as a plasmid encoding the resistance gene, or by mutation¹⁵⁰ (see Table 1) Adaptation resistance is a phenotype resulting from altered gene and/or protein expression and is the result of altered environmental conditions such as stress, nutritional status, growth status, or exposure to antimicrobials below inhibitory levels.^{149,151} The intrinsic and acquired resistance mechanisms are stable and have the potential to be passed on to the next generation. However, adaptive resistance returns when environmental conditions recover.¹⁵⁰ Therefore, endogenous resistance can be recognized as multiple chromosomal genes responsible for resistance, not due to exposure to antimicrobials. However, the acquired resistance is attributed to genetic mutations based on exposure to antimicrobial agents or acquisition of mobile genetic elements conferring resistance.^{149,151}

A plasmid is an extrachromosomal, self-replicating, circular genetic element that contains genes that are not required in the function of the cellular structure.¹⁴⁷ Plasmids are involved in the evolution of bacterial populations and bring benefits to bacteria.¹⁴⁹ The plasmid contains genes that encode the metabolism of rare substances, such as copper and mercury, antimicrobial resistance, migration, replication, and pathogenicity.¹⁰⁵ The plasmid also contains genes encoding proteins for a wide range of functions. Resistance to metal ions and sequestration of ions,^{150,151} sugar fermentation, hydrolysis of urea, production of hydrogen sulphide, decomposition of toxic compounds,¹⁵⁰ production of colicin,¹⁴⁹ proteases, and bacterial appendages.¹⁵² *E. coli* was found carrying the plasmid likely to exhibit antimicrobial resistance,¹⁵³ and the high incidence of plasmid reflects resistance to a wide variety of antimicrobials.¹⁵⁴

The plasmids are divided into similarity groups. Grouping of similarities suggests that plasmids cannot all coexist within the same bacterial host because of the mechanism of replication. This then suggests that plasmids with a similar origin of replication or replication mechanism are unable to exist within the same host.¹⁰⁵ The plasmid replicon type is mostly *E. coli* and determines the similarity group to which it belongs. *E. coli* plasmid belonging to similarity group F.¹⁰⁵ However, Velappan et al¹⁵⁵ mentioned that it was discovered that plasmid similarity is to a greater extent complex, not only emanated from the mechanism of replication but also that plasmids are to a greater extent consentaneous than formally conceptualized. The compatibility of the plasmid depends on other factors, such as plasmid size, number of copies, virulence, and virulence genetic determinants included.¹⁵⁵

It was reported¹⁵⁶ that ETEC from diarrhoea piglets contains a plasmid containing genes encoding resistance to mercury, streptomycin, sulfonamides, and tetracycline, as well as the enterotoxin gene (LT) and thermostability (ST) (pCG86).¹⁵⁶ The study was performed using transconjugation investigations to find out the genetic component of this complex resistance and discovered that the transconjugant contained three plasmids. Further analysis revealed that

a single plasmid was formed from three plasmids formed a single plasmid, transferred to transconjugant cells, and then dissociated into three separate plasmids.^{155,156}

The physical structure of plasmid pCG86 isolated from porcine ETEC was analysed to figure out its origin.¹⁵⁷ It is well acknowledged that there are plasmids containing only the plasmids encoding resistance to many different antimicrobials (R plasmid) and the enterotoxin gene (Ent plasmid). Therefore, it has been hypothesized that the pCG86 plasmid evolved from the reconsolidation of the Ent and R plasmids. Upon additional analysis, recombinant pCG86 plasmid is the product of recombination between the IncFII-R plasmid (resistance plasmid) and the EntP307 (LTST plasmid), or the R determinant and tetracycline transposon can be inserted into the EntP307 plasmid.^{157,158} It was suggested that there is enterotoxigenic *E. coli* plasmid pTC which was isolated from pigs and it was the first plasmid of animal origin to be fully sequenced.¹⁵⁸ The plasmid is 91,019 bp and encodes STa and STb enterotoxin (toxin-specific locus – TSL), ColE1-like origin of replication, and plasmid encoding, self-binding-involved plasmid transfer (tra), and tetracycline resistance (Tn10 transposon). It is composed of genes that are involved in stability and/or maintenance. The TSL region is a fragment of 16,839 bp containing the thermostable toxins *STa* and *STb* genes, 18 transposases, four virtual proteins, and IS elements.¹⁵⁸ The Tn10 transposon region is 9,146 bp long and encodes the resistance genes *tetR*, *tetA*, and *tetC* adjacent to IS10 involved in the independent mobility of the mentioned region.¹⁵⁸ The maximum plasmid transfer region (tra) contains a total of 33,729 bp.¹⁵⁸ It has been hypothesized that the pTC plasmid could be derived from the family of Shigella plasmid. Since the plasmid pTC is self-conjugative, it has a selective advantage and can spread pathogenic and antibacterial factors.¹⁵⁸ Most importantly, pTC from porcine ETEC was found to differ from the pEntH10407 plasmid isolated from human ETEC, suggesting that pTC has minimal zoonotic potential.¹⁵⁸

β-Lactams

Beta-lactam antimicrobials are one of the broader classes of antimicrobials (Table 1) widely used in both human and veterinary medicine.¹⁵⁹ It was reported stated that 11% more β-lactam antimicrobials were used than all antimicrobials marketed in South Africa in 2002 and 2004. Therefore, these antimicrobials are often used in animal production as growth promoters for chickens and pigs.¹⁵⁹ In Europe, β-lactam antimicrobials are the most prescribed in the veterinary sector, and it is said that livestock prescriptions are being reduced to limit the accumulation of resistance gene determinants in livestock-associated pathogens.^{159,160} β-lactam antimicrobials are composed of four major antimicrobial classes, including penicillin, cephalosporins, monobactams, and carbapenems. All antimicrobials in the family contain a β-lactam ring. These classes are distinguished by an additional structural group, the thiazolidine ring of penicillin, and the dihydrothiazolidine ring structure, where the sulfur atom is carbon-modified and has a double bond between carbon atoms number 2 and number 3 of the carbapenem antimicrobial molecule. Cephalosporins have a hydroethidine ring attached to the β-lactam ring, and monobactam constitutes a monocyclic β-lactam molecule.¹⁶¹ Clavulanic acid is no longer a potent antimicrobial by itself, but when mixed with penicillin antimicrobials, it can act as a potent β-lactamase inhibitor.^{161,162}

The mechanism of action of β-lactam antimicrobials is the inhibition of bacterial cell wall biosynthesis by inhibiting peptide transfer.¹⁶³ Bacterial cell wall contains the peptidoglycan layer found in the outer and cytoplasmic membranes of Gram-negative bacteria, which maintains cell shape and protects it from osmosis.^{160,163} The antimicrobial β-lactam ring is a structural analog of the acyl D-alanyl-D-alanine terminus within the N-acetylmuramic acid (NAM) pentapeptide (a component of bacterial peptidoglycan). Due to their similarity, penicillin-binding proteins (PBPs), including transpeptidases, pair with β-lactam antimicrobial agents and cause acylation and inhibition of transpeptidase enzymes.^{163,164} The binding of β-lactam antimicrobial agents to penicillin-binding proteins induces the release and increased permeability of cell wall hydrolases, as well as the final deterioration of the permeable membrane. Beta-lactam antimicrobial agents are composed of the antimicrobial penicillin G¹⁶⁵ in addition to different generations of cephalosporins.¹⁶³

Hydrolysis of β-lactam antimicrobial agents by the β-lactamase enzyme is an important resistance mechanism for such antimicrobial agents.^{163,165} Currently, over 400 β-lactamase enzymes are unique, all of which share the same underlying catalytic activity, β-lactam ring hydrolysis, but with substrate specificity and β-lactamase inhibitors. Several β-lactam antimicrobial agents are used as the first line agents against several infections caused by Gram-positive and

Gram-negative bacteria, however, the increasing trend in resistance to these antimicrobials has predicted that the sensitivity will be reduced to zero by 2023.¹⁶⁷

Tetracycline

Tetracycline antimicrobials fall into different generations, including tetracyclines, oxytetracyclines, and doxycycline, and these antimicrobials can inhibit the growth of both Gram-positive and Gram-negative bacteria. Tetracyclines use a mechanism that blocks the binding of aminoacyl-tRNA to the domain of the 30S ribosomal subunit.¹⁶⁸ Tetracyclines are widely used in agriculture, scientific research, and veterinary medicine because these antimicrobials are beneficial and have minimal side effects.¹⁶⁸ Such widespread use of tetracyclines over the past years has allowed the emergence of resistance primarily due to the genetic acquisition of *tet* genetic determinants.¹⁶⁹ Due to the presence of proteins encoding excretion genes and ribosome-safe proteins, many mechanisms confer resistance to such antimicrobials.^{168,169} Tetracycline efflux proteins are the most common mechanism of tetracycline resistance. The protein encoded by the *Tet* excretion gene is membrane-bound, allowing the transfer of tetracycline-resistance genetic determinants from bacterial mobile genetic elements as intracellular drug concentrations decrease. The genus *Escherichia* includes resistance to *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetI*, and *tetY*.¹⁷⁰ In addition, Gram-negative bacterial efflux genes are generally associated with conjugation plasmids, which often carry multiple other antimicrobial resistance genes. This masks the resistance element because it is encoded in the plasmid. Therefore, plasmid selection may be the main factor behind the increase in multidrug-resistant strains, especially in *E. coli* strains.¹⁷¹ The genetic determinants of tetracycline resistance are usually found in *E. coli* isolated from wild American deer (*tetB*),¹⁷² European wild boar (*tetA* and *tetB*)¹⁷³ and some European wild birds (*tetA* and *tetB*).^{173–175}

Extrachromosomal Genetic Elements Causing Antimicrobial and Heavy Metal Resistance

A plasmid is an extrachromosomal, self-replicating circular DNA that harbours genes that are not required for cellular function.¹⁷⁶ Plasmids are involved in the evolution of bacterial populations and bring benefits to bacteria.¹⁷⁷ The plasmid contains genes that encode migration and replication, antimicrobial resistance, pathogenicity, and metabolism of rare substances such as mercury and copper.^{178,179} The simultaneous presence of antimicrobial resistance and heavy metal tolerance characters was detected without the presence of antimicrobial(s)/heavy metal(s) in the water sources.^{180,181} The induced heavy metal tolerance could promote antimicrobial resistance in bacteria.¹⁷⁵ However, the longer duration of heavy metals in water sources could enhance the selection pressure for the development of tolerance in bacterial isolates.^{175,180} The plasmid also contains genetic determinants for a wide range of functions, including resistance to metal ions and sequestration of ions,^{178,182} fermentation of sugars, hydrolysis of urea, production of hydrogen sulfide, decomposition of toxic compounds,¹⁸³ and production of colicin,¹⁸⁴ proteases and bacterial appendages.¹⁸⁵ *E. coli* carrying plasmids that are likely to exhibit antimicrobial resistance was previously reported,¹⁷⁶ and the high incidence of plasmids reflects resistance to a wide variety of antimicrobials.^{26,177}

Genome-Based Identification and Diversity Mining of Biosynthetic Pathways for Antimicrobials

Microbial-specific metabolites are currently the main source of antibacterial agents used in clinical, agricultural, and food production.^{159,160} Because of the high-speed development and the increase in resistance to these molecules, there is an essentiality to produce new antimicrobial compounds, which can mitigate the ongoing resistant patterns. From the “Golden Age of Antimicrobials” between years the 1960s and the 1970s, the number of new antimicrobial molecules entering the market has been steadily decreasing.^{159,161,162} However, recent developments have raised expectations that computational genomic approaches to natural product discovery can reverse this trend of low production of new antimicrobials. This is because numerous biosynthetic gene clusters (BGCs) were discovered in prokaryotic genomic sequences.^{159,160} Many of the (thousands) BGCs may encode the biosynthesis of previously unknown molecules.¹⁶¹ To help understand these data, many innovative strategies have been established to link them to high-throughput metabolomics data^{162,163} or its synthetic version for heterogeneous.^{164,165} However, the comprehensive biosynthetic pathways and detailed biochemical characterization of their products are nevertheless laborious, with many BGCs encoded in the

production of (useful) non-antibacterial natural products. Therefore, a targeted approach is needed to selectively reduce them.^{162,163}

To have feasible numbers of BGCs that can be tested in the laboratory, a natural product genome that has antibacterial properties and narrows down many of the potentially interesting BGCs.¹⁶¹ It is interesting to note some of the experimental and computational approaches currently emerging for the purpose of identifying the feasibility of BGCs. This can be based on an analysis of biological function and/or mechanism of action, chemical diversity, and ecology and evolution. Genomic data are becoming increasingly important method for distinguishing new biosynthetic pathways to produce antimicrobial agents. Degradation of BGC is particularly useful in identifying the potential for bacteria to produce bioactive natural products. The broad scope of bioinformatics tools such as antiSMASH,¹⁶⁶ BAGEL3,¹⁶⁷ PRISM¹⁶⁸ are accessible to be able to identify these and are mainly found in shared properties amid already well-studied classes of biosynthetic pathways.^{168–170} For instance, the modification enzymes used to produce lanthipeptides are well conserved;^{168–170} as such, they can be used as signatures or anchors for genome mining.^{168,170}

Protein Functionality Domains Utilization for Genome Mining Based on Predicted Function for Antimicrobials

An important step in the strategy for genome-based degradation of antimicrobial agents is recognizing genes that play a role in their biosynthesis.¹⁷¹ A powerful way to execute this is to use a curated model to detect a single or many preserved protein domains, especially for proteins that are less like proteins with known functions. Specific protein domains or combinations of domains exhibit biochemical functions classified as unique to a particular biosynthetic pathway.¹⁷¹ Therefore, it can be used as an “anchor” or “signature” to identify a particular class of BGC. Identification algorithms of the gene cluster for tools including PRISM, BAGEL3, and antiSMASH, are established on this principle.¹⁷¹ As more discoveries are made about new classes of natural product enzymes, researchers can then be able to add various “domain markers” useful in new degradation procedures. In recent years, the comparable strategy was utilized to systematically degrade the genome of the biosynthetic pathway encoding cyanobacteria,¹⁷¹ Thiazole/Oxazole Modified Microcin,¹⁷² and Eneidyne.¹⁷³

Strategy for Antimicrobial Agents Genome Mining

It is already mentioned that it is laborious to speculate from the sequence alone which BGC encodes within the development of natural products and antibacterial activity.¹⁷⁴ However, available are no less than two possible options for this, which are synergistic antimicrobial mining and resistance-based genomic mining. Resistance-based mining¹⁷⁴ has been extensively reviewed by many BGCs encoding antimicrobial biosynthesis that confer self-resistance onto molecules produced to avoid self-destruction. It takes advantage of the fact that it also encodes one or more genes. It has been observed that self-resistance genes appear to be the same as or extremely equivalent to the resistance genes used by other bacteria to avoid antimicrobials and can be gained by antimicrobials through horizontal gene transfer;¹⁷⁵ These include drug remodelling enzymes, transporters, furthermore, the paralogous gene that encodes a “resistant” copy of the housekeeping protein targeted by antimicrobials. Therefore, the detection of BGC self-resistance genes could be a suitable predictor of antimicrobial function in these products. In addition, when the genes become resistant paralogs of housekeeping genes targeted by antimicrobials, they could also be utilized to predict targets.¹⁷⁴

Recently, Tang et al¹⁷⁶ used an approach which is based on the presence of a resistant copy of the fatty acid synthase gene, this approach was used to identify the BGC of thiotetronic acid natural products, including the well-known fatty acid synthase inhibitor thiolactomycin in the *Salinispora* bacterial genome.¹⁷⁶ Similarly, Yeh et al¹⁷⁷ reported that by identifying the gene encoding the proteasome subunit in the gene cluster, a biosynthetic gene for the proteasome inhibitor fellutamide B was also identified. Interestingly, this approach can be utilized to predict natural products with new mechanisms of action.¹⁷⁸ Recently, using resistance-based mining, it was reported that the natural product telomycin family targets the phospholipid cardiolipin. Many excellent libraries of their detection models and known self-tolerance genes are currently available.¹⁸² In particular, the ResFinder database carries a large set of profile-hidden Markov models (pHMMs) which enable the identification of resistance genes against a variety of antimicrobials.¹⁷⁸ These pHMMs were supplemented with an additional resistance gene and 91 models from the literature. In addition, phylogenetic analysis (reminiscent of the EvoMining method has lately been elucidated by Cruz-Morales et al¹⁸³) shows that additional copies

have long-branch lengths and an abnormal number of copies of such genes in the genome are present. This allows the search for further resistance paralogs of housekeeping genes by detecting the BGC. By doing this systematically, we use a comprehensive and wide range of stochastic BGC predictions provided by algorithms such as ClusterFinder.¹⁵⁹

Numerous recent strategies and new classes of antimicrobial biosynthesis could be discovered. The second possible bioinformatics strategy for prioritizing the BGC for its capability to encode new antimicrobial is established based on synergistic interactions.¹⁸⁴ Numerous cases are well studied in which the admixture of two bioactive compounds behaves synergistically to achieve a higher antibacterial effect or circumvent the resistance mechanism of the target strain. A well-known example still in use at the clinic is Augmentin®, a mixture of the beta-lactamase inhibitor clavulanic acid and the beta-lactam amoxicillin.¹⁸⁴ Theoretically, a synergistic set of natural products has great advantages when fighting against antibacterial resistance, as it is increasingly hard for pathogenic bacteria to enhance resistance to both molecules. Synergistic sets of natural substances are found in nature.¹⁸⁴ Moreover, Augmentin® is modelled on a mixture of clavulanic acid and cephamycin, which is naturally produced by *Streptomyces clavuligerus*. Within the *S. clavuligerus* genome, two compounds of the BGCs become intertwined in a “supercluster” configuration, enabling synchronized management of both biosynthetic pathways.¹⁸⁴

Interestingly, other cases have been determined in which both synergistic natural products are co-encoded into such superclusters. Examples are the synergistic antimicrobials lankamycin and lankacidin^{185,186} and the synergistic antimicrobials griseoviridin and viridogrisein that bind to the complementary sites of large ribosome subunits.¹⁸⁸ Therefore, intertwined supercluster configurations may indicate synergistic interactions between natural products that were previously associated only with antimicrobial function.^{186,187} The evolutionary history of genes was tracked and “superclusters” with automated comparative genomic analysis of very sizable “hybrid” BGCs (encoding enzymes that produce many various scaffold types) were created which were estimated by tools such as antiSMASH. You can evaluate whether it is coded or not coded. Generation of several various (and perhaps synergistic) molecules is established on regardless, and the BGCs occurred more not long ago from the fusion before independent gene clusters. In addition, analysis of regulatory motifs of transcription factor-binding sites accidentally encodes such superclusters side-by-side in the genome by evaluating the potential of genes on either side.¹⁸⁷ This can potentially be distinguished from the pair of gene clusters that are present. Of the estimated superclusters, they are co-regulated. Overall, not less than a two-approach based solely on sequence analysis that is available for screening many existing BGCs for new antimicrobials. However, if sequence analysis is supplemented with ecological information, many other identification possibilities, for example, arise from metagenomics.¹⁸⁸

Antimicrobial Agents – the African Perspective

Generally, the control and prudent use of antimicrobials are largely unregulated in the majority of developing countries, including African countries.¹⁸⁹ Per the World Health Organization for Animal Health, various nations (mostly developing countries) are concerned with the conditions allowed when using antimicrobials and veterinary drugs. There is no relevant law or regulation that has been set yet.^{189–191} Sometimes there is no law at all, and if it is in place, oftentimes it is not strictly followed or applied appropriately. Several investigations based in Africa focusing on the usage of antimicrobials in livestock have shown that unreasonable use occurs because of unregulated access and even the administration of veterinary drugs.^{191,192} However, in various nations within the African continent, it is not legal for non-registered veterinarians to administer antimicrobials, but there are no strict controls and often farmers administer antimicrobials to animals without supervision or a veterinary prescription.¹⁹² However, the use of antimicrobials in livestock by a person who is not trained or qualified to do so is not limited to countries that are still developing, but it is a common practice globally (<http://www.oie.int/>). Moreover, the first study to assess the global trends of antimicrobials use in livestock found that global use of antimicrobial agents is likely to increase in the future, and this increase will be driven by increased consumer demand for livestock products. This will occur in middle-income countries and a shift to large-scale farms where antimicrobials are frequently used.¹⁴⁴

Data from various surveys conducted in Nigeria,¹⁵¹ Zambia,¹⁹² and South Africa³¹ have reported that tetracyclines and (mainly penicillin) are the top antimicrobials commonly used in livestock agriculture for the sale of antimicrobial agents for livestock. It shows that it is one of the substances. Sulfonamides and macrolides are also commonly used

antibacterial agents, and this last group (especially concerning tylosin) is also widely marketed in South Africa when it comes to the treatment and prevention of animal diseases.³¹ It has been recorded as a growth promoter registered below therapeutic doses.³¹ Equally concerned is the improper use of fluoroquinolones in veterinary sector in other parts of Africa as reported from a survey in southwestern Nigeria.¹⁹¹ A study conducted in Ghana included 395 livestock farmers engaged in intensive or large-scale agriculture about how to use antimicrobials.¹⁹³ Most farmers use veterinary drugs primarily to prevent illness and then use two purposes, prevention and treatment, treatment only, and rarely growth promotion. Of course, it is of importance to note that the data accumulated from pet owners were self-reported.¹⁹¹⁻¹⁹⁴ This has certain restrictions. Another important outlook to contemplate is the bias in the administration of antimicrobial agents that is common in livestock and is different from that used in human medicine. With regard to a study done in South Africa, the infeed dosage form accounted for almost 70% of all antimicrobial doses sold in the country.³¹ This method favours treating all the animals at the same time, rather than treating the animal individually according to their needs. In addition, Nigeria has recently reported that a significant increase in the consumption of antimicrobial agents in veterinary medicine, which is not like the region's annual livestock rate.¹⁹¹

Regarding livestock species, some studies have shown that chickens have relatively high rates of antibiotic use. This means that the proportion of resistant isolates detected in chickens is high.^{193,195} European reports found on data collected from seven countries show high resistivity of poultry.¹⁹⁶ This can be partially explained by the fact that the use of antibiotics is even more intensive in agriculture and more often in poultry where animals are kept in proximity. In general, the highest rates were observed or detected for ampicillin (60.2–95.7%), trimethoprim/sulfamethoxazole (44.9–80%), and tetracycline (9.5–10.6%), but resistance rates were reported by region and survey. It varies greatly depending on the animal population.¹⁹² African studies on animal-derived foods (retailing carcasses of turkey or chicken or beef and pork) have also reported that resistance to these antimicrobials is most relevant.^{197–199}

Unsurprisingly, these drugs have been used for a long time in veterinary and human medicine since their inception.²⁰⁰ Their integrated resistance, and most of the time, it is the collocation of various factors or genes within the same mobile genetic elements (integrins, transposons, and/or plasmids), contributed to the selection of multidrug resistance of isolates around the world.^{195,200,207} The presence and diversity of integrins in *E. coli* from beef and poultry were investigated, and several reports from Africa²⁰² showed a high prevalence of class 1 and class 2 integrins (60%) containing the common streptomycin (*aad*) and trimethoprim (*dfi*) resistance coding factors. This situation is even more worrisome, as other antimicrobial classes including cephalosporins and quinolones are essential for the treatment of a broad range of human infections, and resistance to them leaves few treatment options. Livestock were identified as an increasing reservoir of ESBL genes worldwide, which significantly contributes to the antimicrobial resistance burden in humans. Several investigations conducted in Nigeria and Tunisia reported an unexpectedly high prevalence of cattle (61–62%)²⁰³ and poultry (42–55%).^{201,204}

One Health Approach

The call for multisectoral and interdisciplinary research to tackle modern environmental challenges and complex health issues is now more substantial than ever. The One Health research strategy ensures that the environmental, animal and human health problems be assessed in a holistic and integrated way, with more problems and potential solutions than possible with an isolated approach. This allows for a more comprehensive understanding of the strategy. Having said that, the One Health strategy is elaborate and has insubstantial guidelines for researchers on the practical design and implementation of One Health research.²⁰⁵ A framework is being developed and aimed at guiding researchers in the planning and design of One Health studies. It describes the fundamental course of action in designing an One Health research study, incorporating conceptualization of research goals and hypotheses, research design options, data sources and collection methods, analytical methods, and identification of collaborators in interdisciplinary research teams. These concepts can be explained by presenting a case study of the health effects linked to land use of biosolids. This approach can be useful to find solutions to current global health problems and there will be a need for interdisciplinary funding to advance the One Health approach in research.²⁰⁵ The One Health approach that has been proposed is important because it emphasizes collaborative research on the relationship between human health, the environment, and

the epidemic of zoonotic pathogens.²⁰⁹ It also requires multidisciplinary cooperation at the local, national, and global levels to achieve optimal human, animal, and environmental health.²⁰⁵

Conclusions

STEC, which carries virulence factors, has a high prevalence in food chain animal production systems.³⁴ Of further concern is that pathogenic isolates have been identified as one or more genetic determinants of pathogenicity and pose a health risk to humans.^{19,34,40} Therefore, this review urges production systems, farm management, and policymakers to develop a proactive approach to mitigate the increased detection rate of *E. coli* O157:H7. Strict hygiene strategies and close livestock monitoring minimize the outbreak potential for STEC strains.^{10,34} It should be implemented at all stages of production and operational control. This should be done throughout the supply process from farms, slaughterhouses, and staff involved in the processing and handling of meat and livestock.¹⁰ Rural farmers should investigate the information and be better informed about the use of antibacterial agents, various diets, and their ingredients.¹⁰ Epidemiological and pathogenic features associated with the O157:H7 strain need to be studied more closely in developing countries, and routine investigations of this pathogen need to be carried out, especially in urban and rural areas.^{10,102}

The large population of South Africa relies on meat, such as beef and pork, as their main food protein source.^{10,18,102} Therefore, many of the reported incidents occur after ingesting contaminated water or poorly cooked food, especially in rural areas. This increases the importance of reducing infection rates and the spread of pathogens, as well as antimicrobial resistance in animals, water, and edible crops.^{22,25,28} It also emphasizes the issue of antimicrobial resistance and the increasing emergence of multidrug resistance.²⁰⁶ These pathogens can cause illness at very low levels of infection, especially in the elderly and young children. Farms that raise livestock and raise them during the slaughter process in their production systems must implement and adhere to proper hygiene practices.^{10,102} Other parts of South African rural areas rely on groundwater. This may be a possible route to human transmission by pathogenic *E. coli* O157:H7, as the microbial ecosystems of livestock, such as cattle, pigs and humans, may be interrelated in these areas. *E. coli* O157:H7 susceptibility is preferred and may be carefully prescribed as the optimal drug for the treatment of O157:H7 infections. This research contributed to the global health vision of the One Health approach. It highlighted the literature gap on antimicrobial and multidrug resistance in South African livestock production system.^{35,100,102} Therefore, such studies will help monitor the prevalence of antimicrobial resistance in South Africa.

Antimicrobial agents have been used in both veterinary and human medicine for more than 70 years. Therefore, antibacterial agents are used in livestock production systems to treat, control, and prevent infections, and to improve foraging and growth efficiency. Over the past years, antibacterial agents have been successfully used to maintain livestock and control the transmission of animals that can infect humans.^{100,102} However, the use of antimicrobials in developing countries requires addressing the challenges of misuse and abuse due to the lack of information on the use of antimicrobials and the consequence of development and spread of antimicrobial resistance.²⁰⁸ However, misuse and abuse have been blamed on antimicrobial resistance, but because antimicrobial resistance is a complex phenomenon that affects the health of both humans and animals, it is not possible to follow resistance patterns. Easy access to healthy and hygienic foods is important, especially in developing countries such as South Africa, where production systems, slaughterhouses, and small-scale farmers regularly adhere to the basic principles of meat inspection.^{101,208} However, in most cases, rural farmers are particularly informed about pathogenic bacterial infections of livestock that pose a threat to human health in terms of contaminated food intake and the environment, as well as the major issues of antimicrobial resistance.^{35,100,101}

Acknowledgments

The authors thank the two anonymous reviewers for their valuable comments, which significantly improved the manuscript. The authors would like to acknowledge the University of KwaZulu-Natal, College of Agriculture, Engineering, and Sciences, School of Life Sciences and The South African National Research Foundation for their support. Dr. M.E. El Zowalaty thanks Carolyn H. Bohach, Ph. D. from the Department of Animal, Veterinary and Food Sciences, College

of Agricultural and Life Sciences, University of Idaho, USA for her permission to re-use the figures in the current manuscript.

Disclosure

The authors report no conflicts of interest in this work.

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