Biology of *Escherichia coli* O157:H7 in human health and food safety with emphasis on sublethal injury and detection

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Approximately 200 foodborne illnesses are recognized worldwide, but enterohemorrhagic *Escherichia coli* (EHEC) is considered among the most important bacterial pathogens to date. EHEC was recognized as a human pathogen in 1982, and still remains important to clinicians, researchers, and the general public. If ingested, *E. coli* O157:H7 can initiate a toxicoinfection in the host, giving rise to abdominal cramping and watery diarrhea, which may lead to hemorrhagic colitis (HC). *E. coli* O157:H7 elicits life-threatening complications such as HC and hemolytic uremic syndrome (HUS). The primary virulence factors of EHEC are the production of one or both phage-encoded Shiga toxins (Stx1 & Stx2), among the most potent bacterial cytotoxins. HC may lead to severe complications, principally HUS, a renal disorder that primarily affects the immunocompromised. HUS is considered to be the leading cause of childhood acute renal failure ultimately requiring dialysis, transfusions, or renal transplantation (all directly from the Stx cytoxin production). The mortality rate varies between 5-17% in HUS patients. Protecting food from contamination by *E. coli* O157:H7, and detecting this pathogen if present in foods, is an area receiving worldwide attention. Several molecular-based detection methods for *E. coli* O157:H7 are available. However, most food microbiology laboratories still rely on traditional culture-based methods that employ appropriate selective media for detection and enumeration of this pathogen. Unfortunately, if the bacteria are sublethally injured following any of several physical stressors during food processing (temperature/acid-shock, or osmotic stress), the cells are unlikey to grow directly on selective bacteriological media, resulting in false negative results and compromising quality control standards. One such method to address injured cell recovery is the selective agar overlay, whereby the *E. coli* O157:H7 is plated initially onto a nonselective medium for a short (2-4h) recovery period that allows cellular repair and resuscitation. Subsequently, a molten agar overlay is applied that allows the medium to then be selective, allowing only for growth of the recovered pathogen from a mixed population. The purpose of this mini-review is to discuss *E. coli* O157:H7 pathogenesis, particularly in light of injured cell recovery methods in use or in development for food safety applications.

Keywords: *Escherichia coli* O157:H7; hemorrhagic colitis; sublethal injury; food safety; selective agar overlay

1. The history and epidemiology of enterohemorrhagic *Escherichia coli* (EHEC)

In 1982, 47 individuals who resided in Michigan and Oregon were afflicted with a severe form of gastroenteritis called hemorrhagic colitis that included intense abdominal cramping in combination with bloody diarrhea [1-3]. An investigation by the Centers for Disease Control and Prevention (CDC) concluded that the outbreak was caused by the ingestion of undercooked, contaminated hamburgers that originated from a fast-food restaurant chain. Curiously, the hamburgers were determined to be contaminated with a novel strain of *Escherichia coli* (*E. coli*) that had not been previously considered a human pathogen [4]. The strain expressed O-antigen 157 and H-antigen 7 and deviated from the other recognized strains of pathogenic *E. coli* in both pathology and in virulence. Because of its association with hemorrhagic colitis, the O157:H7 strain was categorized into a new class of pathogenic *E. coli* called enterohemorrhagic *E. coli* (EHEC) [3]. Then, in 1993, the strain resurfaced in the largest *E. coli* outbreak to date [5]. There were 582 confirmed cases among California, Idaho, Nevada, and Washington and a total of 171 hospitalizations and 4 deaths. Although the isolation of EHEC occurred in 1977 [6,7] and was recognized as a human pathogen in 1982, EHEC still remains an emerging pathogen of great importance.

There are approximately 200 recognized foodborne illnesses [8], but EHEC is considered among the most important pathogens [9,10]. This recognition is not unfounded as EHEC is known to have caused several outbreaks in North America, the United Kingdom, and Japan [11]. In the United States, the CDC reported that there was an annual increase in the number *E. coli* O157:H7 toxicoinfections, which peaked in 1999 with 4,744 confirmed toxicoinfections [7]. Following 1999, the number of reported cases decreased. For example, in 2004 and 2005 there were 2,544 and 2,621 cases confirmed, respectively. The incidence of EHEC infection, especially with *E. coli* O157:H7, significantly differs internationally [12-18]. To illustrate, in 2004 there were an estimated 0.9 infections in the United States per every 100,000 citizens. Internationally, the number of infections per 100,000 citizens appreciably increased in comparison to 4.1 in Scotland. In Japan, there were a moderate 0.87 infections per 100,000, while there were only 0.08 infections reported in Australia per 100,000 of its citizens in 2004. These and other statistics concerning EHEC and *E. coli* O157:H7 are becoming more accurate because of better surveillance and reporting [11]. However, an important caveat still remains; most of the data are collected while investigating outbreaks [1]. Only those infected individuals who seek medical attention are officially diagnosed and reported [7]. Thus, the statistics fail to include those who never seek medical attention and consequently underestimate the total number of cases.

2. EHEC with emphasis on *E. coli* O157:H7

E. coli is a Gram-negative facultatively anaerobic bacillus [7] that colonizes both the intestinal tract of humans [5] and other animals within hours following birth. Most strains of *E. coli* are in fact commensal with their hosts [7], but a considerable number of strains are pathogenic. It is believed that the pathogenic strains acquired virulence factors largely through the transfer and activity of bacteriophages, plasmids, and pathogenicity islands that equipped those strains with the ability to successfully mount an infection [7,9]. Pathogenic strains of *E. coli* are classified according to their specific virulence factors, their pathogenicity, and their specific strain. To date, there are six recognized classes of pathogenic *E. coli*. These classes include the enterotoxigenic *E. coli* (ETEC), the enteropathogenic *E. coli* (EPEC), the enteroaggregative *E. coli* (EAEC), the enteroinvasive *E. coli* (EIEC), the diffusely adherent *E. coli* (DAEC), and the enterohemorrhagic *E. coli* (EHEC).

Among the classes of pathogenic *E. coli*, EHEC is perhaps the most important because of its virulence and its association with life-threatening complications such as hemolytic uremic syndrome (HUS) [7,10]. Moreover, *E. coli* O157:H7 is considered to be the archetypal and most infamous strain of the EHEC, because it is most commonly isolated from patients afflicted with hemorrhagic colitis and HUS [3,7,10,19,20]. However, there are other strains of EHEC that have been associated with hemorrhagic colitis as well including *E. coli* O26:H11 and other lesser known stains [6,19].

Cattle serve as the primary and natural reservoir of *E. coli* O157:H7 [2,7,9], although other animals such as goats and sheep may be carriers as well [24]. In cattle, *E. coli* O157:H7 colonizes the large intestine and rectum [21], and it is estimated that 10-80% of all cattle are colonized [2]. Interestingly, cattle are asymptomatically colonized by *E. coli* O157:H7, but, in rare instances, young calves may experience initial diarrhea and subsequently become asymptomatic. This asymptomatic state and lack of complications in cattle can be best explained by the lack of a critical receptor on the surface of their vascular endothelial cells [3,22] . Furthermore, Hussein and Sakuma recently reported that *E. coli* O157:H7 colonization may even be beneficial to cattle and other ruminants [21].

Unlike cattle, humans are susceptible to *E. coli* O157:H7 infection, which is transmitted most commonly by food [22] *via* the fecal-oral route [24,25]. Because cattle serve as the primary reservoir of *E. coli* O157:H7 [2,7,10], foods originating from cattle, especially undercooked ground beef and unpasteurized milk, are the most common vehicles of transmission [3]. However, other potential sources of infection include ingesting minimally-processed foods such as produce or water [2,7,10] that has come into contact either directly or indirectly with *E. coli* O157:H7 that is shed in animal feces [24]. Furthermore, *E. coli* O157:H7 infection can arise through person-to-person transmission, which is most commonly observed in daycare centers [2,7]. Finally, *E. coli* O157:H7 may be transmitted through the surrounding environment as the microorganism is able to survive in the environment for an extended period of time [24]. Thus, *E. coli* O157:H7 may spread by direct contact, indirect contact, or through contact with the environment.

3. Clinical presentation of *E. coli* O157:H7

If ingested, *E. coli* O157:H7 can initiate a toxicoinfection in the host [1,7]. Following the incubation period of 1 to 8 days after initial exposure to the pathogen, signs and symptoms begin to develop. Although some individuals infected with *E. coli* O157:H7 are fortunately asymptomatic or are only mildly affected, a typical case usually involves an initial onset of abdominal cramping and watery diarrhea, which are often accompanied by nausea and vomiting. Over a period of one to two days, the illness may progress into hemorrhagic colitis [1,7]. At this point, intense abdominal cramping develops in conjunction with grossly bloody diarrhea. Furthermore, the absence of a fever or the presence of only a very low-grade fever is another hallmark of hemorrhagic colitis. Although the illness is agonizing, it is typically self-limiting and resolves itself in approximately one week. Furthermore, because the symptoms of hemorrhagic colitis are primarily caused by the actions of potent cytotoxins called Shiga toxins, antibiotic therapy is often prohibited because of the increased risk of inducing complications [23,27], especially in treating infections caused by *E. coli* O157:H7.

Antibiotic therapy is largely excluded as a means of treatment, because the antibiotics would eliminate the intestinal commensal bacteria and consequently release any preformed Shiga toxins [23]. The increased concentration of Shiga toxins may then enter the circulation and lead to renal insult or other considerable systemic effects. However, severe complications may arise, especially in very young or old populations, as a result of hemorrhagic colitis alone even in the absence of antibiotic treatment [10,25].

Hemorrhagic colitis may lead to severe complications, principally HUS [2,7]. HUS is a renal disorder that primarily affects children [1,5,7,10,23,26] and the elderly and is considered to be the leading cause of childhood acute renal failure. It is estimated that HUS develops in 5-10% of patients suffering from hemorrhagic colitis [7]. The mortality rate of the condition is considerably high as well with an estimated 3-17% of patients expiring [2], and many of those who survive HUS are left with permanent renal consequences, ultimately requiring dialysis, transfusions, or renal transplantation [3,7].

HUS targets renal endothelial cells [10] and is distinguished by the development of microangiopathic hemolytic anemia [1,2,6,7] accompanied by fragmentation of red blood cells, thrombocytopenia [26], uremia, and acute renal failure. It is believed that the potent Shiga toxins are chiefly responsible for renal compromise [6,10,26]. These

cytotoxins prohibit protein synthesis in glomerular endothelial cells that line blood vessels and cause intravascular coagulation of platelets and fibrin in the glomeruli. The coagulation congests the glomeruli and decreases overall filtration, which leads to acute renal failure.

Less frequently, hemorrhagic colitis may progress into a variant form of HUS known as thrombotic thrombocytopenic purpura (TTP)[3,7]. TPP is similar to HUS, except that it more commonly afflicts adults instead of children. In addition to the renal assault characteristic of HUS, TPP has an associated fever with the addition of neurological symptoms including sever headaches, convulsions, lethargy, and encephalopathy.

4. Key virulence factors of *E. coli* O157:H7

The infectious dose of *E. coli* O157:H7 is reported to be as few as one to 100 CFU/mL [2,6, 29-31], which is lower than most other enteric pathogens. The low infectious dose exemplifies the potent virulence of *E. coli* O157:H7, and the virulence of this microorganism stems primarily from the activities of three major virulence factors.

The first and by far most critical virulence factor is the production of one or both phage-encoded Shiga toxins (Stxs) called Stx1 and Stx2 [2,9,26], and these Stxs are among the most potent cytotoxins currently known to affect eukaryotic cells [1]. Each Stx is composed of a single A subunit (or active subunit) and 5 identical B (or binding) subunits [6,9,31] and are therefore members of the AB5 family of toxins [9]. The B subunit binds to globotriaosylceramide (Gb3) receptors located on the surface of a variety of host endothelial and epithelial cells [6, 9], and the A subunit is internalized through endocytosis where it ultimately inhibits protein synthesis in the host cell by exerting its N-glycosidase activity.

Stx1 is nearly identical to the Shiga toxin produced by *Shigella dysenteriae* [25] and differs only by a single amino acid [6]. However, Stx2 is by far the more virulent of the two toxins [9,27]. To illustrate, *E. coli* O157:H7 can produce either one or both of the Stxs [6,32], but the production of Stx2 alone is still more virulent than either the production of Stx1 alone or the production of both Stx1 and Stx2 simultaneously [28]. In addition, there are several variant forms of each Stx including Stx1, Stx1c, and Stx1d as well as Stx2, Stx2c, Stx2d, $Stx2d_{activatable}$, Stx2e, and Stx2f [30], and these variants differ in their activities and potencies as well [9]. For example, Stx2e typically affects swine and binds to Gb4 receptors [6,27], while Stx2c and Stx2d more commonly lead to hemorrhagic colitis and HUS in humans and further demonstrates the greater virulence of Stx2 compared to Stx1. Although the type of Stx produced directly influences the severity of disease, the production of Stxs alone is insufficient for *E. coli* O157:H7 to mount a successful toxicoinfection [2]. Instead, the assistance of the locus of enterocyte effacement (LEE) and the pO157 plasmid is required.

The LEE is a bacterial pathogenicity island, which is a fragment of DNA that encodes a battery of specialized virulence factors [9]. Typically, pathogenicity islands, including the LEE, are transferred between bacteria through lateral gene transfer, insert into the bacterial chromosome, and augment overall virulence [4,9]. The LEE is present in *E. coli* O157:H7 as well as in other pathogenic *E. coli* and is essential for colonizing eukaryotic cells [9]. A number of critical components are encoded within the LEE and include the type III secretion system (TTSS), an adhesin protein known as intimin, the translocated intimin receptor (Tir), and a number of effector proteins [2,3 6,9,23,34]. The expression of these components is stringently regulated and influenced by ambient conditions, multiple regulators, and quorum sensing [19]. When expressed, the components orchestrate the formation of the very characteristic attaching and effacing (A/E) lesions that develop as a result of *E. coli* O157:H7 infection.

Histopathologically, the A/E lesion is distinguished by the destruction (or effacement) of brush border microvilli and by the intimate bacterial attachment to the host epithelial cell [9,19,36]. The formation of the A/E lesion is coordinated by the TTSS, which is a complex, multi-subunit organelle encoded within the LEE [19, 32,33]. Each LEE-encoded subunit of the TTSS is systematically assembled in order to form a complete and functional apparatus [19]. The apparatus ultimately forms a pore in the host cell membrane and also serves as a portal, or channel, through which bacterial effector proteins can pass from the bacterial cell into the host cell. Once inside, the effector proteins influence signal transduction and the phosphorylation of numerous proteins that force actin to polymerize, leading to microvilli effacement and to cytoskeletal rearrangements that form the actin pedestals [4]. Finally, following actin rearrangement, the bacteria transfer a protein called the translocated intimin receptor (Tir) through the TTSS, which embeds into the host cell membrane and serves as the receptor for the intimin adhesin protein. Once intimin interacts with the Tir, intimate attachment of the bacterial cell to the host cell occurs and promotes bacterial colonization of the intestinal tract [4,36-38].

In addition to the LEE, the pO157 plasmid also plays a role in the virulence and pathogenicity of *E. coli* O157:H7 [2,5,10,37]. The pO157 is highly prevalent in *E. coli* O157:H7 compared to other plasmids, and it is postulated that nearly all strains contain the pO157. The specific functions of pO157 in relation to *E. coli* O157:H7 pathogenicity remain largely unknown and are often debated in the scientific literature [7]. Some have asserted that the pO157 is involved in the expression of fimbriae that promote and strengthen bacterial adherence to host cells. Conversely, other research indicates that the pO157 has little influence on bacterial adhesion. Although many of the pO157 functions are disputed, the sequence of the large plasmid is known as well as a handful of its gene products.

The pO157 plasmid is approximately 90kb and contains 100 open reading frames (ORFs) [2,7]. Furthermore, it is known that pO157 encodes a type II secretion system, an enterohemolysin, a serine-protease, a catalase-peroxidase, a lymphocyte inhibitor, potential adhesins, and others. The function and impact of these components is not fully understood, but the pO157 remains a primary area of interest in the scientific domain.

5. Mechanism of *E. coli* O157:H7 infection

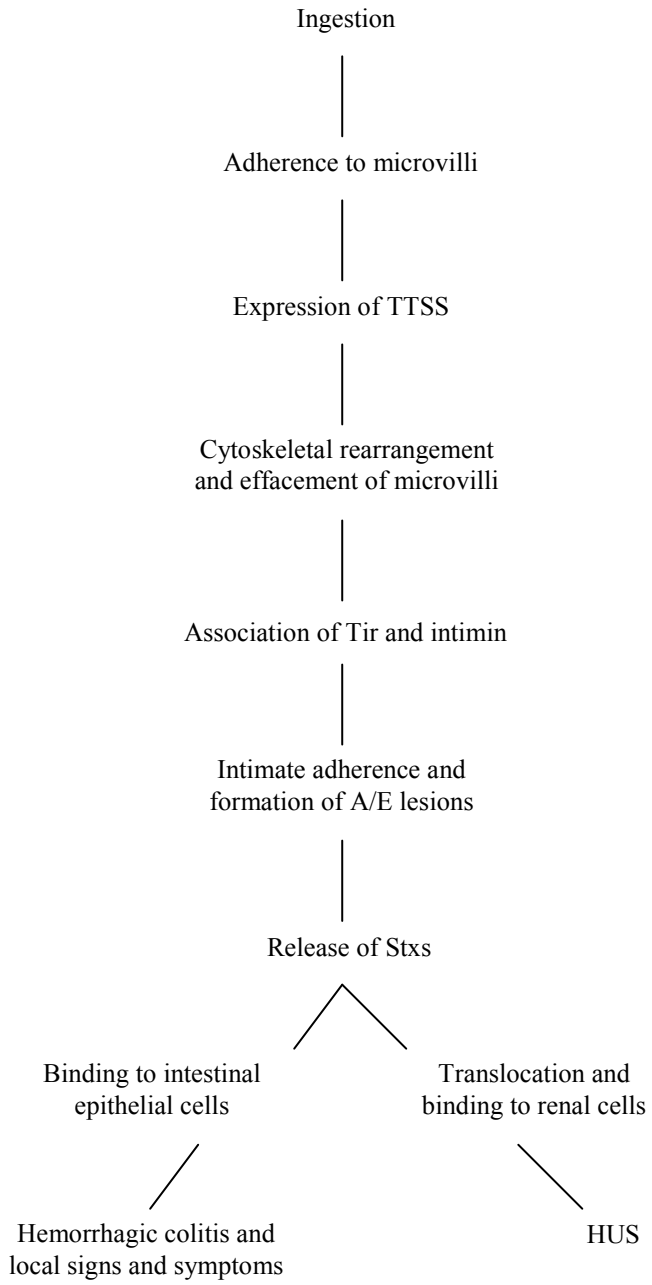

Figure 1: A schematic outline of the progression of *Escherichia coli* O157:H7 infection beginning with ingestion of the bacteria to the development of signs and symptoms.

Following the ingestion of contaminated foods, *E. coli* O157:H7 withstands the acidic environment of the human stomach and begins the arduous and complex process of infection (Figure 1) [26]. From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days [29]. During this time, the bacteria progress through several phases of infection including adherence, colonization, and the production and release of Stxs.

First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells [3]. The association between the bacterial and host cells consequently induces the expression of the TTSS genes located on the LEE. Following their synthesis, the TTSS proteins are systematically assembled [19,20]. The membrane-bound proteins first associate and form the foundation of the TTSS followed then by the proteins that form the extracellular channel and by the proteins that create the pore in the host cell membrane. Once assembled, a multitude of effector proteins are shuttled through the TTSS channel and into the cytoplasm of the host cell.

After the effector proteins invade the cytoplasm, they alter the host cell's normal patterns of signal transduction in order to accommodate bacterial adherence [3]. The alterations in signal transduction are accomplished through the activities of the bacterial effector proteins, and, by selective phosphorylation, the effector proteins force actin to polymerize and the cytoskeleton to reorganize ([9,20,38]. Reorganization results in the effacement of host cell microvilli. At this point, the bacteria may superficially adhere to the host cell while simultaneously preparing for intimate adherence.

Intimate adherence of the bacterial cell to the host cell requires the orchestrated activities of LEE-encoded intimin and the specialized Tir [2-3,6,10,23]. Intimin is an outer membrane protein whose presence and proper activity are critical to intimate adherence and the formation of the A/E lesion. A proposed autotransport system is believed to be responsible for shuttling and inserting intimin into the outer membrane of the bacterial cell where intimin then adopts a β–barrel conformation [19]. Although the N-terminus of intimin is highly conserved, the C-terminus is slightly more variable in comparison. As a consequence, approximately 14 subtypes of intimin are now recognized [3], and each variant has a slightly different tissue tropism for the assorted gastrointestinal cells [18]. On the other hand, Tir is more unique, because it is a protein receptor manufactured by the bacterial cell itself [35,38], which appears to be a distinctive trait of attaching-effacing bacteria.

During infection, Tir is synthesized in the bacterial cell and then translocates through the TTSS to the host epithelial cell [38-40]. After translocation, Tir inserts spontaneously into the plasma membrane and adopts a hairpin loop conformation as it is presented on the surface of the epithelial cell [38]. Once inserted, intimin, located on the surface of the bacterial cell, associates with Tir and causes a more exaggerated rearrangement of the host cytoskeleton. This rearrangement forms the characteristic pedestal of the A/E lesion and enables the bacterial cell to intimately adhere to the host cell. As more *E. coli* O157:H7 intimately adhere to host epithelial cells and form the A/E lesions, the bacteria begin to overwhelm the normal microflora of the intestinal tract and successfully colonize the host [40,41]. However, the exact means by which *E. coli* O157:H7 establishes and sustains colonization in the host remains elusive.

Once it has successfully colonized and established itself within the host, *E. coli* O157:H7 produces and releases its Stxs in the intestinal lumen [2,3]. The B subunits of the Stxs then bind to their corresponding Gb3 receptors on the surface of epithelial and, unlike the LEE-encoded effector proteins, to endothelial cells as well [2, 6]. The A subunit of the Stxs is then internalized into the host cell through the process of receptor-mediated endocytosis and transported to the Golgi apparatus where furin, a serine protease, cleaves the A subunit [3,6]. Next, the A subunit is shuttled to the endoplasmic reticulum where a critical disulfide bond is reduced, releasing the active fragment. The active fragment functions as an RNA N-glycosidase that selectively depurinates an adenine residue in the 28S rRNA of the large ribosomal subunit. This prohibits elongation factors 1 and 2 from binding to the ribosome and inhibits host cell protein synthesis, ultimately forcing the host cell to undergo apoptosis. In addition, it is believed that apoptosis results in the local symptoms of disease. Furthermore, the Stxs can translocate from intestinal epithelial cells into the bloodstream [2,3,6,19]. Here, the Stxs bind to the Gb3 receptors on glomerular endothelial cells. The Stxs injure the glomerular cells and cause platelets and fibrin to deposit within the glomeruli. Eventually, the deposits decrease renal filtration and lead to the acute kidney damage characteristic of HUS.

6. Food safety and its importance in preventing *E. coli* O157:H7 infection

Of the many beef products available to consumers, ground beef is by far the most popular product consumed in the United States [42-46]. However, ground beef is both historically and presently the most common vehicle of *E. coli* O157:H7 infection [24,25]. In fact, a risk assessment conducted in 2002 determined that 2.9% of ground beef packages were contaminated with *E. coli* O157:H7 [47]. Furthermore, in 2002, the United States Department of Agriculture (USDA) recalled approximately 140 tons of potentially contaminated ground beef [48]. Thus, infection with *E. coli* O157:H7 does not only affect the well-being of consumers; it also financially strains the food industry when recalls are issued [49]. In order to avert the consumer health and financial harm that arises from contaminated ground beef, a zero tolerance policy for *E. coli* O157:H7 is now enacted within the food industry [24].

Ground beef, as well as other beef products, typically become contaminated with *E. coli* O157:H7 at food processing plants [50]. Cattle are taken to processing plants where they are eventually slaughtered. The muscle and fat tissues of cattle are sterile in healthy animals, but the hides are not sterile and often harbor *E. coli* O157:H7 [24], which can be potentially transmitted to the sterile tissues during processing [50]. Additionally, the tissues can become contaminated if processing is performed improperly. For example, *E. coli* O157:H7 naturally colonizes the intestinal tracts of cattle, but if the viscera are nicked during slaughter, *E. coli* O157:H7 can spill into the meat of the carcass, leading to contamination. Furthermore, the meat may become contaminated in other instances such as from contacting animal

feces, from the plant environment itself, or from unsanitary employees at the processing plants. Once contaminated, the bacteria can be mixed more thoroughly into ground beef during the grounding process [48]. Moreover, modern production methods further spread the contamination by combining the meat from a large number of cattle into a single lot of ground beef [4]. The severity of contamination is, however, dependent upon the amount of intestinal colonization, fecal excretion, and the overall hygiene of employees [3]. Nonetheless, the necessity of eliminating or, at the very least, decreasing the amount of contamination is undeniable.

In an attempt to eliminate bacterial contamination, foods undergo rigorous processing steps. Common treatments include heating, freezing, or desiccating food products, subjecting them to osmotic or acidic shock, or combining the treatments into a type of medley [51-54]. Of these various treatments, acidic shock merits a more detailed description, because it is often the first treatment applied during food processing [50].

The application of organic acids is a common treatment in food processing [55], in which the organic acids can serve multiple roles [56]. For example, organic acids may function as food preservatives or as chemical treatments designed to curtail bacterial contamination during food processing. For reducing contamination of meat during processing, organic acids are applied to the surface of freshly slaughtered meat surfaces in an attempt to reduce the number of bacterial contaminants [57-60].

Of the many organic acids approved for use, lactic acid is commonly selected, because it is naturally associated with meat and is labeled Generally Regarded as Safe (GRAS) in the food industry [61]. Once applied, lactic acid creates a thin film on the surface of the meat that has a low pH and serves as a disinfectant. Here, the lactic acid enters the bacterial cells by crossing the nonpolar membranes in its undissociated form [62]. Once inside, the lactic acid begins to accumulate inside the cells where it lowers the intracellular pH and inhibits critical metabolic reactions [63]. However, although lactic acid is a suitable disinfectant, its effectiveness ultimately depends upon the amount of original contamination, the strength of bacterial adherence to the meat surface, and the ability to wash away bacteria from the meat [64-68].

Before food products are shipped to consumer markets, they must be inspected for contamination during quality control measures. Because of the importance of quality control, the methods used to detect bacterial contaminants must be sensitive and selective as well as versatile in order to accommodate the dynamic needs of the food processing plant [69]. Currently, there is a wide variety of methods available for quality control testing including traditional plating techniques, molecular-based techniques, and commercial alternatives.

Traditional plating techniques are useful to the food industry during quality control, because they are both cost-effective and familiar [69]. Specific techniques that fall into this category include the standard plate counts, agar overlays, agar underlays, and more classic microbiological approaches. In each technique, media, including agars and broths, serve as the foundation to the quality control procedure. The media selected are based on the pathogen of interest. For example, sorbitol-MacConkey (SMAC) agar is often used to detect *E. coli* O157:H7, as this is one of only a very few pathovars in this species unable to ferment sorbitol, appearing as colorless or transluscent colonies when other *E. coli* would exhibit red colonies from sorbitol fermentation. Traditional plating techniques, to this day, remain an integral aspect of quality control during food processing [70]; however, traditional platings often lack the sensitivity necessary to detect pathogens, especially in lower numbers [69]. Thus, molecular-based approaches are often employed when sensitivity is required.

Molecular-based techniques have the advantages of being both sensitive and comparatively rapid [69]. Techniques included in this category are PCR and DNA-based techniques, immunomagnetic separation, and enzyme-linked immunosorbent assays (ELISAs). Molecular-based techniques are distinctly advantageous because of their sensitivity, selectivity, and their rapid results. However, molecular-based techniques are appreciably more expensive than traditional plating techniques and are also more novel and unfamiliar. Therefore, the integration of molecular-based approaches into quality control procedures depends on the overall needs and resources of the food processing plant.

Finally, a number of commercial alternatives are currently available for detecting numerous bacterial species, including *E. coli* O157:H7. The commercial alternatives include chromogenic agars, dry plate counting products including Petrifilm, bioluminescent assays, manufactured kits [69], and ready-made Petri dishes including Easygel [71]. Commercial alternatives are beneficial, because they often contain all necessary reagents and are convenient. However, like all other techniques, commercial alternatives also have their own limitations including lack of versatility and relative expense [69].

7. Sublethally-injured *E. coli* O157:H7 and the value of selective agar overlays for detection

The harsh treatments applied during food processing kills the majority of bacteria present on the food products, but a small bacterial population may only be sublethally-injured [54,72-76]. Both Gram positive and Gram negative bacteria can be sublethally-injured [76] including *Staphylococcus aureus* [70] and *E. coli* O157:H7, respectively [73-76]. Sublethally-injured bacteria may become viable but nonculturable bacteria [76], which fail to grow and form colonies in selective media in which they normally can in an uninjured state. Sublethally-injured bacteria exhibit different

characteristics than do noninjured bacteria, including an increased susceptibility to organic acids, dyes, and selective media in general, an increased lag time, a compromised ability to divide, and an overall decrease in metabolic activity [76,77]. However, sublethally-injured bacteria are clearly differentiated from dead or dying cells by their gene expression activity and by their level of ATP. Furthermore, although they are nonculturable, sublethally-injured bacteria still sustain their pathogenicity [78-81]. Thus, the detection of sublethally-injured bacteria, including *E. coli* O157:H7, is critical to quality control procedures performed in the food processing industry [72].

Typical quality control measures involve inoculating selective media with food samples in order to detect a specific pathogen of interest, but sublethally-injured bacteria do not readily grow in the selective media and thereby evade detection [54,81]. Therein, the failure of the bacteria to grow in the selective media yields a false-negative result and underestimates the total number of sublethally-injured bacteria [51,81-83]. Therefore, a more sensitive method of detection is demanded.

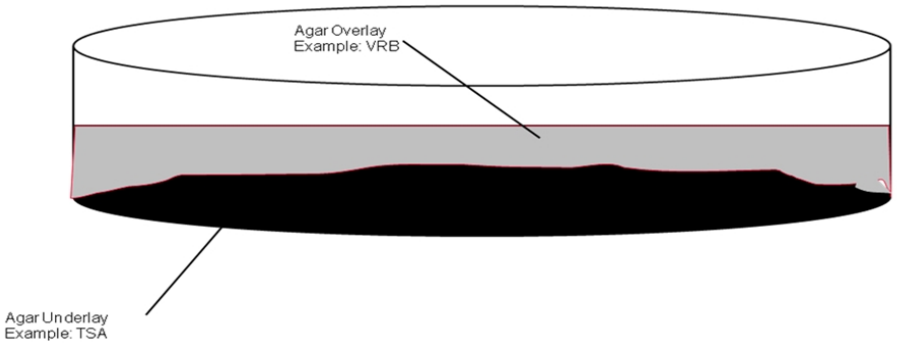

Figure 2: Illustration of a selective agar overlay. A nonselective agar, such as tryptic soy agar (TSA), is poured into a sterile Petri plate already containing an aliquot of bacteria. The agar is allowed to solidify, and the plate is incubated at a favorable temperature for 4 hours to accommodate bacterial resuscitation. This layer of media now serves as the basal layer. After the resuscitation period lapses, a layer of selective agar, such as violet red bile (VRB) agar, is applied on top of the basal layer, is allowed to solidify, and is incubated for 24 hours at the same favorable temperature. This upper layer of media now serves as the selective overlay. Over time, the components of the overlay diffuse into the nonselective basal layer, which either promotes or inhibits the growth of specific microorganisms.

One method available for detecting and enumerating sublethally-injured bacteria is the application of a selective agar overlay (Figure 2) [51,52,84]. This method is unique, because it incorporates a resuscitation period during which the sublethally-injured bacteria revive as well as a selective medium that identifies the pathogen of interest [52 69]. In practice, a bacterial population is first stressed with one of many treatments. Then, samples are serially diluted and allocated into sterile Petri dishes. A molten, nonselective agar, commonly tryptic soy agar (TSA), is poured into the Petri dishes and thoroughly mixed. The agar is allowed to solidify, and the plates are incubated under favorable conditions for a period of two to four hours. During this time, the sublethally-injured bacteria begin to repair themselves and to grow in the nutritive medium. Following the resuscitation period, a molten selective agar such as violet red bile (VRB) agar or sorbitol MacConkey (SMAC) agar is then overlaid onto the nonselective basal layer. The selective overlay now detects the pathogen of interest and simultaneously inhibits the growth of nontarget bacteria.

The selective agar overlay is proving to be a promising technique within the scientific literature. In separate studies, the significance and criticalness of the resuscitation period for sublethally-injured bacteria, including pathogenic *E. coli*, was clearly demonstrated [52,85]. Furthermore, Kang and Siragusa demonstrated that heat shocked populations of *Listeria monocytogenes*, *Salmonella typhimurium*, and even *E. coli* O157:H7 are detectable when using the related agar underlay technique [85]. Therefore, it stands to reason that the selective agar overlay may greatly contribute to the food industry through its detection of sublethally-injured *E. coli* O157:H7.

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