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# THE DETECTION OF SHIGA TOXIN-PRODUCING *Escherichia coli* (STEC) O157 IN MEAT: A SYSTEMATIC REVIEW

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Shiga toxin-producing *Escherichia coli* (STEC) bacteria, including the *Escherichia coli* O157, are described as pathogens producing toxin-like $Stx_1$ and $Stx_2$. These toxins become a significant virulence factor with a vital role in the outbreak of foodborne diseases worldwide. This study therefore aims to compile articles on STEC in several countries, using systematic review methods. **Method**: An online database on PubMed was accessed on 29th October, 2020, to carry out the review, using STEC, *E. coli* O157, PCR, $Stx_1$, and $Stx_2$ as the keywords. This review utilized only full articles, written in English, and published within the past five years. **Results**: According to the results, there are nine related articles on this topic. In addition, primary *E. coli* O157 was found to produce toxins and greatly influence the pathogen's meat detection. All STEC strains were positive for $Stx_1$, while PCR detected Stx2, and the whole-genome sequencing (WGS) provided forensic-level microbiological cross-contamination links from raw to ready-to-eat meat. Also, most of the articles showed cross-contamination occurred due to *E. coli* O157 bacteria's movement from the gastrointestinal tract to carcasses, and hygiene, as well as sanitation levels are the biggest causes of this contamination. **Conclusion**: *E. coli* O157 was identified not only on food like meat and spinach but also on non-food, including the environment. Improving hygiene and sanitation is a possible way to reduce the contamination of *E. coli* O157 on meat.

***Key Words***: *STEC, E. coli O157, PCR, $Stx_1$, $Stx_2$.*

## 1. INTRODUCTION

Foodborne disease is an illness caused by ingesting food contaminated with bacteria, viruses, parasites or toxins, and has become a public health concern around world. The main source of food-borne disease is food, especially livestock products, including beef. Beef is a foodstuff of livestock origin, with high nutritional value, due to the richness in proteins, fats, minerals, and other nutrients required by the body. The increase in meat production and consumption each year has created an awareness for the importance of maintaining the quality of fresh beef, and this quality is evident from the meat's microbiological properties.

Furthermore, the microbiological quality of meat is influenced by the nutritional value, as a factor supporting the growth of microorganisms, whether pathogenic or non-pathogenic. Microorganisms, including, *E. coli*, coliform, *Staphylococcus* sp., *Salmonella* sp. and *Pseudomonas*, were able to contaminate meat[8]. A harmful strain of *E. coli* bacteria with the ability to contaminate meat is *E. coli* O157. *E. coli* O157 is a pathogenic bacterium located in the digestive tract of ruminants, especially cows, and is able to cause food-borne disease[7]. These bacteria are transmitted in humans by the consumption of contaminated food or drink,

interpersonal contact, as well as contact with animal reservoirs [5].

In addition, E. *coli* O157 is a strain of bacteria with toxic gene as a virulent factor on the host's digestive tract, and shiga toxin is one of the main virulent factors[1]. Shiga toxin-producing E. coli (STEC) strain is a group of E. *coli* with the ability to cause serious illnesses, and even death. *E. coli* O157 have the most commonly reported history of causing hemolytic uremic syndrome (HUS) in the community. Shiga-like toxin (SLT) or Shiga toxin (Stx1 and Stx2) also cause other diseases, including hemorrhagic colitis (HC) and kidney failure, as well as death [2]. Therefore, reviewing the contamination by STEC-producing *E. coli* O157, from farm-to-fork, food safety and standard industries, is critical in addressing this issue. In this study, PCR (Polymerase chain reaction) method was used to detect the presence of toxic genes from *E.coli* O157, while WGS (Whole Genome Sequencing) analysis was used to prove the existence of epidemiological linkages between clinical cases, slaughterhouses, and suppliers[14]. The aim of this study is to explore and quantify the database articles on STEC O157 in food products, including meat, as well as to generate a recommendation of preventive measure from STEC O157, based on the outcome of this systematic review.

## 2. METHODS

This study used the systematic review method, a research method of identifying, evaluating, and inter-achieving all relevant research results related to certain research statements, specific topics, or phenomena of concern[10]. The characteristics of systematic boxing are, the possession of clear objectives with reproducible explicit methodologies, involves systematic searches aimed at identifying all studies with the eligibility criteria required by researchers, involve assessing the validity of the included studies' findings and present the characteristics of the findings of the included studies[6]. Meanwhile, the process sequence for conducting a systematic review are identifying a research question, establishing research data-base location as a search area, selecting relevant result, re-selecting results, and presenting the results. In this study, the articles were sourced from PubMed, and the inclusion criteria in the search for this data are the access to online publications, articles written in English, full articles, and published within the last five years, while the exclusion criteria were conference proceedings.

## 3.RESULT AND DISCUSSION

Based on the sequence of the systematic review with the key words STEC, *E. coli* O157, PCR, $Stx_1$, and $Stx_2$, within the inclusion and exclusion criteria, nine articles were obtained. According to these articles, there are numerous Shiga toxin detection methods used in different countries, with different outcomes. Detected virulence genes between O157 and O178 on the faces of western Canadian cattle[13]. The study used to conventional PCR to detect the presence of bacteria, and ddPCR to determine the average position of *E. coli* O157, O178, $Stx_1$ as well as $Stx_2$, and discovered O178 has a greater proportion of GEC, compared to O157. STEC non-O157 on beef, pork and chicken in Korea during 2006-2012, using PCR and PGEF, by observing the pathogenic and phylogenetic characteristic of non-O157 STEC[11].

Based on laboratory tests from fifteen recorded foodborne disease cases reported on 28 June and 19 July (2015) in north-eastern England, 13 were caused by STEC O157. Shiga-toxin producing *E. coli* O157 are responsible for HUS, HC, bloody-diarrhea, and other diseases. Conducted Whole Genome Sequencing (WGS) screening for STEC on lamb in north-eastern England during 2018 and the results provided the forensic microbiological cross contamination from farm to fork links between the farm, the butchers', and the clinical cases[14].

The incidents were caused by consuming food contaminated with STEC O157, including meat and other raw and ready-to-eat (RET) foods. Only one case was reported to be caused by buying raw food, while 6 cases were due to consuming raw food and the remaining 8 were caused by consuming RET food. The case is related to products purchased from butchers during this outbreak. Thus, contaminated meat products from the butchers assumed to be associated with the disease, as well as the incubation period for the cases, were included in the control case study.

The results of the detection of $Stx_1$ and $Stx_2$ with Multiplex real time assay on beef samples showed Multiplex real-time PCR assay is able to rapidly and simultaneously detect *E. coli* O157:H7, while screening for non-O157, effectively[11]. Meanwhile screened pre-harvest cattle faeces, using 16S rRNA gene with comperative analysis diversity, for the presence of O157 and non-O157 *E. coli* [4]. The experimental infection on bovine host with enterohaemorrhagic *E. coli* O157:H7 detected in bovine faeces with RNA-Seq, was used for transcriptome analysis of recto-anal junction tissue and ileal Peyer's patches. In this study, *E. coli* O157:H7 infection was detected by the presence of

the Shiga toxin (Stx), and the first infection detected were bit genes in tissue of recto-anal junction. Only 15 genes were with this method, compared to 1159 genes discovered using the ileal Peyer's patches. These data indicate the primary infection with EHEC possibly suppressed the immune function[9].

In Bangladesh, no official cases have been reported to be caused by STEC O157 as there are no means of surveillance for meat and meat products, but the cases are surely due to pathogen contamination in meat. Cattle are the major reservoir for *E. coli* O157, however, detected and isolated *E. coli* O157 from 42 samples of fresh goat meat. Subsequently, the isolates were screened for major virulence factors, including eaeA, rfbE, fliC, $Stx_1$, as well as $Stx_2$, and these were confirmed biochemically and serologically[15].

The rapid detection of *E. coli* O157 and Shiga toxins by Lateral Flow Immunoassay (LFIA) requires only a few minutes, exhibits color reaction to a specific target, is simple and does not require high costs for interpretation of results stated LFIA is suitable for detecting Stx and *E. coli* O157 in samples from various sources, including food and non-food material (water, environment), as well as any other screening for STEC[16].

The sensitivity and specificity of LFIA showed the highest result after being tested against positive and negative *E. coli* O157 strain (Table 2). Several commercial products are readily available for the identification of *E. coli* O157 and detection of both $Stx_1$ and $Stx_2$ Shiga toxins, as well as other toxins genes of bacterias, however, these products are rather expensive. LFIA is a suitable alternative for screening for *E. coli* O157 and detecting Shiga toxins. The outbreak of foodborne diseases is related to the consumption of undercooked food contaminated with STEC *E. coli* O157, and consequently, with increased virulance[16]. Study by identified *E. coli* O157 in contaminated beef carcasses, using Saltelli Global Analysis and used simulation modelling to reduce the contamination, based on the outcome of study. The outcome of the study was interaction inputs between gastro-intestinal contamination and carcassess, and this was used to improve the hygiene and sanitation of the slaughters. Based on the study, there were transfer and interaction between gastro-intestinal parts and *E. coli* O157, bound to affect the consumers [3]. The saltelli methods for global sensitify analysis are therefore suitable for identifiying the cross contamination and interaction of *E. coli* O157 between beef-harvest and carcasses, as well as other objects.

PCR is a common technology often used in the detection of non-pathogens or even pathogens for clinical, food, and environment samples because the method has enhanced result sensitivity and specificity. Furthermore, PCR methods are a commercial technology available for the detection of *E. coli* O157 for various genes target. According to the multiplex real-time PCR has successfully identified *E. coli* O157:H7 strains (n = 135). The Shiga toxin ($Stx_1$ and $Stx_2$) of these strains were found to be perfectly matched with the counterparts previously determined by conventional PCR as well as *uidA*-based real-time PCR methods, and no cross-reaction was observed from *E. coli* O157 and non-O157. Thus, the Multiplex real-time PCR provided better detection and efficiency for genes target. In addition to the Multiplex real-time PCR, a recently developed technology has been explored as a more efficient and comprehensive approach for STEC detection, and this is the whole-genome sequencing (WGS)[12].

The WGS is able to provide forensic-level microbiological support for the epidemiological links between the farm, the butchers' premises, and clinical cases cross-contaminated to each STEC outbreak. The biggest cross-contamination from the raw meat and ready-to-eat (RTE), at the butchers' premises. Hygiene and sanitation have the most important roles in stopping the cross-contamination in every aspect to reduce the outbreak of foodborne disease. Some control measures suggested to reduce the risk include accepting only clean and healthy cattle only for slaughter, obeying food safety management system based on Hazard Analysis and Critical Control Points (HAACP) at slaughter, cutting and boning, distribution, retail as well as catering levels, maintaining frozen storage, avoiding cross-contamination between raw and RTE food and, fully cooking minced beef products to a core temperature, to kill any bacteria present. Consuming uncooked meat is the major caused of foodborne diseases detrimental to human health, therefore food must be fully cooked to reduce the occurrence of these diseases[14].

The weakness of the systematic reviews is the provision of inadequate summaries on data-base (PubMed), with the keywords of the included articles on STEC O157. Furthermore, the selection of articles based on quality criteria is majorly undetected by the data-base, thus, there are other similiarities with other objects of the study, for instance, STEC O157 and non O157, or between meat and non meat, even in this study. Also, the assessment of study quality was majorly limited. However, the strengths of systematic review are the collection of overall knowledge about a certain topic, for instance, the detection of STCE O157 in meat. Scanning the literature effectively on the data-base using key words related to a certain topics, as well as the inclusion and exclusion criteria.

In the systematic review, the object as well as the inclusion and exclusion criteria was clearly stated by the reviewer. Also, the systematic review identified the best and bad practices, according the articles reviewed, thus simply using data and description to provide a lot imformation on the object to the reader.
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## 4. CONCLUSION

*E. coli* O157 was identified not only on food, for instance, meat and spinach but also on non-food, including the environment, faeces, water, and other surfaces. Currently, there are numerous technologies available for identifying and detecting STEC O157 ($Stx_1$ and $Stx_2$) on meat. People commonly used PCR and multiplex real-time PCR to obtain the highest sensitivity and specificity, with higher exploration, efficiency and comprehensiveness in approaching the genes targets, however, these require expensive product and materials. LFIA, a simple and affordable method was developed as an alternative to these technologies, however, LFIA performs the screening and generates results on a different level. The WGS is suitable for providing forensic-level microbiological support for the epidemiological links between the farm, the butchers' premises, and clinical cases cross-contaminated to each STEC outbreak. Furthermore, hygiene and sanitation play the most important roles in cutting cross-contamination, therefore by improving hygiene and sanitation, people are able to reduce the contamination of *E. coli* O157 on meat.

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