# Research Article



# A Review on Pathogenic Escherichia coli in Malaysia

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**Abstract** | Pathogenic / diarrheagenic *Escherichia coli* is a major foodborne pathogen worldwide, thus of great public health concern. These *E. coli* can be found in human, animals and environment, including soil and water. Infections caused by pathogenic *E. coli* may occur due to direct contact with infected animals and contaminated environment as well as consumption of contaminated or undercooked food and untreated water. This review highlights the occurrence of pathogenic *E. coli* in Malaysia.

**Keywords** | Pathogenic *E. coli*, *E. coli* O157, Diarrheagenic, Foodborne, Public health

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## **INTRODUCTION**

Poodborne illness is an unavoidable public health concern worldwide due to consumption of contaminated food (Jianghong and Carl, 2007). Over 250 bacterial species are reported to cause foodborne illnesses in humans among which *Escherichia coli* (*E. coli*) is considered to be the cause of most of these illnesses (Carlos et al., 2003). *Escherichia coli* is commonly found in the intestinal tract of human which colonises in the gastrointestinal tract of infants within few hours after birth and thus, deemed as one of the first facultative organism to colonise the human gut (Nataro and Kaper, 1998; Fanaro et al., 2003). Maturation of the bacteria however, took several of years and typically confined to the lumen of gut and to the external layer of the intestinal mucous (Mansan-Almeida et al., 2013). *E. coli* is said to be highly versatile, colonizing wide range of mammals as well as birds (Beauchamp and Sofos, 2010).

Escherichia coli is divided in to two types, pathogenic E. coli and non-pathogenic E. coli. The non-pathogenic strains of E. coli described as commensal E. coli are present in the normal microflora of intestine which are harmless, hin-

der the growth of harmful bacteria and produce vitamins (Nataro and Kaper, 1998; Beauchamp and Sofos, 2010). The pathogenic *E. coli* strains can be further classified into intestinal diarrheagenic *E. coli* which causes diarrhea and extraintestinal *E. coli* (ExPEC) which causes wide range of illnesses in humans such as the neonatal meningitis, chronic urinary tract infections, septicemia and hemolytic uremic syndrome (Nataro and Kaper, 1998; Chomvarin et al., 2005; Beauchamp and Sofos, 2010; Croxen and Finlay, 2010).

#### COMMENSAL E. COLI

In spite of the presence of highly diversified and complex microbiota in the gut, *E. coli* is highly adaptable to the gastrointestinal environment and play several important roles in humans, such as performing specific metabolic functions which are absent in humans, modulating the morphology and physiology in the gut as well as assisting in development of the immune system (Mansan-Almeida et al., 2013).

#### PATHOGENIC E. COLI

Intestinal diarrheagenic *E. coli* strains are known to be the



**Table 1:** Types, virulent factors and symptoms of Pathogenic *E. coli* 

Pathogenic E. coli Type	Virulence factors	Diseases and symptoms
Enterotoxigenic E.coli (ETEC)	st, lt, colonization factors (CFs), AAFs and cytotoxins	The most common cause of travellers' diarrhoea, infecting all age group, with mild to severe watery diarrhoea, usually without blood, mucus or pus, sometimes nausea may occur in certain patients, abdominal cramping and mild fever (Beauchamp and Sofos, 2010). Severe cases of diarrhoea in children especially under the age of five years may lead to mortality (Allocati et al., 2013). Besides humans, it is also an important <i>E. coli</i> strain which cause diarrheal disease in piglets as well as other newborn animals (Nataro and Kaper, 1998).
Enteropathogenic E. coli (EPEC)	eae, eaf, bfp, LEE and Intimin	Cause diarrhoea especially among children under poor hygienic conditions and transmission from animals. Fever and nausea may also occur in patients with EPEC infections (Kaper et al., 2004).
Enterohemorrhagic E.coli (EHEC)	Shiga toxins, Intimin, bfp eae, rbf O157, fiCH7	Cause a wide range of diseases such as bloody diarrhea, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Wani et al., 2003). The main target cells for its toxins are the endothelial cells of small arteries, kidney, brain and gastrointestinal mucosa (Mainil and Daube, 2005). Subsequently in the intestine, these toxins result in fluid leakage and or ulcerative lesions leading to hemorrhagic diarrhoea. The STEC is capable of causing chronic kidney damage leading to dialysis and hemorrhagic lesions represented by haemolytic syndrome which is also characterized by micro thrombus formation, thrombocytopenia and haemolytic anemia (Mainil and Daube, 2005).
Enteroinvasive <i>E.coli</i> (EIEC)	<i>Ial</i> , Shiga toxin, <i>Ipa</i> , hemolysin and Cellular invasion	Epidemiological studies indicated that EIEC is responsible for diarrhoea in children above the age of six years even though it may also occur in adults (Nataro and Kaper, 1998).
Enteroaggregative E.coli (EAEC)	AAFs, cytotoxins	EAEC strains have recently been identified as the second most frequent cause of travellers' diarrhoea associated with persistent diarrhoea in humans after ETEC in both developed and developing countries and recently acute diarrheal illness in newborns and children were observed (Croxen et al, 2010). Clinical features of EAEC include watery diarrhoea, sometimes accompany by bloody and mucus, with little to no vomiting along with low fever.
Diffusely Adherent E. coli (DAEC)	Daa, AIDA	DAEC usually infects children under the age of 1 to 5 years (Levine and Edelman, 1984; Nataro and Kaper, 1998). There were few clinical cases for study and most of DAEC patients recorded fecal leukocytes or watery diarrhoea without blood (Poitrineau et al., 1995).
Adherent invasive E. coli (AIEC)	Type 1 fimbriae, cellular invasion	Adherent invasive <i>E. coli</i> (AIEC) has been considered as one of the most important causative agent for Crohn's disease (CD), which when affect the small bowel cause inflammation and is known as inflammatory bowel disease (IBD). Unlike other pathogenic <i>E. coli</i> strains, AIEC pathotype does not express common virulence factors. Therefore, the invasive phenotype and its proinflammatory genetics is not fully understood (Nash et al., 2010). The most prevalent serogroups of AIEC are O6 and O22 with have a high variability of other O:H serotypes (Martinez-Medina and Garcia-Gil, 2014).

Notes: Bfp: Bundle-forming pili; LEE: Locus for enterocyte effacement; CFA: colonization factor antigen; HUS: haemolyticuraemic syndrome; AAF: aggregative adherence fimbria; Ipa: Invasion plasmid antigen; AIDA: adhesion involved in diffuse adherence; Daa: diffuse adhesin

Adapted from Nataro et al., (1998); Allocati et al., (2013) and Croxen et al., (2013).

to mortality among children especially under the age of the disease and presence of virulence factors, at least seven

major contributor of diarrheal diseases worldwide, leading 5 years (Croxen et al., 2013). Based on the mechanism of



classes of diarrheagenic *E. coli* are identified, namely, enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC) include *Shigella*, enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) (Beauchamp and Sofos, 2010; Jafari et al., 2012; Allocati et al., 2013) and the recently emerged, adherent invasive *E. coli* (AIEC) (Allocati et al., 2013; Martinez-Medina and Garcia-Gil, 2014) (Table 1).

Of all diarrheagenic *E. coli* identified, Shiga-toxin or Vero toxin producing (STEC/VTEC) EHEC is the most important pathotype in human diseases (Wani et al., 2003). There are many serotypes in STEC and among them, the EHEC serotype O157:H7 is found to be highly virulent, responsible for causing outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) around the globe. Ruminants are recognized as natural reservoir hosts for *E. coli* O157:H7 (Nataro and Kaper, 1998). No treatment has yet been found for the infections caused by EHEC (Goldwater and Bettelheim, 2012). The non-availability of treatment of EHEC imparts more attention towards the study of epidemiology, pathology and control measures in case of outbreak.

Pathogenic *E. coli* can be found in contaminated environment (water and soil) because they are being shed in the faeces of infected animals and humans. Contamination of animal products may be due to inappropriate practices during slaughtering and dressing process, especially from intestinal contents and faeces during evisceration (Bhunia, 2007).

#### EPIDEMIOLOGY OF PATHOGENIC E. COLI

The epidemiology of each pathogenic *E. coli* was reported to vary according to different species and strains of *E. coli*. The presence of these pathogenic *E. coli* was found in various animal reservoirs and spread within and as well as to other animals (Croxen et al., 2013). Numerous epidemiology studies carried out found that various factors contribute to the shift of prevalence based on different geographical areas, population, age distribution, socioeconomic class and detection methods (Ochoa et al., 2008).

The epidemiology of EHEC has been of a major focus and deem important among the researchers, especially on the detection of EHEC serotype, O157:H7, although other non-O157 strains are also major causes of many outbreaks in many regions including North America, Australia and Europe (Allos et al., 2004; Angulo, 2007). Due to the severity of infections caused by EHEC, surveillance and control measures had taken place such as the establishment of specific program called PulseNET, which was created to provide information necessary in case of sudden breakouts. Currently, PulseNET network is available internationally

except in sub-Saharan Africa (Swaminathan et al., 2001). Based on data presented in PulseNET, in 2011, there were 984 EHEC cases reported in the United States, in which, 463 were of O157:H7 serotype alone with fatality reported as well (Allos et al., 2004). In Canada, cases of O157:H7 has improved in 2010 as compared to 2005, although the overall reported EHEC cases were almost the same for the past 10 years (National Enteric Surveillance Program, 2010). Increase of EHEC incidence were reported in Australia from 2000 to 2010 (Vally et al., 2012) and in European countries, particularly in Ireland and Denmark, where there was also increase of EHEC incidence in 2009 (European Centre for Disease Prevention and Control and European Food Safety Authority, 2011). EHEC also occurred in other developing countries such as Argentina, which was found to have the highest incidence of HUS in children under 5 years of age (Rivero et al., 2010). On the other hand, the neighbouring country, Brazil, showed low incidence of HUS and cases of O157:H7 are rare (Irino et al., 2002). This may be associated with known risk factors such as contaminated meat consumption, playing in contaminated recreational water and poor personal hygiene (Bentancor et al., 2011). Although EHEC infections are detected in many developing countries, the widespread of EHEC still remain unclear due to lack of surveillance and clinical diagnosis especially in the sub-Saharan regions.

In Kenya, case studies by GEMS (Global Enteric Multicenter Study) indicated that EPEC significantly causes moderate to severe diarrhoea in children under the age of 2 years. Continuous studies later revealed that EPEC infections was not strongly related to causing moderate to severe diarrhoea, however, if present, it may increased the risk of death among newly born to 11 months old babies (Kotloff et al., 2013). In another case occurred in the United States, only 4 patients were reported due to EPEC infections (CDC, 2013). It was concluded that the occurrence of EPEC decreases with increase of age and that infections in adults are extremely rare (Nataro and Kaper, 1998). This phenomenon perhaps was associated to the loss of certain EPEC receptors with age or development of the immunity in humans (Nataro and Kaper, 1998). Previously, EPEC was thought to occur predominantly in industrialized countries, however, new findings indicate that EPEC was also reported in developing countries (Afset et al., 2003; Alikhani et al, 2006; Nguyen et al., 2006; Bakhshi et al., 2013). Although, in many countries, EPEC is consider as less important cause of diarrhoea, nonetheless, EPEC still poses serious health concern to children aged 2 and below and the epidemic of this pathogen may well persist in other parts of the world where such epidemiology studies had never been carried out and that the possibility of reemerged of this infection cannot be ruled out.

The outbreak of EIEC has always been under represent-

ed in epidemiology studies due to its less pathogenicity towards human as compared to other pathogenic E. coli pathotypes. Furthermore, due to its genetic, pathogenic and biochemical similarities to Shigella, it is always being misdiagnosed. A comprehensive overall picture on the epidemiology of EIEC is possible only with molecular detection method, targeting specific EIEC gene markers (Croxen et al., 2013). Thus, in the last decade, only a handful of cases was reported in Central and South America, Africa and Asia (Ratchtrachenchai et al., 2004; Okeke, 2009; Perez et al., 2010). The largest outbreak of EIEC was reported in 1985 which affected 370 people in Texas, United States (Gordillo et al., 1992). The epidemiology of Shigella was far more documented and is reported to associate with about 30% to 50% of bacillary dysentery cases worldwide (Pfeiffer et al., 2012).

Due to the limited surveillance implementation globally for all pathogenic E. coli pathogtypes, information on the incidence of EAEC is limited to certain parts of the world such as North and South America and Europe (Nataro et al., 2006). A large scale study carried out in the United States, revealed that EAEC was the most commonly found bacteria in the emergency departments and outpatients clinics of two large academic hospitals (Nataro et al., 2006). A significant large outbreak of EAEC was recorded almost a decade ago, in 1997, which occurred in a school lunch and affected 2,697 school children in Japan (Wanke et al., 1991). In that same year, approximately 15% of a village population in India was affected by EAEC. Subsequently, diarrheagenic EAEC cases was also reported in Mali (Boisen et al., 2011), Libya (Dow et al., 2006), sub-Saharan Africa (Kotloff et al., 2013) and Nigeria (Okeke et al., 2010). Over 900 patients developed HUS and later were found to be infected with hybrid pathogens which carried virulence genes from both EAEC and STEC (Mellmann et al., 2011; Estrada-Garcia and Navarro-Garcia, 2012).

Although ETEC is the primary cause of traveller's diarrhoea, cohort studies in Bangladesh, Argentina, Egypt and Guinea-Bissau, showed that ETEC is significantly associated to morbidity and mortality in children in developing countries (Viboud et al., 1999). Approximately 280 million of children aged 0 to 4 years were reported to experience ETEC induced diarrhoea (Wenneras and Erling, 2004). In many developed countries, diagnosis for ETEC is uncommon for patients presented with diarrhoea. However, it is the most common bacterial related to traveller's diarrhoea, constituting approximately 30% of such cases (Shah et al., 2009). The largest outbreak of ETEC occurred in 1998, in the state of Illinois, Unites States where approximately 3,300 people were believed to have become ill through consumptions of foods prepared by infected personnel (Beatty et al., 2006). Subsequent outbreaks were

also reported in Nevada (Jain et al., 2008) and Illinois (Yoder et al., 2006) from a sushi restaurant and a buffet style lunch respectively. Such outbreaks have also occurred in other developed countries such as in Denmark (Ethelberg et al., 2010), Japan (Konishi et al., 2011) and Israel (Huerta et al., 2000), in which the sources of infection were associated to contaminated water and foods.

The detection for DAEC strain still remains undeveloped and to date, there is no universal protocol for detection of DAEC in clinical laboratory, Hence, the epidemiology and occurrence pattern of DAEC is unclear. However, DAEC isolates were discovered from children with diarrhoea in many countries such as Chile (Levine et al., 1993), Mexico (Giron et al., 1991), Australia (Gunzburg et al., 1993) and the United Kingdom (Knutton et al., 2001). In Brazil, 23% of 1,801 E. coli isolates from 200 children with diarrhoea were identified as DAEC which suggest that it may be more widespread than previously thought (Gomes et al., 1998). One interesting finding from above mentioned studies revealed that DAEC was also identified in healthy individual of the same age control group, suggesting that DAEC may also present in health people without diarrhoea. Thus, there is an urgent need to develop detection methods in clinical setting to specifically differentiate and identified DAED strains accurately and also to discover its mode of transmission as well as responsible reservoir hosts. There are also limited data on the epidemiology of AIEC, however, it was suggested that AIEC is correlated with Crohn's disease (CD), which was evident in several clinical studies that found AIEC isolates in CD patients (Darfeuille-Michaud et al., 2004; Martin et al., 2004). With that, it is essential to carry out more clinical studies to further understand the transmission dynamics of AIEC globally, especially regarding its roles and connections with CD patients.

#### TRANSMISSION OF PATHOGENIC E. COLI

Animal faeces are considered to be the major source of pathogenic *E. coli*. The close contact among animals in the farms may lead to the transmission to other animals. (Karch et al., 2005). Animal wastes, sewages from farming operations, manure/slurries which are frequently used as fertilizers for the crops or silage preparation and cattle grazing also contribute to the infection and re-infection of cattle. (Jiang et al., 2002; Kudva et al., 1997).

The presence and sustainability of Shiga toxin-producing *E. coli* (STEC) in the soil favours its infections in cattle and their presence in the environment also pose risk for human infections (Gagliardi et al., 2002; Howie et al., 2003; Ogden et al., 2002).

Pathogenic E. coli may be found in carcasses meat of infected animals or contaminated by faeces of infected an-

imal through improper slaughtering and processing, and the unhygienic practices of workers in the farm can lead the pathogen to contaminate the milk during milking. Ingestion of raw or uncooked contaminated beef, drinking unpasteurized milk or untreated water can lead to infection by pathogenic *E. coli*, especially serotype O157 (Karch et al., 2005).

EPEC can be transferred through contaminated foods such as vegetables, cheese, tuna fish, potato, macaroni salads, untreated water and through toys, rubber nipples and fomites among children. EAEC can be transmitted through food. The method of transmission of DAEC is not yet identified (Jianghong and Carl, 2007).

Transmission of pathogenic *E. coli* infection from person to person may occur because of unhygienic measures. It can spread within community like families and close contacts through oral route especially among children (Karch, et al., 2005). An outbreak was reported to be caused by transmission of the disease from patients to their family members (Parry, 1998).

Many cases in children infected with pathogenic *E. coli* were due to their contact with the infected animals in the farms, petting zoos or environment contaminated with animal faeces. The infections can occur in persons and children who do not wash their hands (CDC, 2009).

Government regulatory agencies, environmentalists, beach managers and sewage operators are concern over the presence of *E. coli*. Proper risk assessment and control procedures are to be adopted for water control, as *E. coli* in water can originate from human and nonhuman sources, such as farms, wild animals, waterfowl, and pets (Harwood et al., 2000; Krumperman, 1983; Parveen et al., 1997).

## ISOLATION OF E. COLI O157

For qualitative analysis of E. coli O157, direct plating is frequently practiced which later on is improved by using immunomagnetic separation (IMS) technique. According to Šafařiková and Šafařik (2001), the IMS technique performed more sensitive detection of specific microorganism in comparison with direct plating. Small portion of samples can also be sensitively identified on selective media such as Sorbitol MacConkey with Cefixime Tollurite (CT-SMAC), followed by IMS (LeJeune et al., 2006; Khanjar and Alwan, 2014; Dodd et al., 2003; Chapman et al., 1994; LeJeune et al., 2004; Omisakin et al., 2003). The PCR is a sensitive technique for the characterization of different isolates and it can also differentiate pathogenic from non-pathogenic *E. coli*. The pathotypes of *E. coli* can be differentiated by the PCR based upon the existence of virulence genes available in each pathotype. Specific set of primers in the PCR can be used to amplify the virulent

genes (Kalnauwakul et al., 2007). PCR has been used commonly for the epidemiological investigation of pathogenic E. coli infections worldwide (Olsvik et al., 1991). A number of studies showed the use of multiplex PCR (m-PCR) for the detection of pathogenic *E. coli* (Wani et al., 2003; Wani et al., 2005; Kalnanwakul et al., 2007; Fagan et al., 1999). Watterworth, (2005) was able to design an m-PCR by using six sets of specific primers for the detection of four different pathotypes of pathogenic E. coli which were lt and st for ETEC, eaf for EPEC, stx, and stx, for STEC and ial for EIEC. Multiplex PCR is also able to differentiate between pathogenic *E. coli* and other enteric bacteria. Osek, (2001) also designed an m-PCR to differentiate between the ETEC pathotype and other gram negative bacteria using specific primers for the detection of heat stable (st) and heat labile (lt) genes of the ETEC pathotype. Chang et al., (2013) targeted rfbO157 and fliCH7 for the detection of E. coli O157:H7.

OCCURRENCE OF PATHOGENIC *E. coli* WORLDWIDE The occurrence of pathogenic *E. coli* is worldwide is tabulated in Table 2.

Occurrence of Pathogenic *E. coli* in Malaysia Among the pathogenic *E. coli*, the EHEC serotype O157:H7 is of utmost importance due to its serious implications in humans and the increasing reported occurrence in many regions around the globe (Willshaw et al., 1994; Dundas et al., 2001; Effler et al., 2001) specifically in United States and Japan (Rangel et al., 2005; Muto et al., 2008). Thus, most studies on *E. coli* carried in Malaysia, focused mainly on the serotype O157:H7. In Malaysia, the food-borne bacteria such as *Salmonella*, *Listeria*, *Staphylococcus*, *Campylobacter* and *E. coli*, were isolated from animal and animal products (Adzitey et al., 2012; Saleha, 2002;

Table 3 shows some studies conducted on prevalence of *E. coli* and its pathogenic strains among different samples in Malaysia.

# **CONCLUSION**

Arumugaswamy et al., 1995).

There is a high occurrence of *E. coli* in human, animals and their environment. Beef in the markets and milk from cows were also highly prevalent with *E. coli*. The high occurrence of *E. coli* in human, animals and their environment may have occurred because of contaminated environment and by cross-contamination from other animals. Farm management practice, market and stall conditions, environmental factors and workers personal hygiene play an important role in microbial contaminations. This study may serve as a template to investigate the role of human, animal and environmental factors in contamination of *E. coli* and other microbes relevant to food safety.



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Table 2: Occurrence of pathogenic *E. coli* worldwide

Table 2: Occurrence of		
Author	Country	Findings
Hancock et al. (1998)	United States	Samples were collected at 12 cattle. <i>E. coli</i> O157 was isolated from cattle faeces at 2.9% (25/2143), dairy cattle 2.3% (25/1097), equine fecal samples 1.1% (1/90), canine faeces 3.1% (2/65), pooled bird samples 0.5% (1/200), fly samples 3.3% (2/60), and water-trough sample 3.1% (10/320). No <i>E. coli</i> O157 were isolated among rodents (300), cats (33) and assorted wildlife (34).
Tutenel et al. (2002)	Poland	551 cattle faeces samples were collected at beef cattle slaughter house and 0.72% (4) of faecal samples were positive for EHEC O157. All positive samples collected belongs to cattle younger than 2 years.
Blanco et al. (2003)	Spain	A total of 1,300 faecal swabs were collected from healthy lambs. STEC O157:H7 strains were detected at 0.4% (5) and for non-O157 STEC strains at 36% (462).
Omisakin et al. (2003)	United King- dom	589 cattle faeces (rectum) of the slaughtered cattle were collected at abattoirs for identification of <i>E. coli</i> O157, 7.5% (44) were positive for <i>E. coli</i> O157. All the isolates possessed <i>vf2</i> gene, 5 had <i>vf1</i> gene while 39 had <i>eaeA</i> gene.
Berg et al. (2004)	Canada	569 faeces samples of barley and corn fed cattle were examined by both IMS and direct plating techniques for the detection of <i>E. coli</i> O157. 7.4% (42) were positive for <i>E. coli</i> O157 by IMS while 3.3% (19) were positive for <i>E. coli</i> O157 using the direct plating procedure. 225 samples collected from hides of barley-fed cattle, only 3 (1.33%) were positive for <i>E. coli</i> O157:H7 while only 1 (0.44%) of 225 samples collected from the corn fed cattle was positive for <i>E. coli</i> O157:H7.
Fluckey et al. (2007)	United States	60 samples were collected including faeces, hides and carcasses, 20 in each of three separate trial periods. <i>E. coli</i> ranged from 98 to 55%. Highest <i>E. coli</i> presence was recorded from at preevisceration carcass samples at 40.4%.
Nanu et al. (2007)	India	240 raw milk samples were collected from three (3) farmer societies. <i>E. coli</i> was detected in 31.6% (76) of raw milk samples while <i>Staphylococcus aureus</i> at 35% (84). Most of the <i>E. coli</i> isolates were consisted of serotypes 05, 024, 025, 068, 084, 087, 0103, 0116, 0125, 0145, 0157 and 0172.
Ateba and Mbewe, (2011)	South Africa	Among 220 samples collected, the prevalence of <i>E. coli</i> O157:H7 in pork meat, cattle and beef, and water samples was reported at 67.7% (88), 27.7% (36) and 2.3% (3), respectively.
Hajian et al. (2011)	Iran	484 raw meat samples were collected from cattle, camel, sheep, goat, chicken and minced beef. 4.8% (23) samples were positive for <i>E. coli</i> O157. The highest prevalence of <i>E. coli</i> O157 was found in beef minced meat at 11.1% (13/117), followed by beef meat 8.9% (8/90), goat meat 1.7% (1/60) and camel meat 1.3% (1/75).
Chowdhuri et al. (2011)	Bangladesh	Seven (7) different brand types of poultry feed samples were collected from different poultry farm and poultry markets. <i>E. coli</i> were found in 57.14% (4) samples.
Awadallah et al. (2013)	Egypt	400 cloacal swabs, 100 each of wild birds including quails, doves, sparrows and cattle egrets, and 150 stool samples of diarrheic and non-diarrheic humans, were collected. <i>E. coli</i> was isolated at 48% while <i>Salmonellae</i> at 10.75%. The individual prevalence of <i>E. coli</i> among quails was 47%, doves 49%, sparrows 13.2% and cattle egrets 43.6% while <i>E. coli</i> was detected among 56% in humans stool samples.
Hossain et al. (2014)	Bangladesh	100 diarrheic faecal samples were collected from cattle calves. 49 (49%) were positive for <i>E. coli</i> . The antibiogram study revealed that the isolates were 100% sensitive to tetracycline and gentamicin, which is the drug of choice for the treatment of diarrheagenic <i>E. coli</i> in calves.
Mainda et al. (2015)	Zambia	376 cattle faeces samples were collected from 104 dairy farms. <i>E. coli</i> isolates were detected in 98.67% (371) of the sampled animals.
Tanih et al. (2015)	South Africa	176 swab samples from cattle (28) and pigs (16) were collected including samples from rump, flank, brisket, and neck of the animals. 104 (67.5%) samples were positive for <i>E. coli</i> and 50 (32.5%) for <i>S. aureus</i> . Among the total <i>E. coli</i> positive, 14 (13.46%) were observed to be pathogenic strains including enteropathogenic <i>E. coli</i> at 1.9%, enterotoxigenic <i>E. coli</i> (3.8%) and enteroaggregative <i>E. coli</i> (7.6%). All the isolates were find resistant to vancomycin and bacitracin.





OPEN ACCESS

Table 3: Prevalence of *E. coli* and its pathogenic strains among different samples in Malaysia.

Author	Sample Type	Findings	
Radu et al. (1998)	Beef	Beef samples from 25 retail stores were collected, 36% (9) were <i>E. coli</i> O157:H7 positive showing 12 different strains, identified as <i>Shiga toxin</i> 2 baring <i>eae</i> gene and had a plasmid size of 60-MDa.	
Radu et al. (2001)	Beef and chicken meat burger	28 samples were collected from tenderloin beef (25) and chicken meat burger (3), 25 and 3 strains of <i>Escherichia coli</i> O157:H7 were detected in tenderloin beef and chicken meat burger respectively. All the strains carried <i>Xba</i> I genes. All the isolates showed resistance to one or more than three antibiotics among the 14 antibiotics tested.	
Chang, (2003)	Raw beef	88 raw beef samples were collected from markets in Sarawak and Sabah, East Malaysia. 1.1% (5) isolates were <i>E. coli</i> 0157: H7 (Shiga-like toxin producing) positive, 2.3% (4) were non Shiga-like toxin producing <i>E. coli</i> 0157 while 2.3% (2) were non-0157 Shiga-like toxin producing <i>E. coli</i> . The prevalence of <i>E. coli</i> 0157: H7 and STEC in East Malaysia were found to have a link with the location, with 54.5% (6/11) isolated from locations situated in the central region of Sarawak. The STEC 0157: H7 isolates were detected only in frozen imported beef whereas non-0157 STEC in local beef samples.	
Zaliha and Rusli, (2004)	Ducks intestines, wash water, faeces and soil in duck farms and wet markets	Samples collected from ducks intestines, wash water, faeces and soil in duck farms and wet markets showed presence of <i>E. coli</i> at 82%, 50%, 88% and 72% respectively with an overall occurrence of 79% of <i>E. coli</i> while among total samples 29% were <i>E. coli</i> 0157:H7 positive.	
Chye et al. (2004)	Raw cattle milk	930 raw cattle milk samples were collected from 360 randomly sampled dairy milk farmers in Peninsular Malaysia. <i>E. coli</i> was positive in 64.5% (600) samples while 33.5% (312) milk samples were <i>E. coli</i> O157:H7 positive.	
Apun et al. (2006)	Raw beef	Molecular subtyping by pulsed-field gel electrophoresis (PFGE) was performed on 51 previously isolated <i>E. coli</i> isolates from raw beef marketed in Sarawak and Sabah, East Malaysia. <i>E. coli</i> O157:H7 was identified at 9.8% (5), <i>E. coli</i> O157 at 7.8% (4), non-O157 (STEC) at 3.9% (2) while other <i>E. coli</i> isolates (non-STEC) at 78.4% (40).	
Nazmul et al. (2008)	Diarrheic children	It was revealed that 30 confirmed isolates of enteropathogenic <i>Escherichia coli</i> (EPEC) isolated from diarrheic children (Miri hospital, Sarawak, Malaysia, 2003) were carrying verotoxin (VT) gene. 33% (10) of the isolates carried VT1 gene while none carried VT2 gene.	
Sahilah et al. (2010)	Beef	Twenty (20) bacterial strains isolated from beef samples were obtained from laboratory of Food Science and Biotechnology, Universiti Putra Malaysia, Serdang, Selangor. 76% (14) bacterial strains showed presence of <i>E. coli</i> 0157:H7 which were previously collected from four different supermarkets in Selangor and the Federal Territory of Malaysia.	
Sukhumungoon et al. (2011)	Beef	67 beef samples were purchased from local markets in Hat Yai City, Southern Thailand. 25.8% (8/31) of the Malaysian beef samples were <i>E. coli</i> O157:H7 positive showing fourteen strains of <i>E. coli</i> O157:H7 while 11.1% (4/36) of Thai beef samples were <i>E. coli</i> O157:H7 positive showing six strains of <i>E. coli</i> O157:H7. Same pattern of antibiotic resistance against one or more than three antibiotics was observed at 38.5% and 33.3% among Malaysian and Thai isolated strains respectively.	
Lye et al. (2013)	Raw milk	177 raw milk samples were collected from 3 local dairy farms in the state of Selangor, Malaysia. The samples include raw milk collected from cow, goat and buffalo. The highest prevalence of <i>E. coli</i> O157:H7 was detected in raw cow milk at 8.75% (7/80), followed by raw goat milk at 7.32% (3/41) while the lowest prevalence was detected in raw buffalo milk at 1.79% (1/56).	
Chang et al. (2013)	Chicken meat, four-winged bean, tomato, cucumber, white reddish, lettuce, Chinese cabbage and red cabbage	230 samples were randomly collected from two different supermarkets and two organic groceries in Selangor, Malaysia. The samples include, chicken meat (20) and different organic vegetables which were four-winged bean (30), tomato (30), cucumber (30), white reddish (30), lettuce (30), Chinese cabbage (30) and red cabbage (30). <i>E. coli</i> O157:H7 was identified in 5.2% (12) of total organic samples among which the prevalence of <i>E. coli</i> O157: H7 in groceries were higher 8.8% (11/125) in comparison to supermarkets 1.0% (1/105). The highest prevalence of <i>E. coli</i> O157: H7 was detected in chicken meat at 40% (8), followed by four-winged bean at 10% (3) and white radish at 3.3% (1).	

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	Ho et al. (2013)	Nasal swabs, rectal swabs and tongue swabs from pigs	67.5% (345) <i>E. coli</i> isolates were observed in nasal swabs (57), rectal swabs (202) and tongue swabs (86) out of total 511 presumptive <i>E. coli</i> isolates from 110 pigs in 6 pig farms. 2% (7) <i>E. coli</i> positive isolates were positive for verotoxin (VT) while none were positive for LT1, LT2, ST and <i>eaeA</i> genes.
	Bilung et al. (2014)	Cloacal swabs from wildlide hosts in- cluding bats, birds and rodents	Cloacal swabs from 682 wildlife hosts including bats (308), birds (313) and rodents (61) were collected from Sibu and Kapit region of Sarawak, Malaysia for screening of <i>E. coli</i> and <i>E. coli</i> O157:H7. 106 and 259 isolates of <i>E. coli</i> were isolated from wildlife collected from Sibu and Kapit respectively. The overall occurrence rates of <i>E. coli</i> among these hosts were 14% (42), 17% (54) and 54% (33) for bats, birds and rodents, respectively. Isolated <i>E. coli</i> were then screened for <i>E. coli</i> O157:H7 by using a multiplex PCR with four primer pairs <i>slt</i> -I and <i>slt</i> -II, <i>rfb</i> E and fliCH7. Only 3.3% (23) isolates were encoded with fliCH7. It was concluded that the wildlife from different habitats in Sibu and Kapit, Sarawak, Malaysia is free from <i>E. coli</i> O157:H7.
	Ghaderpour et al. (2015)	Water and sedi- ment from rivers	Water and sediment samples were collected from 64 different stations particularly the Sangga Besar, Sepetang and Selinsing Rivers, located in the northwest coast of peninsular Malaysia. <i>E. coli</i> was detected in 85% (148) out of 175 presumptive <i>E. coli</i> isolates obtained from water and sediment samples collected from 8 different stations. All samples were negative for EPEC, EHEC, ETEC, DAEC and EIEC while 2 isolates were identified to be EAEC. 20 (14%) <i>E. coli</i> isolates were observed resistant to all the 15 antibiotics tested.
	Perera et al. (2015)	Faeces of cattle, buffalo, sheep and goat	4% (6/136) ruminant faeces including cattle (96), buffalo (20), sheep (8) and goat (12) samples collected from 6 different farms in Peninsular Malaysia, were STEC O157:H7 positive and all isolates carried 2, eaeA, ebxA and fliC. 1.5% (2) of faecal samples were non-O157 STEC positive carrying a, 2a, 2c, and ebxA. All 6 STEC isolates were positive for the virulence factors stx2, eaeA, and ebxA and also for fliC specific for the H7 antigen indicating they belong to the O157:H7 genotype.
	Cheah et al. (2015)	Food samples including beef, buf- falo meat, chicken, lamb, pegaga, selom, ulam raja, tenggek burung and belacan	176 Escherichia coli isolates were detected in different food sources sampled, including cattle beef (61), buffalo meat (28), chicken (18), lamb (18), pegaga (17), selom (17), ulam raja (11), tenggek burung (5) and belacan (1) collected in Selangor, Malaysia. 47.7% (84) isolates were <i>E. coli</i> O157 positive.

#### **CONFLICT OF INTEREST**

There is no conflict of interest in this review to declare.

## **AUTHORS CONTRIBUTIONS**

All the authors contributed equally for plan of review, article collection and manuscript writing.

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