



Research article

Livestock and environment as potential sources and reservoirs for multi-drug resistant *Escherichia coli* in Malaysia: A Systematic Review

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Abstract

Pathogenic *Escherichia coli* (*E. coli*) is a significant foodborne pathogen that poses a serious global public health risk. These pathogenic strains of *E. coli* can be found in various environments such as soil, water, livestock, livestock products, and humans. This review aims to evaluate the role of livestock and the environment as potential sources and reservoirs of pathogenic *E. coli* in Malaysia over the past two decades. A systematic search was conducted on PubMed, Scopus, Web of Science, and Google Scholar databases using standardized keywords (*Escherichia coli*, Livestock, Abattoir workers, Farm workers, and Malaysia) to identify relevant original research articles published between 2001 and 2023 and our systematic review protocol was published in Prospero (Registration number: CRD42023433199). A total of 20 eligible articles were included in this review. The prevalence of multi-drug resistant *E. coli* among livestock production system and the environment, ranged from 4% to 100%. *E. coli* strains isolated from livestock production systems and environment in Malaysia exhibited high potential for pathogenicity, with diverse enterotoxins, genes associated with antimicrobial resistance, and virulence factors. Phylogroup B and D were noted among the *E. coli* isolates indicating that the strains in Malaysia are highly virulent and extra-intestinal. High levels of resistance to antimicrobials particularly ESBL and colistin which are last resort antimicrobials were noted. Butchering or processing of livestock, contamination of plants via contaminated waterways or livestock manure are the main sources and reservoir for *E. coli* to spread in Malaysia. Continued surveillance, monitoring and a comprehensive 'One Health' approach are recommended to effectively prevent and control livestock drug-resistant *E. coli* strains throughout the country.

Keywords: Antimicrobial resistance, *Escherichia coli*, Livestock, One health

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Article history; received manuscript: 7 July 2023,
revised manuscript: 6 August 2023,
accepted manuscript: 15 September 2023,
published online: 17 October 2023

Academic editor: Korakot Nganvongpanit

INTRODUCTION

Foodborne illnesses resulting from the consumption of contaminated food are significant public health concerns worldwide (Simjee, 2007). *Escherichia coli* (*E. coli*) holds a unique position in the microbial world as it can cause life-threatening illnesses in animals and humans while also comprising a significant portion of the autochthonous microbiota in various hosts (Santos et al., 2020). This bacterium possesses remarkable adaptability, allowing it to persist for extended periods without growth and survive in diverse ecological niches. Such adaptability is attributed, in part, to the acquisition of virulence genes through horizontal transfer of pathogenicity factors, plasmids, bacteriophages, and transposons (Johnson and Russo, 2018).

E. coli can be classified into two distinct categories: pathogenic and non-pathogenic strains. Commensal strains of *E. coli*, which are harmless, aid in preventing the growth of harmful bacteria, synthesize vitamins, and constitute the normal microflora of the intestine (Beauchamp and Sofos, 2010). The pathogenic strains of *E. coli* can be further divided into two main groups: those causing diarrhea, also known as intestinal *E. coli*, and those causing extra-intestinal infections. Six distinct classes of pathogenic diarrhea-genic *E. coli* have been identified based on their epidemiological and clinical characteristics, specific virulence factors, enterotoxin production, and adherence phenotypes. These classes include entero-pathogenic *E. coli* (EPEC), entero-aggregative *E. coli* (EAEC), entero-hemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC), entero-invasive *E. coli* (EIEC), entero-toxigenic *E. coli* (ETEC), and diffuse-adhering *E. coli* (DAEC) (von Mentzer et al., 2014; Baumann et al., 2018).

Livestock products are significant sources of protein in Malaysia. However, due to potential contamination during the various stages of handling from farm to table, these products can serve as vehicles for the transmission of foodborne pathogens (Yusuf et al., 2020). *E. coli* is a dangerous pathogen and one of the leading contributors to global foodborne illnesses (Aklilu et al., 2021). Food poisoning caused by entero-toxigenic strains of *E. coli* producing enterotoxins is a common colibacillosis-related foodborne illness (Devan et al., 2022). Examining the presence of this pathogen in livestock production systems is crucial to ensuring food safety and protecting public health. Therefore, the objective of this literature review was to determine the prevalence of pathogenic and antimicrobial resistant *E. coli* from livestock and to evaluate the potential of livestock production systems and environment in Malaysia as sources and reservoirs of pathogenic *E. coli* that may cause several infections in humans.

MATERIALS AND METHODS

Information sources and search strategies

According to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, this systematic review was carried out and reported (Moher et al., 2015). However, a search protocol was designed and registered on the International Prospective Register of Systematic Reviews (registration number: CRD42023433199). This registration guaranteed that no other systematic review on the same topic in Malaysia is registered.

To find relevant papers published between 1 January 2001 and May 2023, systematic searches of PubMed, Scopus, Web of Science and Google Scholar databases were conducted. Boolean operators (AND, OR) were used to find studies using the keywords: Prevalence, Occurrence, Epidemiology, *Escherichia coli*, Livestock, Poultry, Meat, Dairy cattle, Cattle, Goat, Birds, Pigs, Swine, Pork, Abattoir, Slaughterhouse, Abattoir workers, Farm workers, and Malaysia in the titles, abstracts. Additional studies were looked for in the identified studies reference lists for possible inclusion in the review.

Eligibility criteria

To determine eligibility, the titles and abstracts of the identified studies were screened and evaluated using the following inclusion criteria: any study design except for experimental studies, English language, full-text journal articles published between 2001 and 2023, and studies conducted in Malaysia. Studies were excluded if they did not mention the sample source of the isolates or if *E. coli* was not specifically mentioned as one of the isolates.

Study quality

The quality and reporting bias of the eligible studies were assessed using the McMaster critical evaluation methods for quantitative and qualitative studies. The evaluation considered the year of the study, study purpose, study design, study location, animal type, sample type, and the method of detection (Ducat and Kumar, 2015). This was done by two independent reviewers and discrepancies were resolved through discussion.

Data extraction process

Whenever possible, the following details were extracted from the studies: authors, study design, study location, proportion of *E. coli* isolates, sample type, detection method used, and information on virulence genes, resistance genes, antimicrobial susceptibility profiles, and genotypes of the *E. coli* isolates. These factors were considered important in the data extraction process. Two independent reviewers were employed for this stage and discrepancies were settled by employment of a third independent reviewer through discussion.

RESULTS

Characteristics of eligible studies

For the purposes of this review, a total of twenty publications (Figure 1) from Malaysia were found pertinent. All of the articles (twenty) reported studies from either livestock, livestock products, or environmental source. Most of the studies reported more than one source of sample, and sampling location.

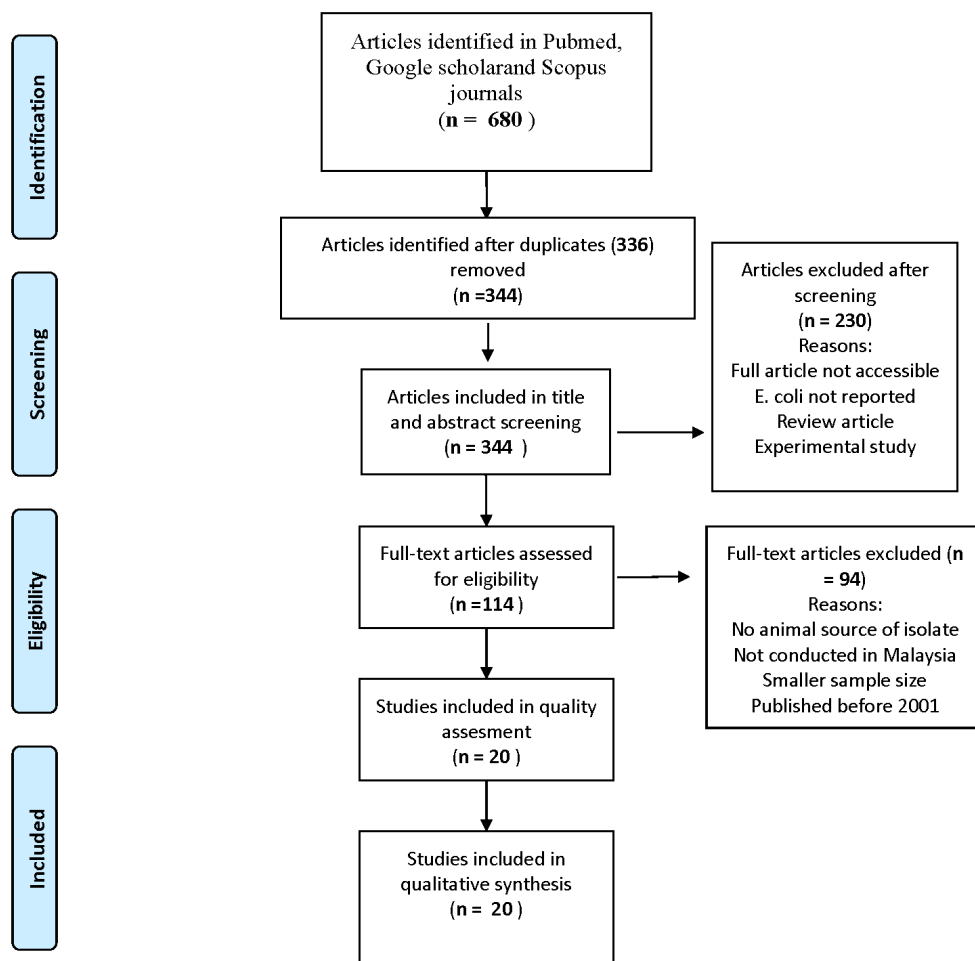


Figure 1 Flow diagram of the study selection process for inclusion in the review

Distribution of percentage prevalence *E. coli* among different States in Malaysia

Twenty studies were considered for this systematic review across all the thirteen States in Malaysia (Figure 2). Geographic distribution of *E. coli* occurrence in Malaysia was conducted in this review. Two studies reported peninsular Malaysia as their sampling location. Kelantan state has the highest number of studies been conducted (seven), followed by Selangor (six), three studies were reported in Pinang, two studies each were reported in Teranganu and Perak while only one study was reported in Sabah and Sarawak States of Malaysia with varying prevalence, virulence factors and antimicrobial resistance (Table 1). Notably, not all of the States reported virulence factor and antimicrobial resistance.

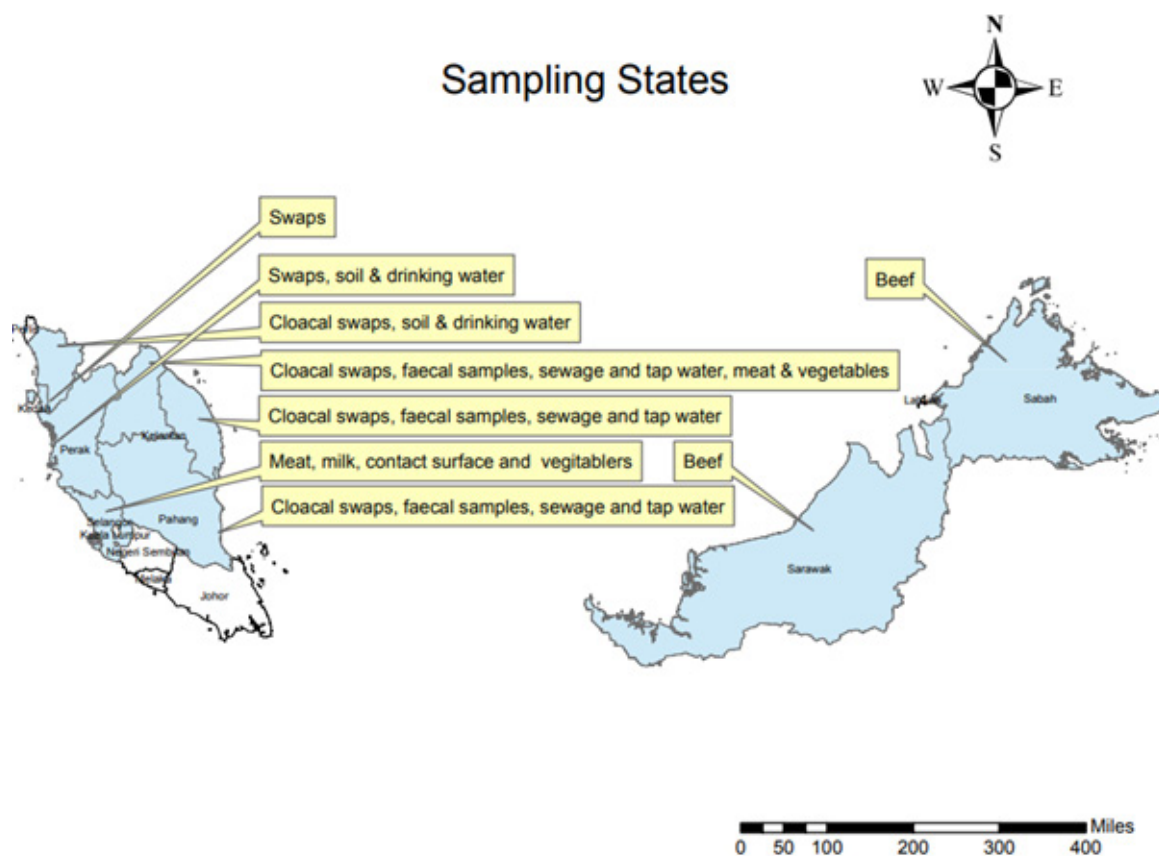


Figure 2 Geographic distribution of studies conducted in Malaysia showing type of samples in each State.

Table 1 Occurrence of pathogenic *E. coli* in Malaysia

No	Author(s), year	Sampling State(s)	Animal/Source	Sample type	Method of detection	% Prevalence	Virulence genes	Resistance genes	Remark
1	(Radu et al., 2001)	Selangor	Cattle and Poultry	Beef and chicken meat	Phenotypic & Molecular	28/28 (100)	-	-	<i>E. coli</i> O157:H7
2	(Chye et al., 2004)	Peninsular Malaysia	Cattle	Raw Milk	Phenotypic	312/930 (33.5)	-	-	<i>E. coli</i> O157:H7
3	(Apun et al., 2006)	Sarawak & Sabah	Cattle	Raw beef	Serology & Molecular	5/51 (9.8)	-	-	<i>E. coli</i> O157:H7
4	(Sahilah et al., 2010)	Selangor	Cattle	Beef	Phenotypic & Molecular	(76)	<i>Stx1</i> , <i>Stx2</i>	-	<i>E. coli</i> O157:H7
5	(Sukhumungoon et al., 2011)		Cattle	Beef	Serology	8/31 (25.8)	<i>Stx1</i> , <i>Stx2</i> & <i>eaeA</i>	-	<i>E. coli</i> O157:H7
6	(Lye et al., 2013)	Selangor	Cattle	Raw milk	Molecular	11/177 (6.21)	<i>Rfb0157</i> , <i>flicH</i>	-	<i>E. coli</i> O157:H7
7	(Chang et al., 2013)	Selangor	Poultry and Environment	Chicken meat, four-winged bean, tomato, cucumber, white reddish, lettuce, Chinese cabbage and red cabbage	Phenotypic & Molecular	-	<i>Rfb0157</i> , <i>flicH</i>	-	<i>E. coli</i> O157:H7
8	(Ho et al., 2013)	Perak & penang	Pig	Nasal swabs, rectal swabs and tongue swabs from pigs	Phenotypic & Molecular	345/511 (67.5)	VTEC	-	<i>E. coli</i> O157:H7
9	(Ghaderpour et al., 2015)	Matang mangrove forest	Environmental	Water and Sediment	Phenotypic & Molecular	148/175 (85)	-	-	<i>E. coli</i> O157:H7
10	(Perera et al., 2015)	Peninsular Malaysia	Ruminant	Faeces of cattle, buffalo, sheep and goat	Phenotypic & Molecular	6/136 (4)	<i>eaeA</i> , <i>ehxA</i> and <i>Stx2</i>	-	<i>E. coli</i> O157:H7
11	(Cheah et al., 2015)	Selangor	Cattle and Poultry	Food samples including beef, buffalo meat, chicken, lamb, pegaga, selom, ulam raja, tenggek burung and belacan	Phenotypic & Molecular	84/176 (47.7)	<i>eaeA</i> , inV, <i>VT1</i> , <i>VT2</i> , LT, ST	-	<i>E. coli</i> O157:H7

Table 1 Occurrence of pathogenic *E. coli* in Malaysia (Cont.)

No	Author(s), year	Sampling State(s)	Animal/ Source	Sample type	Method of detection	% Prevalence	Virulence genes	Resistance genes	Remark
12	(Aliyu et al., 2016)	Selangor	Poultry and Environment	Poultry meat and contact surface	Phenotypic & Molecular	48.8	-	<i>bla</i> _{TEM} , <i>bla</i> _{CTX} , <i>bla</i> _{OXA} , <i>bla</i> _{SHV}	E coli O157:H7
13	(Devan et al., 2022)	Kelantan	Poultry	Cloacal swab	Phenotypic & Molecular	91/320 (28.4)	-	<i>Mcr-1</i>	E coli O157:H7
14	(Akilu et al., 2022)	Kelantan	Poultry	Cloacal swab and raw meat	Phenotypic & Molecular	72/220 (32.73)	-	<i>mcr-1</i> , <i>bla</i> _{TEM-52} , <i>bla</i> _{NDM} , <i>bla</i> _{OXA-48}	E coli O157:H7
15	(Ibrahim et al., 2021)	Kelantan, Teranganu & Pahang	Poultry	Cloacal swab	Phenotypic	199/384 (51.8)	-		E coli O157:H7
16	(Akilu et al., 2021)	Kelantan	Poultry	Cloacal swab	Phenotypic & Molecular	19/78 (24.3)	-	<i>bla</i> _{NDM} , <i>bla</i> _{OXA-48} , <i>bla</i> _{IMP}	E coli O157:H7
17	(Elmi et al., 2021)	Kelantan, Teranganu & Pahang	Poultry & environment	Cloacal swab, feces, sewage, tap water	Phenotypic & Molecular	68/371 (18.3)	-	<i>tetA</i> , <i>tetB</i> , <i>aac</i> , <i>catA</i> , & <i>sul</i>	E coli O157:H7
18	(Mohamed et al., 2022)	Perak & Kedah	Poultry & environment	Cloacal swab, soil and drinking water	Phenotypic & Molecular	48/196 (24.5)	<i>eaeA</i> , <i>inl</i> , <i>bfpA</i>		E coli O157:H7
19	(Devan et al., 2023)	Kelantan	Poultry & environment	Poultry, chicken meat & vegetables	Phenotypic & Molecular	367/517 (71.0%)	-	<i>mcr1</i> , <i>mcr3</i> , <i>mcr6</i> , <i>mcr8</i> & <i>mcr9</i>	E coli O157:H7
20	(Lemlem et al., 2023)	Kelantan	Poultry	Cloacal swab	Phenotypic & Molecular	97/320 (30.3%)	-	<i>mcr-1</i> , <i>bla</i> _{TEM} , <i>bla</i> _{CTX}	E coli O157:H7

Prevalence of *E. coli* among livestock, livestock products, and its environment in Malaysia

Amongst the twenty studies that met the inclusion criteria and sampled, most of the studies involved more than one sample type and some included more than one sampling location (State). Twelve studies reported *E. coli* in poultry, eight studies reported *E. coli* in cattle, six studies reported *E. coli* in the environment while pig, sheep & goat were reported only once (Table 1). The prevalence of *E. coli* amongst livestock, livestock products and environment sampled varied across different sample type. The overall prevalence of *E. coli* ranged from 4% to 100% among all the studies reviewed with poultry prevalence ranging between 18.3% to 100%, cattle prevalence ranging between 4% to 100%, environmental prevalence ranging between 18.3% to 85% while pigs, sheep and goat had a prevalence of 67.5% and 4% respectively (Table 1). Most of the studies reported relatively low to moderately high prevalence rates of *E. coli* ranging from 18.3% to 67.5% (Table 1). One of the studies did not report prevalence rate of *E. coli* as part of its findings. Most of the studies conducted were in Cattle, poultry and environment. Conversely, the prevalence rates of *E. coli* among livestock samples vary even among the livestock species considered from the studies conducted in Malaysia. Notably, most studies used more than one method to identify and detect *E. coli* among the isolates (Table 1).

Virulence factors and antimicrobial resistant genes

Genes to several virulence factors including the entero-invasive, entero-pathogenic, entero-haemorrhagic and entero-toxigenic were reported in a number of the reviewed studies (Table 1). The *Stx1*, *Stx2* & *eaeA*, *Rfb0157*, *fliC_H*, *VT1*, *VT2*, *LT*, *ST* are part of the virulent genes reported in a number of studies reviewed (Table 1). Also, several resistant genes including the colistin and carbapenem genes were also reported in a number of studies reviewed. None of the reviewed studies reported both virulence and resistant genes, while some did not report either of the genes (Table 1).

Antimicrobial resistance profile

Thirteen studies from this review reported susceptibility profile to *E. coli*, although some of these studies did not report the individual susceptibility results of *E. coli* isolates against the respective antibiotics tested (Table 2). There is a high resistance to almost all antimicrobials tested with much emphasis on tetracycline, ampicillin and amoxicillin Clavulanic by the *E. coli* isolates from this review ranging approximately between 60-96%. Seven studies reported susceptibility profile of *E. coli* to tetracycline, six studies reported susceptibility profile to ampicillin and chloramphenicol, five studies reported susceptibility to amoxicillin/clavulanic acid, three studies reported susceptibility profile to imipenem, ofloxacin, ceftazadime, kanamycin, gentamicin and ampicillin/sulbactam, only two studies reported susceptibility results against erythromycin and cefotaxime (Table 2). Even though none of the studies in this systematic review showed 100% susceptibility of antimicrobials considered to be *E. coli*, a study showed 97% and 99% susceptibility to gentamicin and cefotaxime respectively (Table 2).

Table 2 Antimicrobial resistance phenotypes of *E. coli* isolate from Livestock, Livestock product and Environment in Malaysia.

No	Author, year	I	T	O	Cf	E	Amp	K	G	Amk	Sxt	Cfz	Amc	Cfx	Ams	Chl
1	(Devan et al., 2023)	10.2	96.1	ND	ND	ND	94.5	ND	ND	ND	ND	ND	89.8	ND	10.9	ND
2	(Lemlem et al., 2023)	17.5	82.5	59.8	47.4	ND	ND	ND	ND	ND	78.4	61.9	60.8	52.6	37.1	61.9
3	(Mohamed et al., 2022)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	(Devan et al., 2022)	33.33	95.2	76.1	23.81	ND	80.95	ND	ND	ND	85.71	ND	52.38	28.57	42.86	85.71
5	(Aklilu et al., 2022)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	(Ibrahim et al., 2021)	ND	94.6	ND	ND	100	87	57	20.2	ND	83.3	ND	ND	ND	ND	84.5
7	(Aklilu et al., 2021)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	(Elmi et al., 2021)	ND	91.4	ND	ND	ND	51.9	ND	23.3	ND	74.2	ND	21.2	ND	ND	26.3
9	(Aliyu et al., 2016)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	(Ghaderpour et al., 2015)	ND	28	10	ND	ND	33	19	3	ND	19	1	11	ND	ND	16
11	(Ho et al., 2013)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	(Sukhumungoon et al., 2011)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
13	(Radu et al., 2001)	ND	7	37	30	57	7	ND	ND	ND	ND	ND	ND	ND	ND	10

Not Reported (ND), Imepenem (I), Tetracycline (T), Ofloxacin (O), Ceftrazadime (Cf), Erythromycin (E), Ampicillin (Amp), Kanamycin (K), Gentamicin (G), Amikacin (Amk), Ceftriaxone (Cfz), Amoxicillin/Clavulanic acid (Amc), Cefotaxime (Cfx), Ampicillin Sulbactam (Ams), Chloramphenicol (C)

Genotypes of pathogenic *E. coli* isolates from livestock, livestock products and environment

From this review, nine studies reported at least one genotypic characteristic of pathogenic *E. coli* isolates (Table 3). All the nine studies used PCR as a method genotypic characterization of pathogenic *E. coli* isolates. *E. coli* O157:H7 was detected in all the nine studies. One study reported O139:H1, O130:H26, and O168:H21 *E. coli* serotype. Multi-locus sequence typing (MLST) was conducted in two studies and sequence types were reported namely, ST117, ST155, ST69 and ST410, ST155 respectively (Table 3). In addition, RAPD was conducted in two studies, PFGE typing was also conducted in three studies, REP-PCR typing was conducted in two studies and phylotyping was conducted also conducted in three studies (Table 3).

Table 3 Genotypic characteristics of *E. coli* isolates from livestock in Malaysia

No	Author, year	Method	RAPD	Phylotyping	MLST	PFGE	Serotyping	REP-PCR	Integrans
1	(Devan et al., 2023)	PCR	ND	A, B1, C, D F	ND	ND	ND	ND	ND
2	(Lemlem et al., 2023)	PCR	ND	ND	ST117, ST155, ST69	ND	ND	ND	ND
3	(Aklilu et al., 2022)	PCR	ND	A, B1, B2	ST410, ST155	ND	ND	ND	ND
4	(Aklilu et al., 2021)	PCR	ND	A, B1, C, D, E	ND	ND	ND	ND	ND
5	(Ghaderpour et al., 2015)	PCR	ND	A, B1, B3	ND	ND	ND	I, III, V	I, II
6	(Ho et al., 2013)	PCR	ND	ND	ND	A1, A2, A3	O139:H1, O130:H26, O168:H21	B1, B2, B3	ND
7	(Sahilah et al., 2010)	PCR	Done	ND	ND	ND	ND	ND	ND
8	(Apun et al., 2006)	PCR	ND	ND	ND	A-D	ND	ND	ND
9	(Radu et al., 2001)	PCR	Done	ND	ND	Done	ND	ND	ND

Random Amplification of Polymorphic DNA (RAPD), Multi-Locus Sequence Typing (MLST), Pulse Field Gel Electrophoresis (PFGE), Repetitive Extragenic Palindromic PCR (REP-PCR), ND (Not done)

DISCUSSION

The objective of this systematic review was to examine the potential sources and reservoirs of pathogenic *E. coli* in the livestock production systems in Malaysia over the past twenty years. A comprehensive search yielded numerous articles, out of which 20 studies were deemed eligible for inclusion in this review. These 20 studies were conducted in various states across Malaysia, with a majority of them focusing on the Kelantan region in the northeast and the Selangor region in the central part of the Malaysian peninsula.

According to this review, the occurrence of *E. coli* from livestock, livestock products, and the environment in Malaysia varied between 4% and 100%. The prevalence rates reported in different states of Malaysia were relatively low, with ranges as follows: Kelantan (4% to 51.8%), Selangor (4% to 100%), Pinang (4% to 51.8%), Terengganu (4% to 51.8%), Perak (4% to 67.5%), Kedah (4% to 33.5%), Sabah (9.8%), and Sarawak (9.8%). Only one study in Selangor reported a prevalence rate of 100% among the reviewed studies. These rates of *E. coli* prevalence among livestock and the environment in Malaysia were lower than those reported in other parts of the world, such as Nigeria (50.9%), South Africa (67.5%), and Zambia (98.67%) (Mainda et al., 2015; Tanih et al., 2015; Aliyu et al., 2021).

It is worth noting that most of the studies included in this review reported a low occurrence of *E. coli*, generally below 10%, with the highest average being 51.8%. This disparity may be attributed to the under-researched nature of *E. coli* in Malaysia. Moreover, the reviewed studies mostly had small sample sizes. However, a few studies recorded *E. coli* rates above 40%. Surveillance of zoonotic pathogenic *E. coli* has been implemented in hospitals, where it has been found to cause serious infections (33%) in humans (Nazmul et al., 2008). This emphasizes the importance of ongoing surveillance for these strains in Malaysia, in case they emerge in hospital environments and further complicate the treatment of infections in healthcare settings with limited resources. More studies involving large commercial livestock farms should be conducted in Malaysia, especially considering the rapid growth of the livestock industry. The studies analyzed in this review employed multiple tests to identify and detect *E. coli* in the isolates. This suggests that there have been improvements in laboratory diagnostic capabilities in Malaysia, possibly through collaborations. This is significant because misidentification of *E. coli* has been reported in the past. The use of multiple methods ensures more rigorous speciation and identification of *E. coli*. The reviewed studies in Malaysia revealed a high occurrence of *E. coli* in livestock feces and environmental samples. Animal feces are recognized as the primary source of pathogenic *E. coli*. The close proximity between livestock and their environment facilitates the transmission of pathogenic bacteria among animals and between animals and the environment (Gaddafi et al., 2022).

In this review, six studies reported a high prevalence of *E. coli* in environmental samples across peninsular Malaysia. Animal waste, sewage from farming operations, manure/slurries used as fertilizers or for silage preparation, and livestock grazing contribute to the infection and reinfection of livestock. The presence of *E. coli* in the soil promotes its spread among livestock, and its presence in the environment poses a risk of human infections (Mohamed et al., 2022).

Interestingly, the studies included in this review also reported the prevalence of *E. coli* in livestock and livestock products. Pathogenic bacteria can be found in the meat of infected animals or can contaminate carcasses through improper slaughtering and processing. Unhygienic practices by farm workers can lead to the contamination of milk during milking. Ingesting raw or undercooked contaminated beef, consuming unpasteurized milk, or drinking untreated water can result in infections caused by pathogenic bacteria, particularly *E. coli* (Yusuf et al., 2020).

The reviewed studies identified several antimicrobial resistant and virulent genes in *E. coli* isolates from livestock, livestock products, and the environment. Notably, the most commonly isolated *E. coli* virulent genes were *Stx1*, *Stx2*, *eaeA*, *Rfb0157*, *flicH*, *VT1*, *VT2*, *LT*, and *ST*. The presence of these genes suggests that *E. coli* isolates from livestock, livestock products, and the environment in Malaysia pose a significant risk as a cause of foodborne infections, especially for consumers of these products. The detection of genes such as *Stx1*, *Stx2*, *Rfb0157*, and *flicH* indicates the presence of shiga-toxin-producing *E. coli* serotype O157:H7, which can cause severe illness in humans. These serotypes are responsible for foodborne infections in both livestock and humans and are referred to as zoonotic *E. coli*. The detection of Shiga-like toxins is a valuable tool for identifying enterohemorrhagic *E. coli* strains (Vieira, 2010; Mohamed et al., 2022).

Carbapenem and colistin resistance genes in *E. coli* were detected in six studies, and their prevalence rates were relatively high. Genes such as *mcr-1*, *blaTEM-52*, *blaNDM*, and *blaOXA-48* are commonly found in communities where these antibiotics are indiscriminately used. The presence of carbapenem and colistin resistance genes increases the likelihood of multidrug-resistant *E. coli* being transmitted between humans, livestock, and the environment in Malaysia. Malaysia was among the first countries to report the emergence of the colistin gene in Enterobacteriaceae, primarily from livestock and environmental samples, according to the National Surveillance of Antimicrobial Resistance reports (Hsu et al., 2017; Devan et al., 2022). Colistin is considered a last-resort antibiotic for treating life-threatening infections, particularly in the face of global β -lactam and carbapenem resistance. The emergence of resistance to colistin has raised widespread concerns, leading the World Health Organization (WHO) to designate it as the "Highest Priority Critically Important Antimicrobial" (Organization, 2018).

ESBL-resistant *E. coli* is among the most common causes of bloodstream infections, community and hospital-acquired urinary tract infections, and foodborne infections, with associated increased mortality rates, hospitalization costs, prolonged hospital stays, and a limited range of treatment options. Several studies have consistently highlighted the role of livestock and its products in the transmission of multidrug-resistant bacteria among the human population and the environment, posing challenges to food safety and environmental health. Improper handling of livestock during slaughtering, dressing, processing, and retailing has been identified as a major pathway for the horizontal transfer of ESBL-EC, contributing to its global dissemination. ESBL-EC has been reported as a significant concern in the Malaysian healthcare setting, with research demonstrating the potential role of livestock meat as a major reservoir for human exposure to ESBL-EC. The interspecies transmission of these

bacteria poses challenges for the treatment and prevention of infections. The findings of this review indicate that *E. coli* isolates associated with livestock in Malaysia are potentially virulent and can cause infections in humans once they enter the human host.

In this review, we observed that the resistance patterns of *E. coli* isolates against a panel of 15 antimicrobials were generally similar across the states where the studies were conducted (Sarawak, Sabah, Selangor, Perak, Kedah, Kelantan, Terengganu, and Pahang). However, there were significant differences in the antimicrobial resistance profiles between these states. These variations in resistance profiles can be attributed to geographic-specific mis-use on certain classes of antimicrobials (carbapenem, tetracycline, chloramphenicol and cephalosporin). There was a high prevalence of *E. coli* resistance to tetracycline, ampicillin, chloramphenicol, and sulfamethoxazole/trimethoprim, which was consistent across all the states in this review. This resistance pattern reflects the widespread use of these antimicrobials in livestock operations and other agricultural activities (Tang et al., 2017). It is worth noting that most of these antimicrobials are also used in human medicine, particularly tetracycline, chloramphenicol, sulfamethoxazole/trimethoprim, and ampicillin. These findings align with a report by (Gaddafi et al., 2021) that found a similar antimicrobial resistance profile in pigs and farm attendants in Nigeria.

The increasing cases of antimicrobial resistance in humans have been linked, in part, to the transmission of pathogens from animals to humans. This review showed that *E. coli* isolated from livestock, livestock products, and environmental sources in Malaysia exhibited multidrug resistance to commonly used antimicrobials in both animals and humans. Furthermore, the majority of the isolated *E. coli* strains had a Multiple Antimicrobial Resistance (MAR) index greater than 0.2. *E. coli* is the predominant bacterium associated with bacterial infections in livestock, causing significant health problems, increased mortality, reduced production, and higher costs for disease prevention and treatment. A wide range of antimicrobials is commonly used in livestock production, primarily administered orally, to prevent and treat diseases and enhance growth and productivity (Gaddafi et al., 2022).

Notably, only two of the reviewed studies reported the presence of medically important MLST sequence types, namely ST117, ST69, and ST155, in livestock. Both ST117 and ST155 were found in ESBL-producing *E. coli* strains from the community in Malaysia (Dwiyanto et al., 2022), and they have also been reported in humans, food animals, and retail meat sources in Europe (Manges, 2016). This evidence suggests that livestock serve as reservoirs for the zoonotic transmission of pathogenic *E. coli* in Malaysia. Another report highlighted the presence of phylotype B and class I and II integrons in livestock and environmental samples in Malaysia, indicating that livestock and the environment can be sources of human infection with pathogenic *E. coli* strains (Ghaderpour et al., 2015; Aklilu et al., 2022). Therefore, concerted efforts and measures must be implemented to control the spread of these strains.

The current review has few limitations including the fact that only four recognized online databases were searched, and only articles written in English were included, which could have resulted in the omission of relevant studies or publications for this review. Furthermore, there is a lack of data on the risk factors associated with the carriage of zoonotic *E. coli* from livestock to humans in Malaysia. None of the studies included in this review reported sampling

of both livestock, environmental samples, and humans. However, individuals who have direct contact with livestock, particularly cattle, pigs, and poultry, have been identified as being at a higher risk of colonization or infection with pathogenic *E. coli* (Elmi et al., 2020).

CONCLUSIONS

This review examined 20 studies focused on *E. coli* in livestock production systems and the environment in Malaysia. The collective findings from these studies reveal the presence of potentially highly virulent strains of multi-drug-resistant *E. coli* in livestock, livestock products, and the environment. These strains can be transmitted to individuals at high risk who have frequent contact with animals. Adopting an integrated 'One Health' approach is necessary to address zoonotic *E. coli* and ensure effective prevention and control measures against *E. coli* infections, as well as to combat antimicrobial resistance in both animals and humans. Close monitoring and strict regulation of antimicrobial usage in Malaysia's livestock production systems are essential, particularly due to the potential for colistin resistance caused by improper use of colistin sulfate in the poultry industry. This study may serve as a model for investigating the role of human, animal, and environmental factors in the contamination of multi-drug-resistant *E. coli* and other relevant microbes in relation to food safety.

AUTHOR CONTRIBUTIONS

HL, EA: Conceptualize; EA, FK, ZS: Supervision; HL, GMS: Literature search & data extraction; HL, GMS, ML: wrote the initial draft; All authors reviewed and approved the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Aklilu, E., Harun, A., Singh, K.K.B., 2022. Molecular characterization of blaNDM, blaOXA-48, mcr-1 and blaTEM-52 positive and concurrently carbapenem and colistin resistant and extended spectrum beta-lactamase producing *Escherichia coli* in chicken in Malaysia. *BMC. Vet. Res.* 18(1), 190.
- Aklilu, E., Harun, A., Singh, K.K.B., Ibrahim, S., Kamaruzzaman, N.F., 2021. Phylogenetically diverse *Escherichia coli* strains from chicken coharbor multiple carbapenemase-encoding genes (bla (NDM)-bla (OXA)-blai(MP)). *Biomed. Res. Int.* 2021, 5596502.
- Aliyu, A.B., Saleha, A.A., Jalila, A., Zunita, Z., 2016. Risk factors and spatial distribution of extended spectrum β -lactamase-producing- *Escherichia coli* at retail poultry meat markets in Malaysia: a cross-sectional study. *BMC. Public. Health.* 16, 699.
- Aliyu, R.M., Abubakar, M.B., Yakubu, Y., Shuaibu, A.B., 2021. Prevalence of *Escherichia coli* o157: H7 in some animal products sold within Sokoto Metropolis, Nigeria. *Afr. J. Microbiol. Res.* 13(1), 1-6.
- Apun, K., Chang, P., Sim, E., Micky, V., 2006. Clonal diversity of *Escherichia coli* isolates from marketed beef in East Malaysia. *World. J. Microbiol. Biotechnol.* 22, 661-667.
- Baumann, D., Salia, H., Greune, L., Norkowski, S., Körner, B., Uckeley, Z.M., Frankel, G., Guenot, M., Rüter, C., Schmidt, M.A., 2018. Multitalented espb of enteropathogenic *Escherichia coli* (Epec) enters cells autonomously and induces programmed cell death in human monocytic Thp-1 Cells. *Int. J. Med. Microbiol.* 308, 387-404.

- Beauchamp, C., Sofos, J., 2010. Diarrheagenic *Escherichia coli*. In: Juneja, V.K., Sofos, J.N. (Eds.), *Pathogens and toxins in foods*, ASM Press, Washington, pp. 71-94.
- Chang, W., Afsah-Hejri, L., Rukayadi, Y., Khatib, A., Lye, Y., Loo, Y., Shahril, N.M., Puspanadan, S., Kuan, C., Goh, S., 2013. Quantification of *Escherichia coli* O157: H7 in organic vegetables and chickens. *Int. Food Res. J.* 20(2), 1023-1029.
- Cheah, Y., Tay, L., Aida, A., Son, R., Nakaguchi, T., Nishibuchi, M., 2015. Molecular characterization of *Escherichia coli* isolated from different food sources. *Int. Food. Res. J.* 22(1), 31-40.
- Chye, F.Y., Abdullah, A., Ayob, M.K., 2004. Bacteriological quality and safety of raw milk in Malaysia. *Food. Microbiol.* 21, 535-541.
- Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M., Finlay, B.B., 2013. Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin. Microbiol. Rev.* 26, 822-880.
- Devan, S.S., Aklilu, E., Hamdan, R.H., Lemlem, M., Zakaria, Z., 2022. Detection of colistin-resistant *Escherichia coli* isolated from broiler chickens in Kelantan, Malaysia. *Trop. Biomed.* 39(2), 197-202.
- Devan, S.S., Aklilu, E., Zakaria, Z., Hamdan, R.H., Lemlem, M., Kamaruzzaman, N.F., Kamaruzaman, I.N.A., Reduan, M.F.H., 2023. Emergence of Mcr -1, -3, -6, -8 and -9) in *Escherichia coli* isolated from live chickens, raw chicken meat and vegetables from Kelantan, Malaysia. *J. Microbiol. Biotechnol. Food Sci.* 12, E9829.
- Ducat, W.H., Kumar, S., 2015. A systematic review of professional supervision experiences and effects for allied health practitioners working in non-metropolitan health care settings. *J. Multidiscip. Healthc.* 8, 397-407.
- Dwiyanto, J., Hor, J.W., Reidpath, D., Su, T.T., Lee, S.W.H., Ayub, Q., Mustapha, F.B., Lee, S.M., Foo, S.C., Chong, C.W., Rahman, S., 2022. Pan-genome and resistome analysis of extended-spectrum β -lactamase-producing *Escherichia coli*: a multi-setting epidemiological surveillance study from Malaysia. *PLoS. One.* 17(3), e0265142.
- Elmi, S.A., Simons, D., Elton, L., Haider, N., Abdel Hamid, M.M., Shuaib, Y.A., Khan, M.A., Othman, I., Kock, R., Osman, A.Y., 2021. Identification of risk factors associated with resistant *Escherichia coli* isolates from poultry farms in the East Coast of Peninsular Malaysia: a cross sectional study. *Antibiotics (Basel).* 10(2), 117.
- Elmi, V.A., Moradi, S., Harsini, S.G., Rahimi, M., 2020. Effects of lactobacillus acidophilus and natural antibacterials on growth performance and salmonella colonization in broiler chickens challenged with salmonella enteritidis. *Livest. Sci.* 233, 103948.
- Gaddafi, M., Yakubu, Y., Bello, M., Bitrus, A., Musawa, A., Garba, B., Lawal, H., Aliyu, M., Barka, S., Emeka, A., 2022. Occurrence and antibiotic resistance profiles of Methicillin-Resistant (Mrsa) in layer chickens in Kebbi, Nigeria. *Folia. Vet.* 66, 37-45.
- Gaddafi, M.S., Yakubu, Y., Junaidu, A.U., Bello, M.B., Garba, B., Bitrus, A.A., Lawal, H., 2021. Nasal colonization of pigs and farm attendants By *Staphylococcus Aureus* And Methicillin-Resistant *Staphylococcus Aureus* (Mrsa) In Kebbi, Northwestern Nigeria. *Thai. J. Vet. Med.* 51, 119-124.
- Ghaderpour, A., Ho, W.S., Chew, L.L., Bong, C.W., Chong, V.C., Thong, K.L., Chai, L.C., 2015. Diverse and abundant multi-drug resistant *E. Coli* in matang mangrove estuaries, Malaysia. *Front Microbiol.* 6, 977.
- Ho, P., Chan, J., Lo, W., Law, P.Y., Chow, K., 2013. Plasmid-mediated fosfomycin resistance in *Escherichia coli* isolated from pig. *Vet. Microbiol.* 162, 964-967.
- Hsu, L.Y., Apisarnthanarak, A., Khan, E., Suwatarat, N., Ghafur, A., Tambyah, P.A., 2017. Carbapenem-resistant acinetobacter baumannii and enterobacteriaceae in South and Southeast Asia. *Clin. Microbiol. Rev.* 30, 1-22.
- Huijbers, P.M., Larsson, D.J., Flach, C.F., 2020. Surveillance of antibiotic resistant *Escherichia coli* in human populations through Urban Wastewater in ten European countries. *Environ. Pollut.* 261, 114200.
- Ibrahim, S., Wei Hoong, L., Lai Siong, Y., Mustapha, Z., CWS, C.W.Z., Aklilu, E., Mohamad, M., Kamaruzzaman, N.F., 2021. Prevalence of antimicrobial resistance (Amr) salmonella spp. And *Escherichia coli* isolated from broilers in the East Coast of Peninsular Malaysia. *Antibiotics (Basel).* 10(5), 579.
- Johnson, J.R., Russo, T.A., 2018. Molecular epidemiology of extraintestinal pathogenic *Escherichia Coli*. *Ecosal. Plus.* 8(1).
- Lemlem, M., Aklilu, E., Mohammed, M., Kamaruzzaman, F., Zakaria, Z., Harun, A., Devan, S.S., 2023. Molecular detection and antimicrobial resistance profiles of extended-spectrum beta-lactamase (Esbl) producing *escherichia coli* in broiler chicken farms in Malaysia. *Plos One.* 18, E0285743.
- Lye, Y., Afsah-Hejri, L., Chang, W., Loo, Y., Puspanadan, S., Kuan, C., Goh, S., Shahril, N., Rukayadi, Y., Khatib, A., 2013. Risk of *Escherichia coli* O157: H7 transmission linked to the consumption of raw milk. *Int. Food Res. J.* 20, 1001.

- Mainda, G., Bessell, P.R., Muma, J.B., McAteer, S.P., Chase-Topping, M.E., Gibbons, J., Stevens, M.P., Gally, D.L., de, C.B.B.M., 2015. Prevalence and patterns of antimicrobial resistance among *Escherichia coli* isolated from Zambian dairy cattle across different production systems. *Sci Rep.* 5, 12439.
- Manges, A., 2016. *Escherichia coli* and urinary tract infections: the role of poultry-meat. *Clin. Microbiol. Infect.* 22, 122-129.
- Martinez-Medina, M., Garcia-Gil, L.J., 2014. *Escherichia coli* in chronic inflammatory bowel diseases: an update on adherent invasive *Escherichia coli* pathogenicity. *World. J. Gastrointest. Pathophysiol.* 5(3), 213-227.
- Mohamed, M.Y.I., Abu, J., Zakaria, Z., Khan, A.R., Abdul Aziz, S., Bitrus, A.A., Habib, I., 2022. Multi-drug resistant pathogenic *Escherichia coli* isolated from wild birds, chicken, and the environment in Malaysia. *Antibiotics.* 11, 1275.
- Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A., 2015. Preferred reporting items for systematic review and meta-analysis protocols (Prisma-P) 2015 Statement. *Syst. Rev.* 4, 1-9.
- Nazmul, M., Salmah, I., Jamal, H., Ansary, A., 2008. Molecular characterization of verotoxin gene in enteropathogenic *Escherichia coli* isolated from Miri Hospital, Sarawak, Malaysia. *Biomed. Res. J.* 19, 9-12.
- Perera, A., Clarke, C.M., Dykes, G.A., Fegan, N., 2015. Characterization of shiga toxigenic *Escherichia coli* O157 and Non-O157 isolates from ruminant feces in Malaysia. *Biomed. Res. Int.* 2015.
- Radu, S., Ling, O.W., Rusul, G., Karim, M.I.A., Nishibuchi, M., 2001. Detection of *Escherichia coli* O157: H7 by multiplex pcr and their characterization by plasmid profiling, antimicrobial resistance, Rapd And Pfgc analyses. *J. Microbiol. Methods.* 46, 131-139.
- Sahilah, A., Nor'aishah, H., Noraida, I., Ahmad Azuhairi, A., 2010. Detection of shiga toxin 1 and 2 (stx1 and stx2) genes in *Escherichia coli* O157: H7 isolated from retail beef in Malaysia by multiplex polymerase chain reaction (Pcr). *Sains Malays.* 39, 57-63.
- Santos, A.C.D.M., Santos, F.F., Silva, R.M., Gomes, T.A.T., 2020. Diversity of hybrid-and hetero-pathogenic *Escherichia coli* and their potential implication in more severe diseases. *Front. Cell. Infect. Microbiol.* 10, 339.
- Simjee, S., 2007. *Foodborne Pathog. Dis*, Humana Press, Totowa.
- Sukhumungoon, P., Nakaguchi, Y., Ingviya, N., Pradutkanchana, J., Iwade, Y., Seto, K., Son, R., Nishibuchi, M., Vuddhakul, V., 2011. Investigation of stx 2+ eae+ *Escherichia coli* O157: h7 in beef imported from Malaysia to Thailand. *Int. Food Res. J.* 18(1), 381-386.
- Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., Polachek, A.J., Ganshorn, H., Sharma, N., Kellner, J.D., 2017. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. *Lancet. Planet. Health.* 1, E316-E327.
- Tanih, N.F., Sekwadi, E., Ndip, R.N., Bessong, P.O., 2015. Detection of pathogenic *Escherichia coli* and *Staphylococcus aureus* from cattle and pigs slaughtered in abattoirs in Vhembe district, South Africa. *ScientificWorldJournal.* 2015, 195972.
- Vieira, T.B., 2010. Caracterização de *Escherichia coli* isolados de miúdos e carcaça com celulite por rapd e detecção dos genes *iss* e *fela* por pcr.
- Von Mentzer, A., Connor, T.R., Wieler, L.H., Semmler, T., Iguchi, A., Thomson, N.R., Rasko, D.A., Joffe, E., Corander, J., Pickard, D., 2014. Identification of enterotoxigenic *Escherichia coli* (Etec) clades with long-term global distribution. *Nat. Genet.* 46, 1321-1326.
- Yusuf, Y., Aliyu, M.I., Bello, S.A., Fatima, B., Abdullahi, A., Aliyu, M.D., 2020. Occurrence of methicillin resistant *Staphylococcus aureus* (Mrsa) in some retailed animal products in Sokoto, Nigeria. *Alex. J. Vet. Sci.* 64(1), 135-142.

How to cite this article;

Habiba Lawal, Erkihun Aklilu, Nor Fadilah Kamaruzzaman, Zarizal Suhaili, Gaddafi Mohammed Sani and Mulu Lemlem. Livestock and environment as potential sources and reservoirs for multi-drug resistant *Escherichia coli* in Malaysia: A Systematic Review. *Veterinary Integrative Sciences.* 2024; 22(1): 387 - 403