

Review Article

The Epidemiology and Antimicrobial Resistance of *Escherichia coli*: A Review

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Abstract

Antimicrobial resistance (AMR) remains an alarming issue with public-health concern and economic implications on human and animal populations worldwide. These antibiotics have so far been associated with high burden of diseases and the ramifications of veterinary antibiotic resistance of *Escherichia coli* on the Sustainable Development Goals cannot be teased out. This review paper highlights the epidemiology of *Escherichia coli* and antibiotic resistance in livestock productions systems.

INTRODUCTION

Globally, antibiotic use in livestock setting is accounted for approximately 80% of total consumption [1]. Veterinary antibiotics are used for livestock production, growth promotion, prevention and treatment of infections [2]. However, improper use of antibiotics to the livestock can lead to the development of antibiotic-resistant pathogens which can be exposed to the environment and pose a human health risk upon consumption [3]. The persistence and emergence, however, of antimicrobial resistance in bacterial communities in particular faecal form indicators pose a sheer threat to treatment options of microbial infections in a cost effective manner solutions and thus place a burden on health services leading consequences in human and animal health settings [4].

Escherichia coli is considered an indicator of faecal contamination due to the presence of *E. coli* in human and animal feces and its pathogenic strains are responsible for food poisoning and food-related infections and is a serious public health concern [5,6]. The organism naturally occurs in the intestinal tracts of warm-blooded animals including humans, domestic animals, birds, wildlife, and pets [7,8], then released into the environment through deposition of fecal material and typically mixed with watershed, [6,9]. The epidemiology and extent of antimicrobial resistance of *E. coli* in livestock systems has not been comprehensively reviewed. Thus, it is this concern that motivates our review and our approach to this question involves a rapid a review. In this paper, we have conducted overview of the epidemiology and antimicrobial resistance of

E. coli in the wider context and close specific cases pertaining to the themes of interest in Malaysia.

Historical overview of *Escherichia coli*

Escherichia coli was first discovered by Dr. Theodore Escherich in 1885 and named first *Bacterium coli commune* because it was isolated from the stool of infants in Munich and organism which later recognized '*Escherichia coli*' [10]. *E. coli* is an indicator of pollution and is harmless and few microorganisms are highly versatile and frequently deadly pathogen such as O157:H7, enterohemorrhagic and enteroinvasive causing infections in mammals including humans [11]. The microbiologists have faced challenges of differentiating between the strains causing diarrhoea and the harmless gut commensals. Epidemiological investigations were assisted by the description of a serotyping scheme for *E. coli* by [12], and later discovered that 170 serogroups of *E. coli* had been associated as potential cause of epidemic infantile enteritis by [13] as enteropathogenic *E. coli* (EPEC). Recently it was recognized that other *E. coli* strains can produce toxins, and these enterotoxigenic *E. coli* (ETEC) commonly belong to specific serogroups which are different from EPEC. *E. coli* strains belonging to a third serogroups can responsible an illness like shigella dysentery, and these are entero-invasive *E. coli* (EIEC) [14,15].

Classification and nomenclature

Escherichia coli belong to the domain bacteria, phylum proteo bacteria, order Entereo bacteria, class Gamma proteo bacteria, family Entereobacteraceae, and Genus *Escherichia*. The genus

consists of five species: *E. coli*, *E. hermannii*, *E. fergusonii*, *E. vulneris*, and *E. blattae* [16, 17]. *Escherichia coli* can be classified into commensal *E. coli* and pathogenic *E. coli* [18] both in animals and humans. Based on its pathogenicity, *E. coli* can be classified into enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *Escherichia coli* (EPEC), shiga toxin-producing *Escherichia coli*, enteroinvasive *Escherichia coli* (EIEC), Enteroaggregative *Escherichia coli* (EAEC) and diffusely adherent *Escherichia coli* (DAEC) [11]. The most prevalent recognized serotypes are O1:K1, O2:K1 and O78:K80 [19]. The most significant characteristic that leads this serotype to be identified is the antigenic structure of the outer cell membrane. *E. coli* O157:H7 has a Gram-negative cell envelope structure, which means it has an outer membrane with a lipopolysaccharide component separate from the cytoplasmic membrane. The carbohydrate composition and structure of the lipopolysaccharide characterize the O157 antigen. The H7 antigen is determined by the flagella's specific polypeptide composition [20].

General characteristics of *E. coli*

Escherichia coli bacteria is a mobile gram-negative, facultative anaerobic, non-spore rod-shaped, the size of *E. coli* about 0.5 µm in diameter and 1.0-3.0 µm in length and occur as single straight rods, most *E. coli* strains grows a wide range in temperature (approximately 15–48°C) an optimal temperature of 37 °C and can grow a pH range of 5.5-8.0 with optimal growth at neutral, some diarrheagenic *E. coli* strains have the ability to tolerate exposure to pH 2.0 [21]. On blood agar the colonies are relatively large 2-3 mm diameter and fast growing 24 h incubation. The colonies are shiny, smooth, round; grey, rarely mucoid and some strains can be hemolytic. On MacConkey agar *E. coli* ferments lactose with the production of acidic products that reduce the pH and turn the colonies to bright pink. Eosin methylene blue (EMB) agar is sometimes used for identification because *E. coli* colonies have a unique characteristic which is metallic sheen [22-24]. Coliform bacterium of the genus *Escherichia* which primarily lives in the intestinal flora of warm blooded organisms [11].

Antimicrobial resistance of *E. coli*

Antimicrobial resistance (AMR) is an recognized as one of the major threat of public health globally, and usage of antimicrobials as a growth promotion, prophylactic and treatment of infections in animal production is an ultimate driver for the emergence antimicrobial resistance [25, 26]. Livestock especially poultry is one of the most consumed meat types worldwide. Poultry flocks are generally raised under intensive farming conditions using large amounts of antimicrobials in a certain part or all their lives leading the development of antimicrobial resistant pathogens thus may cause failure of the treatment, potentially leading to economic consequences, but also be a source of resistant bacterial strains that pose a risk to animals, human health and the environment [11].

The evolution of AMR bacteria along the food chain posed a serious global health challenges as a result of infections, and contamination of food animals and their products by one or more of the resistant strains mainly zoonotic pathogens such as extended-spectrum beta-lactamase Enterobacteriaceae include *E. coli*, *Salmonella* spp., and *Shigella* spp.; and AMR *Campylobacter* spp., methicillin-resistant *Staphylococcus aureus* (MRSA) [11].

The emergency of antimicrobial resistance in *E. coli* strains is

a serious health problem worldwide and numerous studies about the occurrence of AMR have been conducted in different part of the world. For instance. A 15 years retrospective study on AMR in Malaysia showed that higher prevalence of multidrug resistance *E. coli* phenotypes were from poultry (80.26%), and lower levels from small ruminants (12.28%), pet birds (2.78%), large ruminants (1.90%), pigs (1.90%), companion animals (0.58%), and zoo animals (0.29%). Multidrug-resistant *E. coli* isolates was estimated (29.25%) [27]. Another study on of APEC isolates from several countries identified the plasmid-mediated *mcr-1* colistin resistance gene from China and Egypt. Most of the strains were multidrug resistance to 10 or more antimicrobials [28, 29]. Study conducted in Iran reported that *E. coli* resistance to nalidixic acid was (100%) from broilers and (49.5%) resistance to ciprofloxacin. This associated with the previous history that (52.5%) in flocks had used flumequine and enrofloxacin [30]. Another study from Belgium, [31] recovered ceftiofur resistant *E. coli* from cloacal swabs from broiler chickens from five farms. This high frequency occurred although cephalosporins have not been licensed for use in poultry in Belgium since 2000.

Epidemiology of *Escherichia coli*

Escherichia coli is one of the significant pathogen in the gastrointestinal tract of human and animals and recently emerged as an important public health issue [32]. Coliform bacterium of the genus *Escherichia* which primarily lives in the intestinal flora of warm blooded animals including mammals, birds and reptiles [11] and is released into the environment through deposition of fecal materials. In a typically mixed watershed, host sources of *E. coli* may be from humans, farm animals, wildlife, and pets, among others [6, 9].

The first outbreaks of *E. coli* O157:H7 infections in humans were associated with the consumption of ground beef, and thus cattle were considered the primary reservoir of *E. coli* O157:H7 for human infection, and to a lesser extent, sheep and possibly goats. Other reservoir animals include horses, dogs, cats, rabbits, poultry, pigs and flies [33-34].

Escherichia coli are primarily passed among animals, and it causes diseases in wide range hosts. Humans become infected by coming in contact with animals or animal products, plants and the environment that are contaminated with these pathogens; and can cause disease such as watery diarrhea, bloody diarrhea, urinary tract infection, meningitis, and sepsis to the host by food poisoning via food contamination [11]. *E. coli* O157:H7 may be considered as an environmental pathogen [11].

The avian gut was considered as a reservoir of *E. coli* that could potentially be transmitted from birds to humans [35]. Avian pathogenic *Escherichia coli* (APEC) is the main cause of avian colibacillosis worldwide and is a significant economic burden in animal production mainly within the poultry industry due to the morbidity and mortality rates [36-37]. The prevalence of *E. coli* from poultry meat in Thailand was (25%) [38], and to overall (60%) prevalence was (63.6%) in broiler and (56.4%) in layer in Bangladesh [39], (38.7 %) prevalence of *E. coli* in chicken meat in USA [40].

In livestock, ruminants are the primary natural hosts of *E. coli*, and they are generally healthy carriers. Other hosts include

pigs, cats, dogs, chickens, and wild birds; the organisms can colonize the intestinal tract of animals. The clinical signs depend on a various factors such as virulence factors of the *E. coli* strain, environment, age and immune of the animal, which characterized by diarrhea, septicaemia, colisepticaemia, mastitis, salpingitis, peritonitis, swollen head syndrome, cellulitis, and necrotic dermatitis. Infection of the animals may be through ingestion of contaminated pasture and water and environment with infected animal faeces or direct contact with other infected animals [11]. A study carried out by [41] to estimate the pooled prevalence of *E. coli* O157 in cattle from different part in the world showed a prevalence of 31.20% in Africa, 7.35% Northern America, (6.85%) Oceania, (5.15%) Europe, (4.69%) Asia and (1.65%) Latin America. Livestock prevalence from previous studies was *E. coli* O157 was isolated from (15.7%) cattle, (2.2%) sheep and from (0.4%), but not from chickens [42].

In humans, Enteropathogenic *E. coli* is one among the most significant causative pathogens of diarrheal disease leading cause of mortality and morbidity among children under-five years old in the developing countries. It is responsible for the death of more than 1,400 children every day, and approximately two million deaths annually, about 88% of deaths are intense in South East Asia and sub-Saharan Africa [11].

METHODS OF ISOLATION AND IDENTIFICATION OF *E. COLI*

Conventional culture methods

Conventional culture methods of *Escherichia coli* have recently expanded to a wide range of isolation methods. These methods include MacConkey's agar plates, eosin methylene blue agar (EMB), violet red bile agar, trypticase soya agar, rainbow agar was used for being highly selectivity and sensitivity for the detection of *E. coli* O157:H7 from contaminated meat samples in 24 h. CHROM agar, buffered peptone water with 8 mg/liter vancomycin, 10 mg/liter cefsulodin, and 0.05 mg/liter cefixime (BPW-VCC) also biochemical tests include catalase, Methyl red and indole test, Voges-Proskauer, urease, citrate and TSI. Identification procedure was performed as described by [11].

Molecular and serological confirmation of *E. coli*

Many genuine immunochemical methods have been developed for the detection of foodborne pathogens, including *E. coli* O157:H7 these immunochemical methods included Enzyme-linked immunosorbent assay (ELISA) and latex agglutination methods and Immunomagnetic separation (IMS) which used for routine screening of *E. coli* O157 due to high selectivity, speed, and simplicity of operation and are available commercially. Most of these assays used for reactions between antibodies to O-antigen of the O157 serotype or *E. coli* O157:H7 as a whole antigen [43].

A number of molecular ways have been developed to specifically detect the serotype in food or clinical samples and their applications ranges from diagnosing of the infections and characterization of field strains for epidemiological surveillance and are sensitive, specific, and capable of detecting phenotypic variants of serotype O157:H7. These includes; Restriction fragment length polymorphism (RFLP) and pulsed-field gel

electrophoresis (PFGE) is that the most often used molecular subtyping tools in medicine investigations but it have been shown to be a reliable and specifically selective methodology for subtyping foodborne pathogens include *E. coli* and *Salmonella* spp., as described the method [11]. Polymerase chain reaction (PCR) and DNA probe-based tests may be a wide selection tool for detection, identification and differentiation of *E. coli* O157:H7 in animal and human diseases diagnosing as described the methods a previous studies [11]. Another method includes, ribotyping, and DNA hybridization [11].

Antimicrobial susceptibility testing and resistance profile

According to the guidelines of the Clinical and Laboratory Standards Institute [44] the following three methods for susceptibility testing used including, disk diffusion, broth dilution, and agar dilution. Disk diffusion method refers to the distribution of an antimicrobial agent of an identified concentration from disks, tablets or strips, into the Mueller Hinton Agar (MHA) that has been isolated in a pure culture. This method is based on determination the zone of inhibition proportionally to the susceptibility of the bacteria to the specific antimicrobial disk on the media. Agar dilution and broth methods the broth and agar dilution methods are used to determine the lowest concentration of the assayed antimicrobial that inhibits the visible growth of the bacterium under examination. Both interpretive criteria (susceptible, intermediate, and resistant) for particular bacterium/antibiotic combinations and appropriate quality control reference species should be included in antimicrobial varieties [45, 46]. Various studies have been conducted using these methods for instance as described the method [47].

Global public health significance of *E. coli* infections and its economic implications

Generally foodborne diseases pose a potential public health and economic impact for surveillance and control measures programs within the food industry and the society. Extraintestinal infections caused by *Escherichia coli* are a considerable source of increased, death, and accrued health care charges and became more difficult to treat as a result of the rising spread of resistance to a first-line antimicrobial medicine [48]. *Escherichia coli* is responsible for (25%) of the infant diarrhoea in developing countries [49]. Concurrently, diarrhoeal diseases are the major reason for mortality in the developing countries, [50]. In addition, diarrhoeal disease has caused (3%) mortality in global scale because (30%) of the global population experienced foodborne diseases each year and should be a great concern [51]. Incidence rates has been reported to be (1210) cases per 100,000 inhabitants in France, (2600) cases per 100,000 in the United Kingdom [52], and more than (25,000) cases per 100,000 inhabitants in Australia and the United States [53].

The European Commission (EC) [54], the United States Environmental Protection Agency (USEPA) [55] and the WHO [56] suggest to use of *E. coli* as fecal indicator organism for predicting the presence of pathogens in human consumption and bathing water, and *Enterococcus* sp. because the most relevant for marine waters and as indicator in bathing water moreover [57]. The zoonotic pathogenic *E. coli* are the main cause for waterborne

outbreaks in humans by consumption contaminated water in developing countries and in industrialized countries [34, 58]. The Centre for Disease Control and Prevention (CDC) has estimated that *E. coli* O157:H7 infections cause (73,000) illnesses, (2,200) hospitalizations, and 60 deaths annually in the United States [59].

Surveillance data of Malaysian Health Ministry, 50.33 cases/100,000 people food and water-borne diseases were reported in Malaysia in the year 2016. Among the food and water-borne diseases, the occurrence of food poisoning cases were most prevalent with accounting for 47.34 cases per 100,000 populations in 2016 [60].

***Escherichia coli* in Malaysia**

The prevalence of *Escherichia coli* in Malaysia have been reported from livestock industry ranged 22.6-88.0%. The prevalence of *E. coli* was (68.5%) from the Southern, (57.2%) Central, (72.2%) Eastern and (59.9%) Northern regions. The incidence of *E. coli* O571:H7 serotype was (28.6%), (38.8%), (36.5%) and (35.6%) from the Southern, Central, Eastern and Northern regions respectively [61]. Another reports revealed the prevalence of *Escherichia coli* isolated from rodents, birds and bats was (43%), (18%) and (11%) , respectively in Sarawak, Malaysia [62]. Previously study reported that Avian colibacillosis is one in every of the most causes of morbidity and mortality in poultry globally [29], also has an important economic impact on poultry production worldwide [63]. Poultry is one of products consumed animal one of normally related with outbreaks of foodborne illness that impose a considerable threat on health systems and decrease the economic productivity of the countries [38]. Nevertheless, the infective micro-organisms are transmitted to humans through consumption of contaminated poultry product or by contact with poultry waste. The avian gut considered as a reservoir of *Escherichia coli* that might probably be transmitted from birds to humans [64]. In peninsular Malaysia 3 years study reported the most isolates *E.coli* were from *E.coli* O1:K1 followed by *E.coli* O78:K80 and *E.coli* O2:K1 [63]. Another study, from Selangor, Malaysia reported that (18.5%) imported beef was contaminated *E. coli* O157:H7 and prevalence in local beef samples from wet market at (89.5%) and hypermarkets (44.1%), was positive to *E.coli* [60].

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