Escherichia coli: Animal Foods and Public Health-Review

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Abstract

Foodborne diseases are a growing public health problem all over the world. E. coli is one of the three major food-borne bacterial targets apart from Salmonella spp. and Campylobacter spp. Majority of the E. coli are non-pathogenic; however, few of them are major food borne pathogen of public health significance. The pathogenic E. coli are responsible for watery and bloody diarrhoea, infantile diarrhoea, traveler’s diarrhoea, hemorrhagic colitis, hemolytic uremic syndrome in man. There had been a very rapid development in the detection of various pathogenic microorganisms present in the food and are based on cultural, biochemical and latest DNA based molecular techniques such as polymerase chain reaction (PCR) assay. Further, indiscriminate uses of antibiotics in food animals results in resistance in micro-organisms. Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. Thus, besides judicious use of antibiotics in food animals, there is an urgent need to identify and evaluate the natural food grade antimicrobials that can have potential for the future application to inhibit the growth of pathogenic microorganisms, to protect the consumer from foodborne illness and to extend the shelf life of foods with minimum quality loss. This is essential to give to the consumer and to ensure that the product is free from pathogenic microorganisms is of utmost importance to promote the consumption of products in domestic and export markets. In this review, E. coli in animal foods, their public health significance, their characteristics, serotyping and prevalence, antibiotic resistance pattern, plasmid and hemolysin profile and molecular diagnosis was discussed along with their control.

Keywords: Escherichia coli, Animal foods, Public health, Foodborne pathogens, Shelf life.

1. Introduction

Food is a means to sustain and enjoy life but it is also a vehicle and a medium for transmitting hazards and causing disease and death. Foodborne diseases are a growing public health problem in both developed and developing countries today (Elmi, 2004). Foodborne diseases (FBD) can be defined as those associated with the ingestion of contaminated food and these diseases encompass a wide spectrum of illnesses (WHO, 2006). The World Health Organization (WHO) estimated that in developed countries, up to 30% of the population suffer from food borne diseases each year, whereas in developing countries up to 2 million deaths are estimated per year (WHO, 2007a; WHO, 2007b). To give assurance about the microbial quality to the consumer and to ensure that the product is free from pathogenic microorganisms is of utmost importance to promote the consumption of products in domestic and export markets (Dhanze, 2011; Dhanze et al., 2013). Due to the increase in chicken eggs and meat consumption, the risk of exposure to various animal origin pathogens such as pathogenic Escherichia coli, Salmonella spp., Campylobacter spp. etc. has increased. However, E. coli needs special attention, particularly in the developing countries due to poor hygienic conditions. At slaughter, the organisms enter from the gut, readily soil poultry carcasses and as a result poultry meats are often contaminated with E. coli; likewise eggs become contaminated during laying (Lakhotia and Stephens, 1973; Turtura et al., 1990; Johnson et al., 1996). If not properly handled, E. coli can double every 20 minutes and a single bacterium...
can multiply into more than a million within a very short span of time.

Throughout the 1990s and until today, three major food-borne bacterial targets (Salmonella spp., Campylobacter spp. and E. coli) have persisted, commanding the most research and surveillance attention from government agencies and, to a large extent, the most awareness from the food industry (Newell et al., 2010). In comparison to campylobacteriosis and salmonellosis, pathogenic E. coli cause a relatively low number of cases but the infections caused by E. coli are considered important due to their severity and higher mortality rates (Mataragas et al., 2008). E. coli are generally regarded as part of the normal flora of the human intestinal tract and that of many animals. Most of the strains of Escherichia coli are non-pathogenic; however, some of them are major food borne pathogen of public health importance and responsible for watery and bloody diarrhoea, infantile diarrhoea, traveler’s diarrhoea, hemorrhagic colitis, hemolytic uremic syndrome in man (Johnson et al., 1996; Mead and Griffin, 1998; Shiferaw et al., 2000; Banerjee et al., 2001; Hazarika et al., 2005). Based on their pathogenic phenotypes and the diseases that they cause, diarrhoeageneric E. coli have been classified into 6 groups: enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enteraggregative E. coli (EAEC), diffusely adherent E. coli (DAEC), and Shiga toxin producing E. coli (STEC)/enterohemorrhagic E. coli (EHEC)/verocytotoxin-producing E. coli (VTEC) (Paniagua et al., 1997; Nataro and Kaper, 1998; Matar et al., 2002; Kaper et al., 2004; Bischoff et al., 2005). Other diarrhoeageneric E. coli pathotypes have been proposed, such as cell detaching E. coli (CDEC); however, their significance remains uncertain (Clarke, 2001; Abduch-Fabrega et al., 2002).

There had been a very rapid development in the detection of various pathogenic microorganisms present in the food. These are not only based on cultural and biochemical but also on the molecular techniques such as polymerase chain reaction (PCR) assay, which are based on the nucleic acids. The latest trends shows that the PCR assay with wide variation in its application is rapidly gaining popularity for the purpose of detection of food-borne pathogen and has been recently applied by the various workers for rapid and reliable detection and characterization of E. coli from animal origin food (Toma et al., 2003; Rappelli et al., 2005; Kwon et al., 2008; Oh et al., 2009; Saikia and Saikia, 2011; Canizalez-Roman et al., 2013; Chen et al., 2013; Fujioka et al., 2013). Further, in the process of food production many kinds of antimicrobials are used for preventing and controlling diseases, enhancing growth and increasing feed efficiency in food producing animals (CDC, 2005). The indiscriminate uses of antibiotics in food animals results in resistance in micro-organisms (Philips et al., 2004). Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Levy, 1982; Witte, 1998). Recently, due to the indiscriminate uses of antibiotics the incidence of multiple drugs resistance in E. coli has been increased (Van den Bogaard, 1997; Witte, 1998; Khan et al., 2005; Sharada et al., 2010). Johnson et al. (2007) reported that the poultry meat is one of the major sources of transferring antibiotic resistance to humans. Thus, besides judicious use of antibiotics in food animals, there is an urgent need to identify and evaluate the natural food grade antimicrobials that can have potential for the future application to inhibit the growth of pathogenic microorganisms, to protect the consumer from foodborne illness and to extend the shelf life of foods with minimum quality loss (Dhanze, 2011; Dhanze and Mane, 2012; Dhanze et al., 2013). Plasmid is one of the known most important mediators in facilitating the fast spreading of antibiotic resistance among bacteria (Dale and Park, 2004). Bacteria being genetically flexible have been known to survive in altered environments and have also developed resistance to all different classes of antibiotics discovered to date, because of their capacity to acquire and transfer resistant genes (Frost et al., 2005).

Because many species of bacteria contain plasmids, plasmid profile typing has been used to investigate outbreaks of many bacterial diseases and to trace inter- and intra-species spread of antibiotic resistance (Mayer, 1988). As such, E. coli has a very wide natural distribution (Selander and Levin, 1980) and a propensity for plasmid carriage (Sherley et al., 2003). Resistance to various antibiotics in E. coli strains is frequently plasmid-mediated (Neu, 1992). (Cerquetti et al., 2009; Jan et al., 2009; Chigor et al., 2010) also established a correlation between plasmids and transfer of antibiotic resistance trait.

2. Escherichia coli

E. coli is the head of the large bacterial family, Enterobacteriaceae, the enteric bacteria, which was first described by Theodor Escherich in 1885, as Bacterium coli commune, living in the intestinal tracts of animals in health and disease (Todar, 2005). The species E. coli comprises of gram-negative, rod shaped non-spore forming bacteria, which are about 1 µm in diameter, with a cell volume of 0.6-0.7 µm³. The motility of the organism occurs due to peritrchous flagella, although some non-motile strains have also been recorded. A capsule or microcapsule is often present and a few
strains produce profuse polysaccharide slime (Kubitschek, 1990; Darnton et al., 2006). E. coli is usually a non-pathogenic member of the human colonic flora. However, certain strains have acquired virulence factors and may cause a variety of infections in humans and in animals. Among the pathogenic strains, diarrhoeagenic E. coli (DEC) represents a major public health problem in developing countries (Nataro and Kaper, 1998).

3. Isolation and Identification of E. coli
E. coli can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions. E. coli are most often recovered on MacConkey or eosin methylene-blue agar, which selectively grow members of the Enterobacteriaceae and permit differentiation of enteric organisms on the basis of morphology (Balows et al., 1991). E. coli is a facultative anaerobe that can be recovered easily from clinical specimens on general or selective media at 37°C under aerobic conditions; growth of these organisms has also been observed at 44°C (Edwards and Ewing, 1972). For epidemiological or clinical purposes E. coli strains are often selected from MacConkey agar plates after presumptive visual identification of lactose fermenting pink colonies. However, this method should be used only with caution because only 90 per cent of the E. coli isolates are lactose positive, some diarrhoeagenic E. coli strains are typically lactose negative. The indole test positive in 99 per cent of E. coli strains is the single best test for differentiation from other members of Enterobacteriaceae (Nataro and Kaper, 1998). Identification of different types of DEC includes biochemical test, serotyping, phenotypic assays based on virulence characteristics and molecular detection methods. The serotyping of E. coli based on the somatic (O), flagellar (H) and capsular polysaccharide antigens (K) (Stenutz et al., 2006).

4. Biochemical Characterization of E. coli
Almost all strains of E. coli are reported to produce acid from glucose, lactose, mannitol and arabinose but not from adonitol and inositol and acid from dulcitol, salicin and sucrose varies from different strains. Gas production has been observed in case of glucose only (Sojka, 1965). Other biochemical tests as proposed by Edwards and Ewing (1972), viz. catalase positive, oxidase negative, indole production positive, methyl red positive, Voges-Proskauer negative reaction and inability to grow on Simmon’s citrate medium are considered very useful for preliminary identification of E. coli strains. However, atypical biochemical behaviour of E. coli strains (citrate positive) has also been reported (Ishiguro and Sato, 1979; Lee and Choi, 1983; Kim and Tak, 1984; Dubey and Sharda, 2001, Choudhary, 2012), while few isolates have been reported to produce H₂S (Sutariya, 1993; Mishra et al., 2002). Specifically the citrate positive E. coli were isolated from the disease conditions of animal and humans.

5. Serotyping of E. coli
Serotyping of E. coli occupies a central place in the history of this pathogen. Prior to the identification of specific virulence factors in diarrhoeagenic E. coli strains serotype analysis was the predominant means by which pathogen strains were differentiated. According to modified Kauffm scheme, E. coli is serotyped on the basis of their O (somatic), H (flagellar) and K (capsular) surface antigen profiles (Edwards and Ewing, 1972; Lior, 1996). Whittam et al. (1993) revealed that a specific combination of O and H antigens defines the “serotype” of an isolate. A total of 170 different O antigens, each defining a serogroup, are recognized currently. E. coli of specific serogroups can be associated reproducibly with certain clinical syndromes, but it is not in general the serologic antigens themselves that confer virulence. Rather, the serotypes and serogroups serve as readily identifiable chromosomal markers that correlate with specific virulent clones.

6. Prevalence of E. coli
Foodborne illness is a serious public health threat. More than 200 known diseases are transmitted through food (Bryan, 1982) and foods most likely to cause human illness are animal products such as poultry and eggs, red meat, seafood, and dairy products. Once dismissed as a harmless inhabitant of the intestinal tract, E. coli is now seen as a pathogenic species with remarkable versatility in its ability to cause disease in humans and animals (Nataro and Kaper, 1998). The prevalence of studies on foodborne pathogens indicates that E. coli is one of major foodborne pathogens of animal origin foods with wide variability of virulence (Doyle and Schoeni, 1987; Papadopoulou et al., 1997; Gillespie et al., 2000; Suresh et al., 2000; Zhao et al., 2001; Kobayashi et al., 2002; Johnson et al., 2005; Adesiyun et al., 2006; Singh, 2012; Hasan and Alireza, 2013). Further, the prevalence of virulence E. coli in the poultry and poultry products or the cross contamination of the poultry carcasses from faecal sources were reported by various workers (Jiménez et al., 2003; Musgrove et al., 2004; Hossain et al., 2008; Akond et al., 2009; Keeratipibul et al., 2010; Nzouankeu et al., 2010; Saikia and Joshi, 2010; Ghasemian et al., 2011;
Kagambega et al., 2012a). Specifically 1.3 per cent prevalence of O157 VTEC strains was reported from poultry (Heuvelink et al., 1999); VTEC O157 was isolated (2.2 per cent) from STEC based on eae and VT genes (Schouten et al., 2005); STEC O157:H7 was isolated (17.1 per cent) from the chicken and chicken products (Chinen et al., 2009); STEC O157 was isolated (0.1-54.2 per cent) from the beef carcasses and cuts (Hussain, 2011). The results indicated that there was prevalence of pathogenic STEC in animal foods at higher rates and emphasized the critical need for control measures to assure meat safety. They concluded that poultry can be a source of virulence E. coli strains characteristic of those causing illness in man. Gonzalez et al. (2000) isolated different serogroups of EPEC (O114, O119, O158) from chicken. Similarly Sackey et al. (2000) also isolated enteropathogenic serogroups viz. O158, O125, O25, O28, O159, O15, O126, O63, O143, O26, O78 and O164 from the chicken meat. Kariuki et al. (2002) reported that apparently healthy chicken may carry enteropathogenic E. coli strains isolated from the small scale farms of Kenya. Mishra et al. (2002) reported that predominant serogroups among the E. coli strains isolated from two hundred and fifty poultry samples were O2, O19, O20 and O78.

Chattopadhyay et al. (2001) for the first time isolated verotoxigenic E. coli strains from animal sources in India. Khan et al. (2002) isolated shiga toxin-producing E. coli (STEC) from healthy domestic cattle and raw beef samples collected from the Calcutta city's abattoir and concluded that presence of STEC in domestic cattle and beef samples suggests that this enteropathogen may become a major public health problem in the future. Zhi-Dong et al. (2002) reported that ETEC was the most common pathogen identified from the tourist from Europe and North America who acquired diarrhoea in Mombasa (Kenya), Goa (India), or Montego Bay (Jamaica). Minihan et al. (2003) reported the prevalence of E. coli O157 within the feedlot and subsequent contamination cattle carcasses was 23 per cent. Kijima-Tanaka et al. (2005) assessed the prevalence of STEC among food-producing animals throughout Japan and reported that STEC were isolated from 23 per cent cattle and 14 per cent pig, however no samples was positive in chicken.

Johnson et al. (2006) reported that in the United States, non-O157 E. coli may account for 20 per cent to 50 per cent of all shiga toxin-producing E. coli infections. This study provided a perspective on the non-O157 STEC as human pathogens. Barua et al. (2007) reported that the highest prevalence of E. coli was observed in ready-to-eat (RTE) milk products (76 per cent), followed by RTE meat products (35.21 per cent), RTE vegetable snacks (30 per cent) and water (11 per cent) from animal origin foods. Dhanashree and Mallya (2008) reported that STEC were isolated from diarrhoeagenic stool (73.95 per cent) and meat (77.66 per cent) samples in Mangalore, India by conventional methods and these isolates were further characterized by using PCR. Sehgal et al. (2008) conducted an epidemiological survey of E. coli O157 in different regions of India in last ten years and reported that 0.5, 0.9, 1.8, 8.4 and 1.6 percent samples from human, meat, milk and milk products, seafood and water were positive for E. coli O157. Further, they reported that the isolates were found to be distributed among domestic and wild animals, and the maximum numbers of isolates of E. coli O157 were detected in samples received from coastal belt areas of India. Farooq et al. (2009) conducted a study on the prevalence of enteropathogenic E. coli (EPEC) and shiga toxin producing E. coli (STEC) in avian species in India and reported that 4.24 per cent isolates were STEC and 15.56 per cent were EPEC. Rathore et al. (2010) investigated the prevalence of shiga-toxigenic E. coli (STEC) serotypes in meat and their products from butcher shops and street vendors of Bareilly and Rampur districts of Uttar Pradesh, India. They reported that highest prevalence of STEC was found in raw beef samples and in ready-to-eat beef rolls. Vijayarani et al. (2010) observed that out of total 307 goat and sheep meat samples 101 (32.89 per cent) samples were positive for E. coli by conventional methods. Alonso et al. (2011) detected contamination of chicken by enteropathogenic E. coli at different stages of the chicken slaughtering process by PCR. Arslan and Eyi (2011) reported the prevalence of E. coli isolates to be 42.9 per cent from retail meat samples including chicken meat. Dutta et al. (2013) analysed the trends in the prevalence of different pathogroups of diarrhoeagenic E. coli (DEC) among hospitalized acute diarrhoeal patients and reported that EAEC was prevalent most commonly, followed by ETEC and EPEC based on the multiplex PCR assay. Magwedere et al. (2013) reported the occurrence of STEC serogroups O26, O45, O103, O111, O121, O145, and O157 in retail meat of different animal species.

7. PCR Based Characterization of E. coli
The microbiological safety of meat products is an important public health concern. Numerous epidemiological reports have identified pathogenic E. coli as major cause of disease outbreaks associated with contaminated meat (Olsvik et al., 1991; Meng and Doyle, 1998). Strains of pathogenic E. coli, which have acquired virulence factors, have the ability to cause diarrhoeal disease in human (Lee et al., 2009). Different molecular methods, such as DNA hybridization and PCR, have been developed during the recent years for identification of different categories.
of DEC, and these methods are based on genes related to the pathogenicity of each category (Nataro and Kaper, 1998). One of the methods which have been widely used as a diagnostic method is multiplex PCR (Nataro and Kaper, 1998; Toma et al., 2003; Vidal et al., 2004; Aranda et al., 2007; Vilchez et al., 2009). The application of nucleic acid amplifications requires selecting appropriate oligonucleotide primers and optimizing conditions to maximize sensitivity and specificity. Such multiplex detection is an appropriate solution to the challenge of finding diarrhoeagenic E. coli in food (Lopez-Saucedo et al., 2003). The gene specific simple and multiplex PCR was successfully employed for the identification of pathogenic/virulence genes of E. coli isolates using the primer pairs based on LTI, SII, SLTI and SLTII gene (Tsen and Jian, 1998; Osekk, 2001; Rappelli et al., 2001; Vidal et al., 2005), eae gene (Reid et al., 1999; Rappelli et al., 2001; Paton and Paton, 2002; Toma et al., 2003; Vidal et al., 2005; Wani et al., 2006; Dhanshare and Mallya, 2008; Xia et al., 2010), bfpA (Rappelli et al., 2001; Vidal et al., 2005; Beutin et al., 2007), stxl and stx2 gene (Rappelli et al., 2001; Jothikumar and Griffiths, 2002; Paton and Paton, 2002; Jinneman et al., 2003; Toma et al., 2003; Adwan and Adwan, 2004; Vidal et al., 2005; Watterworth et al., 2005; Wani et al., 2006; Beutin et al., 2007; Dhanshare and Mallya, 2008; Farooq et al., 2009; Xia et al., 2010), ial gene (Rappelli et al., 2001; Watterworth et al., 2005).

PCR assay was found to be convenient, highly specific and sensitive and allowed rapid, specific and simultaneous identification of pathogenic E. coli. It may be used as a method for direct determination of pathogenic E. coli. PCR based prevalence of E. coli isolated from animal foods were employed for the identification of virulence genes and associated pathogenicity (Chattopadhya et al., 2001; Lee, 2009; Cadirci et al., 2010; Islam et al., 2010; Fujioka et al., 2013). These PCR assays allowed rapid, convenient and economical pathogenicity-based identification of the DEC. The multiplex PCR assay was successfully developed and employed for identification of virulence genes for enteroaggregative (EAEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and shiga toxin-producing E. coli (STEC or EHEC) (Chomvarin et al., 2005; Kimata et al., 2005; Rajendran et al., 2010; Ali et al., 2012; Kagambea et al., 2012b; Mohammed et al., 2012; Chen et al., 2013; Hassan and Alireza, 2013; Souza et al., 2013) from animal foods especially poultry meats. Further, Kumar et al. (2001) reported that STEC was prevalent in seafoods’ in India but non-O157 serotype was more common. Khan et al. (2002) reported that all the E. coli isolates from the healthy domestic cattle and raw beef collected from the Calcutta city’s abattoir samples possessed both stx1 and stx2 genes. Johnson et al. (2003) isolated and worked on molecular characterization of nalidixic acid-resistant extra intestinal pathogenic E. coli from retail chicken products to define the prevalence and virulence potential of poultry-associated, quinolone-resistant E. coli in the United States. Kiranmay and Krishnaiah (2010) detected E. coli O157:H7 isolates in foods of animal origin by PCR. Gordillo et al. (2011) developed a multiplex polymerase chain reaction (PCR) procedure based on fliC and rfbE genes for the detection of E. coli O157:H7 in raw pork meat and ready-to-eat (RTE) meat products.

Sharifi et al. (2011) designed a single multiplex polymerase chain reaction (MPCR) for the detection of target genes of stx1/stx2, eae and ipaH in DEC. Oh et al. (2012) concluded that healthy chickens may constitute an important natural reservoir of atypical EPEC strains, and suggested that transmission to humans could not be excluded based on the screening of virulence-associated genes (VT1, VT2, LT, and ST for enterotoxigenic E. coli; eaeA and bfpA for enteropathogenic E. coli; and aggR for enteroaggregative E. coli). Hegde et al. (2012) used two multiplex polymerase chain reaction assays to detect genes of five types of DEC. The targets selected for each category were eae and bfpA (bundle-forming pilus) for Enteropathogenic E. coli (EPEC), hlyA for Enterohemorrhagic E. coli (EHEC), elt and stla for Enterotoxigenic E. coli (ETEC), CVD432 for Enteroaggregative E. coli (EAEC) and iai for Enteroinvasive E. coli (EIEC). Chandra et al. (2013) integrated previously developed multiplex PCR strategies into one single step multiplex that differentiated all the E. coli pathotypes (enteropathogenic (EPEC), enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteroinvasive (EIEC) and diffusely adherent (DAEC) E. coli. Fialho et al. (2013) presented a two-system multiplex PCR for detection of diarrhoeagenic E. coli (DEC). The multiplex-PCR system-1 contained primers for detection of Shiga toxin producing E. coli (STEC; stx1, stx2), enteropathogenic E. coli (EPEC; eae, bfpA), atypical enteropathogenic E. coli (aEPEC; eae), enteroinvasive E. coli (ETEC; iit, st), enteroinvasive E. coli (EIEC; iai), and the internal amplification control 16S rRNA. The system 2 contains primers for EIEC (ipaH), enteroaggregative E. coli (CVD432), diffusely adherent E. coli (dauE), and 16S rRNA.

8. Antibiogram of E. coli

The antimicrobial use in the food animal production was introduced in the late 1940s (Mitchell et al., 1998) to treat sick animals and to ensure safe and

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wholesome meat products (CDC, 2005). Antimicrobial agents are also used for growth promoting and prophylactic purposes in food producing animals. About half of the total amount of antimicrobials produced globally is used in food animals. Among them a large proportion is used not for treating but for preventing disease and for improving efficiency of feed utilization and weight gain (WHO, 2002). The presence of antimicrobial-resistant bacteria in food products is an important public health issue because of the potential for the transfer of antimicrobial-resistant food-borne pathogens to human populations. Moreover, antimicrobial-resistant bacteria may act as a reservoir of resistance genes transferable to pathogenic or commensal bacteria of the human digestive tract (Capita and Alonso-Calleja, 2013). E. coli is the most prevalent bacteria in the intestinal tract in warm-blooded animals and humans, where they constitute approximately $10^6$ to $10^7$ cfu/g of stool. E. coli can easily contaminate food products during animal evisceration after slaughter, through contact with tainted water or during food handling (Álvarez-Fernández et al., 2013). The major factor selecting for antimicrobial resistance in bacteria is antibiotic use, and additionally, crowding and poor sanitation. These three factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in faecal E. coli of poultry (Van den Bogaard and Stobberingh, 1999). Hence, resistant E. coli can infect humans via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora (Van den Bogaard et al., 2001). Furthermore, E. coli of animal origin may act as a donor of antimicrobial resistance genes for other pathogenic E. coli (Hammerum and Heuer, 2009).

In many studies, the varying percentage of multi-antibiotic resistance was reported in E. coli isolated from poultry (Turtura et al., 1990; Singh et al., 1992; Papadopoulou et al., 1997; Suresh et al., 2000; Sackey et al., 2000; Van den Bogaard et al., 2001; Dharani et al., 2003; Johnson et al., 2003; Khan et al., 2005; Zhao et al., 2005; Adesiyun et al., 2006; Diarrassouba et al., 2007; Srinivasan et al., 2007; Huong et al., 2009; Akond et al., 2009; Arslan and Eyi, 2011). While in recent studies, Miles et al. (2006) reported that E. coli isolates showed resistance to tetracycline (82.4 per cent) and trends of higher resistance was observed towards kanamycin and nalidixic acid. Adesiyun et al. (2007) reported high resistance to streptomycin (54.2 per cent) and tetracycline (35.9 per cent), while 55 (46.6 per cent) of E. coli isolates were resistant to three or more antimicrobial agents. Johnson et al. (2007) stated that E. coli isolated from poultry were resistant to trimethoprim-sulfamethoxazole, quinolones and extended-spectrum cephalosporins. Further, they reported that the drug-resistant human isolates were similar to poultry isolates and thus, concluded that many drug-resistant human faecal E. coli isolates may originate from poultry. Smith et al. (2007) detected high prevalence of resistance to drugs such as tetracycline (36 to 97 per cent), sulfonamides (50 to 100 per cent) and streptomycin (53 to 100 per cent) in E. coli isolates obtained from broiler chickens. Yadav et al. (2007) reported the antibiotic resistance pattern of Escherichia coli isolates from mutton as follows: sulphadiazine (93.33 per cent), cephaloridine (80.00 per cent), cephalaxin (33.33 per cent), penicillin G, cefotiofur and norfloxacin, carbenicillin and enrofloxacin (26.67 per cent each), and oxytetracycline and amoxycillin (20.00 per cent each). Arya et al. (2008) reported that the multidrug resistance STEC strains were resistant to kanamycin (100 per cent), cephalaxin (100 per cent) followed by cephalexin (95 per cent), enrofloxacin (85 per cent), amikacin (80 per cent), ampicillin (73 per cent), tetracycline (63 per cent), ceftriaxone (34 per cent), ciprofloxacin (20 per cent), colistin (12 per cent) and cotrimoxazole (5 per cent). Adelaide et al. (2008) detected high resistance levels for most commonly used drugs like tetracycline (75.9 per cent) and cotrimoxazole (72.4 per cent) among E. coli isolates obtained from healthy broiler chicken at Tigoni processing plant, Limuru, Kenya. Hossain et al. (2008) reported that E. coli isolates from apparently healthy broiler and layer chickens in Mymensingh, Bangladesh were highly sensitive to chloramphenicol, ciprofloxacin, kanamycin and cephalaxin and an increasing trend of resistance was recorded in both broiler and layer isolates. Kikuvi et al. (2009) reported multidrug resistance to be highest in the E. coli isolates obtained from healthy broiler chickens (74.0 per cent) as compared to other animals. Various researchers (Ramteke and Tewari, 2007; Sharada et al., 2010; Jakobsen et al., 2010; Thorsteinsdottir et al., 2010; Chen et al., 2013) concluded that the E. coli isolated from various human and poultry sources showed almost similar antimicrobial resistance trends to one or more antimicrobial agents, which supports the hypothesis that poultry, animal and meat reservoirs might exist for pathogenic and antimicrobial resistance E. coli and thus spreading antimicrobial resistance to humans. Lei et al. (2010) reported that 70 per cent of E. coli isolates showed resistance to tetracycline, trimethoprim-sulphamethoxazole, nalidixic acid, and ampicillin. Singh (2012) isolated E. coli from table egg samples and observed that many of the E. coli (30.8 per cent) had multiple-drug-resistance (MDR). Most of the E. coli isolates were resistant to ampicillin and tetracycline but none to enrofloxacin. The study
concluded that MDR E. coli are quite prevalent in poultry eggs. Depoorter et al. (2012) reported that in Belgium, about 35 per cent of the E. coli strains isolated from live broilers are resistant to 3rd generation cephalosporins while over 60 per cent of the broilers are found to be carrier of these 3rd generation cephalosporin resistant E. coli (CREC) after selective isolation. Zhao et al. (2012) reported that compared to beef and pork isolates, the poultry meat isolates had a greater percentage of resistance to all tested drugs, with the exception of chloramphenicol. Álvarez-Fernández et al. (2013) reported that E. coli isolates from poultry carcasses were multi-resistant (91.7 per cent), while the resistance to nalidixic acid being most common (85.0 per cent), followed by resistance to ampicillin (75.0 per cent), ciprofloxacin (73.3 per cent) and tetracycline (61.7 per cent).

9. Interrelationship of Plasmid profiles, Hemolysis, Antibiotic Resistance and Virulence of E. coli

Plasmids are genetic elements that can be transmitted between bacteria. They are not virulence factors, but they can encode genes for a variety of factors that contribute to pathogenesis, including antibiotic resistance, fimbriae, toxins, secretion systems and invasion factors (Prats, 2003). E. coli, perhaps the most studied of microorganism, have been found to possess a variety of plasmid types. Several types of E. coli virulence plasmids exist, including those essential for the virulence of enterotoxigenic E. coli, enteroinvasive E. coli, enteropathogenic E. coli, enterohemorrhagic E. coli, enteroaggregative E. coli and extra intestinal pathogenic E. coli. Despite their diversity, these plasmids belong to a few plasmid backbones that present themselves in a conserved manner (Johnson and Nolan, 2009).

Transmission of plasmids plays a significant role in the growing problem of antibiotic resistance (Prats, 2003). Also, plasmid-encoded virulence genes are important for identification and differentiation of DEC strains from non-pathogenic members, as these are absent in the commensal E. coli (Nataro and Kaper, 1998). The plasmid profile, their association with antibiotic resistance and virulence of E. coli isolated from diverse systems, were investigated by various workers (Caudry and Stanisch, 1979; Ishiguro et al., 1979; Chaslus-Dancla and Lafont, 1985). Son and Gulam (1995) reported that isolates from poultry containing plasmid DNA (size of 1.5 to 64 MDa) were resistant to antibiotics such as ampicillin, erythromycin, streptomycin, sulphonamide, trimethoprim-sulfamethoxazole and tetracycline. Doetkott et al. (1996) concluded that E. coli isolates of chickens frequently contain large plasmids and many of these plasmids are likely to contain virulence related sequences. Kariuki et al. (1999) reported that plasmid DNA of E. coli isolates from chicken and children were found to encode the transferable resistance to co-trimoxazole and tetracycline. Geornaras et al. (2001) isolated E. coli that possessed single and multiple size plasmids while other workers isolated E. coli that had multiple plasmids DNA with different size ranging from 2.3 kb to 26 kb (Jan et al., 2009), 2.3 to 102 kb (Son et al., 1996), 1.0 to 30.9 MDa (Fei et al., 2003), 0.564 kb to >23 kb (Smith et al., 2003), 1 to 68 Kbp (Khoshkhou and Peighambari, 2005), 2.3 kb to 26 kb (Jan et al., 2009), 0.5 to 40 kb (Alam et al., 2010), 48-157 kb (Burgos and Beutin, 2010), 5 to 9 kb (Lulu and Growther, 2012) and very high antibiotic resistance was detected from isolates possessing high molecular weight plasmids (23kb) (Jan et al., 2009). However, Alam et al. (2010) reported that the plasmids were distributed at random in the isolated E. coli strains and no remarkable relationship between antibiotic resistance patterns and plasmids could be established. Saleh (2007) reported that there was a correlation between isolates that had plasmid patterns and maintaining antimicrobial resistance. Al-Bahry et al. (2006) revealed that the isolates contained various size resistant plasmids (R-plasmids) exhibited different antibiotic resistance patterns; some of their plasmids had similar migration patterns on agarose gel electrophoresis.

Santhosh et al. (2009) conducted plasmid profiling and characterization of E. coli. Multiplex PCR revealed presence of iss and fimC genes in 50 per cent of the isolates and plasmids of size ranging between 3 to 4 kb. The E. coli isolates were investigated for their haemolytic activity and its correlation with virulence (Chart et al., 1998; Reingold et al., 1999; Bashar et al., 2011). Bisht et al. (1977) observed association in-between haemolysin and necrotoxin production among the E. coli isolates from cases of acute gastroenteritis, chronic diarrhoea as well as healthy human population. Beutin et al. (1989) observed haemolysin production in verotoxin producing E. coli, while non-verotoxin producing E. coli failed to do so. They further reported that fifteen per cent E. coli strains were positive for alpha haemolysin, however no haemolysin positive strain was found in a group of forty five ETEC strains obtained from humans (42 strains), animals (two strains) or food stuffs (one strain). Blaico et al. (1993) reported that twenty-three (23) strains belonging to classic enteropathogenic E. coli (EPEC) serogroups for the production of alpha-haemolysin (Hly), while four strains belonging to serotypes O118a: H7, O26: H-, O44: H18 , O119: H27 were hemolysin positive. Reingold et al. (1999) reported that haemolysin is one
type of virulence factor that assists in the pathogenesis of *E. coli* and this hemolytic activity has been attributed to haemolysin genes found in enterohemorrhagic *E. coli*. Patil et al. (1999) carried out a study for testing drug resistance and virulence characters viz. haemolysin and enterotoxin production in *E. coli* isolated from diarrhoeal calves. Eight (44.4 per cent) of eighteen *E. coli* isolates were found to produce haemolysin and among these six (75 per cent) were enterotoxigenic (ETEC). Khan et al. (2002) revealed 14 out of 30 STEC isolates from diarrhoeic patients, healthy domestic cattle and raw beef samples showed α-haemolytic activity. However, three STEC strains didn’t produce haemolysin. Bashar et al. (2011) reported that among sixty *E. coli* isolates from poultry chicken faeces; 45 per cent and 14 per cent showed β hemolysis and α hemolysis, respectively. Leverstein-van Hall et al. (2011) assessed the distribution of ESBL genes, plasmids and strain genotypes in *E. coli* obtained from poultry and retail chicken meat in the Netherlands and reported that Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Of these ESBL genes, 86 percent were *bla* (CTX-M-1) and *bla* (TEM-52) genes, which were also the predominant genes in poultry (78 percent) and retail chicken meat (75 percent). Saikia and Saikia (2011) recently characterized the *E. coli* based on ‘*stx* 1’ and ‘*stx* 2’ toxigenic genes for their multiple antibiotic resistant *E. coli* population and phenotypic detection of ESBL producing *E. coli* isolates from local variety of poultry by PCR assay.

10. Conclusions

*E. coli* is one of the most important foodborne pathogens of public health importance. The *E. coli* is mainly originated from animal foods and few of the *E. coli* strains causing fatal infections or diseases in human. Even they are slowly developing resistance for most of the commonly used antibiotic due to indiscriminate use in animal productions systems and this pose a future challenges in treatment of the *E. coli* infections. However, pathogenic *E. coli* infections can be rapidly diagnosed using DNA based PCR assays. Finally it can be concluded that to avoid the resistance development in *E. coli* judicious use of antibiotic is essential along with alternate agents from natural sources having minimum effect on quality attribute of foods.

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