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Study the presence of *Escherichia coli* in broiler chicken meat as a potential public health threat

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Abstract

Escherichia coli is an important pollution indicator and its pathogenic strains is a serious public health concern. A study was conducted to investigate the presence of *E. coli* in Jalalabad city's raw broiler meat retails shops and markets and its antimicrobial sensitivity pattern to common antibiotics. Total number (n = 130) of samples were studied, out of which 34.6 % (45/130) were found contaminated with *E. coli*. The prevalence of pathogenic strain O157 was 3.8% (5/130). In the antibiogram study, 88.9 % (40/45) isolates showed resistance to ampicillin and tetracycline. The resistance against kanamycin were 20% (9/45), whereas 24.5% (11/45) against streptomycin. Several *E. coli* isolates were found resistant to multiple antibiotics. One *E. coli* isolate showed resistance to seven antibiotics (ampicillin, tetracycline, sulfamethoxazole/trimethoprim, gentamicin, chloramphenicol, nalidixic acid and kanamycin) out of nine antibiotics used in the study. The antibiotic resistance of *E. coli* to common commercial antibiotic is a potential threat to food safety and public health of Jalalabad city in Nangarhar province. That is why, Preventing food contamination and human infection from *E. coli* requires control measures at all stages of the food production continuum: from agricultural production, to processing, manufacturing, transporting, storing, and preparation of foods in both commercial establishments and the domestic environment.

Keywords: Poultry meat, *Escherichia coli* O157, antibiotic resistance, food safety

1. Introduction

Food-borne diseases, caused by agents that enter the body through the intake of contaminated food materials are one of the primary public health concerns (Tan *et al.*, 2013) [18]. Meat is consumed by many people as an important source of protein and other nutrients. It has been estimated that 62 billion chickens, 1.5 billion pigs, 545 million sheep, 444 million goats, and 301 million cattle were slaughtered for meat consumption worldwide in 2014. Furthermore, pork is the most consumed meat (average consumption of 16 kg per year in 2013), followed by poultry (15 kg), beef/buffalo (9 kg), and mutton and chevon (2 kg). Meat consumption is known to be highest across high-income countries and lowest in low-income countries (Ritchie and Roser. 2019) [8].

E. coli encompasses a number of strains of the bacterium known as *Escherichia coli*, which normally inhabits the intestines of all animals, including humans. Most strains of *E. coli* are harmless, and, in fact, are necessary for us to develop and function properly. *E. coli* and other bacteria provide us with many necessary vitamins. However, a few strains of *E. coli* are capable of causing illness in humans. Of particular concern is the strain known as *Escherichia coli* 0157:H7, which can cause severe illness such as Enteropathogenic, enteroinvasive and enterotoxigenic types of *E. coli* can be a leading cause of food-borne diarrhoea (Akbar and Anal, 2011) [2].

E. coli 0157:H7 produces large quantities of one or more related potent toxins that cause severe damage to the lining of the intestine. These toxins (verotoxin, or VT, and shiga-like toxin) are closely related (or identical to) the toxin produced by *shingella* dysenteriae. Food Processing: Understanding and Controlling *E. coli* Contamination. Animals, such as dairy and beef cattle, chickens, pigs, and sheep are natural reservoirs for *E. coli* bacteria. However, *E. coli* also grows well outside the animal body, thriving, for example, in unclean food handling equipment. It affects the people's well-being, and imposes economic impacts (Akbar and Anal, 2013) [4]. Direct human or animal fecal contamination is also often a source of this bacteria. *E. coli* 0157:H7 infection, or hemorrhagic colitis, often causes severe bloody diarrhea, abdominal

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cramps, and occasional vomiting. Fever is either absent or of low grade. The illness is self-limiting and lasts for an average of eight days. In some persons, particularly the elderly and children under five years of age, the infection can also cause a complication called hemolytic uremic syndrome (HUS), in which the red blood cells are destroyed and the kidneys fail. About 2% to 7% of *E. coli* infections lead to this complication. In the United States, hemolytic uremic syndrome is the principle cause of acute kidney failure in children, and most cases of HUS are caused by *E. coli* O157:H7. (Oluyeye *et al.*, 2009) [14].

In many developing countries, food-borne disease outbreak from bacteria, such as *Escherichia coli* and *Salmonella* spp. impose a substantial burden on health care systems and can markedly reduce the economic productivity of the countries. A huge number of acute diarrheal cases reported each year, while the reported cases of food poisoning are more than 120,000 per year in Thailand (Minami *et al.*, 2010) [11]. It is hard to identify the pathogen and food vehicle responsible for the majority of food-borne infection. Poultry meat, red meat, desserts and egg can transfer the pathogens like *Salmonella*, *E. coli* and *Campylobacter* (Akbar and Anal, 2014b) [3].

Escherichia coli O157:H7 are mostly associated with food materials. The low infectious dose and life-threatening complication have made this organism an important pathogen and serious threat to public health. Unhygienic practices, use of contaminated instruments and materials in food processing are mainly associated with food-borne diseases (Wilfred *et al.*, 2012) [20].

This is because the muscle of a non-diseased life animal is indispensably sterile. Once the animal is slaughtered, the muscles are exposed and can be contaminated by microorganisms. *E. coli* are known to naturally harbour in the gastrointestinal tract of farm animals. They cross-contaminate meats when the gastrointestinal tract ruptures during evisceration. It was observed during sampling that knives used for cutting meats were not sterilized intermittently (Feng *et al.*, 2017) [15].

The prevalence of antimicrobial resistance among food-borne pathogens increased during recent decades (Akbar and Anal, 2014a) [1]. The frequent and unnecessary use of antimicrobial agents for farming and therapeutic purpose in animals and human are contributing to create resistant strains. *Escherichia coli* is known to be an indicator of faecal contamination, and its presence in food indicates the possible presence of other enteric pathogens. Some of the *E. coli* strains itself are highly pathogenic in human and animals. People with low immunity are the prime target of the pathogenic strains of *E. coli* (Keeratibul *et al.*, 2009; Akbar and Anal, 2011) [2].

The objective of this study was to analyse the presence of *E. coli* and its pathogenic strain O157 in retail poultry meat at consumer counter (shops and markets). AntibioGram of the isolates were also analyzed, in order to investigate the resistance pattern of the isolates to commonly in use antibiotics. For effective food safety management plan, it is necessary to continuously monitor the presence of pathogens in food materials.'

2. Materials and Methods

2.1 Sampling

A total number of 130 broiler meat samples were collected from different open and supermarkets in Jalalabad (city and zones) in sterile polythene bags and kept in a priority disinfected sampling box. The samples were brought to the laboratory in a sampling box maintaining low temperature (\leq

4 °C) using ice pads. The collected samples were processed within six hours of its collection. The samples were collected randomly and each collected sample was marked with the identification code with respect to the date and time of collection. Sampling criteria were limited to 300 g of one sample in open market and one packet in the supermarket. Samples collection criteria were not limited to any specific part.

2.3 Isolation procedure of *Escherichia coli*

Meat with hard pieces or bony samples was first trimmed with sterile knife. Isolation and identification procedure in bacteriological analysis manual (Feng *et al.*, 2002) [8] was followed with slight modification for the isolation and identification of *Escherichia coli*, whereas serological kit was used to confirm the pathogenic strain O157. The meat samples (25 g) were first transferred to the sterile flask containing 225 ml of sterile Tryptic Soy Broth (TSB) (Merck, Germany). The samples were then homogenized with stomacher machine (Bag Mixer, Inter science) for 10 min, and incubated at 37 °C for 16-24 hours. Following the incubation, part (two wire loop full) of the TSB was transferred to Macconkey Agar (MKA) (Himedia, India) and incubated at 37 °C for 24 hours. The lactose fermenting colonies on MKA was transferred to Eosin Methylene Blue Agar (EMBA) (Himedia, India) and incubated at 37 °C for a further 24 hours. Suspected colonies (with a green metallic sheen) were then confirmed by API 20E Kit (Biomérieux, France) along with API web. The pathogenic strain O157 was confirmed with the help of a latex agglutination kit (Oxoid, UK) from the preliminary confirmed *E. coli* isolates. *Escherichia coli* (TISTR 780) was used as control strains for the validation of nutritional media and immunological kits. Antimicrobial susceptibility test All the *E. coli* isolates were exposed to different antibiotics for its antimicrobial susceptibility and drug resistance pattern determination using a disk diffusion assay following the guidelines of clinical and laboratory standard institute. Pre-incubated 24 hours cultures of *E. coli* in sterile buffer peptone water with bacterial count 108 CFU/ml was swabbed over Mueller-Hinton agar (Merck, Germany). After placing the antibiotic discs aseptically, the plates were incubated at 37 °C for 18-24 hours and zone of inhibition were measured subsequently. The commercial antibiotics used in the study were: Ciprofloxacin (5 µg), Ampicillin (10 µg), Tetracycline (30 µg), Sulfamethoxazole/Trimethoprim (25 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Nalidixic acid (30 µg), Streptomycin (10 µg) and Kanamycin (30 µg) (Oxoid, UK).

3. Result and Discussion

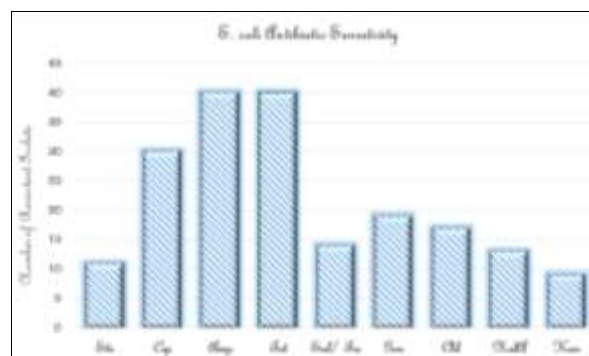


Fig 1: Resistance of *Escherichia coli* isolates against different antibiotics. Streptomycin (Str), Ciprofloxacin (Cip), Ampicillin (Amp), Tetracycline (Tet), Sulfamethoxazole/Trimethoprim (Sul/Tri), Gentamicin (Gen), Chloramphenicol (Chl), Nalidixic acid (NalA), Kanamycin (Kan)

Out of all 130 samples analyzed for the presence of *E. coli*, 34.6 % (45/130) was found contaminated with *E. coli*, in which five were *E. coli* O157 strain making prevalence of 3.8% (5/130) of all samples investigated. The *E. coli* O157 was confirmed with the help of serological kit. The percentage of pathogenic *E. coli* was lower than the overall contamination. Presence of pathogenic strains of *E. coli* in broiler chicken meat is not only a potential threat of cross contamination, but can also lead to become an infectious dose for handlers and consumers. *Escherichia coli* presence in food materials are considered to be an indicator for the presence of other pathogenic bacteria in the respective food items (Shar *et al.*, 2010) [18].

Rasmussen *et al.* (2015) [13] examined locally produced chicken meat and imported chicken thighs into Ghana for *E. coli* and observed that the local chickens 36 (64.29%) and imported chickens 73 (55.30%) were contaminated by *E. coli*. Adzitey (2015) [7] also detected 56% (39/70) of *E. coli* in beef samples sold in the Tamale metropolis of Ghana. Zhao *et al.* (2001) [23] reported 38.7% prevalence of *E. coli* in chicken meat in a similar study in Minami *et al.* (2010) [11] reported zero percent prevalence of *E. coli* O157:H7 in Thailand in a similar study. Hossain *et al.* (2008) [10] recorded 63.6% in broiler and 56.4% in layer equal to the overall 60 % prevalence in a similar study in Bangladesh. Rahimi *et al.* (2012) reported 4.7% *E. coli* O157:H7 prevalence in the raw meat, including beef, camel, sheep, goat, and water buffalo meat in Iran. Handling of meat and animal carcasses, cross contamination from soil, cutting instruments and the use of contaminated water for washing purpose can be a prominent source of contamination. Monitoring of foodborne pathogens in food products are the only means to cope with the problem promptly (Chang *et al.*, 2013) [6].

The *E. coli* isolates including *E. coli* O157 were tested for its antibiotic susceptibility and resistance patterns against nine different commonly in use antibiotics. 24.5% (11/45) isolates were found resistant to antibiotic streptomycin, while the resistance 66.6% (30/45) were noted against ciprofloxacin. The *E. coli* isolates 88.9 % (40/45) were found resistant to ampicillin, while the same percentage of resistance was found against tetracycline. The highest resistance was found against ampicillin and tetracycline. 14 out of 45 (31.1%) *E. coli* isolates showed resistance to sulfamethoxazole /trimethoprim, whereas 42.2% (19/45) were found resistant to gentamicin, and 37.7% (17/45) were found resistant to chloramphenicol, 28.8% (13/45) against nalidixic acid and 20% (9/45) against kanamycin, illustrated in Figure 1.

Hossain *et al.* (2008) [10] reported that the *E. coli* isolates from Bangladesh were found 100% resistant to nalidixic acid and 63% to ampicillin. Out of all nine different antibiotics used against forty five different *E. coli* isolates of poultry meat, streptomycin and kanamycin were found more active as compare to other antibiotics used in the study. Oluyeye *et al.* (2009) [14] reported 91.3% resistance against gentamicin, 34.8% against tetracycline and 8.7% against nalidixic acid in *E. coli* isolates from southwestern Nigeria. The nalidixic acid resistance percentage is in agreement with our study, while in case of tetracycline and gentamicin it varies. The resistance against drugs can be genetically transferred from one bacterium to another by transmissible elements like plasmids (Neu, 1994) [14].

These resistant bacteria can pass their resistance genes to their offspring by replication or to related bacteria through conjugation (Tomasz, 1994) [20]. *Escherichia coli* exchange the resistance genes with the help of conjugation (Madden,

2009) [11]. The study showed that all *E. coli* isolates of poultry meat are resistant to at least three antibiotics, commonly in use against Gram-negative bacteria. Nine *E. coli* isolates showed resistance to almost four antibiotics while fifteen isolates were found resistant to five antibiotics. The highest resistance of one *E. coli* isolate was noted against seven different antibiotics (ampicillin, tetracycline, ulfamethoxazole/trimethoprim, gentamicin, chloramphenicol, nalidixic acid and kanamycin) out of nine used in the study. The study showed that, most of the *E. coli* isolates are multi-drug resistant and majority of the antibiotics were found inactive against them. 15 out of 45 isolates of *E. coli* were resistant to 5 antibiotics. 11 out of 45 isolates were resistant to 4 out of 10 antibiotic used in the study. 20 out of 45 *E. coli* isolates showed resistance to five antibiotics. Akond *et al.* (2009) [5] found multi-drug resistant *E. coli* showed resistant to more than six different types of antibiotics in a similar study in Bangladesh.

Preventing food contamination and human infection from *E. coli* requires control measures at all stages of the food production continuum: from agricultural production, to processing, manufacturing, transporting, storing, and preparation of foods in both commercial establishments and the domestic environment.

4. Conclusion

This study revealed that the presence of *E. coli* and its pathogenic strains are common in the broiler chicken meat. Such contamination can easily lead to cause the infections related to this bacteria. Its presence in food materials is a problem, while the developments of drug resistance by these common pathogens are a more serious matter of concern for food safety and public health. It was concluded that the majority of commonly in use antibiotic are not active against *E. coli* isolates from poultry meat. New strategies and proper food safety management are needed to prevent the contamination of food materials and to reduce the drug resistance. Developing a new and natural antibiotic with a novel mode of action is necessary for the treatment of such multi-drug resistant bacteria.

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