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Presence of *Escherichia coli* in poultry meat: A potential food safety 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* and its pathogenic strain O157 in raw poultry meat and its antimicrobial sensitivity pattern to common antibiotics. Total number (n = 152) of samples were studied, out of which 25% (38/152) were found contaminated with *E. coli*. The prevalence of pathogenic strain O157 was 2% (3/152). In the antibiogram study, 92% (35/38) isolates showed resistance to ampicillin and tetracycline. The resistance against kanamycin were 15.8% (6/38), whereas 23.7% (9/38) 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.

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). It affects the people's well-being, and imposes economic impacts (Akbar and Anal, 2013). In many developing countries, food-borne diseases 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 (Hanson *et al*., 2002; Minami *et al*., 2010). Amongst the food-borne pathogens, *E. coli* and *Salmonella* are the most common and frequent pathogens responsible for food poisoning and food related infections. *Escherichia coli* is responsible for 25% of the infant diarrhoea in developing countries (WHO, 2000). Enteropathogenic, enteroinvasive and enterotoxigenic types of *E. coli* can be a leading cause of food-borne diarrhoea (Akbar and Anal, 2011). 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* (Hughes *et al*., 2007). Unhygienic practices, use of contaminated instruments and materials in food processing are mainly associated with food-borne diseases (Wilfred *et al*., 2012; Akbar and Anal, 2014b). *Escherichia coli* O157:H7 are mostly associated to food materials. The low infectious dose and life-threatening complication has made this organism an important pathogen and serious threat to public health (Akkaya *et al*., 2006). The outbreaks associated to this organism are mostly associated to food of bovine origin, ground meat and raw milk (Bachrouri, 2002).

The prevalence of antimicrobial resistance among food-borne pathogens increased during recent decades (Van *et al*., 2007; Akbar and Anal, 2014a). The frequent and unnecessary use of antimicrobial agents for farming and therapeutic purpose in animals and human are contributing to create resistant strains. Drug resistant bacteria are harder to treat with the common antibiotics (Altekruse *et al.*, 1997). *Escherichia coli* is known to be an indicator of faecal contamination, and its presence in food indicate 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

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the prime target of the pathogenic strains of *E. coli* (Keeratibul *et al.*, 2009; Akbar and Anal, 2011). 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. Antibiogram of the isolates were also analysed, in order to investigate the resistant 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.

Materials and Methods

Sampling

A total number of 152 poultry meat samples were collected from different open and super markets of greater Bangkok region (Bangkok city and suburb) in sterile polyethene bags and kept in priorly disinfected sampling box. The samples were brought to the laboratory in a sampling box maintaining low temperature (≤ 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 identification code with respect to the date and time of collection. Sampling criteria was limited to 300 g of one sample in open market and one packet in supermarket. Samples collection criteria were not limited to any specific part.

Isolation 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) 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, Interscience) for 10 min, and incubated at 37°C for 16-24 h. Following the incubation, part (two wireloop full) of the TSB was transferred to Macconkey Agar (MKA) (Himedia, India) and incubated at 37°C for 24 h. The lactose fermenting colonies on MKA were transferred to Eosin Methylene Blue Agar (EMBA) (Himedia, India) and incubated at 37°C for further 24 h. Suspected colonies (with a green metallic sheen) were then confirmed by API 20E Kit (Biomerieux, France) along with api web. The pathogenic strain O157 was confirmed with the help of latex agglutination kit (Oxoid, UK) from the preliminary confirmed *E. coli* isolates. *Escherichia coli* (TISTR 780) were 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 disk diffusion assay following the guidelines of clinical and laboratory standard institute. Pre-incubated 24 h cultures of *E. coli* in sterile buffer peptone water with bacterial count 10^8 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 h 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).

Results and Discussion

Out of all 152 samples analysed for the presence of *E. coli*, 25% (38/152) were found contaminated with *E. coli*, in which three were *E. coli* O157 strain making 2% (3/152) 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 poultry 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). Zhao *et al.* (2001) reported 38.7% prevalence of *E. coli* in chicken meat in a similar study in Washington D.C., USA. Suthienkul *et al.* (1990) reported 9% of shiga-like toxin producing *E. coli* from beef meat in Thailand. Minami *et al.* (2010) reported zero percent prevalence of *E. coli* O157:H7 in Thailand in a similar study. Voravuthikunchai *et al*. (2002) reported 7% *E. coli* O157 and 8.9% other *E. coli* in food samples in southern Thailand. Hossain *et al*. (2008) recorded 63.6% in broiler and 56.4% in layer equal to overall 60% prevalence in a similar study in Bangladesh, whereas Bhattacharjee *et al*. (1996) reported 40.82% prevalence of *E. coli* in poultry from Bangladesh. Rahimi *et al.* (2012) reported 4.7% *E. coli* O157:H7 prevalence in the raw meat

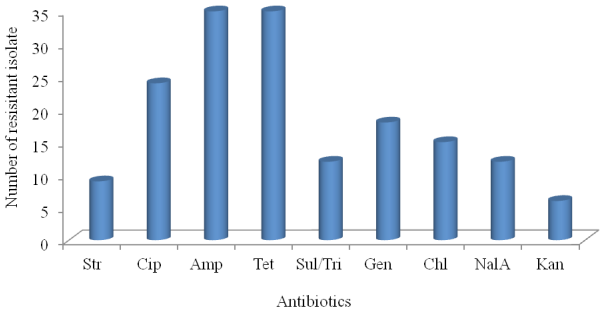

Figure 1. Resistance of *Escherichia coli* isolates against different antibiotics. Streptomycin (Str), Ciprofloxacin (Cip), Ampicillin (Amp), Tetracycline (Tet), Sulfamethoxazole/Trimethoprim (Sul/Tri), Gentamicin (Gen), Chloramphenicol (Chl), Naladixic acid (NalA), Kanamycin (Kan)

including beef, camel, sheep, goat, and water buffalo meat in Iran. Lye *et al*. (2013) reported 18.8, 7.3 and 3.4% *E. coli* O157:H7 prevalence in raw cow, goat and buffalo milk, respectively. 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).

The *E. coli* isolates including *E. coli* O157 were tested for its antibiotic susceptibility and resistance patterns against nine different commonly in use antibiotics. Nine out of thirty eight (23.7%) isolates were found resistant to antibiotic streptomycin, while the resistance 63.2% (24/38) were noted against ciprofloxacin. The *E. coli* isolates 92.1% (35/38) were found resistant to ampicillin, while the same percentage of resistance was found against tetracycline. The highest resistance were found against ampicillin and tetracycline. Twelve out of thirty eight (31.6%) *E. coli* isolates showed resistance to sulfamethoxazole/trimethoprim, whereas 47.4% (18/38) were found resistant to gentamicin, and 39.5% (15/38) were found resistant to chloramphenicol, 31.6% (12/38) against nalidixic acid and 15.8% (6/38) against kanamycin, illustrated in Figure 1. Hossain *et al*. (2008) 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 thirty eight different *E. coli* isolates of poultry meat, streptomycin and kanamycin were found more active as compare to other antibiotics used in the study. Oluyege *et al.* (2009) reported 91.3% resistance against gentamicin, 34.8% against tetracycline and 8.7% against nalidixic acid in *E. coli* isolates from south western 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). These resistant bacteria can pass their resistance genes to their offspring by replication or to related bacteria through conjugation (Tomasz, 1994). *Escherichia coli* exchange the resistance genes with the help of conjugation (Madden, 2009).

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 resistance to five antibiotics. The highest resistance of one *E. coli* isolate was noted against seven different antibiotics (ampicillin, tetracycline, sulfamethoxazole/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. Twelve out of thirty eight isolates of *E. coli* were resistant to three antibiotics. Nine out of thirty eight isolates were resistant to four out of nine antibiotic used in the study. Fifteen out of thirty eight *E. coli* isolates showed resistance to five antibiotics. Akond *et al*. (2009) found multi-drug resistant *E. coli* showed resistant to more than six different types of antibiotics in a similar study in Bangladesh.

Conclusion

This study revealed that the presence of *E. coli* and its pathogenic strains is common in the poultry 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 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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