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Original article

Salmonella, Campylobacter and Escherichia coli IN RAW CHICKEN MEAT, CHICKEN PRODUCTS AND COOKED CHICKEN IN RETAIL MARKETS IN KANDY, SRI LANKA

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SUMMARY: *Salmonella, Campylobacter* and *Escherichia coli* (*E. coli*) are common foodborne zoonotic bacteria with a significant risk of transmission through poultry and related products. Chicken is the most commonly available and consumed meat type in Sri Lanka, hence this study aimed to identify the occurrence of those microorganisms in retail chicken products that may be posing a direct risk to consumers. A total of 124 chicken samples of chilled or frozen raw meat, sausages, meat balls, and cooked chicken curries were purchased from retail outlets in Kandy municipality area. The presence of above organisms and the antimicrobial resistance profiles of *E. coli* isolates were tested utilizing standard methods. All types of samples except chicken curries were contaminated with *Salmonella, Campylobacter* and *E. coli* to different extents. Frequencies of contamination of sausages and meat balls with *Salmonella* and *Campylobacter* were lower than the contamination with *E. coli.* A higher proportion of loose sausages were positive for *E. coli* compared to packaged sausages. Antimicrobial susceptibility patterns of *E. coli* isolates indicated that all were resistant to ampicillin, tetracycline and streptomycin but susceptible to gentamicin, imipenem and amikacin. The study reinforces the importance of adequate cooking of chicken meat and meat products.

INTRODUCTION

Microbiological safety of food was a challenge 20 years ago and continues to be challenging with new ones emerging (Newell *et al*., 2010). It was reported that in 2010 thirty-one foodborne hazards (including viruses, bacteria, protozoa, helminths and chemicals) have caused 600 million foodborne illnesses and 420,000 deaths globally (Havelaar *et al.,* 2015). Among these, foodborne bacterial diseases account for the major part of the burden where *Campylobacter, Salmonella* and *Escherichia coli* (*E. coli*) are reported most frequently (Scallan *et al*., 2011). The Centre for Disease Control and Prevention in the USA (CDC) has identified eight main pathogens as food contaminants and the three named above are the most important of these (CDC, 2014). Among them campylobacteriosis and salmonellosis are the two very important diseases because more than 90% of bacterial originated foodborne cases across the globe represent these two groups (Thorns, 2000). Campylobacteriosis is considered to be the most common bacterial zoonosis in the world (Kaakoush *et al*., 2015). In the case of salmonellosis it is known to be responsible for over 90 million cases associated with diarrhoea, out of which 85% have a link to food (Majowicz *et al.,* 2010). Aggravating the problem, consumption of food contaminated with a strain of bacteria that is resistant to antimicrobials may lead to an infection in humans that cannot be successfully treated with antibacterial drugs (CDC, 2018).

Developed countries conduct regular surveillance studies on foodborne bacterial pathogens and poultry has been identified as a major source of microorganisms leading to foodborne diseases. In developing countries financial and technological constraints limit the ability to conduct regular surveillance and there is much less understanding about the causes of foodborne infections, as highlighted by several authors (Newell *et al*., 2017; Suzuki and Yamamoto, 2009; Newell *et al*., 2010). Nevertheless, production and consumption of chicken meat has significantly increased in the South Asian region in the recent past. The situation in Sri Lanka is no different, and according to the Department of Animal Production and Health (DAPH) poultry meat and egg production now contributes to more than 70% of the livestock sector (DAPH, 2015). Previous studies conducted in Sri Lanka have found contamination of poultry with *Salmonella*, *Campylobacter* and *E. coli* (Kottawatta *et al*., 2017; Kamalika *et al*., 2008; Dissanayake *et al*., 2008), and *Campylobacter* has been identified as a common problem with about 64% prevalence at the farm level (Kalupahana *et al*., 2013). Another study conducted by Kottawatta *et al.* (2014), covering 11 districts of Sri Lanka concluded that 9% of the samples from broiler flocks were positive for *Salmonella*.

The Veterinary Epidemiological Bulletin published by the DAPH in 2012 stated that *Salmonella* had been detected in breeder farms and that one hatchery was positive for *Salmonella enteritidis* (DAPH, 2012). Furthermore, resistance to commonly used antimicrobials was found in many of the bacterial isolates taken during these studies.

Therefore, in the current study the objective was to identify the occurrence of selected bacterial foodborne pathogens among poultry meat and poultry products in

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retail markets and to assess antimicrobial resistance pattern of *E. coli* as an indicator organism.

MATERIALS AND METHODS

Sampling design and samples

A cross-sectional study was conducted from July to October 2014 across the Kandy Municipal Council area. Kandy is the second largest city in Sri Lanka, with a human population of 110,000, and has many different types of retail shops selling a range of different chicken meat and related products. The retail outlets selected for sampling included grocery shops, supermarkets and butcher's shops located in Kandy city and other small towns within the municipality that are easily accessible for customers. Thus, majority of the sampled retail outlets were located in and around the public market and close to the main bus station of the towns. From each selected retail outlet two to three samples were purchased depending on the availability. In the case of packaged products, frequently held in frozen form, 15 packs of sausages and 22 packs of meat balls were purchased, selecting the smallest available packs. Sausages retailed without packaging and displayed in cabinets, usually with other meat products, were purchased from 11 outlets. Since the majority of shops generally sell products belonging to more than one commercial brand special attention was given to include all the brands within each sample type. Chicken curry, which is the most common type of ready-to-eat chicken meat product in the local market, was selected for the cooked meat. Local restaurants and canteens located in areas close to retail outlets sampled for other meat products were randomly selected and from each outlet one portion of chicken curry was purchased.

A total of 124 samples (chilled meat = 28, frozen meat = 23, packaged sausages = 15, loose sausages = 11, packaged meat balls = 22, cooked chicken curry = 25) were purchased during the study. Samples were transported to the research facility within 2-3 hours, without allowing any external contamination or leakage, in ice boxes maintaining a temperature between 0^0C to +4^0C, and were processed immediately upon arrival at the research facility.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella* was carried out according to the standard method as described in ISO 6579:2002. Briefly, 25 g of sample was homogenized in 225 ml of buffered peptone water (BPW) (Oxoid, UK), using a stomacher blender and was incubated for 18 hours for pre enrichment. At the end of incubation 0.1 ml of the culture was inoculated to 10 ml of Rappaport-Vassiliadis enrichment broth (Oxoid, UK) and incubated at 42°C for 24 hours. Then a loopful of this broth was streaked on XLD agar and incubated at 37°C for 24 hours. Presumptive pink colonies with black centre were picked and streaked on nutrient agar. After 24 h of incubation at 37^0C, Gram's staining and biochemical tests were carried out for confirmation.

Isolation and identification of *Campylobacter*

Isolation and identification of *Campylobacter* was carried out according to the standard method given by ISO 10272: 1995 (E) with certain modifications. For this procedure, 10 g of sample was enriched in 90 ml of Preston enrichment broth (Oxoid, UK) and incubated at 42°C for 48 hours. After enrichment, a loopful of broth was streaked on mCDDA (Oxoid, UK) agar, and incubated at 42°C for 48 hours in a microaerophilic environment which was created using a Campy-gen gas pack (Oxoid, UK). Suspected colonies were selected and cultured on blood agar plates. For identification at the genus level Gram's staining, catalase test, oxidase test, aerobic growth at 42°C, anaerobic growth at 25°C and reactions in Triple Sugar Iron (TSI) agar slants were utilized.

Isolation and identification of *E. coli*

For isolation and identification of *E. coli*, only the qualitative identification protocol described by Sri Lanka Standard Institute (SLS 516: part 3:1982) was followed with certain modifications. After preparing an enrichment broth as described earlier (isolation of *Salmonella*), a loopful was streaked on MacConkey agar (Oxoid. UK) and incubated at 37°C for 24 hours. Characteristic colonies from above were sub-cultured on nutrient agar and Eosin Methylene Blue (EMB) agar (Oxoid, UK), incubated at 37°C for 24 hours, and subjected to Gram's staining and biochemical tests for confirmation.

Antimicrobial susceptibility testing of *E. coli*

Antimicrobial susceptibility testing (AST) was performed on *E. coli* isolated from the above samples. The disk diffusion method was performed according to the standard operating protocols described by the Clinical Laboratory Standards Institute (CLSI, 2013). The following antimicrobials were used to determine the sensitivity patterns: ampicillin (10 µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), streptomycin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), and combination of sulphamethoxazole and trimethoprim (23.75/1.25 µg).

RESULTS

The numbers of samples of each type of chicken meat and products that were positive for the three microorganisms tested are given in Table 1. All four raw chicken types, namely chilled and frozen meat, sausages and meat balls, were contaminated with *Salmonella, Campylobacter* and *E. coli* to different extents. As expected, all ready to eat cooked meat samples were free from any of the bacterial pathogens tested.

Table 1: The numbers of different types of samples that were positive for *Salmonella, Campylobacter* and *E .coli*

Sample type (and number)	*Salmonella* n (%)	*Campylobacter* n (%)	*E. coli* n (%)	*Campylobacter* and *E. coli* in the same sample n (%)
Chilled raw meat (28)	3 (10)	6 (21)	3 (10)	3 (10)
Frozen raw meat (23)	4 (17)	2 (8)	2 (8)	2 (8)
Chilled sausages without packaging (11)	1 (9)	1 (9)	6 (54)	1 (9)
Frozen and packaged sausages (15)	Not detected	Not detected	4 (26)	Not detected
Frozen and packaged meat balls (22)	1 (4.5)	1 (4.5)	7 (31)	Not detected
Cooked chicken curries (25)	Not detected	Not detected	Not detected	Not detected

Of the 51 raw meat samples tested, 26 were free from any one of the three microorganisms tested, while 25 samples were positive for at least one of the tested bacteria. Of the chilled meat samples, 32% (9/28) tested positive for *Campylobacter*, 21% (6/28) tested positive for *E. coli* and 10% (3/28) tested positive for *Salmonella*. When frozen meat was considered, 17% of samples tested positive for *Salmonella* and 8% were positive for *Campylobacter*.

Compared to unprocessed raw meat, the frequencies of contamination of meat products (total=48) was lower for *Salmonella* and *Campylobacter* at 4% (2/48) for each. However, higher levels of *E. coli* contamination was found, which was 42% (11/26) for sausages, and 31% (7/22) for meat balls. The levels of *E. coli* contamination identified for packaged sausages and loose sausages were 26% (4/15) and 6/11 (54%) respectively. Additionally, *Salmonella* and *Campylobacter* isolates were present only in unpackaged or loose products.

The results from AST carried out on *E. coli* isolates, ten from raw meat, eleven from sausages and seven from meat balls, are shown in Table 2. The isolates were 100% resistant against ampicillin and tetracycline, and all except one isolate from raw meat showed susceptibility for gentamicin, imipenem, and amikacin.

Table 2: Resistance patterns of *E. coli* for the antimicrobials tested

Antimicrobial	Percentage of isolates resistant to the antimicrobial		
	Raw meat (n=10)	Sausages (n=11)	Meat balls (n=7)
Ampicillin	100	100	100
Imipenem	0	0	0
Amikacin	0	0	0
Gentamicin	10	0	0
Streptomycin	100	82	86
Tetracycline	100	100	100
Nalidixic acid	70	55	86
Ciprofloxacin	70	73	43
Sulphamethoxazole + trimethoprim	90	91	71

DISCUSSION

The present study demonstrated the prevalence of three major bacterial foodborne pathogens and antimicrobial susceptibility patterns of *E. coli* present in poultry meat and meat products within Kandy Municipality area, Sri Lanka. Over 40% of the raw meat samples preserved using either chilling or freezing were contaminated with at least one of the tested bacteria.

Our findings showed that out of 51 raw meat samples tested 7 (13.7%) were contaminated with *Salmonella*. A similar finding (prevalence of 17.91%) was reported in Iran (Jalali *et al.*, 2008). while higher prevalences have been reported in other countries, such as 36.5% in Belgium (Uyttendaele *et al*, 1999), 35.8% in Spain (Dominguez *et al*, 2002), 35.5% in Malaysia (Rusul *et al*., 1996), 34% in Turkey (Yildirim *et al*., 2011) and 39.5% in Greece (Zdragas *et al.*, 2012). The above differences could be due to reasons related to various factors depending on the country, handling, process of slaughter, packaging, methods of distribution and awareness of handlers.

The current study found that 32% of the chilled meat samples tested were contaminated with *Campylobacter*. This prevalence is lower than that reported from Belgium (48%, Habib *et al.*, 2008), Spain (49.5%, Yildirim *et al*., 2011), Kenya (77%, Osano and Arimi 1999), and a previous study from our laboratory in Sri Lanka (59%, Kottawatta *et al*., 2017). A reason for the lower contamination found in our present study could be due to actual improvements in microbiological quality standards of poultry processing that have taken place in the past ten years.

The surface of the carcass during meat processing is highly moist with continuous contact with water during the entire process. *Campylobacter* being ardently hydrophilic, adhere to the surface of the carcass and can reach an average of 1.68 $\log_{10}$ CFU/g ±0.64 (Habib *et al*., 2008). However, *Campylobacter* tolerate environments only above 30^0C and is threatened when temperature is reduced, which limits the growth of this organism (Chan *et al*., 2001).

In comparison to the chilled meat samples (32%), frozen meat (17%) had much lower contamination rate with *Campylobacter*. This could be due to the fact that freezing helps reduce the colony counts by 1.4 $\log_{10}$ CFU/g (Rosenquist *et al*., 2003). But we have to agree with Bhaduri *et al*. (2004) in their hypothesis that even though freezing inhibits microbial growth and causes death of a certain proportion of the microbial population, some will survive the lethal damages and deliver infective organisms to the next stage. That could be why frozen meat still has such considerably high counts. The low *Campylobacter* counts should never be ignored because campylobacteriosis has a very low infective dose (Chen *et al*., 2006). Several scientists have shown that one drop of chicken juice can contain up to 500 *Campylobacter* organisms making it almost impossible to avoid any cross contamination and widespread contamination of meat handling facilities (Acheson and Allos, 2001).

Considering both raw and frozen meat, 20% of samples were positive for *E. coli*. It is a natural inhabitant of intestinal tracts of all warm blooded animals, and therefore is an indicator of faecal contamination (Miranda *et al*., 2008). It is recommended that food should be free from *E. coli* for it to be fit for human consumption (Adeyanju *et al*., 2014), although there is high possibility of contamination with *E. coli* as the predominant organism while slaughtering birds (Jimenez *et al*., 2003). In the current study some samples of all four types of meat tested were contaminated with *E. coli* (chilled 21.4%, frozen 17.4%, sausages 42.3% and meat balls 28%). Based on the specifications stated in Sri Lanka standard for poultry meat, allowable aerobic plate count per gram of meat is upto 10^7 colony forming units, which may include *E. coli* because it is an aerobic bacterium. Nevertheless, according to microbiological quality standards there should not be any *E. coli* in pre-cooked sausages. Generally, the meat mixture is cooked during the process of making sausages and should destroy bacteria. In agreement with this fact, current study showed absence of *Salmonella* and *Campylobacter* in all packaged sausages. Four samples of packaged sausages yielding *E. coli* indicates poor hygiene or improper heat treatment.

The presence of *E. coli* in products retailed without packaging could be due to cross contamination because such products are displayed in cabinets alongside other raw and further processed meat and sometimes fish. This is clearly evident in the present study, where the level of *E. coli* contamination in unpackaged sausages was almost double (54%) the contamination level of packaged sausages (28%). Additionally, three sausage samples, one positive for *Salmonella*, another positive for *Campylobacter* and the remaining one carrying both *Campylobacter* and *E. coli* were unpackaged sausages. The present study revealed that poultry meat sold at retail markets in Kandy municipality area is more frequently contaminated with *E. coli* and *Campylobacter,* and less frequently contaminated with *Salmonella,* which is similar to the findings of another study from Washington, D.C. (Zhao *et al*., 2001).

Other than the intentional mishandling of products at retail there can be number of contributing factors for meat products such as sausages and meat balls to become contaminated with bacteria. One reason for this is that the manufacturing process exposes all the deep tissues to parts like the alimentary tract, which might be already harbouring pathogens. Another reason is the high nutritional composition creating a favourable environment for these pathogens. A study in Pakistan shows that chicken meat balls has the highest water holding capacity (48.18%), fat (10.78%), and moisture content (70%) out of several chicken meat products tested, providing a favourable environment for microbes (Hussain *et al*., 2016). Bacteria which spread into the centre of food are less likely to be destroyed by cooking. Therefore, adequate attention has to be given when cooking meat products since the organisms residing in the middle of the products might remain unharmed if undercooked. A study conducted in

Thailand on the occurrence of *Salmonella* in poultry products such as meat balls and sausages reported 10% of contamination (Jerngklinchan *et al*., 1994). The results of the present study found 4% contamination with *Campylobacter* both in chicken sausages and chicken meat balls. This agrees with findings of Boston *et al.* (1997) on progressive decrease of *Campylobacter* counts from 2.8 - 4.3×10^5 CFU/g in whole meat to <10 CFU/g in meat balls.

Of the *E. coli* isolates tested for AST, majority showed resistance to multiple drugs. However, the pattern of resistance was not very different among raw meat and meat products. This is explainable as all samples are of poultry meat origin and similar types of bacterial strains were isolated. The antimicrobials for which *E. coli* isolates showed high resistance, i.e. ampicillin, sulphamethoxazole + trimethoprim, tetracycline, and nalidixic acid, are the ones which are commonly used by Sri Lankan poultry farmers (personal observations). Imprudent use of important antimicrobials at primary production could be the reason for high levels of resistance seen. However, the susceptibility of isolates to critically important antimicrobials such as gentamicin, imipenem, and amikacin was important because transfer of resistant organisms from animal sector to humans is a topic of interest globally. When considering the literature on antimicrobial resistance (AMR) among human patients in Sri Lanka, it is evident that there is a significant increase in AMR in the recent past. According to Jayatilleke (2014), in *E. coli* isolates from blood samples of patients, only 67% and 58% were susceptible towards amikacin and gentamicin respectively. It seems that the level of resistance already present in human isolates against critically important antimicrobials is higher compared to animal isolates. However, the extent of the current study is too limited to come to any conclusion and it is suggested that a "One Health" approach that includes all responsible sectors should be taken to study and combat this growing problem of AMR.

The finding of this study that even though raw meat and meat products were positive, cooked meat samples did not yield any of the tested pathogens could be due to two reasons; the high internal temperature reached by the centre of the product during the preparation of chicken curries and the spices used in Sri Lankan traditional cooking methods. Researchers have proved that cooking temperature and time have a relationship with bacterial reduction during the cooking process. It is recommended to provide adequate time for the whole meat portion to reach an equal temperature since chicken meat has a solid matrix. The National Advisory Committee on Microbiological Criteria for Foods has provided a margin of safety for cooking poultry. Studies have shown that a temperature of 160^0F (74^0C) held for 15 seconds is sufficient to achieve a 7-log reduction of *Salmonella* and a 50-log reduction of *Campylobacter* (Hussain *et al*., 2016). According to the Sri Lankan method of cooking chicken, this standard is easily achieved and is one major reason behind the safety of chicken curry in the Kandy Municipality area.

The traditional chicken curry earns its desirable taste, aroma, and quality from the spices used such as lemon juice, turmeric powder, crushed garlic and ginger, salt, and black pepper. Researchers who are working on food quality are showing the food preservative potential of these spices, which also have a strong influence over the lower incidence of food allergy from eating chicken meat curry (Witkowska *et al*., 2013). According to recent studies, cinnamon, turmeric, clove, garlic, nutmeg, lemon, green tea and several other plants possess antimicrobial effects (Murali *et al*., 2012; Shekarforoush *et al*., 2014). Absence of tested bacterial species in chicken curry suggest that spices may have the ability to reduce the microbes present in raw meat. Research on garlic extract and clove oil reported an interesting finding that some bacterial isolates which showed antimicrobial resistance were sensitive to these two spices (Arora and Kaur, 1999).

The major limitations of this study can be identified as using convenience sampling instead of random sampling, not performing serotyping for *Salmonella* isolates, and lack of species identification of *Campylobacter*. These were mainly due to the limitations in laboratory resources available.

CONCLUSION

Avoiding the contamination of fresh poultry meat and their products with foodborne zoonotic bacteria is challenging. However, the risk of getting infected with these pathogens can be minimized or completely avoided by practicing appropriate and adequate cooking methods. Because of widespread chicken meat contamination the consumers should be educated to handle chicken and related products with care, including the importance of storing meat separately from other foods, cleaning kitchen equipment and contaminated surfaces after use with chicken meat, and washing hands thoroughly after handling chicken meat and chicken products. Although this study showed the safety of chicken curry in Sri Lanka, the concept of adequately cooking poultry meat and poultry products is applicable globally. Further, the prudent use of antimicrobials at the farm level is emphasized in order to reduce the possibility of anti-microbial resistant strains becoming a risk to human health.

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