Research Article

BACTERIOLOGICAL PROFILE OF RAW CHICKEN MEAT COLLECTED FROM LALITPUR AND THEIR ANTIBIOGRAM

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ABSTRACT

Food-borne disease outbreak have imposed substantial burden on health care systems and have markedly reduced the economic productivity of a country. In developing countries like Nepal, farmers use antibiotics in feed for therapeutic as well as non-therapeutic purpose. This study aims to evaluate bacteriological status of raw chicken meat and their Antibiogram. A comparative study of 25 livers and 25 breast muscles was carried out using standard procedures for isolation and identification of E. coli, Salmonella and their Antibiogram. The prevalence of E. coli and Salmonella in chicken liver was found to be 52% and 36% respectively; and in case of chicken breast, it was 44% and 0% respectively. The isolates from liver showed wider resistance pattern towards in-use antibiotics in comparison to isolates from breast muscles. In addition, 20.83% of Escherichia isolates were found to be multi-drug resistant. The findings of the study indicated emergence of multi-drug resistant bacteria in chicken meat; therefore it is important to control indiscriminate administration of antibiotics to the poultry animals.

Keywords: Escherichia coli, Salmonella, Food- borne disease, Bacteriological status, Antibiogram, Multi- drug resistant

Introduction

Poultry meats are one of the most popular foods as they are wholesome, healthy as well as nutritious . Chicken meat is an ideal culture medium for many organisms because it is high in moisture, rich in complex nitrogenous foods, and plentifully supplied with minerals and accessory growth factors (Frazier & Westhoff, 2009). Many organisms or group of organisms in food have been suggested as indicator organisms . In order to assess the general hygiene status of a food product, a group of bacteria belonging to the family Enterobacteriaceae have been used.

Contaminated food products have been reported to be responsible for numerous food-borne diseases all around the world (Zafar et al, 2016). In many developing countries like Nepal, food-borne diseases outbreak due to bacteria, such as Escherichia coli and Salmonella spp. impose a substantial burden on health care (Hughes et al, 2007). In Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses in meat as well as its invaluable by-products (Joshi, 1991).

In recent years, antibiotics have been used for both therapeutic and non-therapeutic purpose. Non-therapeutically, antibiotics are used as growth promoters in livestock and poultry (McEwen and Fedorka-Cray, 2002). This non-therapeutic use of antibiotics in feed may lead to increased levels of antibiotic resistance in both the pathogens and fecal micro flora of poultry (Atere, 2016). Multi-drug resistant (MDR) strains of Salmonella are now encountered frequently and the cases of MDR have increased considerably in recent years (Majagaiya, 2009). Surveillance data show that resistance in E. coli is consistently highest for antimicrobial agents that have been used for the longest time in human and veterinary medicine (Tadesse et al, 2012).

For effective food safety management plan, it is necessary to continuously monitor the presence of pathogens in food materials (Zafar et al, 2016). This study is targeted to find out the microbial quality of raw chicken meat and the Antibiogram of isolates. And it is believed that this research would be informative and helpful for planners, policy-makers and also those who are interested to know about microbiological quality of poultry in Nepal.

Methods

Sample size and Site

This study was completed within 3 months period from April to June 2018. A total of 50 chicken meat samples (25 livers and 25 breast muscles) were collected from different localities of Lalitpur. The samples were collected from five different sampling sites: Sanepa, Kupondole, Pulchowk, Jawalakhel and Lagankhel.

Sample Collection

The chicken livers and chicken breast muscles were collected in separate sterile zip-lock plastic bags and transported to the

Laboratory of Department of Microbiology at Kantipur College of Medical Science in an ice-cold box within 2 hours of collection.

Microbiological Analysis of Samples

25 grams of raw chicken meat sample was weighed and transferred into 225 ml of sterile buffered peptone water to make a 1:10 dilution. The mixture were homogenized and further processed accordingly. Three main assessments were carried out; enumeration of coliforms, isolation and identification of Escherichia coli and Salmonella spp. and antibiotic susceptibility testing of E. coli and Salmonella.

Enumeration of Coliforms

Coliforms were counted using pour plate technique as mentioned by Feng et al, 2017. Serial dilution of the homogenate was carried out and 1 ml of 3 consecutive dilutions was transferred to petri dishes. The plates were overlaid with violet red bile agar (VRBA) and solidified. Incubation was done at 35°C for 18-24 hours. The plates with 30-300 colonies were selected and the number of colonies was counted and number of organism was calculated.

Isolation and Identification of E. coli

The standard protocol mentioned by Feng et al, 2017 was used. The homogenate was incubated at 37°C for 16-24 hours. A loopful of the pre-enriched broth was inoculated onto Eosin Methylene Blue agar and incubated at 37°C for further 24 hours. Suspected colonies with green metallic sheen were confirmed using gram staining and biochemical tests.

Isolation and Identification of Salmonella spp

The standard protocol mentioned by Abdellah et al, 2008 was used. The homogenate was incubated and a part of it was transferred in selenite cysteine broth and incubated at 37°C for 18-24 hours. A loopful of enriched broth was streaked on Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37°C for 18-24 hours. Presumptive Salmonella colonies were confirmed by gram staining and biochemical assays.

RESULTS AND DISCUSSION

Coliform counts

The average coliform count of raw chicken meat from different location was found to be 4.83*105 CFU/gm with a maximum count of 8.9*105 CFU/gm and minimum count of 6*103 CFU/gm. And, the average coliform count in chicken liver was found to be 2.19*105 CFU/gm and in breast muscle, it was found to be 8.46*104 CFU/gm. The coliform count was found to be higher in chicken liver than chicken breast muscle. The reason behind it may be the difference in anatomical position of liver and breast muscle. The liver of a chicken is much closer to; and has a greater possibility of coming in contact with the enteropathogens and commensals from digestive juices of chicken during slaughtering and evisceration. In addition, the composition of liver and breast muscle also plays a role. Liver contains glycogen and breast muscle is made up of protein and fat. We are familiar with the fact that in a medium containing carbohydrates, proteins and fats, microorganisms first utilize carbohydrates, followed by fat and finally protein. So in a scenario where the coliforms contaminate both liver and breast muscle, the rate of growth in case of liver would be higher than the latter.

Multi-Drug Resistant Isolates

Among 24 isolates of E. coli, 5 isolates were resistant to more than two classes of antibiotics. These were registered as multi-drug resistant organisms. 20.83% of E. coli isolates were multi-drug resistant as shown in figure 3.

Hossain et al (2008) found the isolates were resistant to Ampicillin by 62.85%, Akbar et al (2014) by 92.1%, Rasheed et al (2014) by 13.3% and Guerra et al (2003) by 92%. In the current study, the resistance of E. coli isolates towards ampicillin is higher than all these studies and the reason behind it may be development of bacterial resistance due to production of Beta-Lactamase by the organism. In case of Chloramphenicol, Hossain et al (2008) reported 45.72% resistance and Akbar et al (2014) reported 39.5% resistance which complies with the finding of present study. Akbar et al (2014) found the isolates were resistant to Gentamicin by 47.4% and Guerra et al (2003) by 60% which is similar to the findings of the present study. Akbar et al (2014) found 63.2% resistance to Ciprofloxacin that complies with the finding of current study. In case of Co-Trimoxazole, Akbar et al (2014) found isolates were resistant by 31.6% and Rasheed et al (2014) by 11.3% which are quite higher than the current findings of the study. Akbar et al (2014) and Guerra et al (2014) found higher resistance to Tetracycline i. e. 92.1% and 66% respectively which is similar to the present findings. But Rasheed et al (2014) found comparatively lower resistance of 12.3%.

Atere (2016) reported Salmonella isolates were 100% resistant to Ampicillin, which corresponds with the present finding and 37.5% resistant to Nitrofurantoin which is higher than present finding. This contradiction in result may be due to development of resistance in the bacteria overtime. Kim et al (2012) found Salmonella were 85% resistant to Nalidixic acid and susceptible to ciprofloxacin. In the present study, among 9 isolates of Salmonella, 4 isolates (44.44%) were resistant to at least 3 antibiotics. In a similar study, Kim et al (2012) found 87.2% were resistant to at least 3 antibiotics and were considered to be multi-drug resistant. In this study, the antibiotic resistance pattern of bacteria isolated from liver sample is greater than bacteria isolated from breast muscle. This may be because liver is the organ responsible for elimination and detoxification of various contaminants that enter the body, and liver usually contains residual antibiotic agents.

CONCLUSION

Based on the evidence from this study, it can be concluded that from health and hygiene point of view, the quality of chicken meat sold in retail shops as well as sanitation of slaughterhouses in Lalitpur should be improved. In recent years, poultry farmers have been using antibiotics as growth promoter which has resulted in antibiotic residues in the meat. This in turn is inducing resistance development in the microbiome of chicken as supported by the study. Consuming resistant-bacteria present in raw chicken meat can cause development of resistance in gut microbiome of humans. Therefore, use of antibiotics as growth promoters should be discontinued as soon as possible.

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