Bacteriological Profile of Raw Chicken Meat Collected from Lalitpur and their Antibiogram

Thapaliya Manish[1*] , Shakya. Sastrika[2], Shrestha Upendra Thapa[3], Dangol Astha[2], Shrestha Kusum[4]

[1] *Department of Microbiology, National College, Kathmandu, Nepal*

[2] *Nepal, Department of Microbiology, Kantipur College of Medical Science, Kathmandu, Nepal*

[3] *Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal*

[4] *Department of Microbiology, St. Xavier's College, Kathmandu, Nepal*

Corresponding Author*

Thapaliya Manish, Department of Microbiology, National College, Kathmandu, Nepal, E-mail: manish.thapaliya@yahoo.com

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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 [1]. 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 [2]. Many organisms or group of organisms in food have been suggested as indicator organisms [3]. 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 [4]. 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 [5]. In Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses in meat as well as its invaluable by-products [6].

[7]. Multi-drug resistant (MDR) strains of Salmonella are now encountered frequently and the cases of MDR have increased considerably in recent years [8]. 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 [9].

For effective food safety management plan, it is necessary to continuously monitor the presence of pathogens in food materials [4]. 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.

Materials and 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 [10]. 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 [10] 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 [11] 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.

Antibiotic Susceptibility Test

The antibiotic susceptibility testing of the isolate towards various antibiotic discs was done by modified Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute [12]. Muller-Hinton Agar (MHA) plates were inoculated with the prepared inoculum. Appropriate antibiotic discs were evenly distributed on the surface of plates; the plates were inverted and incubated aerobically at 35°C for 18 to 24 hours. After incubation, the plates were examined to measure the diameter of zone of complete inhibition, including the diameter of disc and compared with standardized zone interpretative chart. Finally, the zone size of each antibiotic was interpreted reporting the organism as 'Resistant', 'Intermediate' and 'Susceptible'. The raw data obtained were entered in MS Excel software program. The data were tabulated and graphs were plotted. The statistical application was done whenever applicable.

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.

Isolation and Identification of E. coli and Salmonella spp.

In the present study, the prevalence rate of E. coli and Salmonella from chicken meat was found to be 48% and 18% respectively, which was in agreement with [13]. Findings of present study revealed 52% of liver samples were positive for Escherichia coli and 36% were Salmonella positive (as shown in figure 1). In a similar study conducted by [14], they reported 8.1% prevalence rate and [15] reported 11.11% prevalence rate, which are much lower than the present findings. The reason behind increase in isolation rate of Salmonella in this study may be improper slaughtering and handling of chicken meat. Findings of current study revealed 44% of breast muscle showed positive cases for E. coli and all the samples (0%) showed negative cases for Salmonella spp (as shown in figure 1). In a similar study conducted by [16], they reported 70% isolation rate of E. coli and [17] reported 73.3% prevalence rate [14]. And, reported 18.48% isolation of Salmonella from chicken breast muscle and [15] reported 6.25% prevalence of Salmonella. The findings of these study are very high than the findings of the present study. The reason behind this decrease in isolation rate of Escherichia coli and Salmonella isolates from chicken breast muscle may be due to development and implementation of good slaughter method, hygienic animal slaughterhouse and proper storage of poultry and poultry products.

In the study, 12 samples were collected in the month of April, 20 in May and 18 in June. The coliform count and the isolation rate of E. coli and Salmonella spp. was found to be highest in the month of June, followed by April and then May as shown in figure 2 and table 1. The highest prevalence rate during the month of June may be because June is one of the warmest months and as we know, high temperature supports the growth of bacteria.

Antibiotic Susceptibility Testing

The antibiotic resistance pattern of all the isolates is shown in table 2. All the isolates showed 100% resistance towards Ampicillin, Cefazolin and Cefepime. Except Salmonella which only showed 77.8% resistance towards Cefepime. All the isolates were 100% susceptible to Nitrofurantoin. The isolates showed different array of resistance towards actively used antibiotics such as Cefotaxime, Chloramphenicol and Co-Trimoxazole. E. coli from liver showed 38.4% resistance towards Chloramphenicol but E. coli from breast muscle and Salmonella from liver showed 100% sensitivity towards Chloramphenicol. Also, E. coli from liver and breast muscle showed resistance towards Gentamicin and Co-Trimoxazole whereas Salmonella was 100% susceptible to these antibiotics. This study suggests that E. coli from liver is the most resistant organism followed by E. coli from breast muscle and finally Salmonella isolated from liver.

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.

[18] found the isolates were resistant to Ampicillin by 62.85%, [19] by 92.1%, [20] by 13.3% and [21] 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, [18] reported 45.72% resistance and [19] reported 39.5% resistance which complies with the finding of present study. [19] found the isolates were resistant to Gentamicin by 47.4% and [21] by 60% which is similar to the findings of the present study. [19] found 63.2% resistance to Ciprofloxacin that complies with the finding of current study. In case of Co-Trimoxazole, [19] found isolates were resistant by 31.6% and [20] by 11.3% which are quite higher than the current findings of the study. [19] and [21] found higher resistance to Tetracycline i. e. 92.1% and 66% respectively which is similar to the present findings. But [20] found comparatively lower resistance of 12.3%.

[22] 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. [23] 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, [23] 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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