

**MICROBIAL PROFILE AND NUTRITIONAL EVALUATION OF BROILER AND DOMESTIC CHICKEN MEAT FROM SELECTED DISTRICTS OF KHYBER PAKHTUNKHWA, PAKISTAN**

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**Abstract:** Poultry meat is considered a favourite meal in Pakistan. It is one of the essential constituents of the human diet daily because it contains protein, vitamins, minerals, and energy. Poultry meat can cause illnesses like food poisoning and typhoid etc. Our study aims to isolate pathogenic microbes and nutritional analysis of different poultry meat samples. Samples of domestic (30) and broilers chicken's (30) meat were collected from different areas (Swat, Malakand, Mardan, Charsadda and Peshawar) of the market randomly. Selective and differential media were used for bacterial growth. The staining techniques and biochemical test were used to identify the bacteria as gram-negative or positive. Different biochemical tests, like Oxidase, Catalase and Triple Sugar Iron (TSI), were performed for the bacterial identification. Disc diffusion procedure was performed to test a panel of antibiotics belongs to different classes against Gram-positive and Gram-negative bacteria. The nutritional analysis was performed by AOAC method. *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perferingens*, *Listiria monocytogenes*, *Yersinia enterocolitica* and *Campylobacter jejoni* were seven different types of bacteria identified from meat sample. *Aspergillus* and *Penicillium*, fungi were identified from broiler chicken meat. From the result of antibiotic sensitivity, the most potent antibiotics found against bacteria were ciprofloxacin and Cefotaxime (54%) respectively, followed by ceftriaxone and Gentamicin (40%), respectively, followed by ceftazidim and Imepenem (19%) while pepracillin and penicillin antibiotic were mostly showed resistant. The result of Nutritional analysis showed that crude protein, crude fats, crude fibers, crude ash and crude moisture in domestic chicken meat ranged from 84.19 to 90.52%, 0.62% to 2.30%, 0.36% to 0.65%, 3.70% to 93% and 1.9% to 3.77% respectively. Similarly, in Broiler chicken meat, the percentage of crude protein, crude fats, crude fibers, crude ash and crude moisture ranged from 75.35% to 86.10%, 4.12% to 7.46%, 0.52% to 0.95%, 4.20% to 6.25%, and 1.92% to 2.78% respectively. The study concludes that domestic chicken meat contained a higher percentage of crude protein and crude moisture than broiler meat while a lower percentage of crude fats, crude fiber and crude ash than those of commercial broilers.

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**Keywords:** Domestic chicken, Broiler chicken, Pathogens, Antibiotics, crude protein

## Introduction

The demand for poultry meat products is increasing worldwide and growing rapidly as a dynamic industry. The chicken species are an important source of human food in the world. Chicken meat consumption increased due to its low cost, broad nutritional value, high-quality protein, easy processing, easily accessible fat, low cholesterol content with tender and fiber. In the minerals chicken, is a good source of selenium and provides zinc, copper, phosphorus, magnesium, and iron (Fakolade, 2015). There is extensive evidence that the feed of animals is habitually polluted with pathogens; as early

as 1948 poultry feed in US was detected for non-Typhi serotypes of *Staphylococcus. Enteric* (Jones, 2007). Human infectivity happens when improperly cooked chicken is eaten (Jamshidi et al., 2008). Important pathogenic microbes like *E. coli*, *S. aureus*, *Campylobacter jejuni/coli*, *Salmonella* spp, *Yersinia enterocolitica* and *Listeria monocytogenes* may find in uncooked meat. Regarding these microbes, *Listeria monocytogenes* mostly considered a risk for public health and can cause many diseases without proper handling and control of these microbes (Nørrung et al., 2009). Broiler chicken meat is very accepted in

consumption by almost all people due to its easy digestion, while human health is greatly affected by food born microbes, which might be caused contamination in it. Normally and integrally, the meat is not comprised of pathogenic individuals but may become polluted during slaughter or from faecal matter. The individuals tend to stay on its surface or inside it (Darshana et al., 2014).

Antibiotics are also used in poultry feed to treat and prevent infection and promote growth. However, regular feeding of antibiotics has serious health hazards to humans because antibiotics residue may accumulate in meat and eating of antibiotics contaminated meat may lead to antibiotic resistance in animals and humans (Zeb et al., 2013). The improvement of antibiotic resistant microbes is that animals fed with antibiotics develop resistant strains of microbial pathogens that may transmit to a person body when foods are not properly cooked. Roxarsone (organoarsenic compound that has been used in poultry production as a feed additive) is also used in poultry feed to increase weight gain. Still, due to arsenic content, this drug is harmful to human. The use of growth hormones in food animals and poultry feed on contaminated meal poses a potential risk to consumer's health (Fisher et al., 2015).

The Chickens are highly vulnerable to spoilage and involved in spreading foodborne diseases. All eatable tissues are subjected to contamination during slaughter and processing from various types of microbes related directly or indirectly to the environment, equipment, and workers (De Melo et al., 2012). These types of pathogens involved in contaminating poultry products are, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter*, *Clostridium perfringens*, *Yersinia enterocolitica* and *E. coli* (Abraham et al., 2012; Rani et al., 2020). The domestic chickens (household chickens) are significant and physically powerful and live on existing food (Kingori et al., 2010). Compared to commercial broilers, they are good in taste, have high competency and are more disease resistant. Domestic chicken meat has low fat, high protein and water-holding capacity (Umaya, 2014). Domestic chickens are grown without antibiotics, steroids, hormones, animal-derived feed and chemical feed; they often seek in the house or near the fields after harvesting for food. The scavenged feed contains green weeds, grains, kitchen waste and insects (Kingori et al., 2010; Umaya, 2014; Ariyaratne, 2010). The present study explored various types of microbial pathogens in meat and showed the nutritional value of broiler and domestic chicken's meat. The research may also have shown microbial contamination during food consumption, which can cause various health problems.

#### Materials and Methods

Samples (60) were randomly collected from domestics and broilers chicken from different areas (Swat, Malakand, Mardan, Charsadda and Peshawar)

market. About twelve samples of both chicken' meats were collected from different retail outlets in each region. The Microbiological analysis was done in Microbiology research laboratory, Abasyn University Peshawar and Nutritional analysis was done at Veterinary Research Institutes (VRI) Peshawar.

#### Processing of collected samples

Different growth Medias like Czapaek Yeast Extract (CYA), along with other growth media like nutrient agar media, potato dextrose agar media were utilized for microbial growth. Nutrient agar media was used primarily for bacterial growth, while potato dextrose media along with (CYA) media were used for fungal growth.

#### Characterization of bacterial isolates and antibiotics sensitivity

The colony morphology, like size, shape, color etc., on different selective and differential media was observed. Gram staining procedure was done to determine the cellular morphology of Gram negative and positive bacteria. Different biochemical tests, like Oxidase, Catalase and Triple Sugar Iron (TSI), were performed for the bacterial identification (Ogunmola et al., 2013). Disc diffusion procedure was performed to test panel of 8 antibiotics belonging to different classes against Gram positive and Gram-negative bacteria. Antibiotics like ceftazidime, ceftriaxone, cefotaxime, pefracilin, penicillin, Gentamycin, ciprofloxacin and imipenem were used for sensitivity assay.

#### Biochemical test

Biochemical tests conducted for isolated bacterial isolation were Catalase, Oxidase, Methyl red test, Citrate, Hippurate and H<sub>2</sub>S test (Okarini et al., 2013).

**Catalase test:** Bacteria were collected from 1 colony by a sterile inoculating loop and 1 drop of 3% H<sub>2</sub>O<sub>2</sub> was added. Bubbles formation indicates the presence of catalase enzyme while no bubble shows the absence of catalase enzyme.

**Oxidase test:** Oxidase reagent was added in Petri dish and dark blue-purple color change within 10-30 sec indicates positive result. No color change or color change after more than 30 sec show negative result.

**Methyl red test:** Inoculated a colony in 0.5 ml of glucose phosphate peptone broth and adding methyl red solution, one night of incubation at 37°C. Red coloration is positive, whereas yellow coloration is negative

**Citrate test:** A small amount of bacteria was inoculated into a tube containing citrate medium and incubated at 30-37°C for 24-48h. Growth showed positive results, while no growth showed negative results.

**Hippurate test:** Inoculating a colony in 0.5 ml of sodium hippurate solution and Incubated at 37°C for 2 h in a water bath. Add 0.2 ml of ninhydrin solution and incubated again at 37°C for 10 minutes. Deep blue indicated positive result while pale blue indicated negative result.

**Hydrogen sulfide production:** Bacteria from one colony deeply inoculated in H<sub>2</sub>S medium and incubated at 30-37°C for 24-48 h. A black precipitate showed a positive results whereas no precipitate showed negative result.

**Characterization of fungal isolates and antifungal sensitivity**

Potato Dextrose Agar was used to observe the growth of fungi. Morphological features of fungal species like colony were ensured. Disc diffusion method was used for antifungal sensitivity to screen the antifungal activity of each antifungal drug against different fungi. The YPG agar plate's surface was used to spread Yeast inoculum in NaCl solution. Sterile filter paper discs with different antifungal drugs like fluconazole, nystatin, and voriconazol were placed on inoculated plates.

**Proximate Analysis**

Nutritional analysis of broiler and domestic chickens meat was performed using the Association of Official

Analytical Chemists (Tobaruela et al., 2018). The samples were sent to Veterinary Research Institute Peshawar for drying using an oven drier at a constant temperature of 80°C. Then dried samples were powdered with a mortar and a pestle.

**Data analysis**

Statistical analysis was performed using MS excel and GraphPad Prism.

**Results**

In this study, we observed that 4(13.3%) samples of domestic chickens meat were infected, and the total bacterial count of the meat samples ranged from 1.5x10<sup>3</sup> to 1.8x 10<sup>6</sup> CFU/g, shown in table 1. The highest colony-forming unit was observed in the samples obtained from Peshawar, while the least count was observed in Malakand samples. The 22 meat samples of broilers were contaminated, and the total bacterial count ranged from 2x10<sup>3</sup> to 2.13x10<sup>6</sup> CFU/g as mentioned in table 1.

**Table.1. Total bacterial count (CFU/g) in commercial broilers meat samples**

Selected area	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	domestic chicken	Commercial broilers	domestic chicken	Commercial broilers	domestic chicken	Commercial broilers	domestic chicken	Commercial broilers	domestic chicken	Commercial broilers	domestic chicken	Commercial broilers
Swat	1.0x10 <sup>4</sup>	-	-	10x10 <sup>4</sup>	-	-	-	8.8x10 <sup>5</sup>	-	-	-	8.7x10 <sup>5</sup>
Malakand	1.5x10 <sup>3</sup>	2x10 <sup>3</sup>	-	NG	-	5x10 <sup>3</sup>	-	3.23x10 <sup>4</sup>	-	-	-	-
Mardan	-	3.4x10 <sup>4</sup>	1.0x10 <sup>3</sup>	2.13x10 <sup>5</sup>	-	-	-	4.1x10 <sup>4</sup>	-	-	-	1.27x10 <sup>5</sup>
Charsadda	-	6.5x10 <sup>3</sup>	-	2x10 <sup>3</sup>	-	1.04x10 <sup>6</sup>	-	1.6x10 <sup>5</sup>	-	1.27x10 <sup>5</sup>	-	-
Peshawar	-	-	1.8x10 <sup>4</sup>	2.13x10 <sup>6</sup>	-	1.9x10 <sup>6</sup>	-	6x10 <sup>3</sup>	-	-	-	5.7x10 <sup>4</sup>

(-) show Null value.

**Isolation of bacteria**

Based on morphology, Gram staining and different Biochemical tests were performed that *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perferingens*, *Listiria monocytogenes*, *Yersinia enterocolitica* and *Campylobacter jejuni* were isolated from meat sample. Some of the fungi were also isolated from the broiler meat sample, as shown in figure 1. The bacteriological analysis revealed that out of 30 meat samples (Broilers), 47% were found to be positive for *Salmonella* and among these positive samples, 13.33% from Peshawar, Mardan (13.33%), Malakand (10%), Charsadda (6.6%) and Swat (3.3%) as shown in table 2. The *Salmonella* was isolated from only 6.6% of domestic chicken meat samples. In the samples of domestic and broiler, the highest prevalence of *E.coli* was found in Mardan (23.3%), followed by Malakand (16.6%), Peshawar (13.3%), Swat (10%) and Charsadda (3.3%). In the broilers meat samples, 43.33% were positive for *Staphylococcus aureus*. The percentage of positive

cases in Peshawar, Malakand, Charsadda, Mardan, and Swat were 13.3, 10, 10, 6.6 and 3.3% respectively, whereas in domestic chicken *Staphylococcus aureus* was detected only 6.6% as shown in the table 2. The Bacterial examination showed that the *Campylobacter jejuni* was isolated 60% from broilers meat. The highest prevalence of *C.jejuni* was found in Peshawar (16.6%) and lowest in Swat (6.6%). In Mardan, *C.jejuni* was 13.3% followed by Malakand (13.3%) and Charsadda (10%), as shown in table 2. Whereas *Campylobacter jejuni* was not found in any sample of domestic chicken. In broiler meat samples, the *Listeria monocytogenes* was reported 10% in Charsadda, Malakand (6.6%), Peshawar (6.6%), Mardan (6.6%), and Swat (3.3%). The *Yersinia enterocolitica* was identified in only 10% of commercial broiler meat samples; among these positive samples (6.6%) were from Peshawar and (3.3%) were from Charsadda. The study showed that 63.3% broiler samples were positive for *Clostridium Perfringens* which 20% from Peshawar,

Mardan (16.6%), Malakand (10%), Charsadda (10%) and Swat (6.6%), whereas in Malakand, *Clostridium Perfringens* was 6.6% from domestic chicken meat.

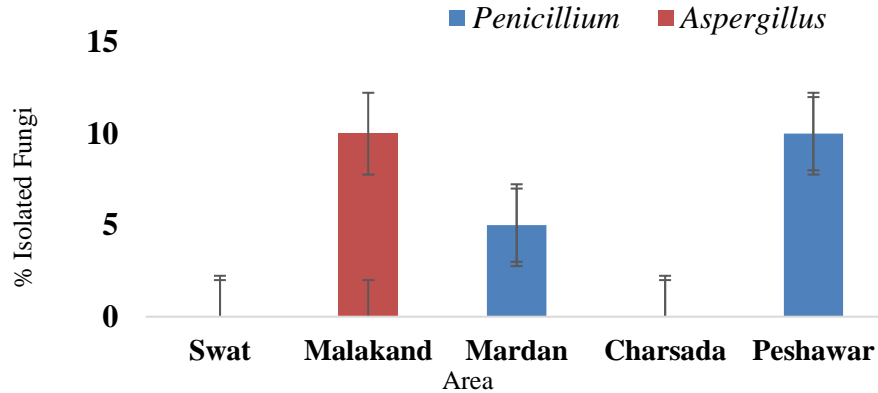


Figure 1. Frequency of positive isolates of fungi from Broiler meat samples

Table.2. Detail of positive isolates in domestic and broiler chicken meat samples from selected areas

Isolated M.O	Swat		Malakand		Mardan		Charsadda		Peshawar		Total percentage %	
	Domestic	Broiler	Domestic	Broiler	Domestic	Broiler	Domestic	Broiler	Domestic	Broiler	Domestic	Broiler
<i>E.coli</i>	0	10%	0	16.6%	0	23.3%	0	3.3%	10%	13.3%	10%	67%
<i>Clostridium Perfringens</i>	0	6.6%	6.6%	10%	0	16.6%	0	10%	0	20%	6.6%	63%
<i>Campylobacter</i>	0	6.6%	0	13.3%	0	13.3%	0	10%	0	16.6%	0	60%
<i>Salmonella</i>	0	3.3%	0	10%	6.6%	13.3%	0	6.6%	0	13.3%	6.6%	47%
<i>Staphylococcus aureus</i>	6.6%	3.3%	0	10%	0	6.6%	0	10%	0	13.3%	6.6%	43%
<i>Listeria monocytogenes</i>	0	3.3%	0	10%	0	6.6%	0	10%	0	6.6%	0	30%
<i>Yersinia enterocolitica</i>	0	0	0	0	0	0	0	13.3%	0	6.6%	0	10%

**Antibiotic susceptibility**

The isolated bacterial species were tested for antibiotics sensitivity profile. The most potent antibiotics found against bacteria were ciprofloxacin, Cefotaxime (54%) respectively

followed by ceftriaxone, Gentamicin (40%) respectively, followed by ceftazidim and Imipenem (19%) while Pepracillin and penicillin antibiotic were mostly resistant as shown in table 3.

Table 3. Different Antibiotic sensitivity test profile

Antibiotics	<i>E. coli</i> (n=14)		<i>Clostridium perfringens</i> pp (n=10)		<i>Listiriamonocytogenes</i> (n=10)		<i>Yersinia enterocolitica</i> (n=11)		<i>Salmonella</i> (n=13)		<i>Staph aureus</i> (n=14)		<i>Campylobacter jejoni</i> (10)	
	S %	R %	S%%	R%	S%	R%	S%	R%	S%	R %	S %	R %	S%	R%
Gentamicin	05	09	04	06	02	08	08	03	05	08	04	10	01	9
Ciprofloxacin	09	05	06	04	05	05	05	06	05	08	10	04	04	06
Pepracilin	01	13	01	09	0	10	01	10	02	11	04	10	01	09
Imipenem	04	11	0	10	05	05	0	11	04	0	02	12	01	09
Penicillin	02	12	02	08	02	08	0	11	0	13	01	13	0	10
Ceftazidime	04	10	0	10	05	05	0	11	04	09	02	12	01	09
Cefotaxime	09	05	06	04	05	05	04	07	05	08	10	04	04	06
Ceftriaxone	05	09	03	07	03	07	10	01	05	08	05	09	02	08

**Nutritional Evaluation of domestic and broiler chicken meat**

The broilers and domestic chicken meat were analyzed for their nutritional status according to parameters such dry matter, crude protein, crude fat, moisture content, ash content and fiber content as shown in the table 4. The percentage of crude protein in domestic chicken meat ranged from 84.19 to 90.52%, in which Malakand, Mardan, Charsadda,

Peshawar and Swat has the value of crude protein, 90.16, 89.95, 87.18, 84.19 and 90.52% respectively. Protein content of commercial broilers meat samples ranged from to 75.35 to 86.10% in which Malakand showed 88.18%, Mardan (83.60%), Charsadda (81.20%), Peshawar (75.35%) and Swat (84.72%). The study indicates that broiler’s protein contents were lower verses domestic chicken as shown in figure 2.

**Table 4. Comparison of Nutrients Contents of domestic and broiler meat**

S.No	Nutrients Contents	Domestic	Broiler
1	Protein contents	84.19%----90.52%	75.35%----86.10%
2	Fats contents	0.62% ----2.30%	4.12%-----7.46%
3	Fibers contents	0.36%----0.65%	0.52%-----0.95%
4	Ash contents	3.70%----4.93%	4.20%----6.25%
5	Moisture contents	1.9%-----3.77%	1.92%----2.78%

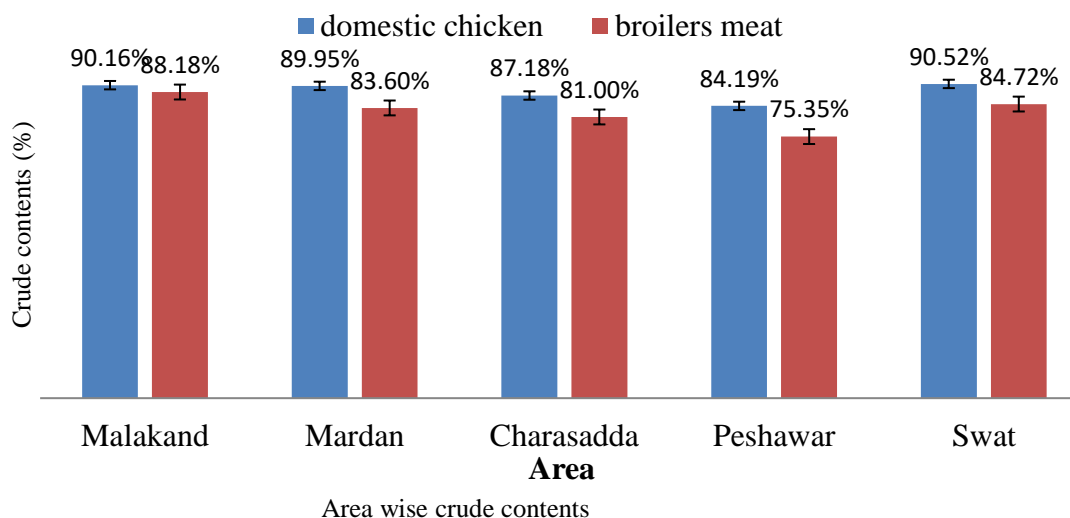


Figure 2: Percentage of Protein crude contents isolated from samples

**Discussion**

The demand for poultry meat product is increasing worldwide, and chicken species is an important source of human food worldwide. Poultry industry plays vigorous role in the economy of Pakistan. In our study, 30 samples from broilers and 30 from domestic chicken were examined for occurrence of bacteria. A total of seven bacteria were isolated and characterized as *Salmonella*, *Campylobacter jejuni*, *Escherichia Coli*, *Clostridium perferngens*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Staphylococcus aureus* from broilers meat samples. Microbial load in broilers chicken meat samples ranged from  $2 \times 10^3$  to  $6.3 \times 10^6$  and  $5 \times 10^3$  to  $1.0 \times 10^4$  CFU/g in domestic. Our study showed similarity with study of *Tobaruela*, who reported total bacterial counts in chicken thighs was  $5.1 \times 10^6$  CFU/g. *Jackson et al., 2006* showed similar results to our current work. The *Salmonella* was isolated 47% of broiler meat samples, whereas from domestic chicken 6.6% of samples. A similar study was performed in Australia where *Salmonella* was isolated 35.5% from chicken meat (*Heetun et al., 2015*). In other previous studies, isolated bacteria were reported less than the

values reported in our study (*Fearnley et al., 2008*; *Angkititrakul et al., 2005*; *Cohen et al., 2005*) and reported high in the research work of *Thanigaivel and Anandhan 2015*. In the current study, *E. coli* were present 67% samples of commercial broiler which is somewhat similar to previous studies *Nisar et al., 2018*; *Cohen et al., 2005*). In another study, samples were contaminated by *E. Coli*, which is lower than our present study (*Angkititrakul et al., 2005*). The *S. aureus* was isolated as 43% from broiler meat samples and 6.6% in domestic chicken meat. In the current study, *S. aureus* percentage from chicken meat was lower than those reported from study of *Abdalla et al., 2009*. and higher in other reported studies (*Jackson et al., 2006*; *Ramya et al., 2012*). In our study *C. jejuni* was isolated 60% *L. monocytogenes* 30% from broiler meat, which is higher than the previous study (*Abdalla et al., 2009*). In another outcome of the study, the nutritional value of domestic chickens and commercial broilers meat significantly differ. Moisture content of domestic meat samples ranged from 1.9% to 3.77% and broiler chicken 1.92% to 2.78%. However, our study supports the study that indigenous chicken biceps



femoris muscle contained less moisture than that of the broiler (Rahimi and Tajbakhsh, 2008). Broiler contained a higher moisture content than Korean native chicken (Jung et al., 2011).

Domestic chicken meat contained higher percentage of protein than those of commercial Broilers. Protein content of domestic chicken and broiler meat samples ranged from 84.19% to 90.52% and 75.35 to 86.10% respectively. Previous findings revealed that protein content observed in the study was in a similar range with my current work (Farooq et al., 2004). In another previous study percentage of protein content of Bali indigenous chicken was higher than broilers chicken (Mund et al., 2017). The protein contents reported in the study are similar to study of *Heetun* in Nigeria. In the current study, ash content was higher than the values reported by another scientist (Wyness, 2016). The fiber content of domestic and broilers chicken ranged from 0.36% to 0.65% and 0.52% to 0.95%, respectively in my study which range is less than from previous reported work of Mund. The percentage of fat content in domestic chicken meat was 0.62% to 2.36% and broilers were 4.12% to 7.6% in current study. Fat content were almost close to the values reported by *Rahimi et al., 2008*. The previous study of *Jaturasitha* revealed that fat content in Thai indigenous chicken was lower than broilers and Bali indigenous chicken also contained lower fat content than broiler (Jaturasitha et al., 2008). However, in the present study, broilers meat contained higher fat than the older slow-growing birds (domestic).

### Conclusion

The current study concludes that *Escherichia coli* is the most predominant bacteria in the chicken meat. The fungal isolation is also observed in which *Penicillium* percentage is mostly found greater as compared to *Aspergillus*. From the result of antibiotic sensitivity, it is concluded that most potent antibiotics found against bacteria are ciprofloxacin and Cefotaxime while most resistant antibiotics are Pepracillin and penicillin. Nutritional analysis of chicken meat is calculated which shows that domestic chicken meat is more significant than broiler meat. The moisture content and protein percentages in domestic chicken meat samples are higher than those of Broilers. Broiler contains higher percentage of Ash as compared to Domestic chicken meat. The study proposes that Fat content in Broiler is higher than domestic chicken meat.

### Conflict of interest

The authors declared absence of conflict of interest.

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