

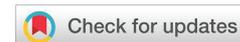
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## Research Article

# Bacteriology of chicken meat samples from Bharatpur, Chitwan, Nepal

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## Abstract

Many food-borne diseases are associated with the consumption of chicken meat and thus are of public health significance worldwide. A cross-sectional study was done to isolate, identify, and characterize bacteria from chicken meat samples of Bharatpur, Chitwan. A total of 102 samples were randomly collected and processed at the Microbiology laboratory of Birendra Multiple Campus, Chitwan for three months (May-July 2016). One gram of each sample was crushed on 9 ml distilled water in a sterile mortar pestle followed by serial dilution and inoculation of 0.1 ml sample into suitable culture media and incubated at 37 °C for 24 hours. Identification of the isolates was done by microscopic examination and biochemical tests and the antimicrobial susceptibility pattern of the isolates was determined by the Kirby Bauer disc diffusion method. Out of 102 meat samples, the growth positivity rate was 94.0% ( $n = 96/102$ ) on all of the culture media. 26/48 fresh and 44/54 frozen samples gave positive growth with 36 isolates from fresh and 60 isolates from frozen meat samples, with the occurrence of *Staphylococcus aureus* 26(27.08%), *Pseudomonas* spp 6(6.25%), *Proteus* spp 4(4.16%), *Escherichia coli* 22(22.91%), *Salmonella* spp 16(16.66%), *Citrobacter* spp 8(8.33), *Acinetobacter* spp 8(8.33%), *Streptococcus* spp 2(2.08%), *Shigella* spp 4(4.16%) and *Vibrio* spp 2(2.08%). *Cefalexin* (92.85%) was the most effective antibiotic against Gram-positive bacteria followed by *Amoxicillin* (71.42%) and *Methicillin* (64.28%). The least effective antibiotic was *Ampicillin* (50%). Similarly, *Gentamicin* (76.47%) followed by *Nalidixic acid* (41.47%) were effective against Gram-negative bacteria, while some showed resistance to all three classes of drugs exhibiting MDR.

## Introduction

The consumption of contaminated food is unsafe for health and its consequences have been one of man's major health problems for a long time. They remain to be a major public health concern globally. Food-borne diseases are responsible for a large occurrence of adult illnesses and deaths; more importantly, as sources of acute diarrheal diseases, they are known to take the lives of many children every day [1]. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. Evidently, in developing countries, up to an estimated 70% of cases of diarrheal disease are associated with the consumption of contaminated food especially meat and meat products [1]. Transmission of entero-pathogenic bacteria is affected directly or indirectly through objects contaminated with feces. These include food and water indicating the importance of fecal-oral

human-to-human transmission [2]. Chicken is a rich source of meat protein and is highly consumed all over the world. However, under a poor hygienic environment, raw chicken meat presents an ideal substrate supporting the growth of pathogenic *Escherichia coli* and coliform bacteria, indicating the potential presence of other pathogenic bacteria; this may even constitute a major source of food-borne illnesses in humans. Chicken is a nutritious, healthy food that is low in fat and cholesterol compared to other meats but an excellent source of protein. Meat must be of high microbiological quality to ensure that the consumer receives a product that is not spoiled or does not carry food-borne diseases [3]. Special attention in poultry meat production is paid to the fact that live animals are hosts to a large number of different microorganisms residing on their skin, feathers, or in the alimentary tract. During slaughter, most of these microorganisms are eliminated, but subsequent contamination is possible at any stage of the production process,

from feather plucking, evisceration, and washing to storage by cooling or freezing [4]. Microorganisms from the environment, equipment, and operators hands can contaminate the meat. The increased prevalence of *Salmonella* contamination in poultry has gained considerable scientific attention during the last few decades. Poultry is one of the most common reservoirs of *Salmonella* and contamination of poultry products can occur during the different stages of poultry production. Poultry is a food that has been highly appreciated by man since time immemorial. It is an important, low-cost source of animal protein, rich in nutrients, phosphorus, other minerals, and B-complex vitamins [5]. Food-borne diseases associated with the consumption of poultry meat and its processed products are of public health significance worldwide [6].

Poultry and poultry meat are often found contaminated with potentially pathogenic microorganisms such as *Salmonella*, *Campylobacter*, *S. aureus*, *E. coli*, and *Listeria*. Microorganisms introduced from environmental exposure, lack of sanitation in slaughtering premises, equipment, and outfits, and operators' hands contaminate the meat product [7]. Chicken meat has higher pathogenic and spoilage bacterial counts than most other foods, where meat can be contaminated at several points throughout the processing operation during scalding, de-feathering, and evisceration as well as cross-contamination from other birds and processing equipment. The meat surface does not normally, inherently contain pathogenic organisms but can acquire the organisms from fecal matter or cross-contamination during slaughter. Ensuring a safe food supply has been a continuous challenge following the recognition of more and more pathogenic bacteria. Despite modern innovations in slaughter hygiene and food production techniques, food safety has been at the forefront of public health issues [8]. The organisms tend to remain on the surface or just under it. Meat is an ideal medium for bacterial growth because of its high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), and a good source of minerals, vitamins, and other growth factors. Furthermore, its pH is favorable for the growth of micro-organisms too [9]. The progressive increase in antimicrobial resistance among enteric pathogens in developed and developing countries has become a critical area of concern [10]. Previous studies have shown that food-borne pathogens, such as *Escherichia coli* and *Salmonella*, are highly prevalent, and have been isolated in stool samples from humans affected by food-borne illnesses, as well as in the meat and poultry products processed for human consumption [11-14]. Ensuring a safe food supply has been a continuous challenge following the identification of more and more pathogenic bacteria. Despite modern innovations in slaughter hygiene and food production techniques, food safety has been at the forefront of public health issues [8]. The safety of commercially processed poultry products is a major area of concern for producers, consumers, and public health officials worldwide for products excessively contaminated with microorganisms are undesirable from the standpoint of public health, storage quality, and general aesthetics [15]. The contamination of chicken meat with microorganisms during processing, handling, and transportation is undesirable, though inevitable. A higher bacterial load on the carcass

could be expected when carcasses are handled unhygienically at the abattoir [16]. Two of the most common etiologic bacterial organisms responsible for causing gastroenteritis, a major public health concern in most regions of Thailand, are *Salmonella* and *E. coli* [17,18]

Several studies have reported an outbreak of infections due to consumption of contaminated food and poor hygiene and in most of the cases, data are loosely based on laboratory isolates which do not reflect the actual ratio of food-borne infections. However, a few community-based reports provide evidence of outbreaks caused by *Salmonella*, *Shigella*, *E. coli*, and *Listeria* spp in different parts of the world [19]. Moreover, antibiotic resistance levels are also elevated among food-borne pathogens such as *Salmonella* and *Shigella* [20]. Meat, a good source of animal protein along with sensory attributes, appeals to consumers very easily.

## Materials and methods

This was a cross-sectional study carried out at the microbiology laboratory of Birendra Multiple Campus, Bharatpur during a period from May to July 2016, and the samples were collected from different wholesalers and retailer meat shops in Bharatpur, Nepal.

All of the slaughter slabs, and wholesale and retail chicken meat shops in Bharatpur ward no. 7, 8, and 10, Chitwan, Nepal were visited and butchers were interviewed.

### Collection of samples

A total of one hundred-two random samples of chicken carcasses were collected from local commercial retail shops and wholesale shops in Bharatpur sub-metropolitan municipality. The collected samples were kept in separate sterile plastic bags and transferred directly to the laboratory in an insulated icebox under complete aseptic conditions without any delay to evaluate their bacteriological quality. Samples with improper labeling and inappropriate collection were also rejected.

### Preparation of samples (USDA 2011)

One gram of the examined samples was removed by sterile scissors and forceps after surface sterilization by a hot spatula, transferred to a sterile polyethylene bag, and 9 ml of 0.1 % sterile buffered peptone water was aseptically added to the content of the bag. Each sample was then homogenized by a mortar and pestle to provide a homogenate of 1/10 dilution.

### Bacterial culture

The collected samples were immediately processed without storage. Samples homogenized with peptone water were incubated at 37 °C for 5 hours, and then one loopful of the culture was streaked on Mannitol salt agar for Gram-positive bacteria, MacConkey agar for Gram-negative bacteria, Eosin Methylene Blue agar for Coliforms, especially, *E. coli* and Xylose lysine deoxycholate agar for the identification of *Salmonella* spp. and incubated at 37 °C for 24 hours. Further, the suspected, isolated colonies were sub-cultured on Nutrient agar and Gram staining for morphological identifications, and

different biochemical tests (IMViC, catalase, oxidase, urease, coagulase, etc.) were carried out.

### Biochemical characteristic tests

Identification of bacterial isolates was carried out based on their cultural (appearance; pigmentation, consistency, margin, and elevation), morphological (Gram's staining, size, and shape), and biochemical characteristics (catalase, oxidase, indole, coagulase tests, citrate utilization, Methyl red and Voges-Proskauer tests, Triple sugar iron, SIM tests, etc.) [21-23].

### Disc diffusion susceptibility test

Susceptibility tests were performed by the disc diffusion method [24,25]. The turbidity of the inoculums should be adjusted to the equivalent turbidity of 0.5 McFarland standards. An 18 hours culture of test organisms incubated at 37 °C was standardized by diluting to 0.5 McFarland turbidity standard before spreading over the surface of Mueller Hinton agar (MHA) (Titan Biotech Ltd. Bhiwadi-301019, Rajasthan, India.) plates using sterile cotton swab/glass spreader [26] and allowed to dry for 2 to 5 minutes. Using sterile tweezers, antimicrobial discs ampicillin (10 mcg), nalidixic acid (30 mcg), nitrofurantoin (300 mcg), trimethoprim (5 mcg), Gentamycin (10 mcg), methicillin (5 mcg), amoxicillin (30 mcg) and Azithromycin (15 mcg) were placed widely spaced aseptically on the surface of MHA plate. Tweezers were re-flamed after the application of each disc. The plates were then incubated at 37 °C for 24 hours. Following incubation, the Diameter of the Inhibition Zone (DIZ) was measured with a transparent ruler and expressed in millimeters (mm).

### Quality control for test

In this study, the quality and accuracy of all tests were maintained by following standard procedures of collection, isolation, and identification. For identification and standardization by the Kirby-Bauer test, a standard culture of *E. coli* ATCC 25922 was used as a reference strain. For quality control, media, antibiotics, and reagents were prepared, stored, and utilized as recommended by the manufacturing company. Antibiotic discs were stored at refrigerator temperature. For each batch of the test, a positive and negative known culture was used for color reaction, biochemical tests, and antibiotic sensitivity tests.

### Statistical analysis

Data entry, management, and analysis were done using SPSS v20. The association between different risk factors and the antibiotic resistivity pattern of isolated bacteria was compared statistically by a Chi-square ( $\chi^2$ ) test.

## Result

### The pattern of growth of chicken meat sample

Out of 102 meat samples, growth was observed in 96 (94.11%) samples, and no growth was observed in 6 (5.88%) samples (Figure 1).

### Differentiation based on gram's reaction

Out of 96 positive cultures, 28 were Gram-positive, while the rest 68 isolates were Gram-negative (Figure 2).

### The growth pattern of total isolates

Out of 102 samples, 40 fresh meat (39.21%) and 56 frozen meat (60.78%) samples were taken. Of which 96 bacteria were isolated i.e., 26(27.08%) *Staphylococcus aureus* spp, 22(22.91%) *E. coli* spp, 14(14.58%) *Salmonella* spp, 4(4.16%) *Proteus* spp, 4(4.16%) *Shigella* spp, 8(8.33%) *Citrobacter* spp, 8(8.33%) *Acinetobacter* spp, 6(6.25%) *Pseudomonas* spp, 2(2.08%) *Vibrio* spp, 2(2.08%) *Streptococcus* spp (Figure 3).

### Antimicrobial susceptibility pattern

The antibiotic discs used for Gram-positive isolates were Cefalexin (CN), Amoxicillin (AMX), Methicillin (MET), Azithromycin (AZM), and Ampicillin (AMP) while for Gram-

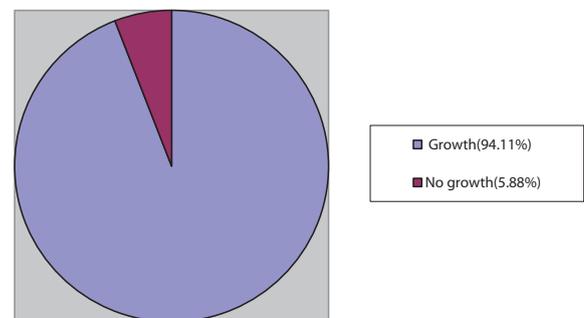


Figure 1: Showing a pattern of growth of meat samples.

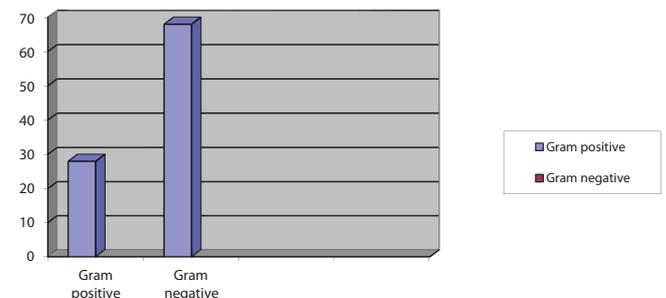


Figure 2: Showing gram-positive and negative from culture.

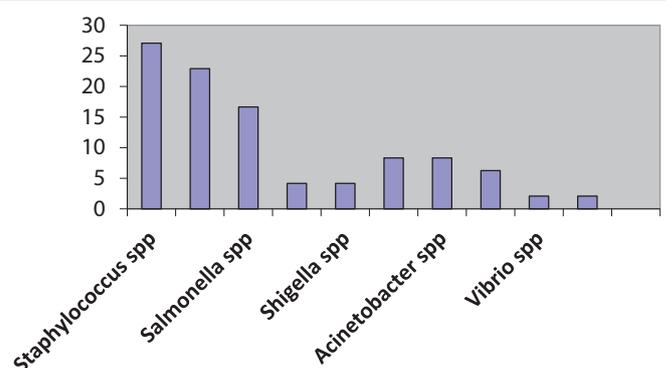


Figure 3: Showing the total growth pattern of the isolates.

negative isolates, Gentamicin (GEN), Nalidixic acid (NA), Trimethoprim (TR), and Nitrofurantoin (NIT) were used. Cefalexin (92.85%) was the most effective antibiotic against Gram-positive bacteria followed by Amoxicillin (71.42%) and Methicillin (64.28%), while the least effective antibiotic was Ampicillin (50.00%). Similarly, Gentamicin (76.47%) followed by Nalidixic acid (41.71%) were effective against Gram-negative bacteria, while the least effective antibiotic was Trimethoprim (36.76%). All the antibiotics used were broad-spectrum antibiotics (Table 1).

### Antimicrobial susceptibility pattern of *Staphylococcus aureus*

26(27.08%) *Staphylococcus aureus* was isolated from the meat sample. Of which, Cefalexin (92.30%) followed by Amoxicillin (69.23%) was the most effective drug against the isolated species (Table 2).

### Antibiotic susceptibility pattern of *Streptococcus* spp

2(2.08%) *Streptococcus* spp were isolated from the meat sample. Of which, Ampicillin (100%) followed by Amoxicillin (100%), Methicillin (100%), Cefalexin (100%), and Azithromycin (100%) was the most effective drug against the isolated species (Table 3).

### Antibiotic susceptibility pattern of *E. coli*

22(22.91%) *E. coli* were isolated from the meat samples. Of which, Nitrofurantoin (72.72%) was the most effective drug followed by Gentamicin (54.54%), Trimethoprim (36.36%), and Nalidixic acid (36.36%) (Table 4).

### Antibiotic susceptibility pattern of *Shigella* spp

4(4.16%) *Shigella* spp were isolated from meat samples. Of which, Gentamicin (100%) was the most effective drug (Table 5).

### Antibiotic susceptibility pattern of *Citrobacter* spp

8(8.33%) *Citrobacter* spp were isolated from meat samples. Of which, Gentamicin (75%) was the most effective drug, followed by Trimethoprim (50%) then Nitrofurantoin (25%), and Nalidixic acid (25%) (Table 6).

**Table 1:** Showing Antibigram of total isolates.

S.N	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Ampicillin	14	50.00	-	-	14	50.00	28
2.	Amoxicillin	20	71.42	-	-	8	28.68	28
3.	Methicillin	18	64.28	-	-	10	35.71	28
4.	Cefalexin	26	92.85	-	-	2	7.14	28
5.	Azithromycin	16	57.14	2	7.14	10	35.71	28
6.	Nitrofurantoin	26	38.23	4	5.88	38	55.88	68
7.	Gentamicin	52	76.47	4	5.88	12	17.64	68
8.	Trimethoprim	25	36.76	-	-	43	63.23	68
9.	Nalidixic acid	28	41.17	14	20.58	26	38.23	68

**Table 2:** Showing antibiogram of *Staphylococcus aureus*.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Ampicillin	12	46.15	-	-	14	53.84	26
2.	Amoxicillin	18	69.23	-	-	8	30.76	26
3.	Methicillin	16	61.53	-	-	10	38.46	26
4.	Cefalexin	24	92.30	-	-	2	7.69	26
5.	Azithromycin	14	53.84	2	7.69	10	38.46	26

**Table 3:** Showing antibiogram of *Streptococcus* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistance		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Ampicillin	2	100	-	-	-	-	2
2.	Amoxicillin	2	100	-	-	-	-	2
3.	Methicillin	2	100	-	-	-	-	2
4.	Cefalexin	2	100	-	-	-	-	2
5.	Azithromycin	2	100	-	-	-	-	2

**Table 4:** Showing antibiogram of *E. coli*.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	16	72.72	-	-	6	27.27	22
2.	Gentamicin	12	54.54	4	18.18	6	27.27	22
3.	Trimethoprim	8	36.36	-	-	14	63.63	22
4.	Nalidixic acid	8	36.36	4	18.18	10	45.45	22

**Table 5:** Showing antibiogram of *Shigella* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	-	-	2	50	2	50	4
2.	Gentamicin	4	100	-	-	-	-	4
3.	Trimethoprim	-	-	-	-	4	100	4
4.	Nalidixic acid	-	-	-	-	4	100	4

**Table 6:** Showing antibiogram of *Citrobacter* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	2	25	-	-	6	75	8
2.	Gentamicin	6	75	-	-	2	25	8
3.	Trimethoprim	4	50	-	-	4	50	8
4.	Nalidixic acid	2	25	4	50	2	25	8

### Antibiotic susceptibility pattern of *Acinetobacter* spp

8(8.33%) were isolated from meat samples. Of which, Gentamicin (100%) was the most effective drug, followed by Nitrofurantoin (50%) and Nalidixic acid (25%) (Table 7).

### Antibiotic susceptibility pattern of *Proteus* spp

4(4.16%) of *Proteus* spp was isolated from the meat samples. Of which, Trimethoprim (100%) was the most effective drug used against *Proteus* spp followed by Gentamicin (50%) and Nalidixic acid (50%) (Table 8).



### Antibiotic susceptibility pattern of *Vibrio* spp

2(2.08%) of *Vibrio* spp were isolated from meat samples. Of which Gentamicin (100%) was the most effective drug (Table 9).

### Antibiotic susceptibility pattern of *Pseudomonas* spp

6(6.25%) of *Pseudomonas* were isolated from the meat samples. Of which Gentamicin (100%) was the most effective drug followed by Nalidixic acid (66.66%) (Table 10).

### Antibiotic susceptibility pattern of *Salmonella* spp

14(14.58%) *Salmonella* spp were isolated from the meat samples. Of which Gentamicin (85.71%) was the most effective drug, followed by Nalidixic acid (71.42%), and Trimethoprim (50%) (Table 11).

### Multi-drug resistant organisms

The highest number of MDR isolates was *Shigella* spp 4(100%) followed by *Citrobacter* 4(50%), *E. coli* (45.45%), *Acinetobacter* 2(25%), *Staphylococcus aureus* 4(15.38%), and *Salmonella* 2(12.50%). The prevalence of MDR among total isolates was found to be 27.08% (Figure 4).

### Age-wise distribution of the butchers and distribution of bacteria among fresh and frozen meat samples

Among 96 isolates, the highest number of isolates 60(62.5%) was recorded from the age group 16 years – 30 years. The lowest number of isolates were detected from age group 46 – 60 accounting for 4(4.167%). Lastly, 32(33.33%) bacterial isolates were detected from the age group of 31 – 45 summing both fresh and frozen meat samples. The prevalence of bacteria in fresh and frozen meats was not significantly affected by the age of butchers ( $p > 0.05$ ) (Table 12).

### Sex-wise distribution of butchers and distribution of bacteria among fresh and frozen meat samples

Among 96 isolates, 22(22.91%) bacteria were detected from fresh meat handled by male butchers, while 14(14.58%) isolates were detected from fresh meat handled by female butchers. Similarly, 42(43.75%) isolates were detected from frozen meat provided by male meat sellers, while 18(18.75%) bacteria were isolated from frozen meat provided by female meat sellers. From the above data collected, meat collected from male butchers was more highly contaminated than meat sold by female butchers as the number of isolates in the case of a male was quite higher than that of a female. There was no significant association between the sex of the respondents and the quality of the products being sold  $p > 0.05$  (Table 13).

Table 7: Showing antibiogram of *Acinetobacter* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	4	50	-	-	4	50	8
2.	Gentamicin	8	100	-	-	-	-	8
3.	Trimethoprim	-	-	-	-	8	100	8
4.	Nalidixic acid	2	25	2	25	4	50	8

Table 8: Showing antibiogram of *Proteus* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	-	-	2	50	2	50	4
2.	Gentamicin	2	50	-	-	2	50	4
3.	Trimethoprim	4	100	-	-	-	-	4
4.	Nalidixic acid	2	50	2	50	-	-	4

Table 9: Showing antibiogram of *Vibrio* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	-	-	-	-	2	100	2
2.	Gentamicin	2	100	-	-	-	-	2
3.	Trimethoprim	-	-	-	-	2	100	2
4.	Nalidixic acid	-	-	-	-	2	100	2

Table 10: Showing antibiogram of *Pseudomonas* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	2	33.33	-	-	4	66.66	6
2.	Gentamicin	6	100	-	-	-	-	6
3.	Trimethoprim	2	33.33	-	-	4	66.66	6
4.	Nalidixic acid	4	66.66	2	33.33	-	-	6

Table 11: Showing antibiogram of *Salmonella* spp.

S.N.	Antibiotics	Sensitive		Intermediate		Resistant		Total isolates
		Number	Percent (%)	Number	Percent (%)	Number	Percent (%)	
1.	Nitrofurantoin	2	14.28	-	-	12	85.71	14
2.	Gentamicin	12	85.71	-	-	2	14.28	14
3.	Trimethoprim	7	50	-	-	7	50	14
4.	Nalidixic acid	10	71.42	-	-	4	28.57	14

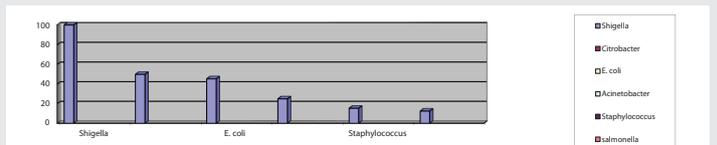


Figure 4: Showing the number of MDR organisms.

Table 12: Showing age-wise distribution of butchers and distribution of isolates among fresh and frozen meat samples.

S.N.	Age	Fresh	Frozen	Total isolates	p - value
1.	16 - 30	26	34	60	0.875
2.	31 - 45	6	26	32	
3.	46 - 60	4	0	4	
Total		36	60	96	

### Distribution of bacteria among the type of shops and distribution among fresh and frozen meat samples

Among 96 isolates, 20(20.83%) bacteria were isolated from fresh meat taken from retailer shops, while 48(50%) isolates were detected from frozen meat samples taken from retailer



shops. Similarly, 16(16.66%) isolates were detected from fresh meat samples collected from wholesale shops, while 12(12.50%) isolates were detected from frozen meat samples taken from wholesale shops. From the above data collected the number of bacterial isolates was higher from frozen meats which were taken from retail shops. However, there was no significant association between the type of shops and the distribution of bacteria among fresh and frozen meat samples  $p > 0.05$  (Table 14).

### Distribution of bacteria among literacy groups and the occurrence of isolates from fresh and frozen meat samples

Among 96 isolates, 20(20.83%) bacteria were isolated from fresh meat provided by literate butchers, while 34(35.41%) bacteria were isolated from frozen meat taken from literate meat sellers. Similarly, 16(16.66%) bacterial isolates were detected from fresh meat provided by illiterate butchers, while 26(27.08%) isolates were identified from frozen meat samples taken from illiterate butchers. The number of bacterial isolates was higher in the case of frozen meat collected from literate meat sellers. There is no significant association between the literacy group and the occurrence of bacteria in fresh and frozen meat samples  $p > 0.05$  (Table 15).

### Discussion

Bacterial contamination of chicken meat can be from the chicken itself, workers, tools, and types of equipment as well as hygiene in the slaughterhouse. In the present study performed, the most frequently isolated bacteria were *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter* spp, *Acinetobacter* spp, *Shigella* spp, and *Salmonella* spp. respectively. Out of 102 samples, 40 fresh lumps of meat (39.21%) and 56 frozen lumps of meat (60.78%) samples were taken. Of which 96 bacteria were isolated i.e. 26(27.08%) *Staphylococcus aureus* spp, 22(22.91%) *E. coli* spp,

14(14.58%) *Salmonella* spp, 4(4.16%) *Proteus* spp, 4(4.16%) *Shigella* spp, 8(8.33%) *Citrobacter* spp, 8(8.33%) *Acinetobacter* spp, 6(6.25%) *Pseudomonas* spp, 2(2.08%) *Vibrio* spp, 2(2.08%) *Streptococcus* spp.

In the study conducted in Chitwan, antimicrobial drugs were used for AST. Ampicillin, Amoxycillin, Methicillin, Cefalexin, and Azithromycin were used against Gram-positive isolates while Nitrofurantoin, Gentamicin, Trimethoprim, and Nalidixic acid were used against Gram-negative isolates. Among 28 Gram-positive isolates, 14(50%) were resistant to Ampicillin, 28.68% were resistant to Amoxycillin, 35.71% were resistant to Methicillin, and 7.41% were resistant to Cefalexin and 35.71% were resistant to Azithromycin. Among 68 Gram-negative isolates, 55.88% were resistant to Nitrofurantoin, 17.64% were resistant to Gentamicin, 63.33% were resistant to Trimethoprim and 38.23% were resistant to Nalidixic acid.

Hence, among all the antibiotics used for Gram-positive isolates, most strains of bacteria were resistant to Ampicillin (50%). And in the case of Gram-negative isolates, most bacteria were resistant to Trimethoprim (63.23%).

Among 26 *Staphylococcus* spp isolated from our study, only 4 of them (15.38%) showed Multi-Drug Resistance (MDR) properties i.e. among 5 antimicrobial drugs used, the isolated spp were resistant to more than 2 classes of drugs. Similarly, out of 22 isolated *E. coli* 10(45.45%) showed MDR, while 14.28% of *Salmonella* were multi-drug resistant. Likewise, 100% *Shigella* spp, 50% *Citrobacter*, and 25% *Acinetobacter* were MDR. The highest number of MDR isolates was *Shigella* spp 4(100%) followed by *Citrobacter* 4(50%), *E. coli* (45.45%), *Acinetobacter* 2(25%), *Staphylococcus aureus* 4(15.38%), and *Salmonella* 2(12.50%).

Among 96 isolates, the highest number of isolates 60(62.5%) was recorded from the age group 16-30 years. The lowest number of isolates were detected in the age group 46 - 60 accounting for 4(4.167%). Lastly, 32(33.33%) bacterial isolates were detected from the age group of 31 - 45 summing both fresh and frozen meat samples. The prevalence of bacteria in fresh and frozen meats was not significantly affected by the age of butchers ( $p > 0.05$ ). Among 96 isolates, 22(22.91%) bacteria were detected from fresh meat handled by male butchers, while 14(14.58%) isolates were detected from fresh meat handled by female butchers. Similarly, 42(43.75%) isolates were detected from frozen meat provided by male meat sellers, while 18(18.75%) bacteria were isolated from frozen meat provided by female meat sellers. From the above data collected, meat collected from male butchers was more highly contaminated than meat sold by female butchers as the number of isolates in the case of the male was quite higher than that of females. There was no significant association between the sex of the respondents and the quality of the products being sold  $p > 0.05$ .

Among 96 isolates, 20(20.83%) bacteria were isolated from fresh meat taken from retailer shops, while 48(50%) isolates were detected from frozen meat samples taken from retailer shops. Similarly, 16(16.66%) isolates were detected from fresh

**Table 13:** Showing sex-wise distribution of butchers and distribution of isolates among fresh and frozen meat samples.

S.N	Gender	Fresh	Frozen	Total	p - value
1.	Male	22	42	64	0.25
2.	Female	14	18	32	
	Total	36	60	96	

**Table 14:** Showing distribution of bacteria among the type of shops and distribution among fresh and frozen meat samples.

S.N.	Shops	Fresh	Frozen	Total	p - value
1.	Retailer	20	48	68	0.5
2.	Wholesaler	16	12	28	
	Total	36	60	96	

**Table 15:** Showing distribution of bacteria among literacy groups and their prevalence in fresh and frozen meat samples.

S.N.	Literacy	Fresh	Frozen	Total	p - value
1.	Literate	20	34	54	1
2.	Illiterate	16	26	42	
	Total	36	60	96	

meat samples collected from wholesale shops, while 12(12.50%) isolates were detected from frozen meat samples taken from wholesale shops. From the above data collected the number of bacterial isolates was higher from frozen meats which were taken from retail shops. However, there was no significant association between the type of shops and the distribution of bacteria among fresh and frozen meat samples  $p > 0.05$ .

Among 96 isolates, 20(20.83%) bacteria were isolated from fresh meat provided by literate butchers, while 34(35.41%) bacteria were isolated from frozen meat taken from literate meat sellers. Similarly, 16(16.66%) bacterial isolates were detected from fresh meat provided by illiterate butchers, while 26(27.08%) isolates were identified from frozen meat samples taken from illiterate butchers. The number of bacterial isolates was higher in the case of frozen meat collected from literate meat sellers. There is no significant association between the literacy group and the occurrence of bacteria in fresh and frozen meat samples  $p > 0.05$ .

Meat is the most perishable of all-important foods since it contains sufficient nutrients needed to support the growth of microorganisms. The annual production of chicken meat in Nepal is 16662 metric tons and the annual production of chicken meat in the Chitwan district is 1422 metric tons. So, Chitwan has an 8.5 percent share of chicken meat production annually in Nepal. Chicken meat can be contaminated at several points throughout the processing operations. Moreover, retail cuts could result in greater microbial load owing to a large amount of exposed surface area, more readily available water, nutrients, and greater oxygen penetration which leads to spoilage of meat. Meat-borne zoonotic diseases such as Salmonellosis, Campylobacteriosis, *E. coli* enteritis, and food poisoning by *Clostridium*, *Staphylococcus*, etc. are the major problems encountered by consumers eating contaminated meat. Only little is known about microbial aspects, shelf life, and food safety of commercial retail chicken meat in Chitwan. The poultry slaughtered and dressed under Chitwan conditions carrying high initial contamination would be exhibited to the point the consumers are offered as retail meat. So, retail meat would harbor all the bacteria that are already present in meat as inherent contamination through infection and that are introduced during handling, improper dressing, cleaning, unsanitary conditions, and retailing. To increase meat quality, assurance following microbial load assessment is deemed necessary. Hence, this study was conducted to assess the microbiological situation of fresh chicken meat which can be the reflection of the hygienic condition of meat consumed and the possible hazards to public health. Chicken meat can also act as a reservoir of drug-resistant bacteria. Antimicrobial resistance among *E. coli*, *Salmonella*, and other species in chicken meat is of increasing concern due to the potential for the transfer of these resistant pathogens to the human population. Most of these genera are known to be of public health concern and have been associated with cases of gastroenteritis and other food-borne diseases [27]. The sources of these contaminations have been linked to poor hygienic conditions of the handlers, the environment, and cross-processing contaminations [28-30]. Among the 102 meat samples, only two did not produce

any microorganisms when incubated at 37 °C. Of the samples, 58 contained Coliform bacteria, 58 contained *S. aureus*, 56 showed *Pseudomonas* growth, and 38 of them contained *E. coli*. Among the samples, 32 out of the 58 samples were *S. aureus*-positive. The susceptibility results of bacteria isolated from meat samples showed that they are highly resistant to all the antibiotics tested. Gram-negative organisms are more resistant than Gram-positives; this is expected because of the intrinsic nature of the gram-negative cell wall. The gram-negative micro-organisms isolated belong to the Enterobacteriaceae family, this group of organisms is always resistant to various classes of antibiotics [31-53].

## Conclusion

A total of 102 chicken meat samples were collected from different retail and wholesale shops, out of which 40 fresh and 56 frozen samples were growth positive while 6 samples were growth negative. The samples were collected from shops with 64 male and 38 female butchers. The results of the present study indicated that the prevalence of common food-borne pathogens in the market samples of chicken meat in Bharatpur, Chitwan was at the higher levels. On antimicrobial susceptibility testing, Ampicillin was the most effective antibiotic against Gram-positive bacteria followed by Amoxicillin, Cefalexin, and Azithromycin. Most of the isolated *Staphylococcus* were Methicillin-resistant and some exhibited MDR too. Similarly, Gentamicin followed by Trimethoprim and Nalidixic acid were effective against Gram-negative bacteria, while some showed resistance to all three classes of drugs exhibiting MDR. The highest MDR organism isolated was *Staphylococcus aureus* followed by *E. coli* and *Salmonella* spp. There wasn't a significant association between the type of meat shops and the condition of the meat samples ( $p > 0.05$ ). Similarly, the association was absent between the age of the meat sellers and the occurrence of pathogens in fresh and frozen meat samples. Hence, we cannot say that the age factor is responsible for causing more contamination of meat products. Likewise, there was no correlation between the sex of butchers and the hygiene of the meat, so we cannot say that male butchers handled meat improperly so the number of isolated bacteria was higher though the collected data suggested that result. The present study provided us with an idea about the occurrence of pathogens in chicken meat in correlation with different risk factors and helped policymakers to build rules and regulations that strengthen the quality of health of people consuming meat and meat products.

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