

Antibiotic Resistance and Occurrence of *Salmonella*, *E. coli*, and *S. aureus* in Chicken Meat, Kathmandu Valley

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Abstract: This paper examines the prevalence of three significant foodborne illness-causing organisms, *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*, in chicken meat in the Kathmandu Valley and their resistance to widely used antibiotics. Twenty chicken meat samples were collected locally in Kathmandu, Bhaktapur, and Lalitpur. Antibiotic susceptibility was determined by the Kirby-Bauer disc diffusion method. The findings revealed that contamination was widespread: *E. coli* was found in 40 to 60 per cent of the samples, *Salmonella* spp. in 30 to 40 per cent, and *S. aureus* in 20 per cent. Antibiotic susceptibility testing was performed to confirm that nalidixic acid, chloramphenicol, and ciprofloxacin were generally susceptible, whereas ampicillin was highly resistant, as commonly observed in *E. coli* and *S. aureus*. More worrying still, more than 50 per cent of *S. aureus* isolates were MRSA. These findings highlight serious food safety concerns in Nepal's poultry sector, where poor hygiene during slaughter, processing, and retail handling allows pathogens to persist, and frequent antibiotic use promotes resistance. Improving hygiene practices, regulating antibiotic use in poultry farming and conducting regular microbiological surveillance are critical steps to reduce the risks of foodborne illness and antimicrobial resistance.

Keywords: Isolation, Identification, chicken, *E.coli*, *Salmonella* spp., *Staphylococcus aureus*

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1. Introduction

Food safety remains a major global public health concern, as foodborne illnesses continue to cause significant health burdens and economic losses worldwide. Despite considerable advances in food science and technology, these diseases persist as a common challenge (Pires et al., 2021). A wide range of microorganisms is responsible for foodborne illnesses, with bacteria originating from humans, animals, or the environment being the predominant causative agents (Aljamali et al., 2021). Among these, *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* are particularly significant due to their potential to cause severe human infections and their frequent association with food contamination, necessitating ongoing surveillance and control measures (Director National Centre for Disease Control, 2017).

Raw meat is a critical vehicle for foodborne pathogens. Contamination often occurs during slaughter, where bacteria from sources such as animal hides, faeces, processing water, intestines, lymph nodes, and equipment can be transferred to the meat (Shilenge et al., 2016). The extent and nature of microbial contamination are heavily influenced by the hygiene standards of the slaughter process, sanitation management, safety protocols, and subsequent handling, storage, and transportation practices (Gill, 2005).

Chicken meat, derived from domesticated poultry, is among the most widely consumed sources of animal protein globally. Its high moisture content, rich in nutrients, proteins, minerals, and carbohydrates such as glycogen, creates an ideal environment for bacterial proliferation. The natural pH of chicken meat further supports the growth of diverse microbes, making it susceptible to contamination if not handled or stored properly (Zhang et al., 2017). Global production and consumption of chicken meat have risen dramatically; in 2024, broiler meat production exceeded 103 million metric tons (Gadzama, 2024). Nepal follows this trend, with commercial chicken production increasing substantially and daily consumption in the Kathmandu Valley alone accounting for nearly half of the national total (Gadzama, 2024).

Despite its popularity, chicken meat is frequently contaminated with pathogenic bacteria, including *Salmonella*, *E. coli*, *S. aureus*, *Campylobacter jejuni*, and *Listeria monocytogenes* (Tahir et al., 2024). Contamination can occur at multiple

points along the supply chain, from the farm and slaughterhouse to retail markets, often due to inadequate hygiene, contaminated water, and poor sanitation (Shakhes et al., 2024). While proper cooking can destroy most pathogens, risks persist through cross-contamination, improper storage, and inadequate reheating, leading to illnesses characterised by symptoms such as nausea, vomiting, abdominal cramps, and diarrhoea (Enteritidis et al., 1981).

A growing and parallel concern is the emergence of antibiotic-resistant bacteria in the food chain. Studies have reported multidrug-resistant (MDR) strains of *Salmonella*, *S. aureus*, and *E. coli* in chicken meat, complicating clinical management and increasing the risk of severe infections (Neupane et al., 2023). Antimicrobial Susceptibility Testing (AST) is therefore crucial for determining resistance patterns, guiding appropriate therapeutic choices, and informing public health interventions to combat antimicrobial resistance (AMR) (Ahmed et al., 2024). Regular surveillance of resistance profiles in foodborne pathogens is essential for effective national health strategies.

In Nepal, particularly in the densely populated Kathmandu Valley, the high consumption of chicken meat coupled with potential lapses in hygiene and antibiotic use in poultry farming underscores the need for localised data. This study aimed to investigate the prevalence of *Salmonella* spp., *E. coli*, and *S. aureus* in chicken meat sold in the Kathmandu Valley and to evaluate their antibiotic resistance patterns. The findings are intended to contribute to the evidence base necessary for improving food safety standards, informing regulatory policies on antibiotic use, and protecting public health in Nepal.

2. Methodology

2.1. Study Design and Site

This research employed a cross-sectional study design. The study was conducted in the Kathmandu Valley, Nepal. Chicken meat samples were collected from retail markets in three districts: Kathmandu (Bagbazar and Putalisadak), Bhaktapur (Sanothimi, Pepsicola, and Lokanthali), and Lalitpur (Kumaripati, Lagankhel, Satdobato, and Dhapakhel).



Figure 1: Locations of the study

2.2. Sample Collection and Processing

A total of twenty ($n=20$) raw chicken meat samples were aseptically collected from the selected local markets. Each sample, weighing approximately 100-150 grams, was immediately placed in a sterile zip-lock bag, labelled, and transported in an icebox maintained at approximately $4 \pm 1^\circ\text{C}$. Samples were transported to the microbiology laboratory of Tri-Chandra Multiple Campus and processed within four hours of collection to preserve sample integrity and minimise microbial changes (Nepal Agricultural Research Council, 2021).

In the laboratory, 25 grams of each sample were homogenised using a sterile mortar and pestle. The homogenate was divided and added to three different enrichment broths: Buffer Peptone Water (BPW) for *Salmonella* spp. enrichment, Selenite F Broth (SFB) for selective *Salmonella* enrichment, and Tryptone Soya Broth (TSB) for *Staphylococcus aureus* enrichment. For *Escherichia coli*, direct plating without prior enrichment was performed alongside enrichment in TSB. All enriched broths were incubated at $35 \pm 2^\circ\text{C}$ for 20-24 hours.

2.3. Isolation and Identification of Bacteria

Following enrichment, standard microbiological techniques were used for isolation and identification. From each positive sample, up to three presumptive colonies per organism were selected to obtain a total of 20 isolates per bacterial species for antibiotic susceptibility testing.

For *Escherichia coli*, a loopful from the enriched broth was streaked onto MacConkey Agar (MA) plates and incubated at 37°C for 24 hours. Pink, lactose-fermenting colonies were considered presumptive for *E. coli*. These isolates were further confirmed through Gram staining and a series of biochemical tests, including Indole, Methyl Red (MR), Voges-Proskauer (VP), Citrate utilisation, Triple Sugar Iron (TSI), Urease, and Oxidative-Fermentative (O/F) tests (Mina et al., 2020).

For *Staphylococcus aureus*, a loopful of enriched broth was streaked onto Mannitol Salt Agar (MSA) and incubated at 37°C for 24 to 48 hours. Yellow colonies indicating mannitol fermentation were considered presumptive. Confirmation was performed through Gram staining, catalase test, coagulase test, oxidase test, and O/F test (Bude & Kidane, 2021).

For *Salmonella* spp., enrichment broth was streaked onto *Salmonella-Shigella* (SS) Agar and incubated at 37°C for 24 hours. Black-centered colonies were considered presumptive. Confirmation involved Gram staining and biochemical tests such as TSI, Urease, Citrate utilisation, IMViC (Indole, Methyl Red, Voges-Proskauer, Citrate), O/F, and motility tests (Tuhin-Al-Ferdous et al., 2013).

2.4. Antibiotic Susceptibility Testing (AST)

Antibiotic susceptibility of the confirmed bacterial isolates was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA). Bacterial suspensions were prepared and adjusted to the 0.5 McFarland turbidity standard. The inoculum was evenly spread on the agar surface using a sterile swab. Commercial antibiotic discs—Ampicillin (AMP 10 µg), Chloramphenicol (C30 µg), Ciprofloxacin (CIP 5 µg), Co-trimoxazole (COT 25 µg), Nalidixic Acid (NA 30 µg), and Cefoxitin (CX 30 µg)—were placed on the inoculated plates. Plates were incubated at 37°C for 18-24 hours. The diameters of the zones of inhibition were measured in millimetres and interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2025).

2.5. Data Extraction and Synthesis

All data obtained from bacterial isolation and antibiotic resistance testing were compiled and analysed. Descriptive statistics, including frequencies and percentages, were calculated to present the prevalence of bacterial contamination and antibiotic resistance patterns for *Salmonella* spp., *E. coli*, and *S. aureus* isolated from the chicken meat samples. The analysis was performed using Microsoft Excel.

3. Results

3.1. Microbiological Analysis of Chicken Meat Samples

A total of 20 raw chicken meat samples were collected from various locations within the Kathmandu Valley, encompassing the districts of Lalitpur, Kathmandu, and Bhaktapur. Microbiological analysis revealed significant contamination with the target foodborne pathogens. A total of 20 isolates each of *E. coli*, *Salmonella* spp., and *S. aureus* were obtained for antibiotic susceptibility testing by selecting multiple colonies per positive sample. The overall findings indicate a concerning level of bacterial presence in the chicken meat supply chain.

3.2. Bacterial Prevalence in Chicken Meat Samples

The contamination rates varied by district and pathogen. A comparative analysis showed that *Escherichia coli* was the most prevalent organism, with the highest isolation rate of 60% in Kathmandu district, followed by 50% in Lalitpur and 40% in Bhaktapur. *Salmonella* spp. were isolated from 40% of samples in Kathmandu and Bhaktapur, and from 30% in Lalitpur. *Staphylococcus aureus* had a consistent prevalence of 20% across all three districts. These distinctions highlight potential variations in hygiene, handling practices, and environmental conditions across the different regions (Thapa et al., 2024; Milk and Meat Safety in Nepal, 2025). The prevalence rates suggest that the chicken meat supply chain in the Kathmandu Valley is subject to significant bacterial contamination, necessitating improved hygiene and overall handling conditions to ensure food safety.

Table 1: Bacterial Prevalence in Chicken Meat Samples across Kathmandu Valley Districts

District	Samples (n)	Salmonella spp. (%)	S. aureus (%)	E. coli (%)
Kathmandu	5	40	20	60
Bhaktapur	5	40	20	40

Lalitpur	10	30	20	50
Total	20	35 (Avg.)	20	50 (Avg.)

These results align with other research findings from Nepal, which indicate high levels of *E. coli* and *S. aureus* contamination in raw chicken meat, with average contamination levels reported at 81.66% and 70%, respectively, in some studies. Similarly, the prevalence of *Salmonella* in chicken meat can reach 40% in local Nepalese markets (Thapa et al., 2024; Milk and Meat Safety in Nepal, 2025). In Bhaktapur, high percentages of *E. coli* (approximately 70%) and *S. aureus* (approximately 75%) have also been reported in other studies (Bacteriological Quality of Poultry Meat, 2018). The persistent prevalence of these pathogens constitutes a critical public health concern, as contaminated poultry remains a major source of human infection (Sun et al., 2021).

3.3. Antibiotic Susceptibility Patterns

Escherichia coli isolates

Antibiotic susceptibility testing for *E. coli* isolates (n=20) revealed varying resistance profiles. Co-trimoxazole and Chloramphenicol were the most effective, with 75% of isolates being sensitive. Ciprofloxacin and Nalidixic Acid showed 70% sensitivity. Ampicillin demonstrated considerable resistance, with only 50% sensitivity, 15% intermediate, and 35% resistant isolates.

Table 2: Antibiotic Susceptibility Pattern of *Escherichia coli* Isolates (n=20)

Antibiotic (Disc Content)	Sensitive n(%)	Intermediate n(%)	Resistant n(%)
Ampicillin (AMP 10 µg)	10 (50)	3 (15)	7 (35)
Ciprofloxacin (CIP 5 µg)	14 (70)	4 (20)	2 (10)
Co-trimoxazole (COT 25 µg)	15 (75)	2 (10)	3 (15)
Nalidixic Acid (NA 30 µg)	14 (70)	0 (0)	6 (30)
Chloramphenicol (C30 µg)	15 (75)	0 (0)	5 (25)

These results indicate that co-trimoxazole, chloramphenicol, ciprofloxacin, and nalidixic acid remain relatively effective against *E. coli*, while ampicillin shows considerable resistance. The varying resistance patterns highlight the need for continuous monitoring and prudent antibiotic use to limit the spread of resistant *E. coli* strains (Kumar et al., 2020; Singh et al., 2022).

Salmonella isolates

For *Salmonella* isolates (n=20), Chloramphenicol was the most effective antibiotic, with 90% sensitivity. Co-trimoxazole and Nalidixic Acid also showed high efficacy, with 85% and 75% sensitivity, respectively. Ciprofloxacin was effective against 65% of isolates, whereas ampicillin was resistant in 50%.

Table 3: Antibiotic Susceptibility Pattern of *Salmonella* Isolates (n=20)

Antibiotic (Disc Content)	Sensitive n(%)	Intermediate n(%)	Resistant n(%)
Nalidixic Acid (NA 30 µg)	15 (75)	2 (10)	3 (15)
Co-trimoxazole (COT 25 µg)	17 (85)	0 (0)	3 (15)
Ciprofloxacin (CIP 5 µg)	13 (65)	6 (30)	1 (5)
Ampicillin (AMP 10 µg)	10 (50)	0 (0)	10 (50)
Chloramphenicol (C30 µg)	18 (90)	0 (0)	2 (10)

These results indicate that chloramphenicol, co-trimoxazole, and nalidixic acid remain effective against *Salmonella*. However, some antibiotics, such as ampicillin and ciprofloxacin, require careful use due to emerging resistance. Monitoring these patterns is important to maintain the effectiveness of antibiotics (Kumar et al., 2020; Singh et al., 2022). The presence of *Salmonella* in chicken meat is a serious public health problem, particularly with studies in Nepal showing high rates on poultry farms and linking it to outbreaks of enteric fever (Fowler et al., 2021).

Staphylococcus aureus isolates

The antibiotic susceptibility profile for *S. aureus* isolates (n=20) indicated that Chloramphenicol was highly effective (90% sensitivity). Co-trimoxazole and Ciprofloxacin showed good efficacy with 80% and 75% sensitivity, respectively. A significant concern was the high level of resistance to Cefoxitin, a surrogate marker for methicillin resistance, with 65% of isolates resistant. Ampicillin also showed lower sensitivity (40%).

Table 4: Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolates (n=20)

Antibiotic (Disc Content)	Sensitive n(%)	Intermediate n(%)	Resistant n(%)
Co-trimoxazole (COT 25 µg)	16 (80)	0 (0)	4 (20)
Ciprofloxacin (CIP 5 µg)	15 (75)	2 (10)	3 (15)
Ampicillin (AMP 10 µg)	8 (40)	6 (30)	6 (30)
Cefoxitin (CX 30 µg)	7 (35)	0 (0)	13 (65)
Chloramphenicol (C30 µg)	18 (90)	0 (0)	2 (10)

These results indicate that chloramphenicol, co-trimoxazole, and ciprofloxacin remain effective for treating *S. aureus* infections. However, the high proportion of cefoxitin-resistant isolates and the resistance to ampicillin highlight the need for ongoing monitoring, particularly for methicillin-resistant *Staphylococcus aureus* (MRSA) (Kumar et al., 2020; Singh et al., 2022). *S. aureus* is known for producing heat-stable enterotoxins that cause food poisoning, and its presence in chicken meat, often due to poor slaughter and processing hygiene, poses a major public health risk (Neupane et al., 2023; Yaddi et al., 2023).

3.4. Multidrug Resistance (MDR) and Methicillin-Resistant *Staphylococcus aureus* (MRSA)

The study found notable rates of multidrug resistance among the isolates. For *Salmonella*, 15% (3 of 20 isolates) were MDR. For *E. coli*, 20% (4 of 20 isolates) were MDR. The highest MDR prevalence was observed in *S. aureus*, with 35% (7 of 20 isolates) showing resistance to multiple antibiotic classes.

Table 5: Prevalence of Multidrug-Resistant (MDR) Isolates

Bacteria	Total Isolates	MDR Isolates (n)	MDR Prevalence (%)
<i>Salmonella spp.</i>	20	3	15
<i>Escherichia coli</i>	20	4	20
<i>Staphylococcus aureus</i>	20	7	35

Most alarmingly, testing against cefoxitin revealed a high prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA). Out of 20 *S. aureus* isolates, 13 (65%) were resistant to cefoxitin, indicating MRSA. Only 7 (35%) isolates were sensitive.

Table 6: Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Total <i>S. aureus</i> isolates	MRSA Strains n(%)
20	13 (65%)

This indicates that more than half of *S. aureus* isolates from poultry meat samples were methicillin-resistant, posing a substantial risk to public health and underscoring the critical need for stringent regulation of antibiotic use and enhanced hygiene throughout the poultry supply chain.

Several limitations of this study should be acknowledged. First, the sample size (n=20) is relatively small and may not be fully representative of the entire Kathmandu Valley chicken meat supply chain. The findings should therefore be interpreted as preliminary, and larger-scale studies are needed to confirm these trends. Second, while cefoxitin disc diffusion is a reliable surrogate for detecting MRSA according to CLSI guidelines, confirmatory testing such as *mecA* gene PCR or oxacillin MIC determination was not performed. Therefore, the 65% MRSA prevalence reported here is presumptive and should be confirmed using molecular methods in future studies. Third, the study did not collect detailed data on antibiotic usage patterns on the farms from which the chickens originated, limiting our ability to directly link resistance patterns to specific agricultural practices. Fourth, sampling was limited to retail markets in three districts and did not include farms, slaughterhouses, or processing plants, where contamination may originate. Future studies should adopt a longitudinal, multi-site approach including molecular characterization of resistance genes to better understand the epidemiology of antimicrobial resistance in Nepal's poultry sector.

4. Conclusion

The chicken meat in the Kathmandu Valley is a significant source of foodborne pathogens, as they were detected at alarmingly high levels across all three survey areas. *E. coli* had the highest contamination (40 to 60 per cent), and second was *Salmonella* spp. (30-40 per cent) and *S. aureus* (20 per cent). The evidence indicates that, at times during the slaughter, processing, and retail stages of food handling, ongoing hygiene failures allow pathogenic bacteria to persist in the local food supply.

The results of antibiotic susceptibility testing indicated that although nalidixic acid, chloramphenicol, and ciprofloxacin were relatively effective, there was significant resistance to ampicillin, particularly among *E. coli* and *S. aureus*. Antimicrobial resistance in *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), is reflected in its prevalence, underscoring the importance of controlling antimicrobial resistance in the poultry industry.

The findings raise a serious public health concern: the presence of bacterial contamination and increased antibiotic resistance in a widely consumed food. There should be heightened hygienic standards within the poultry chain, prudent use of antibiotics (most are used in moderation), and implementation of microbiological surveillance to safeguard consumers. The contamination of meat with drug-resistant pathogens poses a serious threat to food safety and the success of therapeutic procedures in Nepal unless stringent measures are implemented.

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