PREVALENCE AND ANTIBIOGRAM OF *Salmonella* ISOLATED FROM BROILER MEAT OF RETAIL MEAT SHOPS IN RUPANDEHI, NEPAL

ABSTRACT

Salmonella is recognized as a significant foodborne pathogen responsible for causing severe infection. It is one of the main causes of huge economic losses due to mortality and decreased production in poultry sector throughout world. This study was conducted to determine the prevalence and antibiogram of *Salmonella* isolated from retail broiler meat. A cross-sectional study was done from August to September 2023 with a total of 152 samples (106 muscle and 46 liver) from different retail meat shops of Siddharthanagar municipality, Rupandehi and transported to Veterinary Medicine Lab, Paklihawa Campus for further analysis according to standard culture-based methods. Antibiogram of isolated *Salmonella* was evaluated against five different groups of antibiotics by disc diffusion method following CLSI guidelines. Data was analysed in SPSS using Chi-Square test at confidence level of 95%. The overall prevalence of Salmonella was recorded to be 18.42% whereby prevalence from liver sample was 19.57% and muscle sample was 17.92%. Statistical analysis showed no significant difference in prevalence among the sample types. The antibiogram study revealed that none of the antibiotics showed 100% effectiveness. The most resistance was seen with Ciprofloxacin (89.28%) followed by Ceftriaxone (85.71%), Tetracycline (82.14%), Chloramphenicol (57.14%), and Amikacin (53.57%). 26 out of 28 isolates (92.86%) were found to be multidrug-resistant (≥3 antibiotic groups). The study revealed a higher prevalence of *Salmonella* in the retail market and indicates there is a chance for people to be infected with *Salmonella* through poultry meat. Regular

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surveillance on antibiotic resistance and justifiable use of antibiotics in the commercial poultry industry is highly recommended.

Keywords: Antibiogram, Antibiotic Resistance, Prevalence, *Salmonella*, Broiler~~,~~

1. INTRODUCTION

The poultry industry in Nepal has been rapidly expanding in the last decade to meet the increased demand for poultry meat, with an annual growth rate between 17% and 18% and a 261% increase in meat production from 2008 to 2018 (FAO,2020 and Fowler, 2021). In Nepal, poultry farming is important for both economic growth and food security. However, this growth has been met with many challenges, such as bacterial, viral, and protozoal diseases, and ~~a reduction in the effectiveness of medications for the treatment of these diseases~~increased antibiotic resistance by bacterial pathogens, which eventually increases the cost of production of poultry products by the ~~cost of~~ farmers (Gompo, 2019). High bacterial load in poultry meat and their antimicrobial resistance have led to potential health issues (Neupane and Kaphle, 2019). Antimicrobial resistance in bacteria, including *Salmonella* spp. is considered a major food safety concern and the spread of resistant strains along the food chain is to be handled for both veterinary medicine and public health (Telli *et al*.,2022).

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Salmonella is a rod-shaped, flagellated, gram-negative facultative anaerobe which infects multiple animal hosts including human by contaminating a wide variety of foods (Wang *et al.*, 2020). There is an increased risk of bacterial infection like *Salmonella* in poultry with increasing number of ~~commercial~~ unorganized poultry farms, which increases the risk of zoonosis and risk of antibiotic resistance due to overuse of antibiotics as growth enhancers. Salmonellosis is a pathogenic bacterial zoonosis causing substantial public health impacts. Non-typhoidal *Salmonella* (NTS) is responsible for food-borne diarrheal disease while invasive NTS causes major blood stream infection (Sanni *et al.,* 2023). Contaminated meats, mainly from avian origins are the prospective source of human salmonellosis therefore the most important source of meat-borne public health hazard (Buncic *et al.,* 2014). *Sallmonella enterica* subsp. enterica (I) is responsible for major zoonotic diseases.

Tetracyclines, Aminoglycosides and Fluoroquinolones are the most used antibiotic classes among poultry farmers (Subedi *et al.,* 2023). According to Acharya *et al.* (2023) livestock,

particularly poultry sectors are supposed to have the highest burden of antimicrobial resistance. Among six subspecies of *Salmonella enterica*, Typhimurium and Enteritidis demonstrated high virulence and resistance to multiple antibiotics (Alegria-Moran *et al.*, 2017). Antibiotic resistance is a significant public health challenge. In the poultry industry, antibiotics are commonly employed both for treating and preventing (prophylactic use) Salmonella infections. However, *Salmonella* species have been demonstrated resistance to quinolones, nalidixic acid, and their derivatives, including fluoroquinolones. (Yasmin *et al*.,2020)

There are a significant number of commercial poultry farms in the Rupandehi district, but very scanty research has been performed, and no adequate baseline information is available till now. The data obtained from present study will help to alert the veterinarians, ~~and related authorities, including~~ the human health sector, to strengthen one health approach. Furthermore, It is assumed that the AMR problem will cause hundreds of human deaths along with a severe financial crisis and severe damage to livestock production by 2050 (Reference). Thus, this study is intended to provide insights into the prevalence and antibiotic resistance of *Salmonella* in commercial poultry meat of Rupandehi, Nepal. ~~This will ultimately lead to avoiding the inappropriate use of antimicrobials.~~

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3. METHODOLOGY

3.1 Study site

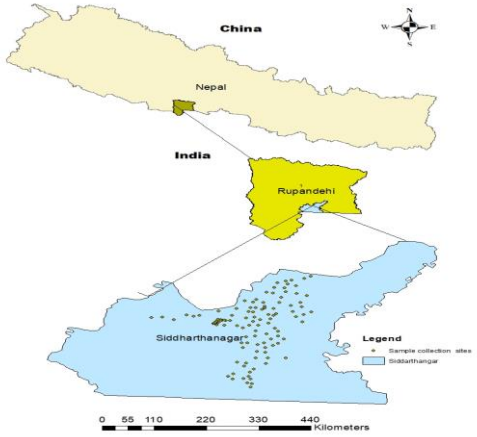


Fig1. Map of Nepal Showing the study site.

The current study was conducted in Siddharthanagar municipality of Rupandehi, Nepal. Samples were collected from retail broiler meat shops. The collected samples were transported to the Medicine lab, Institute of Agricultura and Animal Science, Paklihawa Campus, Rupandehi.

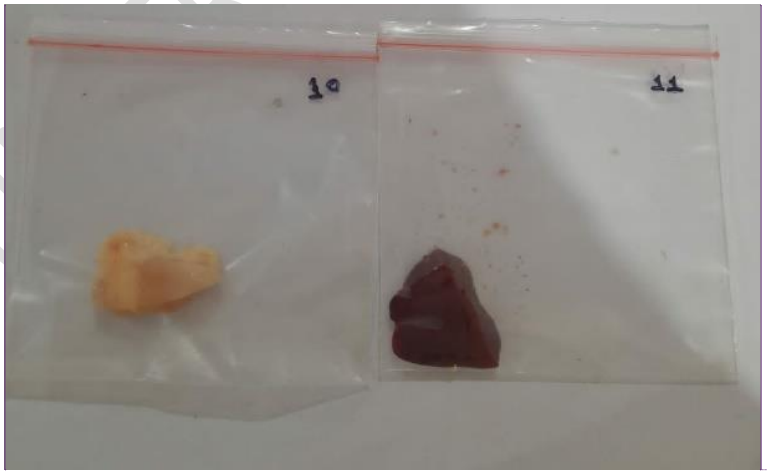
3.2 Study population and sample size

Purposive sampling was conducted in retail broiler meat shops of Siddharthanagar municipality. The sample size for the prevalence of *Salmonella* was calculated using Epi-tools epidemiological calculators by Ausvet. The calculated sample size was with expected precision of 5% and 95% confidence level. Total number of samples collected was 152.

3.5 Sample collection and processing

Collection of samples

For culture, a sample of the liver and muscle of the broiler was taken aseptically in a zip lock bag from different retail meat shops. ~~During the collection process, a cool box and markers were used.~~



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If data available, provide the data.
Because, unorganized unhygienic meat shops are contaminated with many microbes, in comparison to the well organized sector.

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For microbiological study, it should be under sterile conditions.

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Fig 2. Sample collection in Zip-lock bag

Enrichment and selective culturing techniques

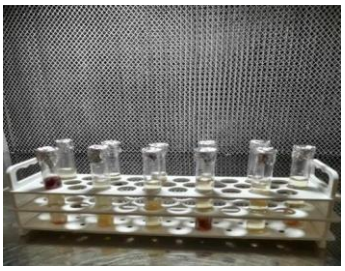

Fig.3**.** Pre-enrichment in Buffer Peptone Water.

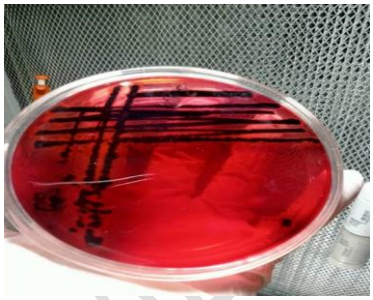

Fig.4. *Salmonella* in XLD agar

All samples were treated aseptically. One gram of minced liver and muscle were ~~individually~~ seperately inoculated in a test tube with 10 ml buffer peptone water(BPW)~~. Inoculated samples were~~ and ~~-~~incubated at 37^0c for 20-24 hrs. ~~1000µl of incubated BPW was transported to 10 ml of Rappaport Vasiliadis with the aid of a micropipette and then~~ A Loopful of the enriched BPW broth was inoculated into Rappaport Vasiliadis broth and incubated at 37^0c for ~~16-20 hrs~~overnight. Selective media XLD was used as the selective media for the isolation of the *Salmonella*. A loopful of ~~the pathogen was taken~~culture from R10 broth ~~with the help of a sterilized inoculating loop and~~ was streaked on the XLD agar and ~~. The streaked plate was~~ incubated at 37~~±1~~^{0}c for 20-24 hrs.

Biochemical tests

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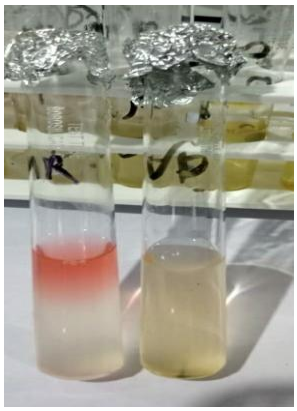

Fig. 5 MR-VP test (+)

Fig. 6 Oxidase test (-)

Fig. 7 Catalase test

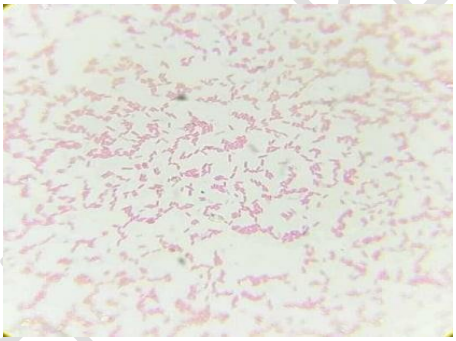

Fig.8 Gram's stain (-Ve rod)

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To confirm suspected *Salmonella* species, a series of biochemical tests were conducted. The primary tests included Gram staining, catalase, and oxidase tests. Gram staining revealed Gram-negative bacteria, catalase testing showed effervescence with 5% hydrogen peroxide, and oxidase testing indicated a positive result with a purple color change on the oxidase disc. Secondary tests for colonies passing the primary tests included the Simmons Citrate test, which indicated citrate utilization with a blue color change, and the Triple Sugar Iron (TSI) test, assessing fermentation and hydrogen sulfide (H_2S) production through characteristic color changes. The Sulphur Indole Motility (SIM) test evaluated motility, indole production, and H2S

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generation, indicated by black precipitate and turbidity. The Methyl Red (MR) test assessed acid production with a red color, indicating a positive result, while the Voges-Proskauer (VP) test indicated butanediol fermentation with no colour change, signifying a negative outcome.

Antibiotic Sensitivity test (AST)

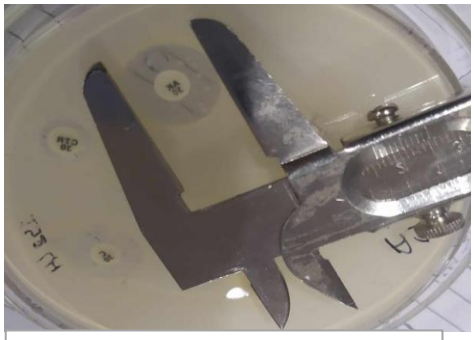

Fig.9 Measurement of zone of inhibition using Vernier calliper scale

Fig.10. Carpet culture in Mueller Hinton Agar using sterile cotton swab

After a biochemical test, 5-6 pure, well-isolated colonies were transferred into a test tube containing 3ml of buffer peptone water from the confirmed culture of Salmonella. Initially, BPW density was measured using a McFarland Densitometer before ~~injection~~ inocularion and then again after inoculation until the turbidity equivalent to a 0.5 McFarland standard was ~~maintained~~obtained. If the turbidity is high, extra buffer peptone is added, whereas if the turbidity is low, more colonies are added.

Fig.11 Zone of inhibition *Salmonella* ~~showing antibiotics~~against selected antibiotic discs

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A sterile non-toxic cotton swab was dipped into the adjustable suspension and spread over the entire agar surface of the Mueller Hinton Agar (MHA) plate four times, turning the plate at a 60^0 angle between each streaking. After that antibiotic discs were placed in the inoculated MHA with the help of an antibiotic dispenser. The antibiotics tested, along with their concentrations, were Ceftriaxone (30 mcg), Chloramphenicol (30 mcg), Tetracycline (30 mcg), Ciprofloxacin (5 mcg), and Amikacin (30 mcg). The plates were incubated at 37^0c for 24 hours after which the zone of inhibition was measured with vernier caliper scale. Using an interpretation chart, according to the zone size of each antimicrobial reporting the organism was interpreted as 'Resistant', 'Intermediate', and 'Sensitive'.

3.6 Statistical analysis

The entry of data and graphical representation was done using Microsoft Office Excel 2019. The statistical association was tested by Chi-square analysis using SPSS version 25 with a significant level defined at $p<0.05$.

RESULT

The prevalence of *Salmonella* was 19.57% in 46 liver samples and 17.92% in 106 muscle samples. Thus, 28 samples (18.42 %) out of the total 152 samples taken were found positive for *Salmonella sps*.

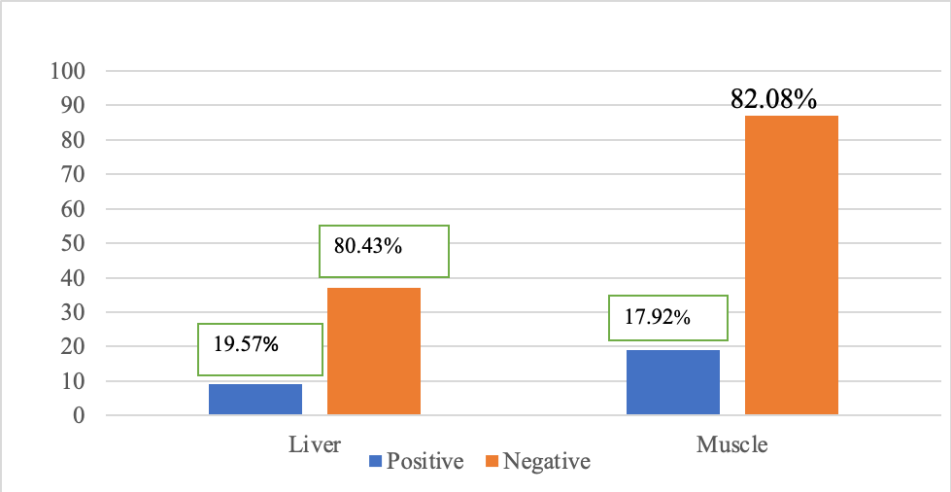

Fig.12. Overall Prevalence of *Salmonella*~~with sample type~~ in muscle and Liver -

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Table 1. Statistical association of *Salmonella* with sample type

Sample type	Number of sample (X)	Number of positive (Y)	Prevalence (Y/X)	Odds ratio	*p*- value	Association
Liver	46	9	19.57%	0.89 (0.37-2.16)	0.82	Statistically non-significant (p>0.05)
Muscle	106	19	17.92%			

The result showed that there is no significant different in prevalence of *Salmonella* with type of sample examined.

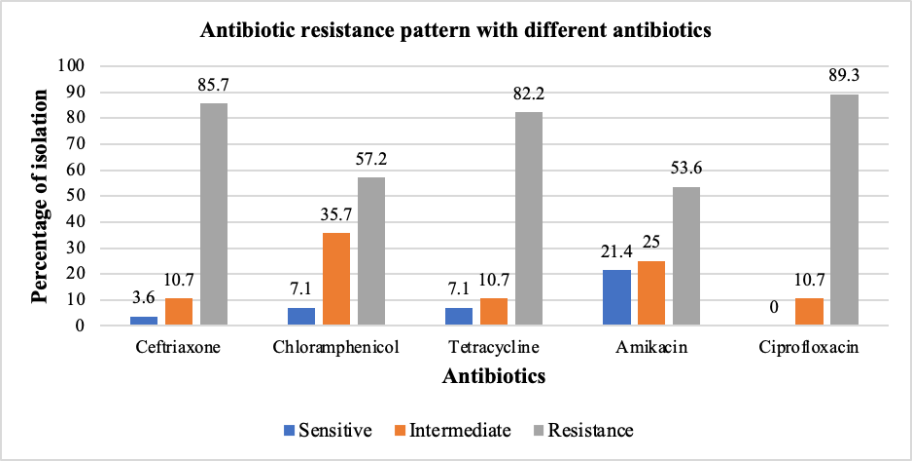

Figure 13. Antibiotic resistance pattern with different antibiotics used

The most resistance was seen with Ciprofloxacin (89.28%) followed by Ceftriaxone (85.71%), Tetracycline (82.14%), Chloramphenicol (57.14%), and Amikacin (53.57%) according to our research.

DISCUSSION:

The prevalence is pretty comparable to the study of Baral *et al.* (2023) and Fowler (2021). Higher prevalence was observed by Bhandari *et al.* (2013), Lamichanne (2018), Bantawa *et al.* (2018), and Mahato (2019), with prevalence of 46.2%, 25%, 60%, and 40%, respectively. The finding obtained was higher than the research conducted by Dhakal and Manandhar (2005) and Maharjan *et al.* (2006), whose findings were 12% and 14.5%, respectively.

Out of 5 antibiotics tested, none of the antibiotics showed 100% effectiveness. ~~Self-prescription, overprescription and irrational prescription of~~Indiscriminate use of antibiotics intended for rapid cure may trigger the development of resistant strains of bacteria, thus reducing the efficacy of antibiotics. Amikacin showed a maximum sensitivity of 42.86% while none of any isolates showed sensitivity with Ciprofloxacin. A similar result was found in the study of Poudel (2021). High percentage of isolates were resistant towards Tetracycline which is similar to the study of Lamichanne (2018), Ellerbroek *et al.* (2010), and Dhakal *et al.* (2016). 57.14% of *Salmonella*

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isolates were resistant to Chloramphenicol, which is comparatively lower than 87.50% (R. Selvaraj *et al.*, 2010) but higher than 7.69% (Dhakal *et al.*, 2016).

Table 2. Multidrug resistance profile of isolated *Salmonella*

Number of antibiotics group resistant	Number of isolates resistant	Percentage (%) of isolates resistant	MDR
1	0	0	-
2	2	7.14%	-
3	13	46.43%	+
4	4	14.29%	+
5	9	32.14%	+

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26 out of 28 isolates (92.86%) were found to be MDR positive (resistant to ≥ 3 antibiotic groups).

Previous study by Crump *et al.* (2023) showed 80% of non-typhoidal *Salmonella* isolates to be multidrug resistant. But lower percentage of MDR (45.16%) was found in the study of Adhikari *et al.* (2023). ~~Misuse and overuse~~Indiscriminate use of antibiotics for preventive, metaphylactic, therapeutic and as growth promoters might be the main causative factor for increased MDR (Subedi *et al.*, 2023).

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WHAT IS YOUR CONCLUSION?

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