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EXTENDED SPECTRUM BETA LACTAMASES (ESBL) PRODUCING *SALMONELLA* ISOLATED FROM RAW GOAT MEAT AND WATER FROM SLAUGHTERHOUSES IN CHITWAN, NEPAL

Bibek PUDASAINI[1], Parisha THAPA[2*], Amar Nath CHAUDHARY[3], Supriya SHARMA[4]

[1]Agriculture and Forestry University (AFU), Rampur, Nepal
[2]Department of Livestock Production and Management, AFU, Rampur
[3]Department of Anatomy, Physiology and Biochemistry, AFU, Rampur
[4]Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal
***Corresponding Author's Email**: parishathapa@afu.edu.np

ABSTRACT

Salmonella contamination in raw meat and water used in slaughterhouses poses a significant health risk, particularly with strains producing Extended Spectrum Beta-Lactamases (ESBL). This study aims to determine the prevalence of *Salmonella* and its ESBL activity in raw goat meat and water used in slaughterhouses, examining risk factors associated with contamination. In this cross-sectional study conducted between November 2022 and January 2023, a total of 50 raw goat meat and 50 water samples were collected from slaughterhouses in Bharatpur Metropolitan city, Nepal. Microbiological and biochemical techniques were used to identify *Salmonella* isolates, and ESBL production was confirmed by combined disc test. Risk factors, including slaughterhouse type, water quality, and cleaning practices, were analyzed using chi-square tests. *Salmonella* was isolated from 8% of meat and 44% of water samples. Among Salmonella-positive samples, ESBL activity was observed in 50% of meat and 40.9% of water samples. Significant associations were found between Salmonella presence and risk factors such as open slaughterhouse type, direct cash transactions, use of non-potable water, and lack of regular cleaning ($p < 0.05$ for each).The findings underscore the need for improved sanitation, regular cleaning, and water quality management in slaughterhouses. Implementing these measures and enhancing public awareness can mitigate *Salmonella* contamination risks and reduce the spread of antimicrobial resistance. Regulatory oversight and best practice guidelines should be developed and enforced in Nepal's slaughterhouses to protect public health and address growing antimicrobial resistance challenges.

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INTRODUCTION

Raw meat, particularly goat meat, is a critical source of protein and other nutrients essential for human health. It is rich in proteins, essential fatty acids, vitamins, and minerals, making it a staple in many diets worldwide (Pereira and Vicente, 2013). However, raw meat is highly perishable due to its susceptibility to contamination by microorganisms, including pathogens like *Salmonella.* The conditions under which animals are slaughtered play a crucial role in the microbial load of meat; poor hygiene during slaughtering and improper handling can lead to significant contamination (Klaharn et al., 2022*)*. In developing countries like Nepal, the absence of modern slaughterhouses and regulated processing facilities often results in the contamination of meat with pathogenic microorganisms. Many butcher shops lack proper sanitary practices, leading to cross-contamination through tools, water, and hands (Ovuru et al., 2024). *Salmonella* is a major foodborne pathogen that causes gastrointestinal illnesses worldwide. It is a Gram-negative bacterium that can infect both humans and animals, often through contaminated food products (Lamichhane et al., 2024). The most common strains involved in foodborne outbreaks are *S. enterica* serovars Typhimurium and Enteritidis (Vencia et al., 2015). The muscles of healthy animals are usually free of pathogens; however, contamination occurs during slaughter due to contact with animal hides, gastrointestinal contents, and environmental sources (Heredia and García, 2018). This contamination can extend to the post-harvest stage during transportation and retail sale, posing a significant risk to public health (Lenzi, Marvasi and Baldi, 2021). A major concern is the increasing prevalence of antimicrobial-resistant strains of *Salmonella*, particularly those that exhibit Extended Spectrum Beta-Lactamase (ESBL) activity (Pulingam et al., 2022). ESBLs are enzymes that confer resistance to a broad spectrum of beta-lactam antibiotics, including third-generation cephalosporins (Castanheira, Simner and Bradford, 2021). This resistance limits treatment options for severe *Salmonella* infections, especially in vulnerable populations such as children and immunocompromised individuals (Kariuki et al., 2015). The improper and extensive use of antibiotics in animal agriculture is a significant factor contributing to the emergence of these resistant strains (Singh, Bhat and Ravi, 2024). This study aims to assess the prevalence of *Salmonella* and its ESBL activity in raw goat meat in Bharatpur, Nepal. The findings will contribute to understanding the contamination sources and provide insight into the public health risks associated with consuming contaminated meat.

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MATERIALS AND METHODS

Study design: This was a cross-sectional study conducted from November 2022 to January 2023 in Bharatpur Metropolitan City, Chitwan, Nepal. Chitwan, a prominent agricultural region in Nepal, is known for its substantial meat production, particularly goat and poultry, driven by a growing demand for animal protein within local and regional markets.

Study population: Samples were collected from slaughterhouses/butcher shops in 10 wards of Bharatpur metropolitan city based on convenient sampling. These shops included both open and closed premises to account for different levels of exposure to environmental contamination.

Sample size: A total of 50 samples of raw goat meat including thigh & breast meat, part of liver and heart and another 50 water samples used in slaughterhouse for different purposes like dressing, washing, cleaning etc of meats, slaughter slab, knife, chop board etc. were included in the study.

Sample collection and transportation: Raw meat samples were collected aseptically, and stored in sterile containers. Water samples, used for cleaning tools and meat, were also collected in sterile containers. All samples were transported to the Microbiology and Parasitology Laboratory, Agriculture and Forestry University (AFU), within four hours, maintaining proper cold chain conditions.

Laboratory Methods

Pre-enrichment: In the laboratory, 10 ml water samples were added to 90 ml of Selenite F broth and properly mixed. Similarly, 25 gm of meat samples were weighed, properly minced with sterile blades, and added to 225 ml of Selenite F broth. The samples were then kept at 37°C overnight under aerobic incubation.

Isolation and identification: After enrichment, the samples were inoculated into Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 hours. Identification of *Salmonella* was based on the observation of colony characteristics, Gram's staining and biochemical tests, including catalase, oxidase, indole, motility, Methyl Red Voges Proskauer, Triple Sugar Iron Agar, citrate utilization and urease production test (Makwana et al., 2015).

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Antibiotic susceptibility testing (AST): Each isolate was subjected to AST by following CLSI (Clinical Laboratory Standard Institute) guidelines. Briefly, the pure culture of isolate was inoculated into nutrient broth, the turbidity of which was matched to that of 0.5 Mc Farland Standard. The suspension was then swabbed into Mueller Hinton Agar (MHA) and antibiotic discs were placed onto the swabbed agar surface. After incubation at 37°C for 24 hours, the zone of inhibition around each disc was measured and reported as susceptible, intermediate or resistant.

Detection of ESBL production: The screening test for the production of ESBL producing isolates was performed by measuring the diameters of zone of inhibition around Ceftazidime (30µg) and Ceftriaxone (30 µg)/Cefotaxime (30µg) on MHA media by disc diffusion method following CLSI guidelines. The suspected ESBL producing isolates i.e. screen test positive isolates were subjected to phenotypic confirmation by combination disc method (CLSI, 2020).

Data analysis: Data were analyzed using SPSS software (version 25). Chi-square test determined associations between contamination and risk factors, with statistical significance at $p < 0.05$.

RESULTS AND DISCUSSION

Out of 100 samples, *Salmonella* was isolated from 26 samples. The prevalence of *Salmonella* among the meat samples was 8% (4/50) and that among the water samples was 44% (22/50). Out of 26 isolates, 11 were ESBL producers (Table 1).

Table 1. Isolation of Salmonella from different samples from slaughter houses (n=100)

Source of samples	No. of samples	No. (%) of samples positive for *Salmonella*	ESBL production	
			Positive No. (%)	Negative No. (%)
Meat	50	4 (8%)	2 (50%)	2 (50%)
Water	50	22 (44%)	9 (40.91%)	13 (59.09)

Salmonella was isolated from 8% of meat samples only, 44% of water samples only and 4% of both meat and water samples. Open slaughterhouses, direct cash transactions, non-potable water, lack of regular cleaning, use of shared utensils, and improper evisceration practices were each significantly correlated with increased Salmonella contamination rates (Table 2).

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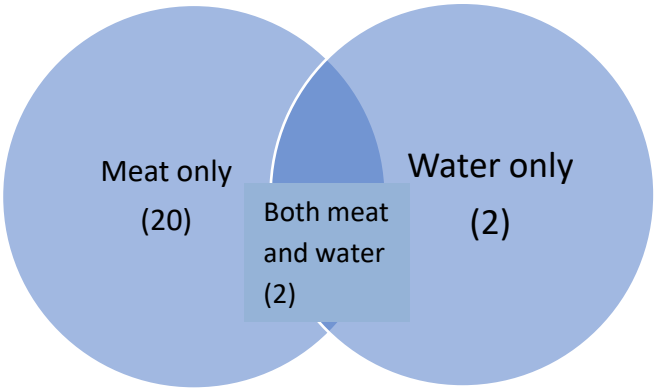

Figure 1. Venn diagram showing the number of samples positive for *Salmonella*

The high prevalence of *Salmonella* contamination observed in water samples (44%) and moderate contamination in meat samples (8%) from slaughterhouses in this study aligns with similar findings from regions with limited sanitation infrastructure. For instance, a study from India found comparable contamination rates in water sources used in slaughterhouses, highlighting the role of untreated water in spreading foodborne pathogens (Makwana et al., 2015). Studies in Nepal and India further underscore that open slaughterhouses are at elevated risk for contamination, as these environments are more exposed to environmental contaminants due to insufficient physical barriers and limited regulatory oversight (Klaharn et al., 2022; Lamichhane et al., 2024).The contamination rates here also reflect findings from lower- and middle-income countries, such as Thailand, where inadequate sanitation practices increase bacterial contamination risks in slaughterhouse environments (Klaharn et al., 2022).

Notably, our study found that non-potable water use was significantly associated with Salmonella presence, a factor similarly highlighted by Ovuru et al. in developing countries, where contaminated water is a major contributor to microbial load (Ovuru et al., 2024).Comparatively, developed countries with stricter hygiene protocols and potable water use report lower contamination rates in meat-processing facilities (Heredia and García, 2018), which suggests that enforcing potable water use and implementing sanitation controls could drastically reduce Salmonella contamination in Nepal's slaughterhouses.

The prevalence of ESBL activity (50% in meat and 40.9% in water samples) among Salmonella isolates is concerning, especially given that ESBL-producing bacteria pose

a significant public health risk due to their resistance to beta-lactam antibiotics. These findings are similar to studies from India and Bangladesh, where high levels of ESBL activity among foodborne pathogens were attributed to the widespread, unregulated use of antibiotics in livestock production (Pulingam et al.,2022; Singh, Bhat and Ravi,2024). However, this prevalence contrasts with lower ESBL rates observed in developed nations, such as the European Union, where stricter antibiotic use policies have effectively reduced antimicrobial resistance among foodborne pathogens (Castanheira, Simner and Bradford, 2021). The disparity highlights the critical need for regulatory interventions in Nepal to monitor and control antibiotic use in agriculture to limit the spread of resistant strains.

Table 2. Risk factors associated with isolation of *Salmonella* from slaughter houses

Type of slaughter house	**Total number**	**Culture positive for *Salmonella* No. (%)**	**p-values**
Open	30	22 (73.33)	0.0007
Closed	20	4 (20)	
Direct connection of cash between seller and customer			
Yes	30	22 (73.33)	0.0007
No	20	4 (20)	
Water used in slaughterhouses			
Potable	15	1 (6.67)	0.0002
Non-potable	35	24 (68.57)	
Regular cleaning of slaughterhouses			
Yes	15	1 (6.67)	0.0002
No	35	24 (68.57)	
Use of common utensils or knife to cut different species of meat at a time			
Yes	40	25 (62.50)	0.0088
No	10	1 (10.00)	
Follow instruction while evisceration of carcass to reduce cross contamination			
Yes	10	1 (10.00)	0.0088
No	40	25 (62.50)	

Operational practices were also significantly associated with contamination levels in this study. For instance, using common utensils across different meat types and inadequate evisceration practices both increased contamination risks, echoing findings from South Asia and Africa where cross-contamination from shared utensils is prevalent due to limited resources (Makwana et al., 2015). In contrast, developed countries with stringent food safety regulations typically report lower contamination

rates, as proper cleaning and separation protocols are followed to prevent cross-contamination (Lenzi, Marvasi and Baldi, 2021). These operational practices indicate that simple yet critical interventions, such as separate utensils for different species and strict adherence to evisceration guidelines, could lower contamination rates significantly in Nepal's slaughterhouses.

CONCLUSION

The findings underscore the need for improved sanitation, regular cleaning, and water quality management in slaughterhouses. Implementing these measures and enhancing public awareness can mitigate *Salmonella* contamination risks and reduce the spread of antimicrobial resistance. Regulatory oversight and best practice guidelines should be developed and enforced in Nepal's slaughterhouses to protect public health and address growing antimicrobial resistance challenges.

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