

## Prevalence of Multidrug-Resistant and Biofilm-Forming *Salmonella enterica* in Beef from Peshawar

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### Abstract

*Salmonella enterica* is a major public health concern and is frequently linked to beef contamination, particularly in developing countries where the emergence of multidrug-resistant (MDR) strains has increased health risks associated with the consumption of contaminated meat. This study aimed to determine the prevalence, seasonal distribution, antimicrobial resistance patterns, and biofilm-forming ability of *S. enterica* isolated from different points of the beef supply chain in Peshawar, Pakistan. A total of 250 beef samples were collected from retail markets across 23 locations between February 2021 and February 2023. Isolates were identified using standard biochemical tests, and antimicrobial susceptibility was evaluated against 16 antibiotics using the Kirby-Bauer disk diffusion method. Biofilm-forming ability was assessed using a quantitative microtiter plate assay. The overall prevalence of *S. enterica* was 68.0% (170/250), and 150 isolates were further subjected to molecular analysis. A clear seasonal pattern was observed, with the highest prevalence in summer (85.7% in August) and the lowest in winter (43.8% in January), showing a strong positive correlation with temperature ( $r=0.89$ ,  $p<0.001$ ). Spatial analysis showed higher odds of contamination near slaughterhouses (OR=2.21, 95% CI: 1.15-4.23) and in urban centers (OR=2.41, 95% CI: 1.38-4.22) compared to rural areas. High resistance levels were detected against azithromycin (87.3%), tetracycline (61.3%), and streptomycin (48.0%). The MDR phenotype was observed in 56.7% of isolates with a mean MAR index of 0.342. Biofilm formation was detected in 87.3% isolates, and strong producers exhibited a higher prevalence of MDR.

**Keywords:** Antimicrobial resistance, beef contamination, biofilm formation, food safety, *Salmonella enterica*

### Introduction

*Salmonella enterica* is one of the most important foodborne bacterial pathogens in the world. It is a facultative anaerobic, Gram-negative rod-shaped bacteria that belonging to the Enterobacteriaceae family.<sup>1</sup> The global burden of non-typhoidal *Salmonella* infections is estimated at approximately 94 million cases annually, resulting in over 150,000 deaths.<sup>2</sup> The pathogen is commonly transmitted through contaminated food products, particularly meat and poultry, due to its natural presence in the intestinal tract of animals.<sup>3</sup> Most human infections are caused by a limited number of non-typhoidal serovars (Typhimurium, Enteritidis and Newport) which are frequently found in food-producing animals such as poultry, cattle, and pigs.<sup>4,5</sup>

Contamination of meat products occurs at various stages of the food supply chain. In many developing countries, inadequate food safety systems, weak regulatory enforcement, and inconsistent hygiene control during slaughtering and retail handling increase the likelihood of contamination. Factors such as poor animal husbandry, unsanitary slaughterhouse conditions, and disruptions in the cold chain further exacerbate this risk.<sup>5</sup> Contamination may originate from the pre-harvest sources, such as, contaminated feed, water, transportation environment, or from post-harvest stages including processing, storage, and distribution processes.<sup>6</sup> In addition, improper consumer practices, such as mishandling refrigeration practices and extended exposure of the raw meat protein to ambient temperatures may further contribute to the risk of bacterial proliferation. Although proper cooking is an effective way to eliminate *Salmonella*, raw meat remains an important reservoir for the pathogen and can lead to cross-contamination during the

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preparation of food-products.<sup>4,7</sup>

Pakistan is a major meat-producing country with steadily increasing consumption. However, food safety monitoring and hygienic control in the meat supply chain is still variable in many areas.<sup>1</sup> Several studies conducted in Pakistan have reported pathogenic bacteria contamination (*Salmonella*) in meat and poultry products, indicating any lack of sanitation and quality control in the slaughterhouses and retail markets.<sup>8</sup> International food safety authorities such as the Codex Alimentarius Commission, recognize *Salmonella* to be an important hazard in meat production systems. Despite this recognition, data on the distribution, prevalence, and antimicrobial resistance pattern of *Salmonella* in meat supply chains in Pakistan is still lacking since it remains limited on the basis of comprehensive data.<sup>9</sup>

The public health impact of *S. enterica* is further exacerbated by its ability to develop antimicrobial resistance and form biofilms. Biofilms are structured microbial communities that enhance bacterial survival under adverse conditions and reduce susceptibility to antimicrobial agents.<sup>10,11</sup> In environments with inadequate sanitation, the formation of biofilm increases the persistence of the pathogen in meat processing environments and the susceptibility of retail products to contamination. Concurrently, antimicrobial resistance in *Salmonella* has emerged as a major global health concern. The genus contains more than 2,500 serovars, of which *S. enterica*, is the most clinically relevant strains that cause infections in humans. Resistance mechanisms can occur because of chromosomal mutation or via the acquisition of resistance genes carried on mobile genetic elements such as plasmids.<sup>12</sup> The emergence of multidrug-resistant *Salmonella* strains complicates treatment and contributes to increased morbidity, mortality and healthcare costs.<sup>13</sup>

Despite reports of *Salmonella* contamination in retail meat in several developing countries, detailed data on prevalence, AMR, and biofilm-forming capabilities within the beef supply chain of Peshawar remain scarce. Peshawar, a major city in Khyber Pakhtunkhwa province, contains numerous slaughterhouses and retail outlets with varying hygienic conditions and regulatory oversight. Therefore, this study aimed to determine the prevalence, antimicrobial resistance patterns, and biofilm-forming ability of *Salmonella enterica* isolated from beef supply chains in Peshawar, Pakistan.

## Methods

This laboratory-based cross-sectional study was conducted to isolate and characterize *Salmonella enterica* from retail beef samples in Peshawar, Pakistan. The investigation focused on determining pathogen prevalence, antimicrobial susceptibility patterns, and biofilm forming ability. Meat samples were collected from various retail stores and slaughterhouse-associated markets across Peshawar, which were operationally categorized as urban, peri-urban, or rural based on population density and market settings.

Prior to sampling, verbal permission was obtained from shop owners, and data confidentiality was strictly assured. A total of 250 beef samples were collected aseptically from randomly 200 selected meat shops from February 2021 to February 2023. Samples were performed repeatedly across the study period to account for seasonal variations (summer, autumn, winter, and spring), with monthly prevalence values generated by pooling all samples collected in the same calendar month across the full two-year period. A multistage random sampling approach was used; first, 21 sampling locations across Peshawar were identified after which eligible meat shops were listed and selected using simple random sampling. From each selected outlet, one 100 g sample of freshly displayed beef was collected during the morning hours (08:00–11:00). Samples showing visible signs of spoilage, frozen samples, or those weighing less than 100 g were excluded. Each 100 grams sample was placed in a sterile zipper sealed bag and transported to the microbiology laboratory under chilled conditions (approximately 4 °C) using sterile carriers to prevent cross-contamination, and processed within two hours of collection.

For microbiological analysis, two grams of each meat sample was aseptically transferred into 50 mL of Buffered Peptone Water (BPW; Oxoid, UK) and incubated at 37°C for 18–24 hours for pre-enrichment. Following incubation, serial dilutions were prepared, and aliquots of 10<sup>-4</sup> and 10<sup>-5</sup> dilutions (100µL) were plated onto Xylose Lysine Deoxycholate (XLD) agar using the spread plate technique. Plates were incubated at 37°C for 24–48 hours. Colonies exhibiting pink coloration with black centers, indicative of the production of hydrogen sulfide, were considered presumptive isolates of *Salmonella* bacteria. These colonies were subculture on *Salmonella*-Shigella (SS) agar for the preparation of pure

cultures. Colonies that were colorless with black center on SS agar were picked for further identification.

Presumptive isolates were Gram stained and Gram-negative rods compatible with the *Salmonella* morphology were selected for biochemical confirmation. A series of biochemical tests were performed as per standard microbiological protocol tests including catalase, oxidase, indole, utilization of citrate, Triple Sugar Iron (TSI) agar, urease, motility, and Methyl Red-Voges Proskauer (MR-VP) tests. Isolates showing catalase positive and oxidase negative reactions, no indole production, was able to utilize citrate, and produced an alkaline slant and acid butt with hydrogen sulfide production on TSI agar, negative urease activity, motility positivity, and showed MR positive/VP negative reactions were identified as *S. enterica*. Bacterial load was determined by counting colony-forming units (CFU) on SS agar plates using a digital colony counter. Plates containing between 25 and 250 colonies were considered valid, and CFU per milliliter was calculated using standard formulas based on colony counts, dilution factors, and plated volume.

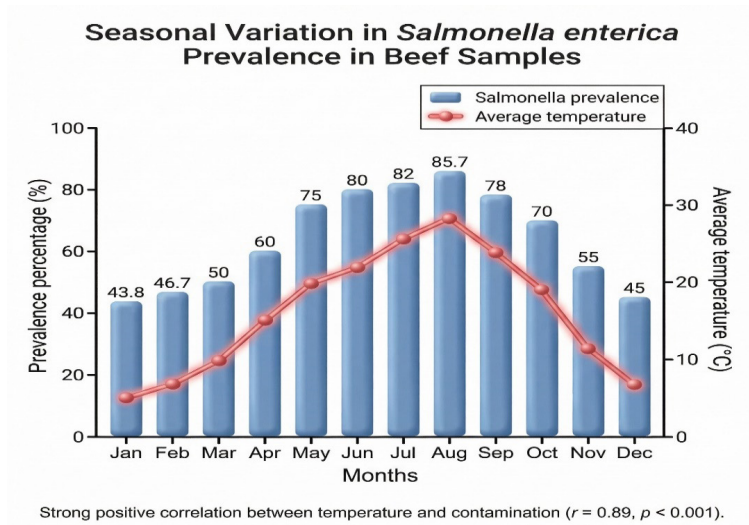
The biofilm forming ability was assessed using qualitative and quantitative methods. For qualitative screening, isolates were inoculated onto Congo Red Agar prepared with Brain Heart infusion agar supplemented with sucrose along with Congo red dye and incubated at 37°C for 24–48 hours. Colonies with black, dry crystalline morphology were considered as biofilm producers, whereas red color were considered as non-producers. In addition, the tube adherence method was done using Tryptic Soy Broth with different concentrations of glucose. After incubation at 37°C for 24 hours, tubes were stained with 0.1% safranin and examined for the presence of a visible film on the inner walls as an indication of biofilm formation. Quantitative evaluation was performed by using the microtiter plate assay in sterile 96-well plates. Bacterial suspensions adjusted to 0.5 McFarland Standard were inoculated into the wells containing Tryptic Soy Broth and incubated at 37°C for 24 hours. Wells were then washed, fixed with methanol, stained with safranin, and optical density was determined at wavelength 570 nm using microplate reader. Biofilm production was classified as non-producer, weak, moderate, or strong based on optical density values relative to the cut-off optical density (OD<sub>c</sub>) calculated from the negative control. All assays were performed in triplicate to ensure reproducibility.<sup>14</sup>

Antimicrobial susceptibility testing was performed via the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar following the 2022 Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial suspensions equivalent to the 0.5 McFarland turbidity standard were prepared in sterile saline, and approximately 100 µL of each suspension was spread evenly on Mueller-Hinton agar plates. Sixteen antimicrobial agents (Oxoid, UK) from commonly used classes of antibiotics were tested. Plates were incubated at 37°C for 16–18 hours and inhibition zone diameters were measured and interpreted according to CLSI breakpoints. The multiple antibiotic resistance (MAR) index was determined for each of the isolates using the formula  $MAR = a/b$ , where “a” represent the number of antibiotics the isolate was resistant to and “b” represent the total number of the antibiotics tested. MAR index values greater than 0.2 were considered as indicative for isolates to have originated from environments that have been contacted frequently by antibiotics. Confirmed isolates of *S. enterica* bacteria were maintained for subsequent analysis by storage of overnight cultures in nutrient broth supplemented with 20% sterile glycerol at -80°C. For each isolate, 800 microliters (µL) of bacterial culture were mixed with 200 µL of glycerol in sterile cryovials and saved under frozen conditions.

Data analysis was conducted using IBM SPSS Version 27, including descriptive statistics and chi-square tests for location differences. Associations between biofilm-forming capacity and antimicrobial resistance were determined and statistical significance at p-value less than 0.05 level was defined. Optical density values from microtiter plate assays represented mean values with a standard deviation of triplicate values. Crude odds ratios (ORs) with 95% confidence intervals were calculated to compare contamination levels across location categories, using rural sites as the reference group. Ethical approval was not required, as the study involved retail food samples and did not include human participants or live animals.

## Results

A total of 250 beef samples were taken from slaughterhouses, butchers, and retail outlets across 23 locations in Peshawar. Following enrichment and culture on Xylose Lysine Deoxycholate (XLD) and *Salmonella*-Shigella (SS)



**Figure 1** Seasonal Variation in the Prevalence of *Salmonella enterica*

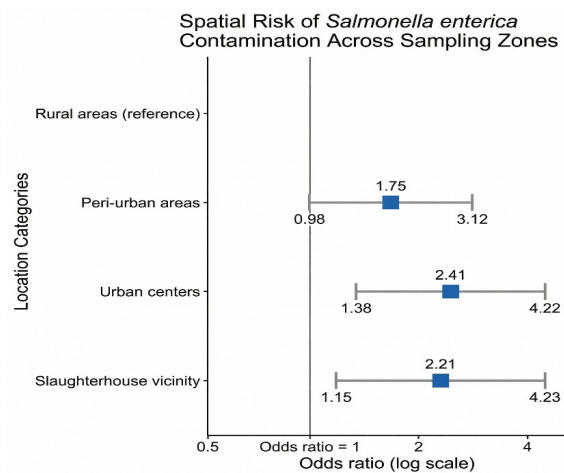
agar, *Salmonella enterica* was confirmed in 170 samples representing an overall prevalence of 68.0%. From these positive samples, 150 isolates were selected for comprehensive analysis, ensuring representative distribution across all sampling locations.

Monthly prevalence values represent pooled data across 2021–2023. The highest contamination rate was observed in August (85.7%), while the lowest was recorded in January (43.8%). A strong positive correlation was observed between the environmental temperature and contamination rates ( $r=0.89$ ,  $p<0.001$ ) (Figure 1).

Spatial analysis further showed that contamination rates were varied significantly

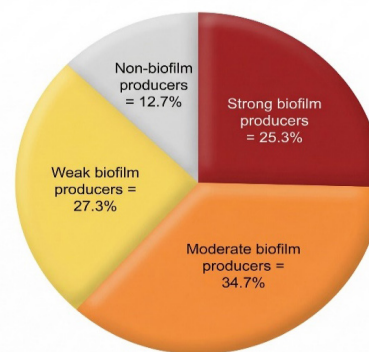
by locations. Samples collected from regions adjacent to slaughterhouses and with a high-density urban area had significantly higher odds of *Salmonella* contamination compared to rural areas. Analysis showed higher odds of contamination near slaughterhouses (OR=2.21, 95% CI: 1.15–4.23) and in urban centers (OR=2.41, 95% CI: 1.38–4.22) compared with rural areas. In contrast, peri-urban areas showed a borderline associated with contamination risk. Quantitative enumeration of bacterial loads showed that samples from close to slaughterhouses showed significantly higher bacterial loads when compared to other locations ( $p<0.001$ ) (Figure 2).

The biofilm-forming capacity of all the 150



**Figure 2** Spatial Distribution of *Salmonella enterica* Contamination Risk

**Distribution of Biofilm Formation Among *Salmonella enterica* Isolates.**



**Figure 3** Distribution of Biofilm Formation among *Salmonella enterica* Isolates

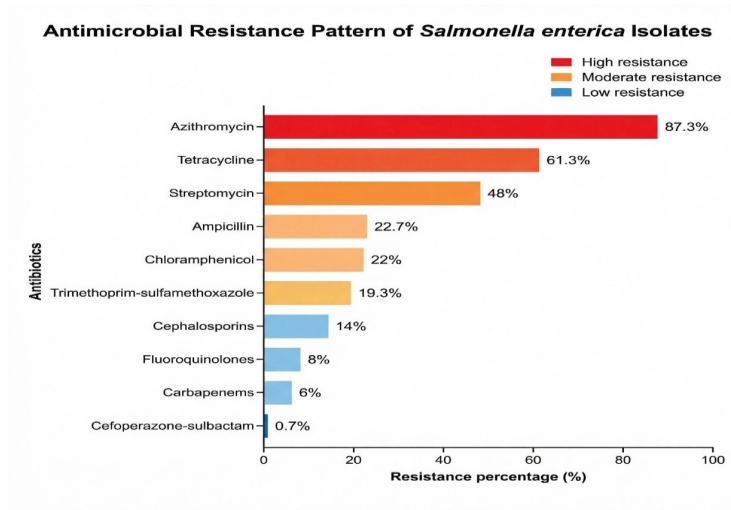


Figure 4 Antimicrobial Resistance Pattern of *Salmonella enterica* Isolates

confirmed isolates was evaluated using the microtiter plate assay. The majority of the isolates (87.3%) demonstrated the capacity to produce biofilms. Among them, 25.3% were classified as strong biofilm producers, 34.7% as moderate biofilm producers, and 27.3% as weak biofilm producers, while 12.7% of isolates exhibited no detectable biofilm formation (Figure 3).

Antimicrobial susceptibility test was performed on all isolates using Kirby-Bauer disc diffusion method against 16 antimicrobial agents of different groups of antibiotics. The highest resistance rates were recorded for azithromycin

(87.3%), tetracycline (61.3%), and streptomycin (48.0%). Moderate resistance was observed for ampicillin (22.7%), chloramphenicol (22.0%), and trimethoprim-sulfamethoxazole (19.3%). Low resistance rates were found for cephalosporins (14.0%), fluoroquinolones (8.0%) and carbapenems (6.0%) (Figure 4). The cephalosporin and beta-lactamase inhibitor combination cefoperazone-sulbactam had the best susceptibility, with only 0.7% resistance among the isolates.

Multidrug resistance (MDR), defined as resistance to three or more antimicrobials

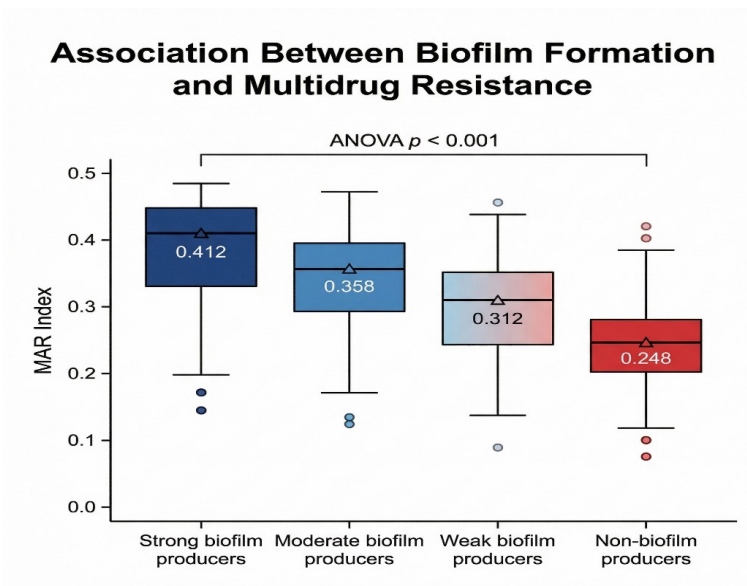


Figure 5 Association Between Biofilm Formation and Multidrug Resistance

classes, was observed in substantial proportion of isolates. The mean Multiple Antibiotic Resistance (MAR) index of isolates was 0.342. A positive link was observed between biofilm-forming capacity and antimicrobial resistance. Strong biofilm-producing isolates had the highest prevalence of multidrug resistance (73.7%) and the highest mean MAR index (0.412). In comparison, moderate biofilm producers showed MDR prevalence rate of 61.5% with mean MAR index of 0.358 while weak biofilm producers showed MDR prevalence rate of 46.3% with mean MAR index of 0.312. Non-biofilm forming isolates had much lower levels of resistance, with MDR prevalence of 31.6% and Mean MAR index of 0.248. Statistical analysis confirmed whether there were significant differences in the levels of resistance according to the type of biofilm ( $p < 0.001$ ) (Figure 5).

Analysis of resistance profiles identified 37 distinct antibiograms among the 150 isolates, indicating significant diversity in the AMR patterns of *Salmonella enterica* in the study area.

## Discussion

The present study revealed a high prevalence of multidrug-resistant *Salmonella enterica* (68.0%) in beef samples collected from different points of the supply chain in Peshawar. This prevalence is considerably higher than reports from other regional studies, such as those in Lahore. These discrepancies may be attributed to differences in sampling strategies and environmental conditions between retail and slaughterhouse settings.<sup>15</sup> The high contamination rates suggest these environments serve as significant reservoirs for bacterial dissemination. Similar trends have been observed in other low- and middle-income countries where challenges in maintaining the cold chain contribute to meat product contamination.<sup>16</sup> Although hygiene practices were not directly assessed in the present study, the higher contamination burden observed in slaughterhouse-associated and urban retail settings may reflect differences in handling conditions, equipment sanitation, carcass exposure, and cold-chain maintenance reported in similar settings.

A distinct seasonal trend was observed, with significantly higher contamination rates during warmer months. The strong correlation between environmental temperature and contamination ( $r = 0.89$ ,  $p < 0.001$ ) is consistent with the global epidemiological evidence showing the

existence of a positive relation between ambient temperatures and the survival and multiplication of *Salmonella* in food products and surrounding environments. Several studies have reported that the rise in environmental temperature is linked with the rise in number of non-typhoidal *Salmonella* infections and higher contamination rate in food supply chains.<sup>17,18</sup> Warmer conditions may enable the growth of bacteria on the meat surface, multiplied mechanical vectors such as flies, and reduce the efficiency of the cold chain of preservation of the food during transport and retail display. These mechanisms overall add up to seasonal enhancement of foodborne pathogens.

Spatial analysis revealed significantly higher odds of contamination among samples collected round slaughter-houses and in urban retail environments in comparison with rural areas. These results show the important role that slaughterhouse environments have as contamination hotspots in the meat production chain. Slaughtering processes are often associated with the extensive handling of carcasses, exposure of these carcasses to contaminated equipment and cross-contamination of carcasses by intestinal contents during evisceration. Previous investigations have also indicated slaughterhouses as significant points for transmission of food borne pathogens and antimicrobial-resistant bacteria.<sup>19,20</sup> The considerably higher bacterial loads reported in samples collected near to slaughterhouses is an additional indication of these environments as major sources of contamination.

The antimicrobial resistance patterns identified in this study indicate a substantial burden of resistance among *Salmonella enterica* isolates. High resistance rates were observed for azithromycin, tetracycline, and streptomycin. Similar resistance patterns have been reported in studies investigating foodborne *Salmonella* isolates from livestock and poultry production systems in South Asia.<sup>21,22</sup> The widespread use of tetracyclines and other antibiotics in livestock production is considered a major driver of antimicrobial resistance development in foodborne pathogens. Continuous exposure of bacterial populations to sub-therapeutic concentrations of antimicrobials used for growth promotion or disease prevention may select for resistant strains that subsequently enter the food chain. The detection of multidrug resistance in more than half of the isolates in this investigation study suggests that the meat supply chain may represent an important reservoir for

resistant bacteria.

An important finding of this study was the high prevalence of biofilm-forming isolates and the strong association between biofilm formation and antimicrobial resistance. More than 80% of the isolates demonstrated the ability to form biofilms, with strong biofilm producers exhibiting significantly higher multidrug resistance and MAR index values compared with non-biofilm-forming isolates. Biofilm formation provides several adaptive advantages for bacterial survival in food processing environments. The extracellular polymeric matrix produced during biofilm development acts as a physical barrier that limits the penetration of antimicrobial agents and disinfectants. In addition to that, the cells inside biofilms often have decreased metabolic activity and a divergent pattern of gene expression that make the cells more tolerant to antimicrobial compounds.<sup>23,24</sup> High cell density within biofilms is also a condition that allows rapid horizontal transfer of resistance genes via plasmids or other mobile genetic elements, accelerating the dissemination of antimicrobial resistance.

The spatial distribution of biofilm forming isolates evidenced in these findings further supports the hypothesis that the environmental conditions in slaughterhouses may be conducive to the selection of biofilm producing strains. Strong biofilm producers were found more often in the samples around slaughterhouses than in the rural areas. For one, slaughterhouses and meat procession facilities are places where bacteria are constantly exposed to disinfectants and cleaning agents, not to mention more variable environmental conditions. Such stresses can facilitate the choice of bacterial populations with the capacity to create stable biofilms in equipment surfaces and carcasses. These biofilms can be permanent after formation and be the source of continuous contamination in the processing environment even after normal sanitation practices.<sup>25</sup>

The implications of such findings on population health are great. The prevailing prevalence coupled with multidrug resistance and the ability to form biofilms implies that contaminated beef may pose a significant source of transmission of drug-resistant pathogens to humans of isolates of *Salmonella enterica*. Multidrug-resistant bacterial foodborne infections may result in more treatment failures, longer stay and more expensive medical care. In addition, the existence of the resistant bacteria in the food supply chain helps to further spread

the antimicrobial resistance in the community due to the presence of the environmental and human exposures pathways.<sup>26</sup>

This study has several limitations. First, the absence of serotyping limits identification of specific *Salmonella* serovars and their distribution, which may vary in virulence and resistance profiles. Second, the lack of molecular characterization of antimicrobial resistance genes and biofilm-associated determinants restricts understanding of the genetic mechanisms underlying the observed phenotypes. Third, the cross-sectional design captures contamination patterns at a single point in time and does not allow evaluation of temporal trends or causality. Future studies should prioritize molecular characterization of isolates to resistance genes, mobile genetic elements, and biofilm-associated genetic determinants. Utilizing advanced techniques such as whole-genome sequencing (WGS) and serotyping will be instrumental in elucidating complex transmission routes between animal reservoirs, food products, and human infections. Longitudinal surveillance studies to monitor dynamic trends in antimicrobial resistance within food supply chains. Such data will be vital for evaluating the long-term effectiveness of targeted intervention strategies and improving food safety frameworks in the region.

In conclusion, the beef supply chain in Peshawar is a significant source of multidrug-resistant *Salmonella enterica*. The high prevalence of biofilm-forming isolates, especially among resistant strains, underscores a heightened risk of environmental persistence. Strengthening cold chain maintenance and implementing stricter antimicrobial stewardship in livestock production are crucial to reducing the public-health burden of foodborne antimicrobial resistance.

## References

1. Han J, Aljahdali N, Zhao S, Tang H, Harbottle H, Hoffmann M, et al. Infection biology of *Salmonella enterica*. *EcoSal Plus*. 2024;12(1):eesp00012023. doi:10.1128/ecosalplus.esp-0001-2023
2. Galán-Relaño Á, Valero Díaz A, Huerta Lorenzo B, Gómez-Gascón L, Mena Rodríguez MÁ, Carrasco Jiménez E, et al. *Salmonella* and salmonellosis: an update on public health implications and control strategies. *Animals (Basel)*. 2023;13(23):3666. doi:10.3390/

- ani13233666
3. Abebe E, Gugsu G, Ahmed M. Review on major food-borne zoonotic bacterial pathogens. *J Trop Med*. 2020;2020:4674235. doi:10.1155/2020/4674235
  4. Ehuwa O, Jaiswal AK, Jaiswal S. *Salmonella*, food safety and food handling practices. *Foods*. 2021;10(5):907. doi:10.3390/foods10050907
  5. Zeng H, Yang D, Huang N, Li Y, Chen J, Yu Z, et al. Prevalence and antimicrobial susceptibility of salmonella in retail meat collected from different markets in Sichuan, China. *Pathogens*. 2025;14(3):222. doi:10.3390/pathogens14030222.
  6. Majeed Y, Waseem M. Postharvest Handling Systems. In: Zhang Q, ed. *Encyclopedia of smart agriculture technologies*. Cham: Springer International Publishing; 2022. p. 1–9. doi: 10.1007/978-3-030-89123-7\_125-1
  7. Nazir J, Manzoor T, Saleem A, Gani U, Bhat SS, Khan S, et al. Combatting *salmonella*: a focus on antimicrobial resistance and the need for effective vaccination. *BMC Infect Dis*. 2025;25(1):84. doi:10.1186/s12879-025-10478-5
  8. Galán-Relaño Á, Valero Díaz A, Huerta Lorenzo B, Gómez-Gascón L, Mena Rodríguez MÁ, Carrasco Jiménez E, et al. *Salmonella* and salmonellosis: an update on public health implications and control strategies. *Animals (Basel)*. 2023;13(23):3666. doi:10.3390/ani13233666
  9. Ahmed MJ, Hossain MI, Bhuiyan MIH, Rahman MA, Kaderi IJ, Nirob AI, et al. Antimicrobial resistance in meat and meat products from Asia: An urgent one health challenge- a systematic review and meta-analysis. *Poultry Science*. 2025;104(11):105811. doi:10.1016/j.psj.2025.105811
  10. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: from definition to treatment strategies. *Front Cell Infect Microbiol*. 2023;13:1137947. doi:10.3389/fcimb.2023.1137947
  11. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. *Microorganisms*. 2023;11(6):1614. doi:10.3390/microorganisms11061614.
  12. McDermott PF, Zhao S, Tate H. Antimicrobial resistance in nontyphoidal *salmonella*. *microbiol spectr*. 2018;6(4):10.1128/microbiolspec.arba-0014-2017. doi:10.1128/microbiolspec.ARBA-0014-2017
  13. Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA. Worldwide epidemiology of *salmonella* serovars in animal-based foods: a meta-analysis. *Appl Environ Microbiol*. 2019;85(14). doi:10.1128/AEM.00591-19
  14. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS*. 2007;115(8):891–9. doi:10.1111/j.1600-0463.2007.apm\_630.x
  15. Fatima A, Saleem M, Nawaz S, Khalid L, Riaz S, Sajid I. Prevalence and antibiotics resistance status of *Salmonella* in raw meat consumed in various areas of Lahore, Pakistan. *Sci Rep*. 2023;13(1):22205. doi: 10.1038/s41598-023-49487-2
  16. Yang X, Huang J, Su Y, Cai S, Zhang J, Guo W, et al. Incidence and antimicrobial resistance of *Salmonella* serovars in fresh retail aquatic products from China. *LWT*. 2022;171:114123. doi:10.1016/j.lwt.2022.114123
  17. Lake IR, Gillespie IA, Bentham G, Nichols GL, Lane C, Adak GK, et al. A re-evaluation of the impact of temperature and climate change on foodborne illness. *Epidemiol Infect*. 2009;137(11):1538–47.
  18. Li B, Webster TJ. Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections. *J Orthop Res*. 2018;36(1):22–32. doi:10.1002/jor.23656
  19. Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules*. 2018;23(4):795. doi:10.3390/molecules23040795
  20. Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: a developing country-perspective. *Front Microbiol*. 2016;7:1881. doi:10.3389/fmicb.2016.01881
  21. Rahman M, Rahman A, Islam M, Alam M. Detection of multi-drug resistant *Salmonella* from milk and meat in Bangladesh. *Bangladesh J Veterinary Med* 2018;16(1):115–20. doi:10.3329/bjvm.v16i1.37388
  22. Kumar R, Adeyemi NO, Chattaraj S, Alloun

- W, Thamarsha A, Anđelković S, et al. Antimicrobial resistance in *Salmonella*: One Health perspective on global food safety challenges. *Sci One Health*. 2025;4:100117. doi:10.1016/j.soh.2025.100117.
23. Xia Y, Hu Z, Jin Q, Chen Q, Zhao C, Qiang R, et al. Structural characteristics, functions, and counteracting strategies of biofilms in *Staphylococcus aureus*. *Comput Struct Biotechnol J*. 2025;27:488–500. doi:10.1016/j.csbj.2025.01.021.
24. Aleksandrowicz A, Carolak E, Dutkiewicz A, Błachut A, Waszczuk W, Grzymajło K. Better together-*Salmonella* biofilm-associated antibiotic resistance. *Gut Microbes*. 2023;15(1):2229937. doi:10.1080/19490976.2023.222993
25. Carrascosa C, Raheem D, Ramos F, Saraiva A, Raposo A. Microbial biofilms in the food industry-a comprehensive review. *Int J Environ Res Public Health*. 2021;18(4):2014. doi:10.3390/ijerph18042014
26. Samtiya M, Matthews KR, Dhewa T, Puniya AK. Antimicrobial resistance in the food chain: trends, mechanisms, pathways, and possible regulation strategies. *Foods*. 2022;11(19):2966. doi:10.3390/foods11192966