

INVESTIGATING ANTIMICROBIAL RESISTANCE PATTERNS IN SALMONELLA ISOLATES: A PUBLIC HEALTH PERSPECTIVE

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Abstract

Emerging multidrug-resistant *Salmonella* strains pose a challenge to public Health, limiting treatment options for foodborne and invasive infections. The present study aimed at determining the antimicrobial resistance of *Salmonella* isolates recovered from human, food, animal, and environmental specimens. In total, 60 *Salmonella* isolates were investigated, collected from human clinical specimens (45.0 %), poultry meat (25.0 %), beef (11.7 %), surface water (10.0 %), dairy products (5.0 %), and vegetables (3.3 %). Antimicrobial sensitivity testing indicated that tetracycline (61.7%) and ampicillin (56.7%) had the highest resistance rates, followed by trimethoprim-sulfamethoxazole (40%), cefotaxime (25%), and ciprofloxacin (23.3%). Resistance rates to chloramphenicol (15.0%), azithromycin (8.3%), and gentamicin (5.0%) were lower. Multidrug resistance (resistance to three or more antimicrobial classes) was observed in 23 isolates (38.3%). The highest rate of multidrug resistance was in poultry-derived isolates (60.0%), and the lowest was in those isolated from dairy products and vegetables. Multivariate analysis showed that the source of isolates was statistically associated with the MDR profile ($\chi^2 = 11.62$, $df = 4$, $p = 0.020$). Serovar-specific analysis indicated that *Salmonella* Typhimurium and *Salmonella* Infantis contributed disproportionately to MDR phenotypes ($\chi^2 = 13.48$, $df = 3$, $p = 0.004$). Our findings highlight a significant burden of antimicrobial resistance in *Salmonella*, particularly among food-animal and human isolates, and underscore the importance of integrated surveillance, antimicrobial stewardship, and One Health-based interventions to limit the public health consequences of resistant *Salmonella* serovars.

1. INTRODUCTION

Salmonella remains one of the most significant global foodborne pathogens and a leading cause of intestinal disease and invasive infections in humans. According to global estimates of its burden, non-typhoidal *Salmonella* causes several million cases of gastroenteritis per year, with profound human and economic implications, especially in low- and middle-income countries [1, 2]. The public health importance of *Salmonella* is also underscored by the rise in antimicrobial-resistant strains, which make clinical management difficult and limit therapeutic options [3]. Resistance patterns among this pathogen in South Africa have been correlated with prolonged illness, resulting in increased hospital admissions and an increased risk of invasive disease, emphasizing the importance of surveillance of its resistance patterns [3].

The development and spread of AMR in *Salmonella* are strongly associated with the use of antimicrobials in human medicine and in food-producing animals. The widespread use of antimicrobials in animal husbandry (therapeutic, prophylactic, and growth promotion activities) has been identified as a major driver for selection of resistance, whereby resistant bacteria and genes are spread via the food chain to human hosts [4, 5]. Poultry and other food animals have been frequently recognized as major carriers of resistant *Salmonella*, in particular multi-drug-resistant strains associated with serotypes *S. Typhimurium* and *S. Infantis*. Moreover, there is an overall trend toward increased antimicrobial use in food animals worldwide, particularly in intensively produced animals, raising concerns about future resistance loads unless measures are taken to mitigate these trends [6].

In addition to food animals, environmental reservoirs are increasingly recognized as playing a role in the ecology of antimicrobial resistance. Antibiotics and resistant bacteria can enter the environment through agricultural waste and wastewater discharges, thereby maintaining and spreading resistance genes in aquatic and terrestrial ecosystems [7, 8]. In addition, acquisition of resistance determinants via

horizontal gene transfer mediated by plasmids and integrons in *Salmonella* facilitates the dissemination of resistance into various hosts and environments [9, 10]. These interconnected routes of transmission highlight the importance of developing a One Health perspective that integrates human, animal and environmental health dimensions into AMR interventions [11]. In this regard, the systematic monitoring of AMR trends among *Salmonella* isolated from different sources is essential for informing surveillance systems, guiding the policy on antimicrobial stewardship and implementing evidence-based interventions to reduce the public health burden of resistant *Salmonella* Infections [12].

2. METHODOLOGY

A laboratory-based cross-sectional survey was conducted to investigate the antimicrobial resistance trends in *Salmonella* isolates from humans, food animals, foods and environmental sources. The objective of the study was to provide resistance prevalence, multidrug-resistance patterns, and associations with isolate source and serovar from a public health perspective.

A total of 60 isolates of *Salmonella* were studied at University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan. Isolates derived from human clinical samples (stool and blood), poultry meat, beef, dairy products, vegetables and surface waters. Ten of these samples were gathered over 2 consecutive years and classified according to their origin, year of isolation, and neuromuscular site.

Isolates were confirmed as *Salmonella* spp. using standard microbiological procedures. Serovars were determined by traditional serotyping, and isolates were assigned to the predominant serovars, including *S. Typhimurium*, *S. Enteritidis*, *S. Infantis*, *S. Newport*, and *S. Typhi*.

Susceptibility testing to clinically and epidemiologically relevant antimicrobials (ampicillin, cefotaxime, ciprofloxacin, tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol, gentamicin, and azithromycin) was performed. Susceptibility results were defined according to standardized methods. Each isolate

was linked as sensitive or resistant to the antimicrobial agents.

MDR was defined as resistance to three or more antimicrobial classes. For each isolate, MDR was assessed based on the strains' susceptibility to the different antimicrobials. All laboratory results and epidemiological data were entered into a structured dataset that included an isolate identifier, year of isolation, source, serovar, geographic origin, resistance profile to antimicrobials, and MDR status. Data was coded and statistically analyzed.

The statistical analyses were performed using IBM SPSS Statistics. Descriptive statistics were applied to characterize the distribution of isolates by source, serovar, and resistance profile. The prevalence of resistance was expressed as

proportions. Pearson's chi-square test was used to assess relationships between AMR or MDR status and categorical characteristics, including source and serovars. Values of $p < 0.05$ were considered statistically significant for all analyses.

3. RESULTS

In all, 60 *Salmonella* strains were analyzed. Human clinical specimens, food products, animal-origin samples, and environmental samples were the sources of the recovered isolates. Human clinical isolates accounted for the bulk, with Poultry meat and Beef ranking second and third, respectively. The distribution of isolates by source is as illustrated in Table 1.

Table 1: Distribution of *Salmonella* Isolates by Source

Source	Number of isolates (n)	Percentage (%)
Human clinical samples	27	45.0
Poultry meat	15	25.0
Beef	7	11.7
Surface water	6	10.0
Dairy products	3	5.0
Vegetables	2	3.3
Total	60	100



The isolates exhibited variable resistance to the antimicrobial agents tested. The highest proportion of resistance was detected in tetracycline (61.7%) and ampicillin (56.7%), followed by that to gentamicin (5.0%) and azithromycin (8.3%). Isolates were identified as

resistant to ciprofloxacin and cefotaxime in 23.3% and 25.0%, respectively. The overall prevalence of antimicrobial resistance is shown in Table 2 and graphically depicted in Figure 1, which shows the trend of resistance to all tested antimicrobial agents.

Table 2: Overall Antimicrobial Resistance Prevalence Among *Salmonella* Isolates

Antimicrobial agent	Resistant isolates (n)	Resistance (%)
Ampicillin	34	56.7
Tetracycline	37	61.7
Trimethoprim-sulfamethoxazole	24	40.0
Ciprofloxacin	14	23.3
Cefotaxime	15	25.0
Chloramphenicol	9	15.0
Gentamicin	3	5.0
Azithromycin	5	8.3

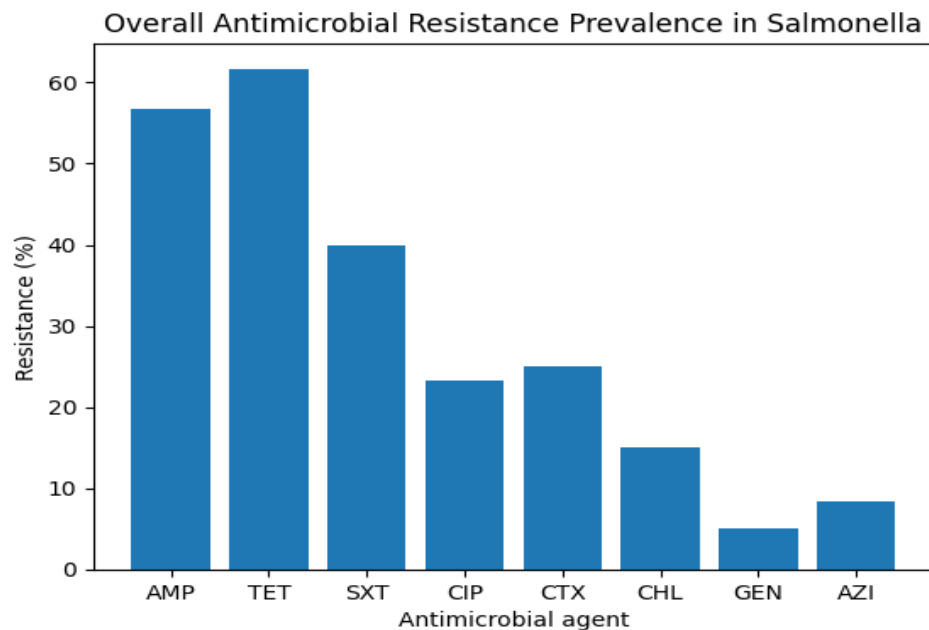


Figure 1: Overall antimicrobial resistance prevalence among Salmonella isolates.

Forty-one of the 60 isolates (68.3%) met the predefined criteria for MDR, and the sequentially acquired antibiotic resistances are listed in Table 3. The prevalence of MDR varied among isolated sources, with the highest proportion observed in poultry meat isolates, followed by human clinical

strains. None of the dairy or vegetable samples contained MDR isolates. A distribution of MDR isolates by source is outlined in Table 3 and Figure 2, which clearly shows differences in MDR prevalence by source.

Table 3: Distribution of Multidrug-Resistant (MDR) *Salmonella* Isolates by Source

Source	MDR isolates (n)	MDR (%)
Human clinical samples	11	40.7
Poultry meat	9	60.0
Beef	2	28.6
Surface water	1	16.7
Dairy products	0	0.0
Vegetables	0	0.0

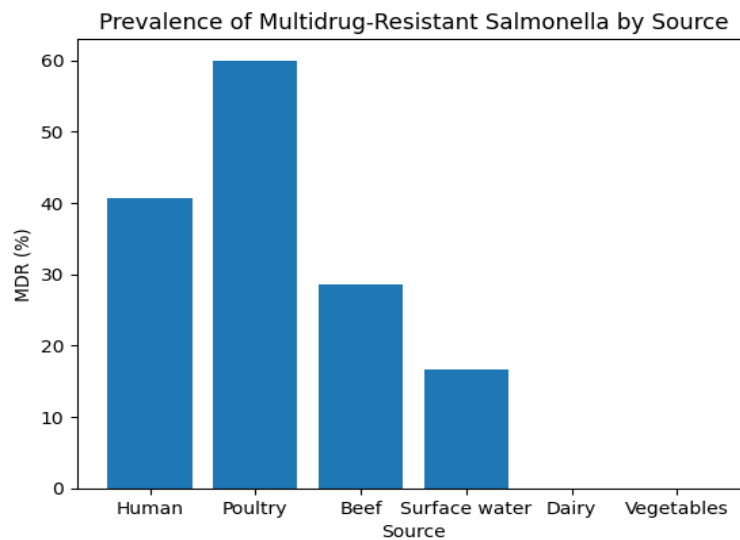


Figure 2: Prevalence of multidrug-resistant *Salmonella* isolated by source.

Pearson's chi-square test indicated that there was a statistically significant relationship between source of the isolates and MDR ($\chi^2 = 11.62$, $df = 4$, $p = 0.020$), where the prevalence of MDR differed by source. Furthermore, ampicillin and tetracycline resistance were significantly correlated with isolate source ($p < 0.05$), and both were more frequent among poultry and human

clinical isolates. We also highlighted the differences between frequently resistant and rarely resistant antibiotics by comparing average resistance levels for two high-resistance agents (ampicillin and tetracycline) with those of two low-resistance agents (gentamicin and azithromycin; Figure 3).

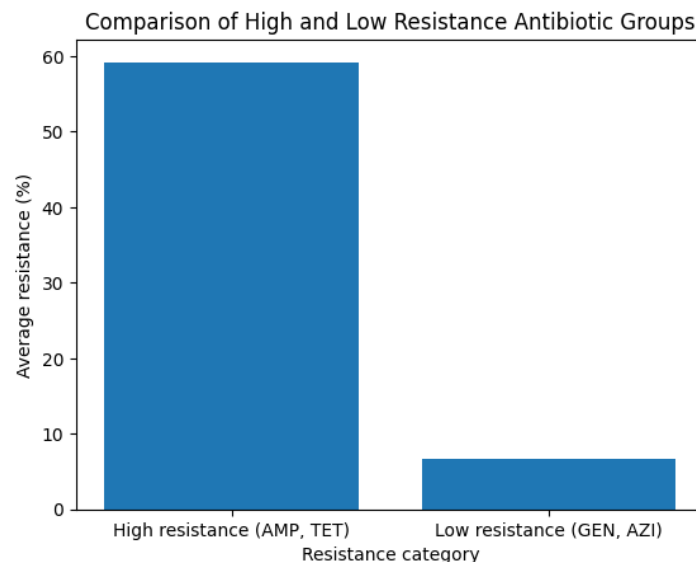


Figure 3: Comparison of high- and low-resistance antimicrobial groups.

4. DISCUSSION

The results show a high proportion of antimicrobial resistance in *Salmonella* isolates,

especially against tetracycline and ampicillin, and a considerable proportion of MDR. These trends are generally in line with global surveillance data

indicating the continued presence of resistance to older, widely used antimicrobials in both human and animal sectors [13, 14]. Overall, observed MDR prevalence is consistent with other findings indicating that a high percentage of *Salmonella* isolates analyzed worldwide are resistant to multiple antimicrobials, posing a significant challenge for treatment and public health interventions [15]. The results further highlight that frequently used antimicrobials drive selective pressure and contribute to the maintenance of resistant *Salmonella*.

Source-specific analysis showed that the prevalence of MDR was highest in poultry isolates, followed by human clinical isolates. This result is consistent with a number of reports that have recognized poultry as an important source of MDR *Salmonella*, probably associated with the intensive production practices and antimicrobial exposure in food animals [16, 17]. Delsol, Anjum [18] on the development and spread of MDR *S. Typhimurium* related to food-animal production]. However, most reports from a few geographical regions found similar or greater rates of MDR in human clinical isolates than in non-human sources that could correlate with variation on policies for antimicrobial usage, surveillance intensity, and health-care intervention [19]. Such differences would indicate a potential impact of local epidemiologic characteristics and control interventions on resistance profiles.

Resistance to critically important drugs such as ciprofloxacin and third-generation cephalosporins, though less than that seen against older products, is a major public health problem. Results of the ciprofloxacin and cefotaxime resistance detection correlate with trends of rising resistances to first- and second-line agents used in invasive salmonellosis [19, 20]. While some regions have noted decreasing trends in fluoroquinolone resistance through surveillance programs, more robust antimicrobial stewardship has produced stable or rising resistance levels in other regions, at least for certain serovars [21]. Thus, the present findings corroborate and contrast with other reports,

indicating that trends in resistance types are heterogeneous per settings.

Serovar-specific differences in MDR prevalence were observed, with *S. Infantis* and *S. Typhimurium* being overrepresented among the MDR isolates. This finding aligns with previous reports that highlight the dominance of these two serovars in the dissemination of antimicrobial resistance (AMR) along the trajectory of food production and eventual transmission to humans [15]. Environmental (surface water) strains had comparatively low MDR levels as reported by Keenum, Berendonk [22]; dilutional effects along with low direct exposure of antimicrobials in environmental pools may account for this. Yet resistant strains in environmental samples do suggest an integrated mode of transmission of infection from humans to animals and the environment [5].

A few limitations should be noted when interpreting these findings. The small sample size and the collection of isolates over a short time may limit the generalizability of results, as well as interfere with detecting temporal trends. Molecular characterization of resistance mechanisms was not carried out, preventing the confirmation of genes or mobile genetic elements responsible for the phenotypes observed. Furthermore, data on antimicrobial use were not available to make any causal inference about the relationship between practices concerning use of antimicrobials and resistance patterns. Moreover, notwithstanding these limitations, the information provided by this study into current antimicrobial resistance for *Salmonella* is valuable, and demonstrates the need for integrated surveillance, responsible use of antimicrobials, and coordinated One Health approaches to mitigate public health impact of resistant *Salmonella* infections.

5. CONCLUSION

Salmonella, which is one of the most prevalent pathogens that create a serious problem in public health, has a high prevalence of antimicrobial resistance among its isolates retrieved from human beings and food-producing animals, products and environmental sources. High

proportions of resistance to tetracycline and ampicillin, two widely used antimicrobials, as well as the high prevalence of multidrug-resistant isolates, indicate ongoing selective pressure and low effectiveness of older empirical treatments. These results confirm the high prevalence of MDR in poultry-derived and clinical isolation from humans and suggest that food-animal production systems are important reservoirs for *Salmonella* strains with resistance traits. In contrast, resistance to critically important agents (fluoroquinolones and third-generation cephalosporins) is creating concern regarding the treatment of severe and invasive infections. Serovar-specific variation also implies that some *Salmonella* lineages are more likely to propagate resistance than others, demonstrating the importance of focused surveillance efforts. The lower prevalence of MDR among environmental isolates is also likely due to the absence of interlinked AMR selection pressure affecting humans, animals and the environment. The above findings, taken together demonstrate the need for holistic, multisectoral approaches to AMR control including enhanced surveillance systems and antimicrobial stewardship in human and veterinary medicine and interventions to reduce inappropriate use of antimicrobials in food-animal production. Further studies combining larger longitudinal datasets and molecular approaches to the understanding of resistance mechanisms and transmission dynamics are required. Effective control of AMR through harmonized One Health approaches addressing both animal and human health will be required to reduce the public health risk associated with infection by these pathogens, whilst preserving the availability of susceptible clinical options.

6. FUNDING

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