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ISOLATION AND ANTIBIOTIC RESISTANCE PROFILING OF SHIGELLA-SPECIES FROM POULTRY MEAT

Original Research

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ABSTRACT

Background: Food-borne bacterial pathogens remain a significant public health concern globally, particularly in developing regions where meat handling and hygiene practices are often substandard. Poultry meat is a well-recognized reservoir for enteric pathogens such as *Salmonella*, *Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter*, *E. coli*, and *Shigella* spp. Of particular concern is *Shigella*, a Gram-negative, rod-shaped, unencapsulated bacterium from the *Enterobacteriaceae* family, known for its resistance to multiple antibiotics and its association with severe gastrointestinal infections.

Objective: This study aimed to isolate *Shigella* spp. from poultry meat and evaluate their antibiotic resistance profiles in the Hazara region of Khyber Pakhtunkhwa, Pakistan.

Methods: A total of 50 raw chicken meat samples were collected from butcher shops in Haripur between October 2021 and May 2022. Samples were processed in the microbiology laboratory at the University of Haripur. Isolation and identification of *Shigella* spp. were performed using MacConkey and Salmonella-Shigella (SS) agar, Gram staining, and a series of biochemical tests including citrate utilization, oxidase, and triple sugar iron (TSI) tests. Antibiotic susceptibility was assessed using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar with six antibiotics: azithromycin, ciprofloxacin, levofloxacin, cefotriaxone, gentamicin, and tobramycin.

Results: *Shigella* spp. were isolated in 15 out of 50 samples, indicating a 30% prevalence. High resistance was observed against cefotriaxone (86%), levofloxacin (80%), ciprofloxacin (73%), and azithromycin (60%). Moderate resistance was noted for tobramycin (26.6%), while gentamicin showed the lowest resistance (13.3%).

Conclusion: The high prevalence of antibiotic-resistant *Shigella* spp. in poultry meat highlights the urgent need for routine surveillance, stricter food safety practices, and rational use of antibiotics in food animals to mitigate public health risks.

Keywords: Antibiotic Resistance, Enterobacteriaceae, Food Safety, Poultry Meat, Public Health, *Shigella* spp., Zoonotic Infections.

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INTRODUCTION

Food-borne infections continue to pose a significant threat to global public health, particularly in resource-limited settings where food safety infrastructure is inadequate. Poultry and meat products serve as major reservoirs of pathogenic microorganisms, with contamination often occurring during slaughtering, processing, and transportation, despite the fact that meat from healthy animals typically contains minimal microbial load (1). According to the World Health Organization, 22 of the most common food-borne diseases result in approximately 600 million illnesses and 420,000 deaths each year, disproportionately affecting immunocompromised individuals (2). These infections, whether localized to the gastrointestinal tract or resulting in systemic disease, impose not only clinical burdens but also considerable economic challenges through healthcare costs and productivity losses (3). Among the key bacterial pathogens responsible for these illnesses are *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, *Listeria monocytogenes*, *Campylobacter*, and *Shigella* (4). These microorganisms are responsible for over 200 different gastrointestinal diseases and are a leading cause of morbidity and mortality, especially in developing nations where an estimated 1.8 million deaths occur annually due to food-borne infections (5). Even in high-income countries, these infections account for millions of gastrointestinal illness cases each year, translating into billions of dollars in healthcare expenditure and economic losses (6).

Shigella, in particular, is one of the most prevalent bacterial causes of food-borne illness globally. It is a Gram-negative, non-motile, non-spore-forming rod-shaped bacterium belonging to the *Enterobacteriaceae* family, commonly transmitted through the fecal-oral route, direct contact, or ingestion of contaminated food or water (7). The genus is classified into four serogroups: *S. dysenteriae* (Group A), *S. flexneri* (Group B), *S. boydii* (Group C), and *S. sonnei* (Group D) (8). The pathogen primarily affects the colonic epithelium, where it invades and multiplies within cells, leading to mucous-laden and often bloody diarrhea (9). Notably, *Shigella* has been isolated in poultry, highlighting the potential for zoonotic transmission (8). The cornerstone of shigellosis management has long been antimicrobial therapy, which not only reduces the duration and severity of illness but also minimizes the risk of complications and transmission. However, widespread misuse and overuse of antibiotics have led to a troubling rise in multidrug-resistant (MDR) *Shigella* strains, significantly complicating treatment strategies (10). In some regions, up to 50% of clinical isolates show resistance to commonly used antibiotics, including first-line agents like ciprofloxacin and azithromycin (11). Resistance in *Shigella* is mediated through various mechanisms such as efflux pumps, enzymatic drug inactivation, mutations in target sites, and acquisition of resistance-conferring genetic elements like R plasmids and transposons (12). These mechanisms collectively reduce antibiotic efficacy, leading to prolonged illness and increased mortality in vulnerable populations (13). Given the alarming rise in MDR *Shigella* and the narrowing therapeutic options, continuous surveillance of antimicrobial resistance patterns is imperative. This will not only guide appropriate empirical therapy but also inform public health policies on antibiotic stewardship and infection control. Therefore, the present study aims to investigate the antimicrobial resistance profiles of *Shigella* species, with a focus on identifying prevailing resistance trends to support evidence-based management of shigellosis in clinical and community settings.

METHODS

The present study was conducted in Haripur, located in the Khyber Pakhtunkhwa (KPK) province of Pakistan, with laboratory procedures carried out at the Microbiology Laboratory, University of Haripur. A cross-sectional design was employed to investigate the presence and antimicrobial resistance of *Shigella* species in meat samples. Between October 2021 and May 2022, a total of 50 raw meat samples were collected from various retail meat shops across Haripur. The samples were obtained between 9:00 AM and 11:00 AM to maintain consistency, and collection was carried out using sterile gloves and forceps to avoid cross-contamination. Each sample was placed in a sterile zip-lock plastic bag and promptly transported to the laboratory under controlled conditions at 4°C until further processing. Upon arrival at the laboratory, chicken meat samples were subjected to microbial isolation procedures. Sterile cotton swabs were used to swab the meat surface, and the samples were inoculated onto selective media, particularly MacConkey agar, for the preliminary isolation of *Shigella*. Suspected colonies were further examined using standard microbiological identification techniques, including morphological characterization, Gram staining, and a series of biochemical tests to confirm the presence of *Shigella* species. Ethical approval for this study was granted by the Institutional Review Board of the University of Haripur and all sample handling was conducted following biosafety and ethical guidelines. Since this study involved no human or animal participants, informed consent was not applicable.

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Gram's Staining: For Gram staining, a small smear of the bacterial culture was prepared on a sterile glass slide using a 24-hour-old isolated colony. A loopful of the culture was mixed with distilled water on the slide, spread in a circular motion, and heat-fixed gently. The slide was stained with crystal violet for one minute, rinsed with tap water, followed by application of Gram's iodine for another minute. After rinsing, the slide was treated with a decolorizer for 30 seconds, then counterstained with safranin for one minute. Following a final rinse, the slide was air-dried, a drop of immersion oil was added, and the slide was examined under a compound microscope using the 100x objective lens (14).

Catalase Test: To perform the catalase test, a drop of 3% hydrogen peroxide (H_2O_2) was placed on a sterile glass slide. Using a sterile loop, a small amount of bacterial colony from an 18-hour culture was mixed with the drop of H_2O_2. The appearance of visible bubbles indicated a positive catalase reaction, while no bubble formation indicated a negative result (15).

Oxidase Test: In the oxidase test, a drop of oxidase reagent was applied to a piece of filter paper. A small quantity of the 18-hour-old culture was transferred to the reagent using a sterile loop. The development of a dark blue or purple coloration within 10 seconds indicated a positive oxidase reaction; the absence of color change within this timeframe was interpreted as a negative result (16).

Citrate Utilization Test: Citrate utilization was evaluated using Simmon's citrate agar. A total of 23.3 g of the medium was dissolved in 1000 mL of distilled water, sterilized by autoclaving at 121°C, and dispensed into sterile test tubes in a slanted position. The inoculated tubes were incubated at 35–37°C for up to four days. A color shift from green to blue was considered indicative of citrate utilization by the organism (17).

Triple Sugar Iron Test: The triple sugar iron (TSI) test was used to assess the fermentation of glucose, lactose, and sucrose, as well as gas and hydrogen sulfide production. The medium was prepared by dissolving 64.4 g of TSI agar in 1000 mL of distilled water, followed by autoclaving at 121°C. The sterilized medium was dispensed into slanted test tubes and allowed to solidify. Bacterial cultures were inoculated by streaking the slant and stabbing the butt of the agar. Tubes were incubated at 35–37°C for 18 to 24 hours. Observations were made regarding color changes in the slant and butt, gas formation, and black precipitate indicative of H_2S production (18).

Antibiotic Susceptibility Test: The Kirby-Bauer disk diffusion method was utilized to assess the antibiotic resistance profiles of the isolated *Shigella* species. Mueller-Hinton agar (MHA) was prepared by dissolving 38 g of medium in 1000 mL of distilled water and sterilizing it by autoclaving. The media was poured into sterile Petri dishes and solidified under aseptic conditions. Fresh *Shigella* cultures, revived from glycerol stocks on Salmonella-Shigella (SS) agar, were incubated for 18 hours at 37°C. Colonies were picked and evenly spread on the MHA plates to create a lawn culture. Commercial antibiotic discs—levofloxacin, ciprofloxacin, cefotaxime, and azithromycin—were placed on the plates according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimeters to determine the susceptibility pattern (19).

RESULTS

Out of the total 50 meat samples collected from local butcher shops between October 2021 and May 2022, *Shigella* species were successfully isolated from 15 samples, indicating a prevalence rate of 30%. The isolates were first identified on MacConkey agar, where they appeared as colorless colonies, indicative of non-lactose fermenting Gram-negative bacteria. These colonies were further sub-cultured on Salmonella-Shigella (SS) agar for confirmation, showing similar colorless morphology. Microscopic examination of Gram-stained smears revealed that all 15 isolates were Gram-negative rods, consistent with the morphology of *Shigella* species. This morphological and biochemical characterization confirmed the presence of *Shigella* in the tested meat samples. The isolates underwent a series of confirmatory biochemical assays to determine enzyme production and substrate utilization, providing additional validation of identity.

Catalase Test: All 15 *Shigella* isolates demonstrated positive catalase activity, as evidenced by rapid bubble formation upon exposure to 3% hydrogen peroxide. This indicated the enzymatic breakdown of hydrogen peroxide, confirming catalase production.

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Oxidase Test: None of the tested *Shigella* isolates exhibited a color change when oxidase reagent was applied, confirming oxidase-negative status for all 15 isolates, a result consistent with members of the *Enterobacteriaceae* family.

Citrate Test: The isolates failed to utilize citrate as a sole carbon source during the four-day incubation period at 35–37˚C. No color change was observed in the Simmon's citrate agar, supporting a citrate-negative result.

Triple Sugar Iron Test: All isolates tested positive in the TSI test. Results showed sugar fermentation, evident by color changes, and gas production in the medium after incubation. This indicated the metabolic activity typical of *Shigella* species.

Antibiotic Susceptibility Profiling of Shigella: Antimicrobial susceptibility testing was performed on all 15 isolates using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar. Six antibiotics were tested: Azithromycin (15 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), and Tobramycin (10 µg). The highest resistance was observed against Ceftriaxone, with 86% (13 out of 15) of isolates exhibiting resistance. Levofloxacin resistance was observed in 80% of isolates, followed by Ciprofloxacin (73%) and Azithromycin (60%). In contrast, Gentamicin showed the highest susceptibility, with only 13.3% resistance, while Tobramycin resistance was 26.6%. Notably, isolates 5 and 7 showed multi-drug resistance to all four major antibiotics tested, whereas isolate 14 demonstrated the most favorable susceptibility profile, being either susceptible or intermediate to all tested drugs.

Table 1: Results of SHIGELA Isolates

Sr. No	Isolates	Colony	Microscopy	Catalase	Oxidase	Citrate	TSI	Azithromycin	Ciprofloxacin	Levofloxacin	Ceftriaxone	Gentamicin	Tobramycin
1	S-1	Colorless Colonies	Gram negative Rod	+	-	+	+	S	S	R	I	I	R
2	S-2	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	I	I	S	S
3	S-3	Colorless Colonies	Gram negative Rod	+	-	+	+	S	R	R	R	S	S
4	S-4	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	R	R	S	I
5	S-6	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	R	R	S	R
6	S-12	Colorless Colonies	Gram negative Rod	+	-	+	+	S	R	R	R	S	R
7	S-19	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	R	R	I	R

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Sr. No	Isolates	Colony	Microscopy	Catalase	Oxidase	Citrate	TSI	Azithromycin	Ciprofloxacin	Levofloxacin	Ceftriaxone	Gentamicin	Tobramycin
8	S-24	Colorless Colonies	Gram negative Rod	+	-	+	+	S	R	R	R	R	S
9	S-29	Colorless Colonies	Gram negative Rod	+	-	+	+	R	I	R	R	S	I
10	S-30	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	I	R	S	I
11	S-36	Colorless Colonies	Gram negative Rod	+	-	+	+	R	I	R	R	R	I
12	S-39	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	R	R	I	S
13	S-43	Colorless Colonies	Gram negative Rod	+	-	+	+	S	I	R	R	I	S
14	S-47	Colorless Colonies	Gram negative Rod	+	-	+	+	S	R	I	R	I	I
15	S-50	Colorless Colonies	Gram negative Rod	+	-	+	+	R	R	R	R	I	I

Table 2: Antibiotic zone diameter breakpoints as determined by CLSTI guide lines.

Sr. No	Antibiotics	Concentration	S	I	R
1	Azithromycin	15µg	≥ 13	-	≤12
2	Ciproflaxacin	5µg	≥26	22-25	≤21
3	Levoflaxacin	5µg	≥21	17-20	≤16
4	Cefotriaxone	30µg	≥23	20-22	≤19
5	Gentamicin	10µg	≥15	13-14	≤12
6	**Tobramycin**	**10µg**	**≥15**	**13-14**	**≤12**

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Table 3: Antimicrobial results of Shigela isolates

Isolates	Azithromycin	Ciproflaxacin	Levoflaxacin	Cefotriaxone	Gentamicin	Tobramycin
1	S	S	R	I	I	R
2	R	R	I	I	S	S
3	S	R	R	R	S	S
4	R	R	R	R	S	I
5	R	R	R	R	S	R
6	S	R	R	R	S	R
7	R	R	R	R	I	R
8	S	R	R	R	R	S
9	R	I	R	R	S	I
10	R	R	I	R	S	I
11	R	I	R	R	R	I
12	R	R	R	R	I	S
13	S	I	R	R	I	S
14	S	R	I	R	I	I
15	**R**	**R**	**R**	**R**	**I**	**I**

Table 4: Comparison of antimicrobial profiling results of Shigella

Results Total Percentage						
Sr. No	Antibiotics	S	I	R	Resistance	
1	Azithromycin	6	0	9	15	60%
2	Ciproflaxacin	1	3	11	15	73%
3	Levoflaxacin	0	3	12	15	80%
4	Cefotriaxone	0	2	13	15	86%
5	Gentamicin	7	6	2	15	13.3%
6	**Tobramycin**	**5**	**6**	**4**	**15**	**26.6**

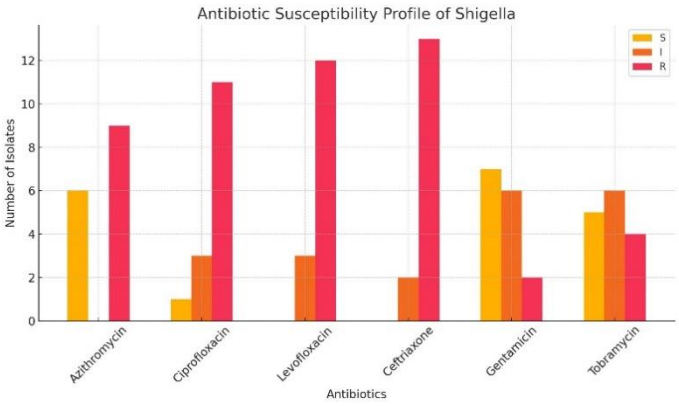

Figure 1 Antibiotic Susceptibility Profile of Shigella

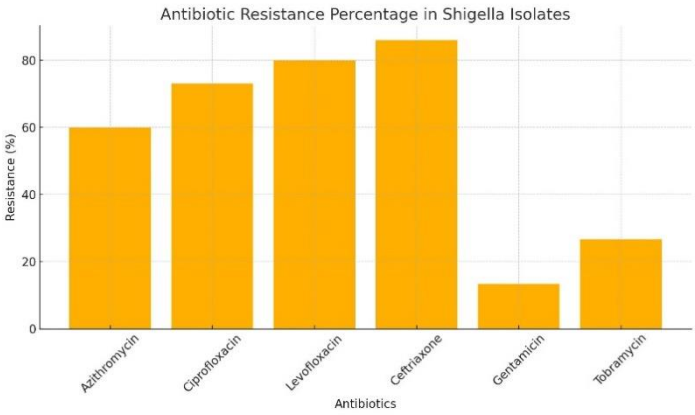

Figure 2 Antibiotic Resistance Percentage in Shigella Isolates

DISCUSSION

Shigellosis remains one of the most prevalent food-borne bacterial diseases globally, particularly in low- and middle-income countries, where inadequate sanitation and food safety practices persist. In this study, *Shigella* spp. were isolated from 30% of meat samples collected from local butcher shops in Haripur, a prevalence significantly higher than previously reported in similar investigations conducted in Tunisia (2.14%), Egypt (1.7%), and Iran (1.4%) (16,17). This elevated detection rate suggests potential lapses in hygienic meat handling, cross-contamination, or environmental sanitation in the studied region. The findings emphasize the critical need for strict hygiene enforcement along the entire meat production and distribution chain to mitigate public health risks. The data align with existing literature showing that meat and fresh food products, particularly those handled manually and served raw, are frequent vehicles for *Shigella* contamination. Such contamination typically results from improper food handling practices or direct fecal contamination during processing or transportation. Studies have consistently reported food as a primary route for *Shigella* transmission, alongside the well-established person-to-person spread, especially in endemic regions with compromised water and sanitation systems (18,19). Differences in isolation rates between this and previous studies can be attributed to variations in geographical region, sampling methodologies, seasonal factors, sample size, and differences in the quality of health surveillance and hygiene services.

A major concern raised by the study is the emergence of antibiotic-resistant *Shigella* strains, with alarming resistance levels detected against commonly used antibiotics. The isolates demonstrated high resistance to ceftriaxone (86%), levofloxacin (80%), ciprofloxacin (73%), and azithromycin (60%), while resistance to gentamicin (13.3%) and tobramycin (26.6%) remained relatively lower. These resistance patterns are not only consistent with earlier regional reports but, in some instances, exceed previously documented resistance frequencies. Comparable studies from other regions of Pakistan and neighboring countries reported lower resistance rates to azithromycin and fluoroquinolones, indicating that resistance dynamics may be evolving rapidly depending on regional antimicrobial usage trends (20). Notably, the current study's resistance profile reflects a shift from earlier therapeutic norms, wherein fluoroquinolones like ciprofloxacin and levofloxacin were considered the frontline treatment for shigellosis in both adults and children. Recent resistance trends now limit their clinical efficacy, necessitating the reevaluation of treatment guidelines (21). The high multidrug resistance (MDR) rate observed in the isolates underscores the implications of indiscriminate antibiotic usage in veterinary and agricultural practices, including growth promotion and prophylaxis in food animals. This indiscriminate use facilitates horizontal gene transfer and the emergence of resistant strains in the human food chain. Resistance to third-generation cephalosporins such as ceftriaxone further complicates therapeutic options, particularly in regions where access to newer or combination therapies is limited (22). These findings highlight the need for integrated One Health approaches targeting antimicrobial stewardship across human, animal, and environmental health sectors.

This study offers important insights into the local prevalence and resistance patterns of *Shigella* spp., marking it as one of the first in the Haripur region to comprehensively report such trends in retail poultry meat. A key strength lies in the standardized microbiological and biochemical identification procedures used for confirmation of isolates. However, several limitations are notable. The relatively small sample size may not fully represent broader contamination trends across the region. The study also lacks molecular-level analysis, such

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as detection of resistance genes or strain typing, which would have offered deeper insights into resistance mechanisms and epidemiological links. Seasonal variation, specific hygiene practices in different butcher shops, and meat processing conditions were also not documented, which limits the scope of causal inference. Future research should expand sampling across various seasons and include molecular profiling of resistant strains to map genetic determinants and resistance pathways. Integrating hygiene audits at sample collection sites could offer further context on contamination sources. Additionally, longitudinal surveillance across food supply chains would be beneficial to assess the temporal evolution of resistance trends and the impact of intervention strategies. Overall, the findings stress the urgency of strengthening food safety protocols and implementing regional antibiotic stewardship programs. Without such coordinated interventions, the ongoing rise in MDR *Shigella* poses a serious threat to public health, especially in communities with limited access to healthcare and clean water infrastructure.

CONCLUSION

This study highlights a concerning prevalence of *Shigella* spp. in poultry meat, accompanied by a high level of antimicrobial resistance, particularly to commonly used antibiotics. The findings underscore the urgent need for robust food safety measures, especially in low-resource settings where food-borne illnesses like shigellosis remain endemic. Effective control strategies should prioritize regular surveillance of food products for multidrug-resistant pathogens and reinforce hygiene practices along the food supply chain. Expanding the scope of research through larger sample sizes and incorporating molecular analyses would provide deeper insights into the epidemiology and genetic mechanisms underlying antibiotic resistance. These actions are critical for informing public health interventions and ensuring safer food systems.

AUTHOR CNTRIBUTION

Author	**Contribution**
Muqadas Hameed	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Waseem Sajjad	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Sehrish Hameed	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Muhammad Naeem	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Kinza Khalid	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Sohail Ahmad Shah	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
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Sobia Nisa	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published

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