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Multidrug resistance in enteric pathogens from smallholder poultry farms in peri-urban Dhaka: One Health implications

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Abstract

Background Antimicrobial resistance (AMR) in food animal pathogens poses a growing public health threat, particularly in low-resource settings where zoonotic transmission is poorly monitored.

Objective This study investigated the occurrence, spatial distribution, and AMR profiles of enteric pathogens isolated from chickens reared in smallholder commercial poultry farms in Bangladesh.

Methods Cecal samples (n=100) were collected from broiler and layer chickens across ten peri-urban locations within 50 km radius of Dhaka city. Bacterial isolation and identification were performed according to standard protocols. Antibiotic susceptibility was assessed using disk diffusion against 12 commonly used antibiotics. The multiple antibiotic resistance (MAR) index and prevalence of multidrug resistant (MDR) were calculated. Statistical analyses included chi-square tests, analysis of variance (ANOVA), and t-tests.

Results A total of 270 bacterial isolates were recovered, predominantly *Escherichia coli* (55.9%) and *Salmonella* spp. (16.7%). Resistance was highest to Amoxicillin (93.3%), Streptomycin (52.6%), and Tetracycline (46.3%), while Ceftriaxone showed the lowest resistance (6.7%). Overall, 71.5% of isolates were multidrug-resistant with broiler-derived strains showing significantly higher MDR prevalence (78.8%) and MAR index (0.41 ± 0.11) than layer-derived strains (62.2%, 0.30 ± 0.09; $p < 0.001$). Spatial analysis revealed significant variation in isolate counts and resistance burden across locations, with Narsingdi, Savar, and Arai-hajar identified as high-risk zones ($p < 0.05$).

Conclusion The high prevalence of MDR enteric pathogens and elevated MAR indices in broiler farms highlight the zoonotic risk posed by poultry feces. These findings emphasize the need for targeted antimicrobial stewardship and surveillance within the One Health framework.

Keywords *Escherichia coli* | *Salmonella* spp. | MAR index | antibiotic resistance | Bangladesh

Introduction

Poultry production in Bangladesh has expanded rapidly to meet the growing protein demand; however, intensive farming practices have led to widespread antibiotic misuse, fueling antimicrobial resistance (AMR) in enteric pathogens.

This poses a risk to human health via zoonotic transmission (Islam *et al.*, 2016). Smallholder commercial poultry farms, serve as critical nodes in the supply chain, providing affordable animal protein to urban and peri-urban populations (Col-lignon, 2003). However, the intensification of poultry production has been accompanied by growing concerns over mi-

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crobial contamination and the emergence of AMR pathogens, which pose serious risks to both animal and human health (Apata, 2009).

AMR in food-producing animals is widely recognized as a global health crisis. The misuse and overuse of antibiotics in poultry, often for growth promotion, prophylaxis, and metaphylaxis, has accelerated the selection of resistant bacterial strains (Caprioli *et al.*, 2000; Kolář *et al.*, 2002). These resistant organisms, particularly enteric pathogens such as *Escherichia coli* (*E. coli*), *Salmonella* spp., and *Campylobacter* spp., can be transmitted to humans through direct contact, environmental exposure, or the food chain, contributing to the global burden of AMR infections (Doyle, 2006; Donoghue, 2003). In Bangladesh, where veterinary oversight and antibiotic stewardship are limited, the risk of zoonotic transmission is particularly acute (Islam *et al.*, 2016).

Several global frameworks have been established to address the growing threat of AMR. The World Health Organization (WHO) launched the Global Antimicrobial Resistance Surveillance System (GLASS) to standardize AMR data collection and promote evidence-based policies across human and animal health sectors (WHO, 2015). Complementing this, the WHO-FAO-OIE Tripartite Collaboration advocates a One Health approach, emphasizing the interconnectedness of human, animal, and environmental health in tackling AMR (WHO-FAO-OIE Tripartite Report, 2022). These frameworks highlight the urgent need for integrated, cross-sectoral surveillance systems, particularly in low- and middle-income countries (LMICs) where antibiotic use in livestock is often unregulated and poorly documented.

Bangladesh exemplifies these challenges. The poultry sector is characterized by dense farm networks, informal antibiotic procurement, and limited veterinary oversight. Despite the growing recognition of AMR as a national priority, surveillance systems remain fragmented, and farm-level data on resistance patterns are limited. This gap is particularly pronounced in smallholder commercial farms, which dominate the poultry landscape but often lack biosecurity infrastructure and access to veterinary guidance. The absence of spatially resolved data on the resistance burden further limits the ability to implement targeted interventions and monitor zoonotic risks.

Our research group has been actively engaged in AMR and One Health research in Bangladesh, contributing foundational evidence to inform national and regional policies. Our team documented the first comprehensive assessment of antibiotic usage patterns in Bangladeshi broiler farms (Islam *et al.*, 2016), revealing widespread and unregulated application of critically important antimicrobials. This seminal study established the basis for subsequent research on AMR dynamics in poultry production systems. We subsequently recorded veterinary antibiotic residues in chicken eggs, highlighting the risk of chronic dietary exposure (Islam *et al.*, 2020). Recently, we identified key risk factors for *Campylobacter* colonization in broiler farms and reported high levels of multidrug resistant (MDR) *Salmonella enteritidis* and *E. coli* in raw chicken meat and market-sourced broilers (Al-Maruf *et al.*, 2024; Islam *et al.*, 2025; Karim *et al.*, 2025). Collectively, these investigations emphasize the zoonotic potential of re-

sistant strains circulating within the food system of Dhaka. In Bangladesh, AMR in poultry production is increasingly recognized as a potential contributor to foodborne exposure and the overall human AMR burden within the One Health framework (Sultana *et al.*, 2012; Sarker *et al.*, 2019).

Despite these advances, there are still critical knowledge gaps. No comprehensive studies have systematically compared the resistance burden between broiler and layer systems or examined the spatial variation in resistance patterns. Most existing research has focused on market-level contamination or single-species profiling, with limited integration of farm-level sampling, spatial epidemiology, and quantitative resistance metrics such as the Multiple antibiotic resistance (MAR) index. Moreover, the identification of geographic hotspots for MDR pathogens remains underexplored, limiting the ability to implement targeted interventions. To the best of our knowledge, this study is the first of its kind in Bangladesh to compare AMR burdens between broiler and layer chickens while integrating spatial epidemiology and quantitative resistance metrics.

The present study addresses these gaps by systematically characterizing the phenotypic resistance profiles, MAR indices, and MDR prevalence of enteric pathogens isolated from chickens reared in smallholder poultry farms across ten peri-urban locations surrounding Dhaka. By integrating microbiological analysis with spatial distribution data and comparisons between broiler and layer production systems, this study aimed to identify geographic hotspots of resistance and quantify farm-level risk. These insights are intended to inform targeted antimicrobial stewardship strategies, strengthen farm-level biosecurity, and support the development of integrated AMR surveillance frameworks within the One Health context. Our findings contribute to national AMR action plans and align with global initiatives such as WHO GLASS and the WHO-FAO-OIE Tripartite Collaboration, emphasizing the need for coordinated interventions across animal, environmental, and human health sectors (WHO-FAO-OIE Tripartite Report, 2022).

Methods

Ethical approval

This study involved the collection of cecal samples from chickens reared in smallholder commercial poultry farms. No endangered species were involved, and no live animals were subjected to experimental procedures beyond routine veterinary sacrifice. All sampling was conducted in accordance with the ethical standards for non-invasive microbiological research. Approval for the study was obtained from the Departmental Research Review Board, Department of Medicine and Public Health, Faculty of Animal Science and Veterinary Medicine, and the Sher-e-Bangla Agricultural University Research System (SAURES), Dhaka, Bangladesh. Informed consent was obtained from all participating farm owners prior to sample collection.

Spatial coverage and demography

General The study was conducted across ten peri-urban locations surrounding Dhaka, the capital and most populated

city of Bangladesh. These sites are all situated within a 50 km radius of Dhaka (**Figure 1**) and form a major poultry production belt supplying meat and eggs to the city food chain. As summarized in **Table 1**, the selected locations represent a range of smallholder poultry production environments, including industrial, suburban, and semi-rural clusters with high poultry density and active market linkages, ensuring broad spatial representation for AMR assessment.

Study design and sampling framework

A cross sectional design was employed to investigate AMR in poultry origin bacteria across ten peri-urban sites surrounding Dhaka, Bangladesh. The geographic coordinates of each farm were recorded and incorporated into GIS based spatial analysis to visualize clustering patterns and assess location specific resistance trends. At each location, one broiler farm and one layer farm were selected, resulting in 20 small-

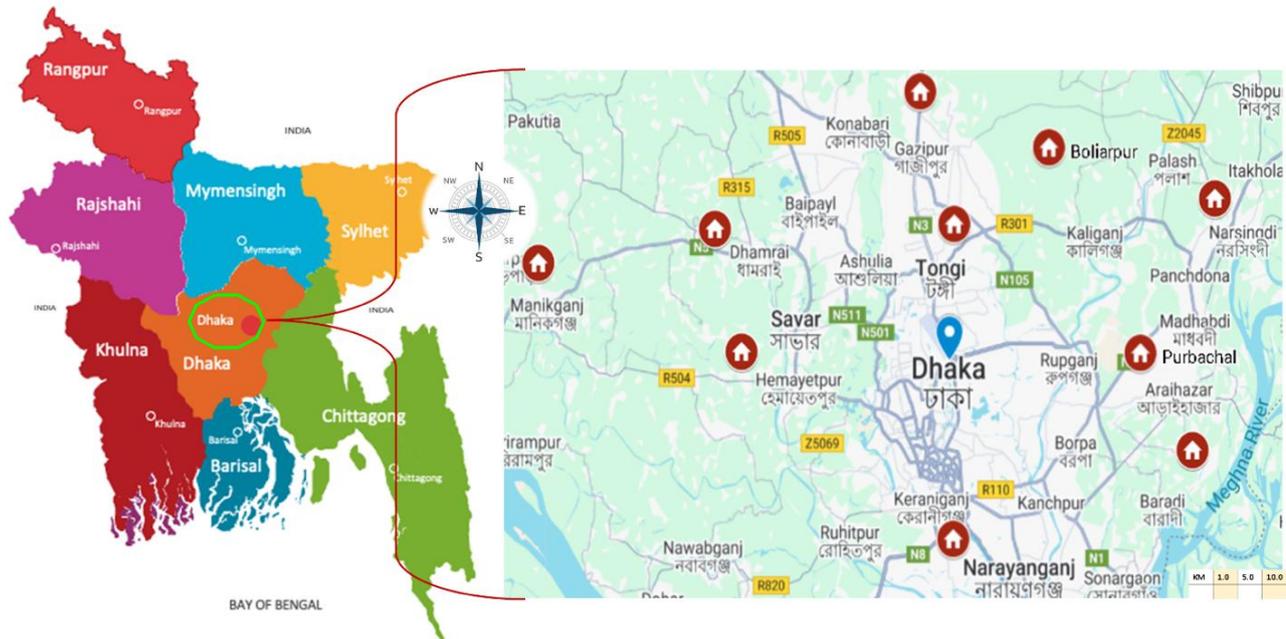


Figure 1 Left: Map of Bangladesh showing the capital Dhaka city. Right: Geographic distribution of selected smallholder poultry farm study sites in peri-urban surrounding Dhaka within a 50 km radius. Red house icons indicate farm locations; Dhaka is marked with a blue pin. Major roads and rivers are labeled for orientation. Scale bar and directional arrows are included for spatial reference.

Table 1 Demographic and poultry profile of study locations around Dhaka, Bangladesh

Location	Demography type	Distance (km) and direction from Dhaka	Poultry Farm Profile
Savar	Peri-urban, industrial, agricultural	~28 W	High poultry density; feed trading hub
Keraniganj	Urban-industrial, peri-urban farming	~25 S	Numerous hatcheries, feed mills, large poultry clusters
Gazipur	Urban-industrial, peri-urban farming	~35 N	Poultry integrated with mixed farming
Dhamrai	Semi-urban, agricultural	~45 NW	Smallholder poultry farms, mixed agriculture
Tongi	Urban peri-urban interface	~23 N	Smallholder poultry near market corridors
Purbachal	Rapidly urbanizing peri-urban	~20 E	Emerging poultry clusters within new town area
Boliarpur	Semi-rural, agricultural	~25 NE	Smallholder poultry; mixed farming
Manikganj	Semi-rural, agricultural	~50 NW	Growing poultry clusters, Smallholder poultry supporting regional markets
Narsingdi	Semi-rural, agricultural	~40 NE	Smallholder poultry; mixed agriculture
Araihazar	Peri-urban, rapidly urbanizing	~35 SE	Growing poultry clusters, Poultry farms integrated into food supply chains

Demographic classifications and poultry farm profiles were compiled through field surveys and local government records. Distances and directions are approximate and measured from central Dhaka. Poultry density, farm types, and integration levels reflect observed production characteristics during the study period.

holder commercial farms and ensuring equal spatial coverage across production systems. Five chickens were selected from each farm using simple random sampling, yielding ten birds per location and an overall sample size of 100 chickens (50 broilers and 50 layers). Farm selection was based on accessibility, willingness to participate, and the representation of typical smallholder commercial operations. Following humane sacrifice under veterinary supervision, the cecal contents were aseptically collected using sterile instruments. Each sample (~1 g) was transferred into pre-labeled sterile containers containing peptone water and transported under cold chain conditions to the Laboratory of Medicine and Public Health, Sher e Bangla Agricultural University (SAU) within three hours of collection. The samples were then processed for bacteriological analysis following standard protocols.

Bacterial isolation and identification

Isolation and identification of bacterial strains were conducted following standard procedures described by Biswas *et al.* (2020). Briefly, samples were homogenized and serially diluted (10^{-1} to 10^{-7}) using sterile distilled water. Aliquots (1.0 ml) from dilutions 10^{-5} , 10^{-6} , and 10^{-7} were plated on nutrient agar and selective/differential media including *Salmonella-Shigella* agar, Eosin Methylene Blue agar, MacConkey agar, Mannitol Salt agar, and Cysteine Lactose Electrolyte Deficient agar. Plates were incubated aerobically at 37°C for 24 hours. Colonies were examined morphologically and subcultured for purity. Presumptive identification was performed using standard biochemical tests as described by Cheesbrough (2006), including catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, and triple sugar iron tests.

Antibiotic susceptibility testing

The antimicrobial susceptibility of all isolates was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. The following 12 antibiotics were tested: Amoxicillin (30 µg), Azithromycin (15 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Cloxacillin (5 µg), Cotrimoxazole (25 µg), Erythromycin (10 µg), Gentamicin (20 µg), Ofloxacin (30 µg), Streptomycin (10 µg), and Tetracycline (30 µg). The antibiotic panel was selected to include drugs commonly used in poultry pro-

duction in Bangladesh, along with critically important antimicrobials for human medicine, to capture both farm-level exposure and public health. The zones of inhibition were measured and interpreted as susceptible, intermediate, or resistant.

Data analysis

Descriptive statistics were used to summarize the bacterial counts and isolate frequencies. Inferential analyses included: Paired t-tests to compare bacterial loads and isolate counts between broiler and layer samples; One-way ANOVA with Tukey's HSD post-hoc test to assess location-wise variation; Chi-square tests to evaluate differences in species distribution and MDR prevalence across locations. Ninety-five percent confidence intervals (CIs) for proportions were calculated using the Wilson score method. Differences in MDR prevalence were assessed using Pearson's χ^2 test with continuity correction; and odds ratios and 95% CIs were computed. MAR indices were compared using independent samples t-test or one-way ANOVA followed by Tukey's HSD post-hoc test. The MAR index was calculated for each isolate using the formula: MAR Index=Number of antibiotics to which the isolate is resistant/Total number of antibiotics tested. Isolates resistant to ≥ 3 antibiotic classes were classified as MDR. Statistical significance was set at $p \leq 0.05$. All analyses were performed using the IBM SPSS Statistics (software Version 29.0; IBM Corp., Armonk, NY, USA).

Quality control

E. coli and *Staphylococcus aureus* strains of poultry origin, previously characterized and validated against ATCC 25922 and ATCC 25923 respectively, were included as quality-control organisms in every run to ensure reproducibility according to CLSI (2020) guidelines.

Results

Total bacterial counts in broiler and layer farms

The HI Across all locations, broiler farms consistently exhibited higher bacterial loads than layer farms (Table 2). The highest broiler count was observed in Purbachal (10.8 ± 1.6 cfu/g $\times 10^7$), while the lowest was in Arahajar (9.7 ± 1.5 cfu/g $\times 10^7$). Layer farms showed lower counts overall, ranging from 7.1 ± 1.9 to 8.3 ± 1.6 cfu/g $\times 10^7$. A paired t-test

Table 2 Total bacterial count in chickens from smallholder farms across ten locations around Dhaka, Bangladesh

Sample locations	Bacterial count (cfu/g) $\times 10^7$	
	Broilers	Layers
Savar	10.5 \pm 1.5	8.3 \pm 1.6 ^a
Keraniganj	9.8 \pm 1.2	7.9 \pm 1.2 ^{ab}
Gazipur	9.9 \pm 1.2	8.2 \pm 1.4 ^a
Dhamrai	10.4 \pm 1.9	7.5 \pm 1.1 ^b
Tongi	10.1 \pm 1.6	7.1 \pm 1.9 ^b
Purbachal	10.8 \pm 1.6	7.7 \pm 1.2 ^{ab}
Boliarpur	9.9 \pm 1.8	8.2 \pm 1.1 ^a
Manikganj	10.2 \pm 1.7	7.8 \pm 1.9 ^{ab}
Narsingdi	9.9 \pm 1.2	8.1 \pm 1.2 ^a
Arahajar	9.7 \pm 1.5	8.3 \pm 1.4 ^a

The values represent mean bacterial counts (cfu/g $\times 10^7$) \pm SEM based on triplicate measurements. Different superscript letters within a column indicate statistically significant differences among locations ($p < 0.05$, Tukey's HSD test).

confirmed that the bacterial load was significantly higher in broilers (10.12 ± 0.35) than in layers (7.91 ± 0.39) [$t(9)=9.12$, $p<0.001$, Cohen's $d=2.89$]. For broiler farms, one-way ANOVA indicated no significant variation in bacterial counts across locations [$F(9,20)=1.23$, $p=0.32$]. For layer farms, ANOVA revealed significant variation across locations [$F(9,20)=2.87$, $p=0.03$, $\eta^2=0.56$]. Tukey's HSD identified two statistically distinct groups, as indicated by superscripts in **Table 2**: Savar, Arai-hajar, Gazipur, Boliarpur, and Narsingdi formed the higher-count group ("a"), while Tongi and Dhamrai formed the lower-count group ("b").

Distribution of bacteria across different sample locations and farm types

A total of 270 isolates were recovered from 100 samples, with location-wise distribution presented in **Table 3**. Broiler farms yielded significantly more isolates (151; 55.9%) than layer farms (119; 44.1%) [$t(9)=2.31$, $p=0.04$, Cohen's $d=0.73$], indicating a moderate effect size for the difference in isolate burden between production types. Isolate counts varied significantly across locations [$\chi^2(9)=21.67$, $p<0.01$; $F(9,90)=3.12$, $p<0.01$, $\eta^2=0.24$], suggesting that approximately 24% of the variance in isolate counts was attributable to location.

Narsingdi, Arai-hajar, and Savar contributed the highest proportion of isolates, and these spatial differences indicated clustering of bacterial burden in specific peri-urban zones.

Species composition and spatial distribution of bacterial isolates

The species composition of bacterial isolates recovered from ten study locations is illustrated in **Figure 2**. *E. coli* was the predominant organism (151 isolates; 55.9%), followed by *Salmonella* spp. (45 isolates; 16.7%) and *Clostridium* spp. (18 isolates; 6.7%). The remaining 56 isolates (20.74%) comprised a diverse group of enteric bacteria, including *Shigella* spp. (5.6%), *Enterobacter* spp. (5.2%), *Pseudomonas* spp. (3.7%), *Micrococcus* spp. (3.7%), and *Serratia* spp. (2.6%). The distribution of species varied across locations, with *E. coli* and *Salmonella* spp. being present at all ten sites. The proportion of *E. coli* isolates was highest in Savar (59.4%) and Narsingdi (58.8%), whereas *Salmonella* spp. were most frequently recovered in Manikganj (20.7%) and Arai-hajar (21.9%). Broiler samples contributed a greater share of *E. coli* (58.3%) and *Salmonella* spp. (60.0%) compared to layer samples. The remaining species were more evenly distributed between the farm types, although *Clostridium* spp. and *Shigella* spp. were slightly more prevalent in broiler samples. Chi square analysis

Table 3 Distribution of bacterial isolates by poultry type from smallholder farms across ten locations around Dhaka, Bangladesh

Sample Location	Number of Samples Collected (5 broiler + 5 layer)	Broiler Isolates (n, %)	Layer Isolates (n, %)	Total Isolates (n, %)
Savar	10	18 (6.67%)	14 (5.19%)	32 (11.85%)
Keraniganj	10	14 (5.19%)	11 (4.07%)	25 (9.26%)
Gazipur	10	13 (4.81%)	11 (4.07%)	24 (8.89%)
Dhamrai	10	15 (5.56%)	11 (4.07%)	26 (9.63%)
Tongi	10	10 (3.70%)	7 (2.59%)	17 (6.30%)
Purbachal	10	15 (5.56%)	12 (4.44%)	27 (10.00%)
Boliarpur	10	13 (4.81%)	11 (4.07%)	24 (8.89%)
Manikganj	10	16 (5.93%)	13 (4.81%)	29 (10.74%)
Narsingdi	10	19 (7.04%)	15 (5.56%)	34 (12.59%)
Arai-hajar	10	18 (6.67%)	14 (5.19%)	32 (11.85%)
Total	100	151 (55.93%)	119 (44.07%)	270 (100.00%)

The values represent the number and percentage of bacterial isolates recovered from each location.

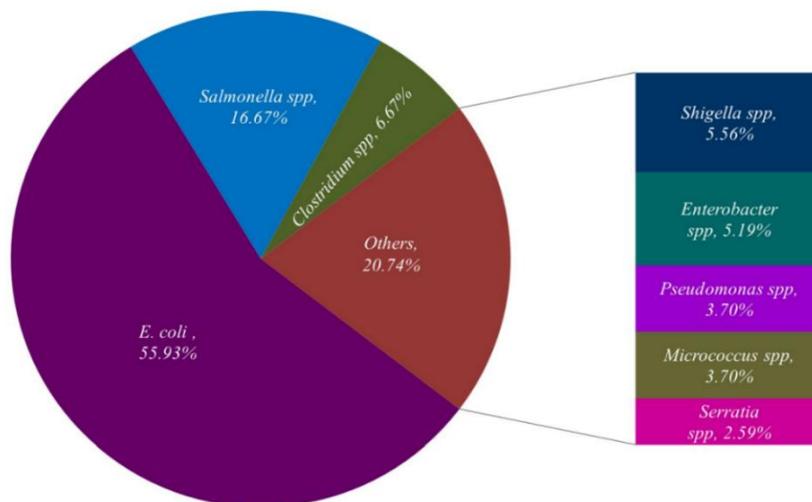


Figure 2 Species composition of bacterial strains isolated from poultry cecum in smallholder farms in peri-urban surrounding Dhaka, Bangladesh

confirmed that species distribution differed significantly across locations $\chi^2(18)=24.83$, $p<0.04$, with *E. coli* and *Salmonella* spp. showing the most pronounced geographic clustering.

AMR profiles of isolated strains

The phenotypic resistance patterns of all isolates are presented in **Figure 3**. Resistance was highest to Amoxicillin (93.3%), followed by Streptomycin (52.6%) and Tetracycline (46.3%). Moderate resistance was observed to Ciprofloxacin (44.4%) and Gentamicin (36.7%), while Ceftriaxone exhibited the lowest resistance (6.7%). Broiler-derived isolates consistently showed higher resistance proportions than layer-derived isolates, and this difference was statistically significant [$t(268)=4.12$, $p<0.001$, Cohen’s $d=0.50$], indicating a medium effect size. These patterns highlight the substantial AMR pressure across both production systems.

MDR prevalence

A total of 193 out of 270 isolates (71.5%, 95% CI: 65.9–76.7%) were MDR (resistant to ≥ 3 antibiotic classes). *E. coli* exhibited the highest MDR prevalence (118/151, 78.1%, 95% CI: 71.3–84.3%), followed by *Clostridium* spp. (13/18, 72.2%, 95% CI: 49.1–87.5%), *Salmonella* spp. (30/45, 66.7%, 95% CI: 52.0–78.9%), and the remaining genera combined (“Others”; 32/56, 57.1%, 95% CI: 44.1–69.2%) (**Figure 4**). Broiler-derived isolates had a significantly higher MDR prevalence (78.8%) than layer-derived isolates (62.2%) [$\chi^2(1)=11.42$, $p<0.001$; OR=2.26, 95% CI: 1.39–3.67], indicating that broiler isolates were more than twice as likely to be MDR. These findings demonstrate a substantial MDR burden in the peri-urban poultry sector.

MAR index

MAR index values ranged from 0.14 to 0.71, with an overall mean of 0.36 ± 0.12 , as shown in **Table 4**. A total of 81.1% of isolates had MAR indices above the critical threshold of

0.2, indicating frequent exposure to multiple antibiotics. Broiler-derived isolates had significantly higher MAR indices (0.41 ± 0.11) than layer-derived isolates (0.30 ± 0.09) [$t(268)=6.84$, $p<0.001$, Cohen’s $d=1.09$], representing a large effect size. These results suggest that broiler production environments may exert a greater antimicrobial selection pressure.

Spatial distribution of isolates and resistance burden

Table 4 summarizes isolate counts, MDR proportions, and MAR index values by location and farm type. Isolate counts varied across the ten locations, ranging from 17 to 34 per location (mean 27 ± 4.7), although this variation was not statistically significant [$\chi^2(9)=8.37$, $p<0.497$]. Location-wise analysis revealed that isolates from Narsingdi had the highest MAR index (mean= 0.44 ± 0.10), followed by Savar (0.42 ± 0.11) and Arai-hajar (0.41 ± 0.12). The lowest MAR indices were observed in Tongi (0.28 ± 0.09) and Gazipur (0.30 ± 0.08). These differences in MAR index were statistically significant [$F(9,260)=4.12$, $p<0.001$, $\eta^2=0.13$], indicating that 13% of the variance in MAR index was attributable to location. The proportion of MDR isolates also varied significantly across locations [$\chi^2(9)=18.76$, $p<0.027$], with the highest burden in Narsingdi (85.3%) and the lowest in Tongi (52.9%). Post-hoc Tukey HSD tests identified Narsingdi, Savar, and Arai-hazar as high-risk zones with elevated MAR indices (means 0.48–0.52, $p<0.05$ compared to overall mean) and higher MDR proportions (85.3%, 80.6%, and 78.1%, respectively). **Figure 5** illustrates the spatial distribution of resistance burden, overlaying MDR prevalence (bars) and MAR index values (line graph) across the ten study sites.

Discussion

AMR in livestock animal production systems remains a critical global health challenge, with profound implications for food safety, environmental integrity, and public health.

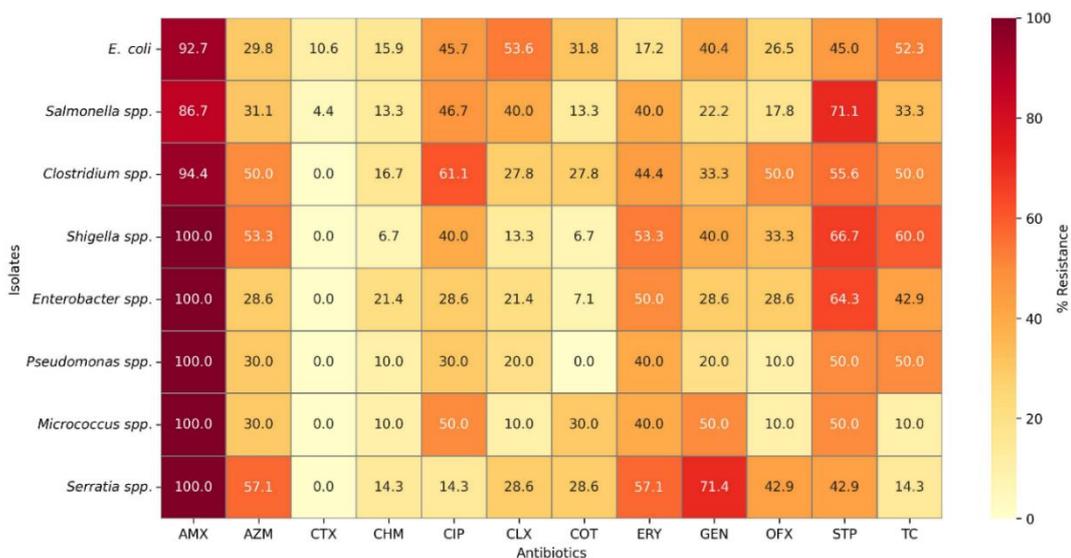


Figure 3 Phenotypic antibiotic resistance patterns of bacterial isolates recovered from poultry ceca across ten smallholder farms near Dhaka. The color bar represents the percentage of resistance, ranging from yellow (low resistance) to dark-red (high resistance). The values annotated within each cell of the heatmap correspond to the exact percentage of resistance of a specific isolate to a particular antibiotic. The x-axis and y-labels represent the different antibiotics tested and bacterial isolates, respectively.

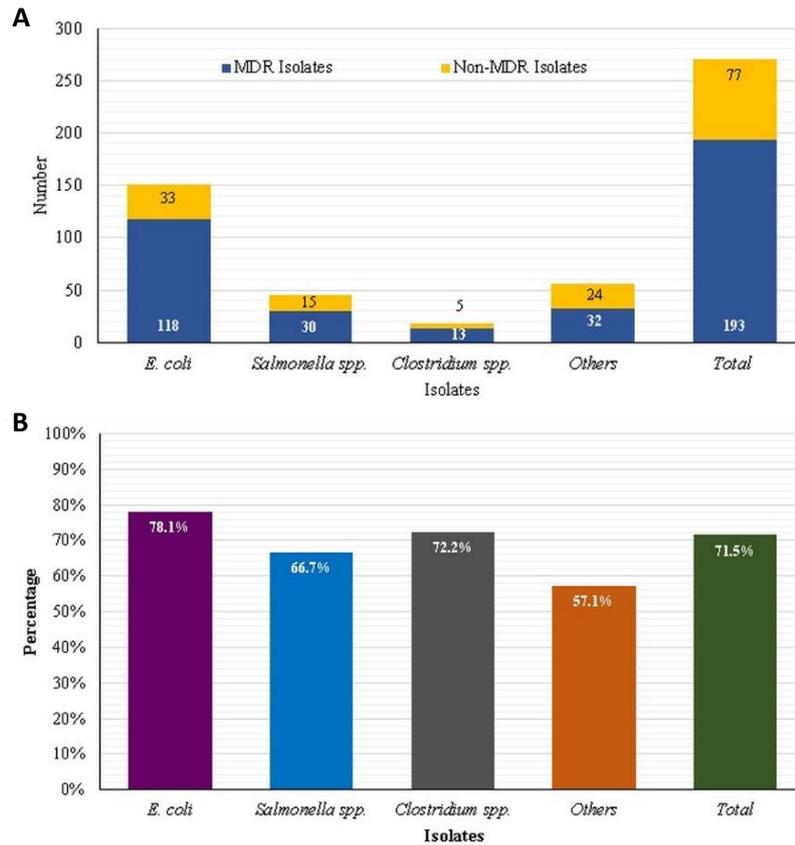


Figure 4 (A) Distribution of MDR and non-MDR bacterial isolates among *E. coli*, *Salmonella spp.*, *Clostridium spp.*, and other enteric species recovered from smallholder poultry farms. (B) MDR prevalence (%) across the same groups.

Table 4 MDR burden and MAR index by farm type and sample location around Dhaka, Bangladesh

Category	Group/Location	No. of Isolates	MDR Isolates (%)	MAR Index (Mean ± SEM)
Farm Type	Broiler	151	78.8	0.41 ± 0.11 ^a
	Layer	119	62.2	0.30 ± 0.09 ^b
Location	Savar	32	81.3	0.42 ± 0.11 ^a
	Keraniganj	25	72.0	0.35 ± 0.08 ^b
	Gazipur	24	62.5	0.30 ± 0.08 ^c
	Dhamrai	26	73.1	0.36 ± 0.09 ^b
	Tongi	17	52.9	0.28 ± 0.09 ^c
	Purbachal	27	74.1	0.37 ± 0.11 ^b
	Boliarpur	24	70.8	0.34 ± 0.09 ^b
	Manikganj	29	75.9	0.38 ± 0.10 ^b
	Narsingdi	34	85.3	0.44 ± 0.10 ^a
	Araihajar	32	78.1	0.41 ± 0.12 ^a

The values represent MDR proportions and mean MAR index ± SEM for each farm type and location. Different superscript letters indicate statistically significant differences in MAR index values across groups ($p < 0.05$, Tukey's HSD).

This study provides a location-wise assessment of bacterial burden and resistance patterns in smallholder poultry farms surrounding Dhaka, Bangladesh, revealing substantial MDR and elevated MAR indices across both broiler and layer operations. These findings highlight the interconnected nature of poultry management practices, antimicrobial exposure, and environmental dissemination of resistant bacteria within the One Health Framework.

Building on existing evidence, this work advances the national AMR knowledge base by incorporating farm type stratification together with spatial clustering analysis and MAR based quantification of resistance risk. The combined use of

these approaches offers a more granular understanding of geographic AMR patterns and production linked risk gradients in peri urban poultry systems; an analytical perspective that has been largely absent from previous studies in Bangladesh. This integrated framework supports more targeted interpretation of resistance dynamics and provides a stronger foundation for policy design and surveillance planning.

Broiler farms consistently exhibited higher bacterial loads, MDR prevalence, and MAR indices than layer farms, reflecting short production cycles, high stocking densities, and frequent antimicrobial use for prophylaxis or growth promotion. Such conditions create selective pressure favoring resistant enteric

Multidrug resistance in poultry-origin enteric pathogens

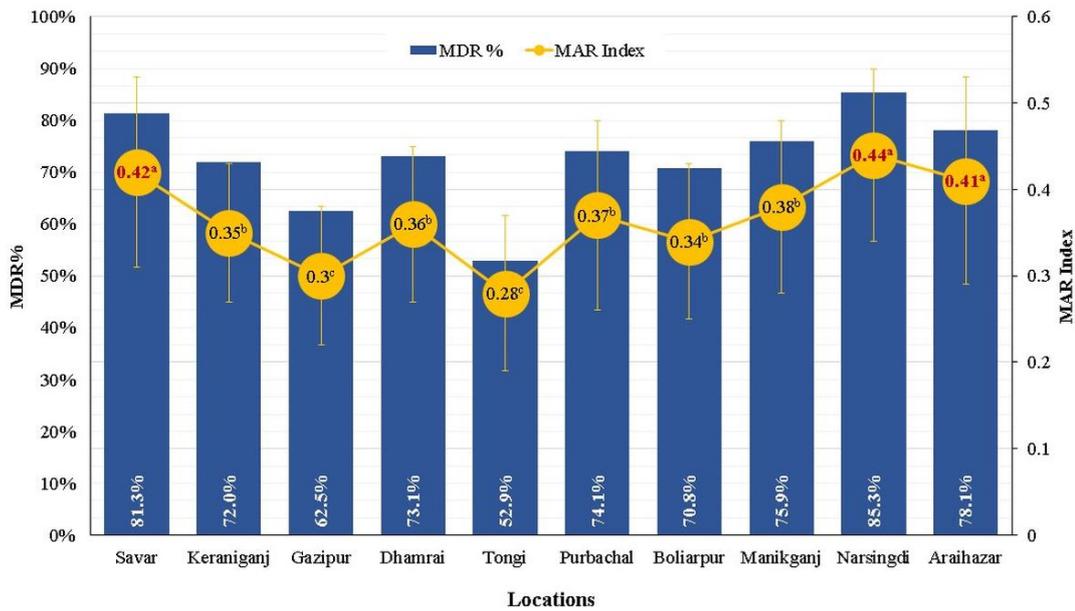


Figure 5 Location-wise comparison of MDR prevalence and MAR Index among bacterial isolates from smallholder poultry farms surrounding Dhaka. Bars represent MDR prevalence (%), and the line graph shows mean MAR Index \pm SEM. Superscript letters indicate statistically significant differences ($p < 0.05$) based on Tukey's HSD post-hoc test.

bacteria, particularly *E. coli* and *Salmonella* spp., which dominate the isolate pool (Amjad *et al.*, 2005; Alo & Ojo, 2007). These patterns align with reports identifying intensive broiler systems as AMR hotspots in LMICs (Van Boeckel *et al.*, 2019). In contrast, lower bacterial counts and greater spatial variability in layer farms may be attributed to differences in management intensity, antimicrobial exposure, and housing conditions.

The uniformity of bacterial load across broiler farms suggests broadly similar husbandry practices, feed sources, and antibiotic use patterns among peri-urban producers in Bangladesh. Spatial variation in layer farms may reflect differences in biosecurity, age related immunity, and hygiene. High bacterial loads in broiler operations have important One Health implications, as fecal waste frequently contaminates soil, surface water, and agricultural fields, facilitating environmental dissemination of resistant organisms and resistance genes (Caprioli *et al.*, 2000; Pei *et al.*, 2006).

The dominance of *E. coli* and *Salmonella* spp. among the isolates aligns with their known ecological roles as commensal and pathogenic bacteria in the poultry gut and their frequent use as sentinel organisms in AMR surveillance (Kolář *et al.*, 2002; Adegunloye, 2006). The presence of *Clostridium*, *Shigella*, *Enterobacter*, *Pseudomonas*, *Micrococcus*, and *Serratia* spp. indicates a diverse microbial community influenced by fecal contamination, environmental persistence, and interspecies transmission dynamics (Ajayi & Egbebi, 2011; Omojowo & Omojola, 2013). The widespread detection of *E. coli* and *Salmonella* across all locations supports earlier findings from Bangladesh documenting these genera as dominant carriers of resistance genes in live birds and market meat (Islam *et al.*, 2016; Karim *et al.*, 2025). Higher isolate recovery in Narsingdi, Savar, and Arahazar may be attributed to hygiene conditions, water quality, litter reuse, and antibiotic procurement. These peri-urban hubs host dense poultry and feed trading networks with limited regulatory oversight, facilitat-

ing pathogen exchange and resistance dissemination. Their clustering suggests they function as AMR hotspots where animal, environmental, and human health interfaces are tightly interlinked.

The resistance patterns observed in this study reflect the widespread and largely unregulated antimicrobial use in smallholder poultry systems. High resistance to Amoxicillin, Streptomycin, Tetracycline, Ciprofloxacin, and Cloxacillin mirrors the commonly used antimicrobials in Bangladeshi farms, where over the counter access and limited veterinary oversight drive indiscriminate use (Islam *et al.*, 2016). These findings align with previous studies indicating high antimicrobial consumption in poultry, often without adherence to withdrawal periods or dosage guidelines (WHO-FAO-OIE Tripartite Report, 2022; Islam *et al.*, 2020). The relatively lower resistance to Ceftriaxone and Chloramphenicol likely reflects restricted availability and higher cost, resulting in reduced selection pressure. High resistance among *E. coli* is particularly concerning, given its capacity for horizontal gene transfer and its role as a donor of plasmid-mediated resistance to both commensal and pathogenic bacteria in humans and animals (Kolář *et al.*, 2002; Adegunloye, 2006).

The MAR index findings further reinforce the high risk nature of the study sites, indicating frequent exposure to multiple antimicrobials (Raghunath, 2008; Apata, 2009). Broiler isolates exhibited significantly higher MAR values than layer isolates reflecting differences in production intensity, antibiotic exposure, and microbial ecology between the two systems (Donoghue, 2003; Doyle, 2006). Similar trends were reported in *E. coli* and *Salmonella* isolates from retail chicken meat and live bird markets in Dhaka (Islam *et al.*, 2025; Karim *et al.*, 2025), reinforcing the continuity of resistance from farm to market.

Spatially, the highest MAR indices and MDR burdens were observed in Narsingdi, Savar, and Arahazar; locations characterized by dense poultry production and suboptimal waste

management. These results are consistent with the geographic clustering of total bacterial counts and isolate frequencies, suggesting a convergence of factors such as antibiotic overuse, inadequate biosecurity, and environmental cross-contamination. These patterns reflect the association between environmental antibiotic exposure and MAR elevation reported in aquatic and soil ecosystems (Schwartz *et al.*, 2003; Amy *et al.*, 2006). The presence of resistant bacteria in poultry feces, if used untreated as fertilizer or feed in aquaculture, can perpetuate environmental cycling of AMR genes, exacerbating public health risks.

The patterns observed in this study closely mirror global trends in which intensive poultry systems are recognized as important sources of MDR pathogens (Caprioli *et al.*, 2000). Detection of MDR *E. coli* and *Salmonella* spp. raises direct public health concerns, as these organisms can enter the human population through contaminated meat, occupational exposure, and informal wet market handling. In Bangladesh, where live bird markets and household slaughtering are common, the risk of zoonotic spillover is amplified, and resistant bacteria have been documented in retail poultry meat and market surfaces (Sarker *et al.*, 2019; Al Masud *et al.*, 2020). These findings emphasize the need to integrate poultry AMR surveillance with environmental and human health.

Spatial identification of high risk zones can guide targeted interventions, including antimicrobial stewardship training, improved manure management, and wastewater treatment. Adoption of the WHO GLASS framework would further support coordinated data sharing and evidence based policymaking aligned with the One Health approach (WHO, 2015). Although this study focused on poultry isolates, the high MDR and MAR indices observed in broiler farms indicate potential spillover risks to humans and the environment, reinforcing the importance of integrated One Health surveillance.

Taken together, these findings offer important insights into the dynamics of AMR in peri-urban poultry systems in Bangladesh. However, several methodological considerations must be acknowledged. First, the sample size of 100 birds across ten locations, may limit the generalizability of the findings to broader regional or national contexts. Second, farm selection was based on accessibility and willingness to participate, and detailed antibiotic use histories were not available for all sites. This introduces potential selection bias, as farms with different antimicrobial practices or biosecurity levels may have been underrepresented. Third, the cross-sectional design captured resistance patterns at a single time point, limiting causal inference regarding antimicrobial use and resistance emergence. These constraints should be considered when interpreting the findings and designing future research.

Conclusion

This study presents the first integrated assessment of phenotypic AMR, MAR indices, and MDR prevalence among enteric pathogens from smallholder poultry farms in peri-urban Bangladesh. The high AMR and MDR rates, coupled with MAR indices above the risk threshold, confirm substantial an-

tibiotic exposure and identify poultry farms as important reservoirs of resistant bacteria. These findings provide a practical framework for identifying high-risk zones and guiding targeted interventions. They emphasized the urgency of coordinated AMR surveillance, rational antibiotic use, and strengthened farm-level stewardship under a One Health approach.

The results can directly inform Bangladesh's national AMR action plan by supporting the regulatory control of veterinary antibiotics, prioritizing stewardship programs in high-burden areas, and integrating poultry surveillance with environmental and human health monitoring. Future research should include molecular characterization of resistance genes, plasmid and genomic analyses, and longitudinal monitoring to clarify transmission dynamics. Incorporating environmental sampling of water, soil, and market surfaces will further improve our understanding of AMR dissemination and support evidence-based policymaking across sectors.

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Author contribution KBMSI: Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Visualization; Writing original draft; Writing review & editing; Supervision; Project administration. SSUM2: Literature review; Formal analysis; Writing original draft; MRK: Investigation; Data curation; Resources. MKI: Investigation; Data curation; Resources. AZ1: Investigation; Data curation; Resources. AZ3: Literature review; Writing review & editing. SSUM4: Literature review; Writing review & editing. FT: Formal analysis; Writing original draft; Writing review & editing. All authors: Validation; Writing review & editing; Approval of the final manuscript.

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