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Distribution, Antimicrobial Resistance Profiles, and Biofilm-Forming Capacity of Gram-Negative Bacteria Isolated from Poultry Wholesale Markets in Dhaka, Bangladesh

Sabbir Hossain[1*], Md Atiqur Rahaman[2], Ariba Siddique[1], Emon Hawlader[1], Sumaiya Akter[1], Shahida Parvin Roja[1], Mst. Jannatul Ferdousi[1], Jannatul Ferdous Sadia[1], Mst. Mim Akter[1], Md. Fatha Ahmmed Mahi[1], Kamrul Hasan[1], Md Rabby Shekh[1]

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ABSTRACT

Live-bird wholesale markets in Dhaka often contain poultry, people, and waste, making them a hotspot for antimicrobial-resistant bacteria. However, resistance and biofilm formation of non-indicator Gram-negative genera are rarely studied together. This exploratory study sampled three Dhaka poultry wholesale markets. Three samples from each of six sources (eggs, water, feces, cloacal swabs, air, and feed) per market (9–23 July 2026) yielded 119 isolates. Disk diffusion (CLSI M100) assessed susceptibility to nine antibiotics, and MDR, XDR, and MAR were computed. Biofilm was screened in all isolates by the tube method and quantified in tube-positive isolates by microtiter plate assay. Seven taxa were identified, with *E. coli* (31.9%), *Klebsiella pneumoniae* (18.5%) and *Pseudomonas aeruginosa* (15.1%) being most frequent (isolate-level, not sample prevalence). Resistance was highest to ampicillin (97.5%) and lowest to meropenem (10.1%); 117 isolates (98.3%) were MDR when intrinsic resistance was included, 55 (46.2%) were XDR, and the median MAR index was 0.67. Biofilm formation was detected in 53 (44.5%) of the isolates (probably underestimated), with 24 of the 25 strong biofilms being formed by *K. pneumoniae*, *P. aeruginosa* and *A. baumannii*. Strong biofilm was more common in XDR than in non-XDR MDR isolates (34.5% vs 9.7%, Holm-adjusted $p = 0.006$); this was mainly taxon-driven. Among these isolates, *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* were most often XDR and strong biofilm producers. This study was limited by its inclusion of only three markets, reliance on phenotypic identification, and possible overestimation of MDR/XDR prevalence. Surveillance should include non-indicator genera, molecular confirmation and biofilm testing, with market sanitation and judicious antimicrobial use.

INTRODUCTION

Wholesale live-bird markets are key hubs in the poultry value chain, one of Bangladesh's fastest-growing livestock sectors. Birds are collected from various districts and kept in small cages until they are sold to retailers, restaurants, and supermarkets, and holding, slaughter, and selling are not always separated in Dhaka (Parvin *et al.*, 2022). This means workers, customers, and the community are in close contact with the animals and their waste, and that blood, wash water, and droppings are often flushed into drains that flow into nearby waterways (Parvin *et al.*, 2022). A single market can therefore gather bacteria from many farms and spread them into food products and the urban environment.

This matters because antimicrobial resistance (AMR) is already a major global health threat. In 2019, bacterial AMR was associated with 4.95 million deaths, of which 1.27 million were directly attributable to resistance (Murray *et al.*, 2022). In 2021, bacterial AMR was associated with 4.71 million deaths, of which 1.14 million were attributable to resistance, and it is projected to cause 1.91 million attributable deaths annually by 2050 (Naghavi *et al.*, 2024). Carbapenem-resistant *Acinetobacter baumannii* and third-generation cephalosporin- or carbapenem-resistant *Enterobacter*ales are included in the critical-priority group in the World Health Organization (WHO) priority list 2024 (WHO, 2024). Food animals contribute to this pressure: global antimicrobial use in food animals is expected to increase by approximately two-thirds between 2010 and 2030 (Van Boeckel *et al.*, 2015). High antimicrobial use, limited veterinary oversight, and poor biosecurity in low-resource poultry production systems promote the emergence of resistant bacteria (Hedman *et al.*, 2020).

The evidence from Dhaka indicates that the poultry trade may contribute to this problem. Multidrug resistance was detected in 93.2% of *Escherichia coli* isolates and in all *Salmonella* isolates from cloacal swabs in five wholesale chicken markets, and 97.7% of the *E. coli* isolates were extended-spectrum β-lactamases (ESBL) producers (Parvin *et al.*, 2022). In addition, azithromycin resistance was associated with *mphA* in 39 of 83 *E. coli* isolates collected from live bird markets in Dhaka, and third-generation cephalosporin resistance was associated with plasmid-borne *blaCTX-M-55*, *blaCTX-M-65*, or *blaDHA-1* in six isolates (Davies *et al.*, 2024). Multidrug-resistant *E. coli* were common in cloacal swabs of live broilers (Al Azad *et al.*, 2019). These studies, however, have focused on a few indicator organisms, mainly *E. coli* and *Salmonella*. Other Gram-negative genera found in the poultry gut and market environment, such as *Klebsiella*, *Enterobacter*, *Proteus*, and *Pseudomonas*, may also harbor resistance genes

[1] Department of Microbiology, Primeasia University, Dhaka-1213, Bangladesh
[2] Department of Immunology, World Health Organization, Dhaka, Bangladesh
[*] Corresponding author's e-mail: rifatmia10978@gmail.com

and cause opportunistic infections but have largely been ignored in Dhaka's poultry markets, to our knowledge. Without this broader context, surveillance could underestimate the pool of resistance genes that could be transferred to bacteria more commonly associated with human infections.

Another gap concerns biofilm formation. A biofilm is a community of bacteria attached to a surface and surrounded by a self-generated matrix, and bacteria living this way are much more resistant to antimicrobials and environmental stresses (Donlan & Costerton, 2002; Hall-Stoodley *et al*., 2004). Bacteria can withstand routine cleaning, spread from carcasses to equipment and between surfaces, and share resistance determinants on wet, nutrient-rich, infrequently cleaned surfaces like market surfaces, cages, drains, and wash-water containers (Galié *et al*., 2018). Resistance and biofilm capacity are therefore a greater concern than resistance alone, but the two traits are hardly ever reported together for Gram-negative bacteria from poultry markets in Bangladesh, so it remains to be seen whether the two traits are linked.

We isolated Gram-negative bacteria from poultry wholesale markets of Dhaka and identified them by cultural and biochemical methods. We determined their antimicrobial resistance by standardized disk diffusion; multidrug resistance was defined according to international consensus (Magiorakos *et al*., 2012), and biofilm-forming ability was determined by the tube method and microtiter plate assay. This study aimed to characterize the occurrence, antimicrobial resistance, and biofilm-forming ability of Gram-negative bacteria and assess whether antimicrobial resistance was associated with biofilm-forming ability, thereby establishing a baseline for risk assessment and future surveillance in this segment of the poultry supply chain.

MATERIALS AND METHODS

Study Sites and Sample Collection

This study was conducted at three poultry wholesale markets in Dhaka, Bangladesh: Kaptan Bazar, Karwan Bazar, and Tejgaon. These were selected because they are among the largest poultry wholesale markets in Dhaka and attract very large numbers of buyers; besides wholesale trade, some retailers and occasional individual customers also buy poultry there at lower prices, so the markets are crowded and bird–human contact is extensive. Sampling these three major markets was therefore considered sufficient to give a baseline picture of the wholesale poultry trade of Dhaka city. Sampling was conducted from 9 to 23 July 2026. A total of 54 samples were collected, comprising three samples from each of six sources (eggs, water, feces, cloacal swabs, air, and poultry feed) at each market, yielding 18 samples per market. This balanced design (three samples × six sources × three markets) was chosen to represent the main bird-associated and environmental sources in each market. No formal sample-size calculation was performed because the study was designed as an exploratory baseline survey and not to estimate prevalence. Eggs and cloacal swabs were sampled using sterile cotton swabs moistened with sterile buffered peptone water (BPW); approximately 1 g of feces or feed was collected in sterile containers; and 100 mL of water was collected in sterile containers. Passive air sampling was performed by exposing open MacConkey agar plates to ambient air for 30 min at approximately 1 m above ground level. All samples were labeled, kept in an insulated box with ice packs (2–8 °C), and brought to the laboratory within 2 h. A total of 119 isolates were recovered from the 54 samples, with 46 from Kaptan Bazar, 43 from Tejgaon, and 30 from Karwan Bazar.

Ethical Approval

The Life Science and Veterinary Research Laboratory, Dhaka-1230, Bangladesh approved the study (approval ID: T/LSVR/EA/2026/020).

Sample Preparation

Swabs were added to 9 mL of BPW and vortexed for 30 s. One gram of feces or feed was added to 9 mL BPW, and 1 mL of well-mixed water was added to 9 mL BPW. All samples (with the exception of air-exposed plates) were pre-enriched at 37 °C for 18–24 hours. The samples were then serially diluted 10-fold in sterile 0.85% saline for plating. Air-exposed plates were incubated directly.

Isolation and Identification

Aliquots (100 µL) of the enriched cultures and dilutions were spread on MacConkey agar and eosin methylene blue (EMB) agar and incubated aerobically at 37 °C for 24 h. Colonies were differentiated by colony morphology and lactose fermentation and purified by streaking on nutrient agar. One colony of each distinct colony type was retained per sample; repeated colonies of the same type from the same sample were not kept. Gram-negative isolates were verified by Gram staining and were identified to genus or species level using standard biochemical tests: catalase, oxidase, triple sugar iron (TSI) agar, indole, methyl red, Voges-Proskauer, citrate utilization, urease and motility, which were interpreted using standard identification schemes (Barrow & Feltham, 1993). The final collection included 119 isolates representing 7 taxa (*Escherichia coli*, *Klebsiella* pneumoniae, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Salmonella* spp., *Proteus* mirabilis and *Enterobacter* cloacae). *Salmonella* isolates were only identified to genus level and not serotyped. Pure cultures were stored in tryptic soy broth with 20% glycerol at −80 °C until further analysis.

Antimicrobial Resistance Profiling

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method (Bauer *et al*., 1966) on Mueller–Hinton agar as per the CLSI guidelines (Mathers *et al*., 2026). Bacterial suspensions were adjusted to 0.5 McFarland turbidity and spread evenly on the agar surface. Antibiotic disks were placed on the plates and incubated for 16–18 hours at 35 ± 2 °C. Zone

diameters were measured and interpreted as susceptible, intermediate, or resistant based on the CLSI M100 (36th edition) breakpoints (Mathers *et al.*, 2026). The panel included nine antibiotics from nine antimicrobial classes: ampicillin (AMP, 10 µg), amoxicillin-clavulanate (AMC, 20/10 µg), ceftazidime (CAZ, 30 µg), meropenem (MEM, 10 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 10 µg), tetracycline (TET, 30 µg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), and chloramphenicol (CHL, 30 µg). To allow comparison, the same panel and interpretive criteria were applied to all taxa, including the non-*Enterobacter*ales (*P. aeruginosa* and *A. baumannii*), for which CLSI M100 does not provide disk breakpoints for some of these agents. Categories for these two taxa should therefore be regarded as provisional screening categories rather than clinical interpretations. *Escherichia coli* ATCC 25922 was used as the quality control strain. The isolates were categorized based on Magiorakos *et al.* (Magiorakos *et al.*, 2012), in which each antibiotic was considered as a different antimicrobial category. An isolate was considered multidrug-resistant (MDR) if it was resistant to one or more drugs in three or more categories, and extensively drug-resistant (XDR) if it was resistant to at least one agent in all but two or fewer of the nine categories. Pan-drug resistance was not assessed because the panel did not cover all antimicrobial categories; intermediate results were considered non-resistant, which is more conservative than the non-susceptible criterion of Magiorakos *et al.* Multiple Antibiotic Resistance (MAR) index was determined for each isolate by dividing the number of antibiotics to which the isolate was resistant by the number of antibiotics tested (nine), and a MAR index > 0.2 was considered to indicate a high-risk source of contamination (Krumperman, 1983). A sensitivity analysis was conducted by classifying intermediate results as non-susceptible. Intrinsic resistance was not excluded, so the MDR and XDR classifications include the intrinsic resistance of each taxon (Leclercq *et al.*, 2013).

Biofilm Formation Assays

Biofilm formation was qualitatively screened in all 119 isolates as described by Christensen *et al.* (Christensen *et al.*, 1982; Hassan *et al.*, 2011). A loopful of overnight culture was added to 10 mL of tryptic soy broth (TSB) in glass tubes and incubated at 37 °C for 24 h. The broth was removed, the tubes were washed with phosphate-buffered saline (PBS, pH 7.3) and dried, and then stained with 0.1% crystal violet for 15 min. The tubes were rinsed to remove excess stain and dried upside down. The presence of a visible film lining the wall and the bottom of the tube was scored on the usual 0 (absent/weak), 1 (moderate), and 2 (strong) scale (Mathur *et al.*, 2006). Isolates that scored 1 or 2 were considered tube-positive, and those that scored 0 were considered tube-negative. Every isolate was tested twice, and uninoculated TSB was used as a negative control.

To quantify biofilm formation, the crystal violet assay was performed with tube-positive isolates in 96-well flat-bottom polystyrene plates, as described by Stepanović *et al.* (Stepanović *et al.*, 2000; STEPANOVIĆ *et al.*, 2007). Overnight cultures were diluted 1:100 in TSB to ~106 CFU/mL, and 200 µL was added to each of three wells per isolate. Negative controls were wells with sterile TSB. After incubation at 37 °C for 24 h, wells were washed gently three times with sterile PBS to remove non-adherent cells, fixed with methanol for 15 min, and stained with 0.1% crystal violet for 15 min. The excess stain was washed off, the bound dye was dissolved in 33% glacial acetic acid, and absorbance was measured at 570 nm. The mean OD of the replicate wells was used for each isolate. The cut-off optical density (ODc) was taken as the mean OD of the negative control plus three standard deviations and was 0.094. Isolates were categorized as non-biofilm producers (OD ≤ ODc), weak (ODc < OD ≤ 2×ODc), moderate (2×ODc < OD ≤ 4×ODc) or strong biofilm producers (OD > 4×ODc) (Stepanović *et al.*, 2000; STEPANOVIĆ *et al.*, 2007). Tube-negative isolates were not tested further by microtiter assay and are listed as non-producers under this screening scheme.

Data Analysis

Data were entered in Microsoft Excel and analyzed in Python 3 (pandas, SciPy). Categorical data are presented as numbers and percentages at the isolate level (not sample-level prevalence), and MAR indices as medians and ranges. Relationships between resistance to different antibiotics were summarized using pairwise phi correlation coefficients. Proportions between two groups were compared using Fisher's exact test, and MAR indices across groups using the Kruskal–Wallis test (two-sided, $p < 0.05$). Because some taxon and source groups were small, only a limited set of comparisons was tested, and Holm adjustment was applied across all eight tests (two Kruskal–Wallis and six Fisher's exact). Because isolates from the same sample or market are not fully independent, p-values are interpreted as exploratory, and taxon-stratified analyses were used to limit confounding by taxon.

RESULTS AND DISCUSSION

Isolate Collection

A total of 119 Gram-negative isolates were analyzed. All percentages in this study are proportions of isolates (n = 119) and not sample-level prevalence. Seven taxa were identified (Figure 1): *Escherichia coli* (38 isolates, 31.9%), *Klebsiella* pneumoniae (22, 18.5%), *Pseudomonas aeruginosa* (18, 15.1%), *Acinetobacter baumannii* (15, 12.6%), *Salmonella* spp. (11, 9.2%), *Proteus* mirabilis (9, 7.6%), and *Enterobacter* cloacae (6, 5.0%). A total of 86 (72.3%) isolates were members of the order *Enterobacter*ales, and 33 (27.7%) were the two non-fermenting species, *P. aeruginosa* and *A. baumannii*. *Salmonella* isolates were only determined to the genus level. The isolates were recovered from 54 samples (three per source in each of the three markets) and came from Kaptan Bazar (46 isolates), Tejgaon (43), and Karwan Bazar (30) and from six sample sources: eggs

(37 isolates), water (20), feces (18), cloacal swabs (17), air (17), and feed (10). Most *Salmonella* isolates (9/11, 81.8%) came from eggs, whereas the largest share of *P. aeruginosa* isolates (8/18, 44.4%) came from air.

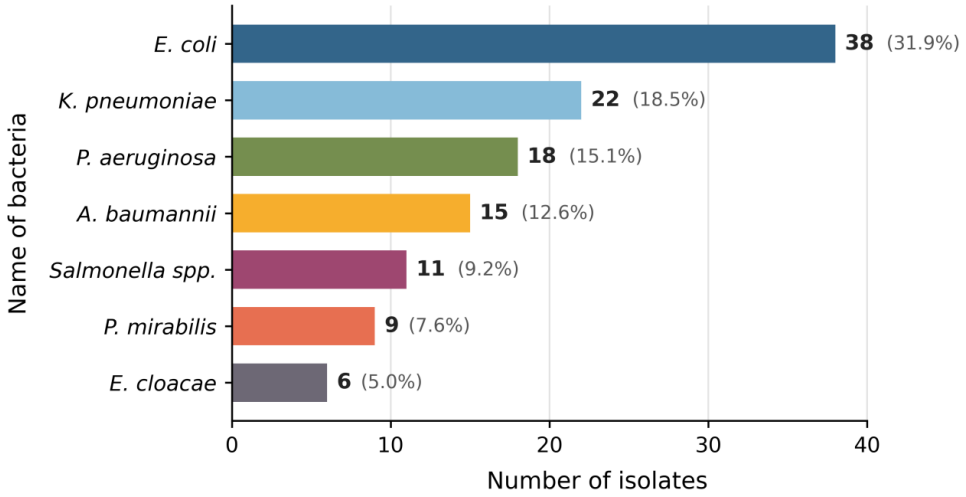


Figure 1: Taxonomic distribution of the 119 Gram-negative bacteria isolated from three poultry wholesale markets of Dhaka. The number of isolates of each taxon is indicated by horizontal bars (percentage in parentheses).

Antibiotic Resistance by Sample Source and Taxon

Resistance was highest to ampicillin (116 isolates, 97.5%), followed by tetracycline (101, 84.9%), trimethoprim-sulfamethoxazole (95, 79.8%), ciprofloxacin (93, 78.2%), chloramphenicol (89, 74.8%), and amoxicillin-clavulanate (86, 72.3%) (Table 1). The third-generation cephalosporin ceftazidime (73, 61.3%) and gentamicin (68, 57.1%) showed lower resistance, but most isolates remained resistant. Meropenem was the most active agent, with 93 isolates (78.2%) susceptible, 14 (11.8%) intermediate, and 12 (10.1%) resistant. Gentamicin (16.8%) and amoxicillin-clavulanate (16.0%) had the highest intermediate results.

Table 1: Antibiotic susceptibility of the 119 isolates, ordered by frequency of resistance.

Antibiotic	Antimicrobial class	Resistant, n (%)	Intermediate, n (%)	Susceptible, n (%)
Ampicillin (AMP)	Penicillins	116 (97.5)	2 (1.7)	1 (0.8)
Tetracycline (TET)	Tetracyclines	101 (84.9)	10 (8.4)	8 (6.7)
Trimethoprim–sulfamethoxazole (SXT)	Folate pathway inhibitors	95 (79.8)	13 (10.9)	11 (9.2)
Ciprofloxacin (CIP)	Fluoroquinolones	93 (78.2)	13 (10.9)	13 (10.9)
Chloramphenicol (CHL)	Phenicols	89 (74.8)	14 (11.8)	16 (13.4)
Amoxicillin-clavulanate (AMC)	Penicillin + β-lactamase inhibitor	86 (72.3)	19 (16.0)	14 (11.8)
Ceftazidime (CAZ)	Cephalosporins (3rd gen.)	73 (61.3)	15 (12.6)	31 (26.1)
Gentamicin (GEN)	Aminoglycosides	68 (57.1)	20 (16.8)	31 (26.1)
Meropenem (MEM)	Carbapenems	12 (10.1)	14 (11.8)	93 (78.2)

Interpretation followed CLSI M100 (36th edition) disk diffusion breakpoints.

Resistance profiles were generally similar across sample sources (Figure 2a). Resistance to ampicillin was 94–100% across all sources, and resistance to tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin, chloramphenicol, and amoxicillin-clavulanate was at least 60% across all sources. Air isolates showed the highest resistance to several agents: 100% were resistant to tetracycline and amoxicillin-clavulanate, and 94% were resistant to chloramphenicol. Ciprofloxacin resistance was highest in water isolates (90%) and lowest in feed (60%) and cloacal swab (65%) isolates. Meropenem resistance was detected at five of the six sources: eggs (5/37, 14%), water (3/20, 15%), cloacal swabs (2/17, 12%), feces (1/18, 6%), and air (1/17, 6%); none of the 10 feed isolates were resistant. Percentages are descriptive only, because several sources yielded 10–20 isolates.

Resistance patterns differed more between taxa than between sources (Figure 2b). All *P. aeruginosa* and *A. baumannii* isolates were resistant to ampicillin and amoxicillin-clavulanate, and ceftazidime resistance was also high (77.8% and 86.7%, respectively). Ciprofloxacin resistance was highest in *A. baumannii* (86.7%) and *K. pneumoniae* (86.4%). Meropenem resistance occurred in four taxa: *A. baumannii* (4/15, 26.7%), *P. aeruginosa* (4/18, 22.2%), *E. coli* (3/38, 7.9%), and *K. pneumoniae* (1/22, 4.5%). None of the *Salmonella* spp., *P. mirabilis*, or *E. cloacae* isolates were resistant to meropenem. Ten of the 12 meropenem-resistant isolates were also resistant to ceftazidime. *P. mirabilis* was the least resistant taxon, with low resistance to amoxicillin-clavulanate (11.1%), ceftazidime (22.2%) and gentamicin (11.1%), although all nine isolates were resistant to tetracycline.

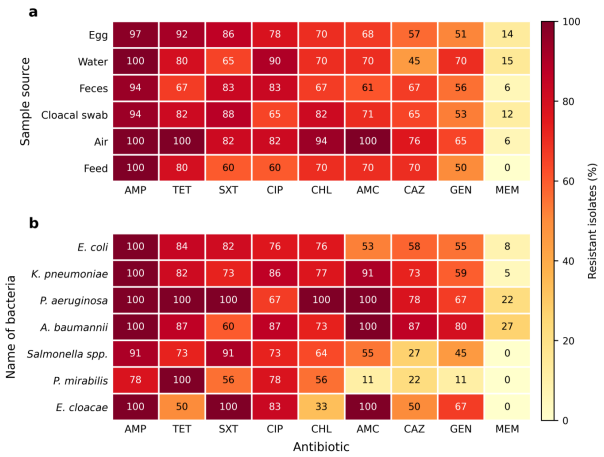


Figure 2: The percentage of resistant isolates for each of the nine antibiotics (a) by sample source and (b) by bacterial taxon. The percentages of isolates resistant to the antibiotic listed below each column are shown in the cells; the color scale is from 0% (pale yellow) to 100% (dark red). The definitions of antibiotic labels are given in Table 1.

Multidrug Resistance, Extensive Drug Resistance, and MAR Index

Of the 119 isolates, 117 (98.3%) were multidrug-resistant (MDR) when intrinsic resistance was included. The two non-MDR isolates were both *P. mirabilis*, which were resistant to only two antibiotics. Of the 117 MDR isolates, 55 (46.2% of all isolates) were extensively drug-resistant (XDR), and 62 (52.1%) were MDR but not XDR. Most isolates (110, 92.4%) were resistant to 4–8 of the 9 antibiotics; 31 (26.1%) were resistant to 7 antibiotics, and three *P. aeruginosa* isolates were resistant to all 9 antibiotics. The XDR proportion was highest in *A. baumannii* (11/15,

Table 2: Resistance and biofilm characteristics of the 119 isolates by taxon.

Taxon	n	Median MAR	MDR (non-XDR), n	X D R , n (%)	Ceftazidime-resistant, n (%)	Meropenem-resistant, n (%)	Tube-positive, n (%)	Strong biofilm, n
Escherichia coli	38	0.67	25	13 (34)	22 (58)	3 (8)	8 (21)	1
Klebsiella pneumoniae	22	0.78	10	12 (55)	16 (73)	1 (5)	16 (73)	9
Pseudomonas aeruginosa	18	0.83	5	13 (72)	14 (78)	4 (22)	14 (78)	9
Acinetobacter baumannii	15	0.78	4	11 (73)	13 (87)	4 (27)	11 (73)	6
Salmonella spp.	11	0.56	8	3 (27)	3 (27)	0 (0)	2 (18)	0
Proteus mirabilis	9	0.44	6	1 (11)	2 (22)	0 (0)	1 (11)	0
Enterobacter cloacae	6	0.67	4	2 (33)	3 (50)	0 (0)	1 (17)	0
All isolates	119	0.67	62	55 (46)	73 (61)	12 (10)	53 (45)	25

MAR, multiple antibiotic resistance index; MDR, multidrug-resistant; XDR, extensively drug-resistant. MDR (non-XDR) isolates were resistant in three to six categories; the two non-MDR P. mirabilis isolates are not shown in a separate column. Percentages are rounded to whole numbers. Strong biofilm, OD > 4×ODc (ODc = 0.094).

73.3%) and *P. aeruginosa* (13/18, 72.2%), intermediate in *K. pneumoniae* (12/22, 54.5%), and lower in *E. coli* (13/38, 34.2%), *E. cloacae* (2/6, 33.3%), *Salmonella* spp. (3/11, 27.3%), and *P. mirabilis* (1/9, 11.1%) (Table 2).

The median multiple antibiotic resistance (MAR) index was 0.67 (range 0.22–1.00), and all 119 isolates had values > 0.2, which indicates origin from a high-risk source of contamination. The MAR index varied significantly among the different taxa (Kruskal–Wallis $p < 0.001$). The median value was highest in *P. aeruginosa* (0.83), followed by *K. pneumoniae* and *A. baumannii* (0.78 each) and lowest in *P. mirabilis* (0.44) (Figure 3).

The classification was sensitive to how intermediate results were treated. If intermediate results were considered non-susceptible, then all 119 isolates were MDR and 88 (73.9%) were XDR. These figures include the intrinsic

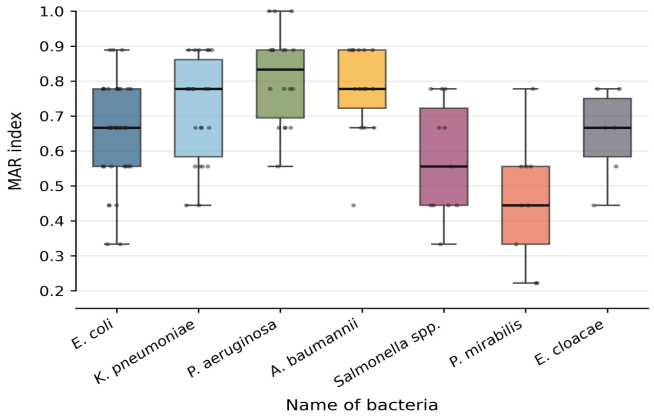


Figure 3: Multiple antibiotic resistance (MAR) index of the 119 isolates by bacterial taxon. Boxes indicate the interquartile range, horizontal lines the median, whiskers the data within 1.5 times the interquartile range, and dots individual isolates.

resistance of each taxon. Consequently, the XDR rates of *P. aeruginosa* and *A. baumannii* may partly reflect the antibiotic panel used and should not be interpreted as reflecting acquired resistance only.

Resistance Patterns and Co-Resistance

The isolate-level resistance map (Figure 4) indicates a strong taxon-based structure of resistance. *P. aeruginosa* and *A. baumannii* isolates were nearly homogeneous in their resistance profiles, except for meropenem and, to a lesser degree, ciprofloxacin, gentamicin and (in *A. baumannii*) trimethoprim-sulfamethoxazole. *K. pneumoniae* isolates showed a similar pattern, with more susceptible results for ceftazidime and gentamicin. *E. coli* isolates were more variable: all were resistant to ampicillin, but there were more mixed results for amoxicillin-clavulanate, ceftazidime and gentamicin. *Salmonella* spp., *P. mirabilis*, and *E. cloacae* isolates showed the most heterogeneous patterns. The resistance classes (MDR and XDR) and biofilm categories for the same isolates are shown in the resistance matrix; strong biofilm producers were enriched among K. pneumoniae, P. *aeruginosa*, and *A. baumannii* isolates.

The 119 isolates exhibited 58 different resistance patterns

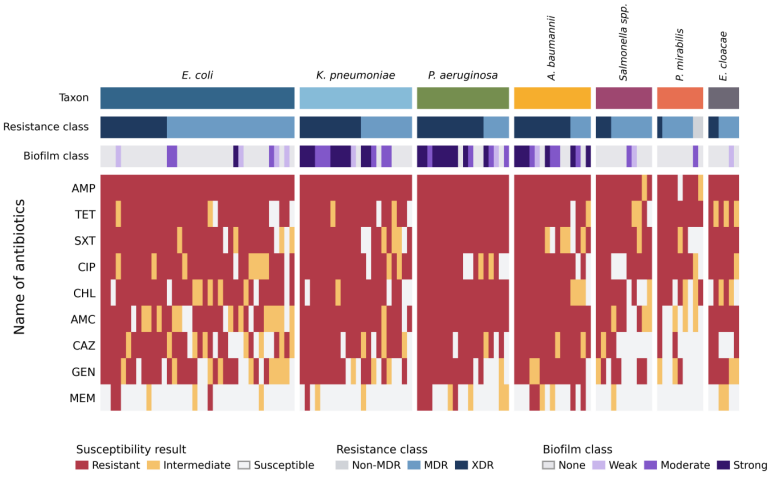


Figure 4: Isolate-level resistance map. Isolates (n = 119) are grouped by bacterial taxon and, within each taxon, ordered by the number of resistances. The three bands above the matrix indicate each isolate's taxon, resistance class (non-MDR, MDR, XDR), and biofilm class (none, weak, moderate, strong). The nine antibiotics tested (labels as defined in Table 1) are listed as rows, and cells are colored according to susceptibility results (resistant, intermediate, or susceptible).

(Figure 5b). The most common pattern was resistance to all agents except meropenem (16, 13.4%), followed by the same pattern except for gentamicin resistance (10, 8.4%). Eleven patterns were shared by three or more isolates (58 isolates, 48.7%), and 64 (53.8%) were resistant to ampicillin, tetracycline, trimethoprim-sulfamethoxazole and ciprofloxacin. Thirty-six isolates (30.3%) were co-resistant to ceftazidime, ciprofloxacin and gentamicin, representing third-generation cephalosporins, fluoroquinolones and aminoglycosides, respectively.

Although the majority of isolates were resistant to multiple drugs simultaneously, correlation between resistance to different antibiotics was weak (Figure 5a). Pairwise (phi) coefficients between antibiotics ranged from −0.13 to 0.28 (the diagonal of 1.00 just represents each antibiotic correlated with itself). The highest coefficient was observed for amoxicillin-clavulanate and ceftazidime (0.28), ampicillin and amoxicillin-clavulanate (0.26), and trimethoprim-sulfamethoxazole and amoxicillin-clavulanate (0.25), while a few pairs were slightly negative, such as chloramphenicol and meropenem (−0.13). This suggests that multiple resistance largely reflects high background resistance to older agents, leaving little scope for resistance to different antibiotics to co-occur more often than expected. Meropenem resistance was rare, but nearly always accompanied resistance to other agents: all 12 meropenem-resistant isolates were resistant to ampicillin and amoxicillin–clavulanate, 11 to ciprofloxacin, 10 to ceftazidime and trimethoprim-sulfamethoxazole, 9 to tetracycline and gentamicin, and 7 to chloramphenicol.

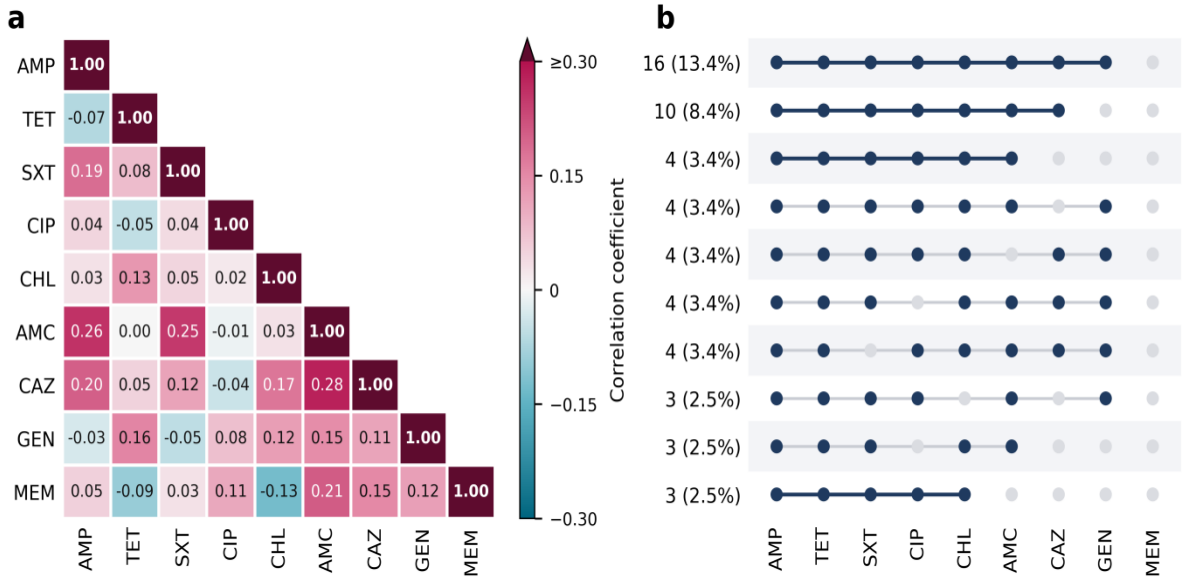


Figure 5: Co-resistance between the 9 antibiotics. (a) Pairwise correlation (phi) coefficients between resistance to each pair of antibiotics (resistant versus not resistant; 119 isolates) shown as a lower-triangle heatmap. The diagonal represents each antibiotic's correlation with itself (1.00); plum cells indicate resistance that tends to co-occur, and teal cells indicate resistance that tends not to co-occur (as indicated by the vertical color bar). (b) The 10 most common exact resistance profiles (4 profiles were shared by 3 isolates each and 3 of these are represented); filled dots indicate the antibiotics to which the isolates with the same profile were resistant and the number (and percentage) of isolates is indicated on the left. Antibiotic labels are described in Table 1.

Biofilm-Forming Capacity

The tube method was positive for 53 of 119 isolates (44.5%), and all were above the microtiter cut-off (ODc = 0.094), with OD values ranging from 0.109 to 0.819 (median 0.325). Of these, 25 isolates (21.0% of all isolates) were strong, 19 (16.0%) moderate, and 9 (7.6%) weak biofilm producers. The remaining 66 isolates (55.5%) were tube-negative and were classified as non-producers, although weak biofilm formation may have been missed because they were not retested by microtiter assay (Figure 6a).

Biofilm formation differed markedly among taxa (Table 2; Figure 6). It was frequent in *P. aeruginosa* (14/18, 77.8%), *A. baumannii* (11/15, 73.3%), and *K. pneumoniae* (16/22, 72.7%), and infrequent in *E. coli* (8/38, 21.1%), *Salmonella* spp. (2/11, 18.2%), *E. cloacae* (1/6, 16.7%), and *P. mirabilis* (1/9, 11.1%). Biofilm formation was observed in 74.5% (41/55) of isolates from these three biofilm-prone taxa, whereas only 18.8% (12/64) of isolates from all other taxa were biofilm-positive ($p < 0.001$). These three taxa also accounted for 24 of the 25 strong biofilms (9 K. pneumoniae, 9 P. *aeruginosa*, and 6 *A. baumannii*), and a single *E. coli* isolate was the only other strong producer. The median OD of the tube-positive isolates was highest for *P. aeruginosa* (0.51), followed by *A. baumannii* (0.42), *K. pneumoniae* (0.41) and *E. coli* (0.19) (Figure 6b).

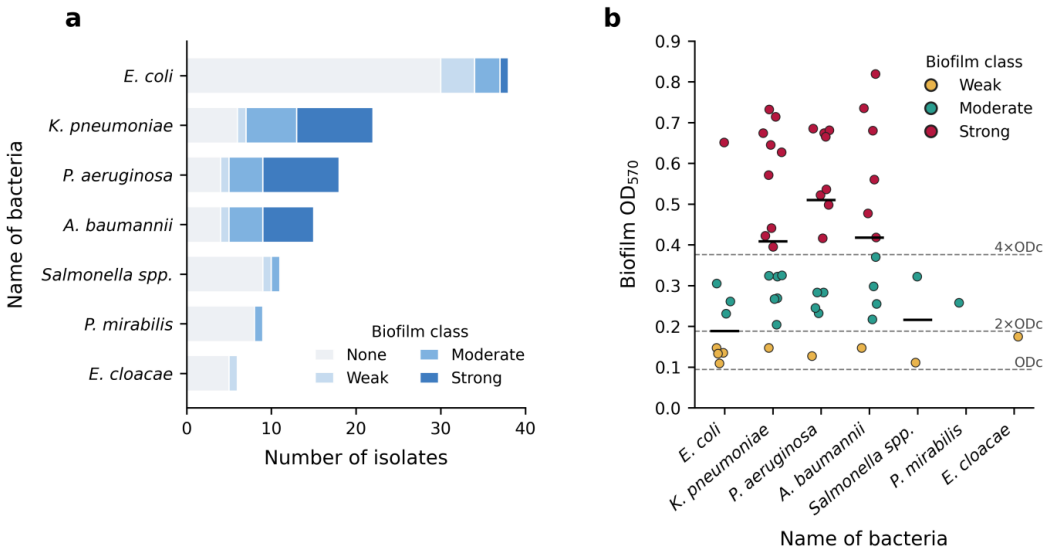


Figure 6: Biofilm formation of the 119 isolates. (a) Distribution of isolates by biofilm class (none, tube-negative; weak, moderate, strong by microtiter plate OD) by bacterial taxon. Darker shades represent stronger biofilm formation. (b) Biofilm OD570 of the 53 tube-positive isolates by taxon; each dot represents one isolate, horizontal bars represent medians (not plotted for taxa with one isolate), and dashed lines represent cut-off OD (ODc, 0.094), 2×ODc and 4×ODc.

Relationship Between Resistance and Biofilm Formation

The median MAR index was 0.89 for strong biofilm producers, 0.67 for moderate biofilm producers and non-producers, and 0.56 for weak biofilm producers (Kruskal–Wallis $p < 0.001$; Figure 7a). XDR isolates tended to form biofilm more often than MDR isolates that were not XDR (30/55, 54.5% vs 22/62, 35.5%; $p = 0.04$, Holm-adjusted $p = 0.13$, not significant), and strong biofilm was far more frequent among XDR isolates (19/55, 34.5%) than among non-XDR MDR isolates (6/62, 9.7%; $p = 0.001$, Holm-adjusted $p = 0.006$) (Figure 7b). Neither of the two non-MDR isolates was a strong producer, and 9 of 12 meropenem-resistant (MDR) isolates (75.0%) were biofilm-positive.

This association was largely linked to taxon. The three taxa with the highest biofilm prevalence were also the most resistant, with 36 XDR isolates out of 55 (65.5%) compared with 19 XDR isolates out of 64 (29.7%) in the other taxa ($p < 0.001$, Holm-adjusted $p < 0.001$). Within the three biofilm-prone taxa, the frequency of biofilm production was not significantly different between XDR and MDR non-XDR isolates, although there was a trend for higher frequency of biofilm production in the XDR isolates (29/36, 80.6%) than in the MDR non-XDR isolates (12/19, 63.2%; $p = 0.20$, Holm-adjusted $p = 0.30$). In all other taxa, numbers were small, and the difference was reversed but not significant: 1 of 19 XDR isolates (5.3%) formed biofilm compared with 10 of 43 MDR non-XDR isolates (23.3%; $p = 0.15$, Holm-

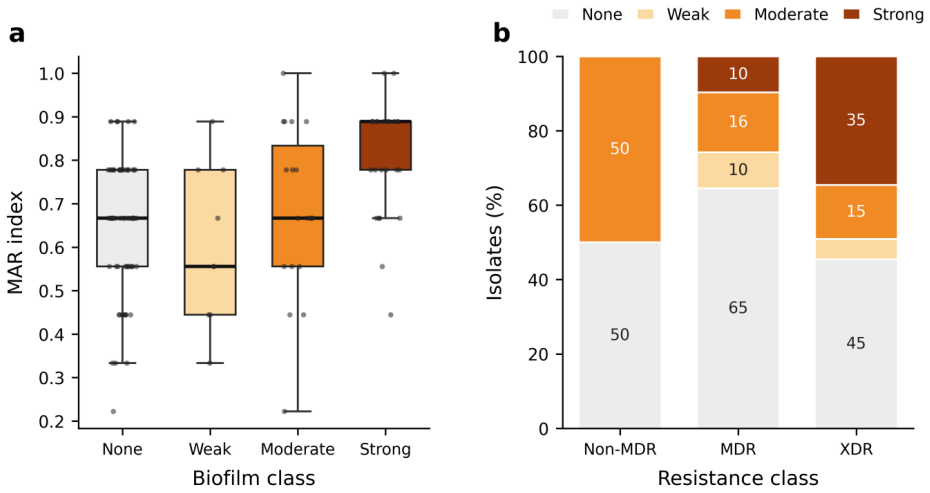


Figure 7: Correlations of resistance and biofilm formation. (a) MAR index by biofilm class; boxes indicate the interquartile range, horizontal lines the median, and dots individual isolates. (b) Proportion of isolates in each biofilm class in each resistance class (non-MDR, n = 2; MDR, n = 62; XDR, n = 55); percentages shown within the bars.

adjusted p = 0.30). The overall association of XDR status and biofilm formation is therefore primarily attributable to the co-occurrence of both traits in K. pneumoniae, *P. aeruginosa* and *A. baumannii*.

Of the 119 isolates, 19 (16.0%) were XDR and strong biofilm producers (8 P. *aeruginosa*, 7 K. pneumoniae, and 4 *A. baumannii*). None of the *E. coli*, *Salmonella*, *P. mirabilis* or *E. cloacae* isolates belonged to this group.

Discussion

The findings indicate that the Gram-negative isolates recovered from three poultry wholesale markets in Dhaka were diverse, highly resistant, and often biofilm-forming, and were not confined to *E. coli* and *Salmonella*. Nearly all (98.3%) were MDR, almost half (46.2%) were XDR, and all exceeded the MAR threshold of 0.2, which indicates contamination from sources with high antimicrobial exposure (Krumperman, 1983).

Isolates from non-bird sources were also highly resistant. Water, air, and feed isolates were as resistant as isolates from feces and cloacal swabs, suggesting that the market environment, as well as the birds themselves, is a reservoir of resistant bacteria. Water isolates had the highest resistance to ciprofloxacin (90%) and meropenem (15%). In addition, nine of the 11 *Salmonella* isolates were from eggs. Although the number of isolates per source is small, these observations suggest that market surveillance should extend beyond birds to determine source-specific risk.

The resistance burden is consistent with previous studies of poultry in Bangladesh. Multidrug resistance was observed in 93.2% of *E. coli* and all *Salmonella* from cloacal swabs in Dhaka chicken markets (Parvin *et al.*, 2022). Plasmid-borne blaCTX-M genes were identified in cephalosporin-resistant *E. coli* from live bird markets (Davies *et al.*, 2024) and multidrug-resistant *E. coli* were prevalent in broiler cloacal swabs (Al Azad *et al.*, 2019). A recent survey found ESBL-producing *Enterobacter*ales in 88% of fecal samples from urban live bird markets compared to 48% on rural farms, with *K. pneumoniae* the most common species other than *E. coli* (Amin *et al.*, 2025). Our findings are consistent with this: ceftazidime resistance (61.3%) was highest among the *Enterobacter*ales in *K. pneumoniae* (72.7%). However, ceftazidime resistance alone does not confirm ESBL production. The high resistance to ampicillin, tetracycline, and ciprofloxacin is not surprising, as these antibiotic classes are heavily used in broiler production (Imam *et al.*, 2020; Roth *et al.*, 2019), and antimicrobial use is poorly regulated in Bangladesh, where resistance is high in both the human and animal sectors (Ahmed *et al.*, 2019; Hoque *et al.*, 2020). Similar resistance has been reported in poultry in other LMICs (Founou *et al.*, 2016; Nhung *et al.*, 2017) and globally (Ewers *et al.*, 2012). However, direct numerical comparisons between studies are limited by differences in antibiotic panels, breakpoints, and sample types.

Although meropenem was the most active agent, 12 isolates were resistant, including 26.7% of *A. baumannii* and 22.2% of P. *aeruginosa*, and 10 of these were also resistant to ceftazidime. Carbapenems are not used in food animals, but carbapenem-resistant *Enterobacter*ales have been detected in poultry and other food-producing animals (Köck *et al.*, 2018), and NDM-producing bacteria have been detected in Chinese poultry production (Wang *et al.*, 2017). As the genes for carbapenemases were not specifically investigated, our disk results do not distinguish between acquired and intrinsic mechanisms. Plasmid-mediated mcr-1 has also been described in animals and humans (Liu *et al.*, 2016) but was not tested here. These isolates warrant genotypic follow-up, given the WHO critical-priority status of carbapenem-resistant *A. baumannii* and cephalosporin- or carbapenem-resistant *Enterobacter*ales (WHO, 2024).

MAR indices and the XDR percentages were highest for *P. aeruginosa* and *A. baumannii*, and these values should be interpreted with caution. Our panel included agents to which these species are intrinsically resistant (Leclercq *et al.*, 2013) and these were counted in the MDR/XDR classification. Much of this baseline resistance is attributable to low outer-membrane permeability, efflux and chromosomal AmpC (Lister *et al.*, 2009). However, these taxa are well adapted to market settings: *A. baumannii* is known to survive on dry surfaces and tolerate desiccation (Harding *et al.*, 2018; Peleg *et al.*, 2008), and *K. pneumoniae* is a ubiquitous environmental organism with a capacity to share resistance genes with clinical strains (.Wyres & Holt, 2018; Podschun & Ullmann, 1998). Wet stalls, wash water and air in crowded markets thus offer both niches and opportunities for gene exchange. The finding that 8 of the 18 *P. aeruginosa* isolates were from air may reflect aerosolization of wash water and dust, although our study design cannot test this.

Biofilm formation was observed in 44.5% of the isolates, with the highest frequencies in *P. aeruginosa* (77.8%), *A. baumannii* (73.3%) and *K. pneumoniae* (72.7%). This is consistent with the known biology of these taxa (Ciofu & Tolker-Nielsen, 2019; Gedefie *et al.*, 2021; Vuotto *et al.*, 2017). The lower frequency in *E. coli* (21.1%) and *Salmonella* (18.2%) aligns with the fact that biofilm formation in these species is strain-dependent and varies with test conditions (Naves *et al.*, 2008; Stepanovic *et al.*, 2004).

Strong biofilm producers had the highest median MAR index (0.89), and XDR isolates tended to be biofilm producers more often than non-XDR MDR isolates (54.5% versus 35.5%; p = 0.04, Holm-adjusted p = 0.13), whereas strong biofilm production was significantly more frequent in XDR isolates (Holm-adjusted p = 0.006). This association was mainly driven by taxon; the biofilm-prone species were also the most resistant, and outside them, only 1 of 19 XDR isolates formed biofilm. The data therefore indicate that the two traits coexist in specific taxa, rather than demonstrating a general association between resistance and biofilm formation. Mechanistically, matrix-limited penetration, slow growth, and persister cells make biofilm bacteria tolerant to

antibiotics (Hall & Mah, 2017; Høiby *et al*., 2010; Mah & O'Toole, 2001) and biofilms favor horizontal gene transfer (Madsen *et al*., 2012); relationships between virulence-related traits and resistance may reflect co-selection or trade-offs (Schroeder *et al*., 2017). These explanations are not distinguishable from our phenotypic data. The greatest concern is the 19 isolates that were XDR and strong biofilm producers, as they may survive on surfaces even after routine cleaning (Galié *et al*., 2018) and disk diffusion testing of planktonic cells may not reflect biofilm cell tolerance (Høiby *et al*., 2010).

These findings have public health implications. Birds and their waste are part of the market environment, as are workers and customers, and human, animal, and environmental factors all contribute to resistance in low-resource settings (Rousham *et al*., 2018). The strength of evidence linking animal antimicrobial use to human disease varies by pathogen and transmission pathway (Hoelzer *et al*., 2017), but transfer could occur through shared water, drains, and food chains. Practical measures include regular cage, block, and drain cleaning and disinfection, including removing biofilm, separating species, treating wash water before discharge and minimizing non-therapeutic antibiotic use on supplying farms. Since resistance and biofilm formation were clustered in the same three taxa, it would be feasible to monitor risk in these markets by surveillance of K. pneumoniae, *P. aeruginosa* and *A. baumannii*, including carbapenem-resistant isolates.

The study was limited by the lack of molecular confirmation of bacterial identification and resistance gene analysis to better understand resistance mechanisms. Because the same panel and interpretive criteria were applied to all taxa, resistance may have been overestimated in some species (notably *P. aeruginosa* and *A. baumannii*): intrinsic resistance can lead to misclassification as MDR/XDR, and the categories for these taxa are provisional. Biofilm formation was assessed under a single set of conditions, potentially overlooking weak biofilm producers (tube-negative isolates were not retested by microtiter assay, so biofilm prevalence is probably underestimated), and biofilm-associated antibiotic tolerance was not investigated. In addition, the prevalence estimation, generalizability, and ability to infer transmission were limited by the small, cross-sectional sample from three markets, enrichment, and lack of data on antibiotic use in supplying farms. Isolates from the same sample or market are not fully independent, so p-values should be regarded as exploratory. Further research is needed to better understand resistance mechanisms and biofilm-associated persistence using whole-genome sequencing and biofilm inhibition assays.

CONCLUSION

This study indicates that the Gram-negative isolates recovered from three poultry wholesale markets in Dhaka were taxonomically diverse, frequently multidrug-resistant, and often biofilm-forming, and were not restricted to *E. coli* and *Salmonella*. Nearly all isolates (98.3%) were multidrug-resistant when intrinsic resistance was included, with 46.2% being extensively drug-resistant and at least 44.5% producing biofilm. K. pneumoniae, P. *aeruginosa*, and *A. baumannii* were the taxa with the highest resistance and strongest biofilm formation, and 16.0% of all isolates were both XDR and strong biofilm producers, which was mostly due to these taxa; control and surveillance efforts in these markets could prioritize these three taxa, rather than assuming that resistance and biofilm formation are correlated in all bacteria. Although meropenem was the most active agent, resistance was observed in 10.1% of isolates, mainly *A. baumannii* and P. *aeruginosa*, which is of concern, although carbapenemase genes were not investigated. Given the limited scope of this study (three markets, phenotypic identification, single-step biofilm screening), these findings are a baseline and should not be generalized beyond the sampled markets; nevertheless, surveillance in poultry markets should include non-indicator Gram-negative genera, genotypic confirmation of ESBL and carbapenem resistance, and biofilm assessment, along with improved market hygiene, regular surface disinfection, wash-water treatment, and more judicious antimicrobial use across the poultry supply chain.

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Data Availability

The datasets generated and/or analyzed during the current investigation are accessible from the corresponding author on reasonable request.

Competing Interests

The author(s) declare no competing interests.

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Declaration of Generative AI and AI-assisted Technologies

The authors acknowledge using Grammarly and ChatGPT (OpenAI, GPT-5.2) to improve language, correct grammar, and enhance clarity during manuscript preparation. After using these tools, the authors reviewed and revised the manuscript as needed and accept full responsibility for the published article's content.

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