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RESEARCH ARTICLE

Prevalence and Multidrug Resistance Patterns of *Pseudomonas aeruginosa* from Captive Wild and Migratory Birds in Sylhet, Bangladesh

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A B S T R A C T

Antimicrobial resistance (AMR) is increasingly reported in wild and migratory birds, yet its drivers, ecological impacts, and transmission dynamics remain poorly understood due to limited non-epizootic surveillance. This study investigated the prevalence and resistance profiles of *Pseudomonas aeruginosa* isolated from fecal samples of captive wild and migratory birds in Sylhet region of Bangladesh. Of the 219 samples tested, *Pseudomonas* spp. were estimated at 18.72% (95% CI:13.78-24.53), with a higher prevalence in migratory birds (19.64%) compared to wild birds (17.76%) as detected through cultural and molecular (PCR) testing. The prevalence of *Pseudomonas aeruginosa* was 10.05%, significantly (P<0.001) higher in migratory birds (11.67%) compared to captive wild birds (8.41%). Phenotypically, all *P. aeruginosa* isolates shown 100% sensitive to levofloxacin, ciprofloxacin, tetracycline, colistin sulphate, meropenem and gentamicin, while 73% exhibited resistance to ampicillin. Multidrug resistance (MDR) was found in 63.64% of the isolates. Genotypically, the resistance gene *bla*TEM was detected highest (90.90%), whereas *bla*OXA and *bla*SHV were detected in lowest percentage (11.24%). Sulfonamide (*sul1*) resistance gene was the most prevalent at 22.73% for *P. aeruginosa*. These findings underscore the potential role of wild and migratory birds as reservoirs and disseminators of AMR genes, highlighting risks to environmental and public health. The study emphasizes the need for integrated surveillance programs under the One Health framework to mitigate AMR's spread across ecological boundaries.

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INTRODUCTION

Nearly 75% of globally reported re-emerging infectious diseases are zoonotic in origin, emphasizing the crucial role of animals in the transmission of pathogens to humans (Sharan *et al.*, 2023). As a result, it is critical to identify and monitor possible disease reservoirs, such as birds, whose mobility and diverse exposure to a variety of environment could facilitate the spread of infections (Sharan *et al.*, 2023). The rise of communicable diseases and the potential harm they pose to wildlife has increased interest in the roles of birds as monitors, carriers, and sources of AMR (Vezeau & Kahn, 2024). Because of the influence of the world's rapidly growing populace and environmental shifts that result in more frequent and closer interactions between humans and wildlife, zoonoses are becoming a significant issue (Smoglica *et al.*, 2023).

The global rise of AMR presents a significant threat to public health, with projections estimating that by 2050, AMR could cause approximately 10 million deaths per year, surpassing all other causes of mortality (Tanni *et al.*, 2025). In spite of the fact that AMR is often connected with increased antibiotic consumption, this applies not only the case among wildlife but also in migratory birds; thus, its existence might be utilized as a signal of anthropogenic activities that influence the entire ecosystem (Smoglica *et al.*, 2023). Septic water treatment plants, farm manure, and slurry, for example, are all significant homes for birds and

other animals, but they can become contaminated with antibiotics (and/or their metabolites), AMR-causing bacteria, and other materials that can function as specific AMR drivers. Although determining the directionality of this spread is a difficult issue, previous research has found a link between the physical closeness of wild animals to humans and resistance tendencies (Smoglica *et al.*, 2023; Shoaib *et al.*, 2025a; Shoaib *et al.*, 2025b). Wild birds, particularly migratory species, play a distinct role in the global dissemination of AMR due to their vast geographical range, exposure to diverse environments, and interaction with contaminated habitats, including agricultural lands, urban areas, and wetlands (Ahmed *et al.*, 2019; Shoaib *et al.*, 2025c).

Unlike companion birds, which are often confined to controlled environments, wild birds encounter antibiotics and resistant bacteria in natural ecosystems, such as polluted water bodies or areas with high anthropogenic activity (Mukerji *et al.*, 2019). This exposure increases the likelihood of acquiring and spreading AMR genes, such as *mcr-1* or *bla*TEM, through horizontal gene transfer (Hernando-Amado *et al.*, 2019). Additionally, wild birds serve as reservoirs and vectors for resistant pathogens, transmitting them to native wildlife, livestock, and humans, thereby amplifying the One Health implications of AMR (Begum *et al.*, 2024; Rahman *et al.*, 2024). These avian species suffering from enteric diseases, likely caused by *Staphylococcus aureus*, *Klebsiella* species, *Campylobacter* species, *Pseudomonas* species, and *Salmonella* species, may have significant public health implications (Hernando-Amado *et al.*, 2019; Navarro-Gonzalez *et al.*, 2020).

Due to its high mortality rate, incidence, increasing resistance trends, significant health burden, and transmissibility, *P. aeruginosa* has been classified as a high-priority pathogen in the WHO Priority Pathogen List (Sati *et al.*, 2025). Previously, from 2017 to 2023, it was categorized as a critical-priority pathogen (Sati *et al.*, 2025). Additionally, *P. aeruginosa* is recognized as one of the ESKAPE pathogens, exhibiting high levels of antibiotic resistance and posing a serious global public health threat. Previous studies investigating naturally occurring bacteria associated with birds have largely focused on microorganisms of importance to human and food animal health (Haesendonck *et al.*, 2016; Ahmed *et al.*, 2019; Navarro-Gonzalez *et al.*, 2020; Hao *et al.*, 2024). However, limited information is available on the non-epizootic occurrence of *P. aeruginosa* in avifauna, apart from reports describing multidrug-resistant *Pseudomonas* spp. isolated from wild birds during disease outbreaks associated with high mortality (Vasconcelos *et al.*, 2017; Vidal *et al.*, 2017; GC Rodrigues *et al.*, 2021). Overall, surveillance of *P. aeruginosa* in wildlife, including avian species, remains an underexplored area with potential implications for human, animal, and environmental health (O'Neill, 2016; Hernando-Amado *et al.*, 2019). In Bangladesh, this study represents one of the first investigations specifically targeting *P. aeruginosa* isolated from the feces of migratory and captive wild birds. The aim of this study was to determine the prevalence and antimicrobial resistance profiles of *P. aeruginosa* in these avian populations, identify key antimicrobial resistance genes, and evaluate the potential role of birds in the dissemination of antimicrobial resistance across ecosystems. The findings are expected to contribute to integrated One Health strategies for monitoring and mitigating AMR at the wildlife-human-environment interface.

MATERIALS AND METHODS

Collection of fecal samples and epidemiological data: From January 2022 to July 2022, a cross-sectional investigation was conducted at Horipur, Hakaluki Haor of Moulovibazar, Tanguar Haor, and other locations within Tahirpur upazila of Sunamganj district of Sylhet division in Bangladesh. Additionally, samples of wild birds in captivity were taken at the Bangladesh Bannyaprani Sheba Foundation in Srimongol and the Tilagor Eco Park in Sylhet (Fig. 1). The required sample size was calculated using the standard formula (Rahman *et al.*, 2024): $n = (Z^2 \times p \times (1 - p)) / d^2$, where n represents the required sample size, Z = 1.96 for a 95% confidence level, p = expected prevalence (0.50), and d = desired absolute precision (0.05). The expected prevalence was set at 50% in the absence of prior data, as this assumption yields the maximum sample size and ensures adequate statistical power. Based on this calculation, the minimum required sample size was 384. However, due to logistical and field constraints, including limited accessibility to sampling sites and variability in target bird availability, a total of 219 fecal samples were collected among them 112 are migratory birds and 107 were captive wild birds (Table 1). Although this number is lower than the calculated minimum, the sample size was still considered sufficient to provide meaningful preliminary insights into the prevalence and antimicrobial resistance patterns of *P. aeruginosa* in the study area. Birds were selected using a stratified random sampling approach to ensure representation across different habitats, and migratory species. The study area was stratified based on habitat categories (e.g., wetlands, forest areas, and captive settings) and bird classification (migratory vs. resident). Within each stratum, birds were selected opportunistically based on availability during sampling visits, due to practical constraints in field conditions. Furthermore, efforts were made to minimize sampling bias by covering multiple geographic locations, selecting diverse species, and maintaining standard sampling protocols. Efforts were made to minimize stress by quickly releasing birds post-sampling. Sampling was carefully designed to avoid duplicate collection from the same individual birds by ensuring single-time fecal sample collection per bird and applying field-level identification and separation procedures. Fecal swabs were collected aseptically to prevent contamination and were individually packed in sterile plastic containers containing Buffered Peptone Water (BPW) (Hi Media, India) as a non-selective pre-enrichment step to resuscitate stressed or sub-lethally injured bacteria and enhance overall recovery prior to selective isolation. For consistency, all samples were processed within 24 hours to ensure data reliability.

Table 1: Fecal sample collection across different sampling sites

Sl. No	Study Area	No. of Sample
1	Tanguar Hoar	94
2	Horipur	18
3	Srimongal	71
4	Ecopark, Sylhet	36
Total		219

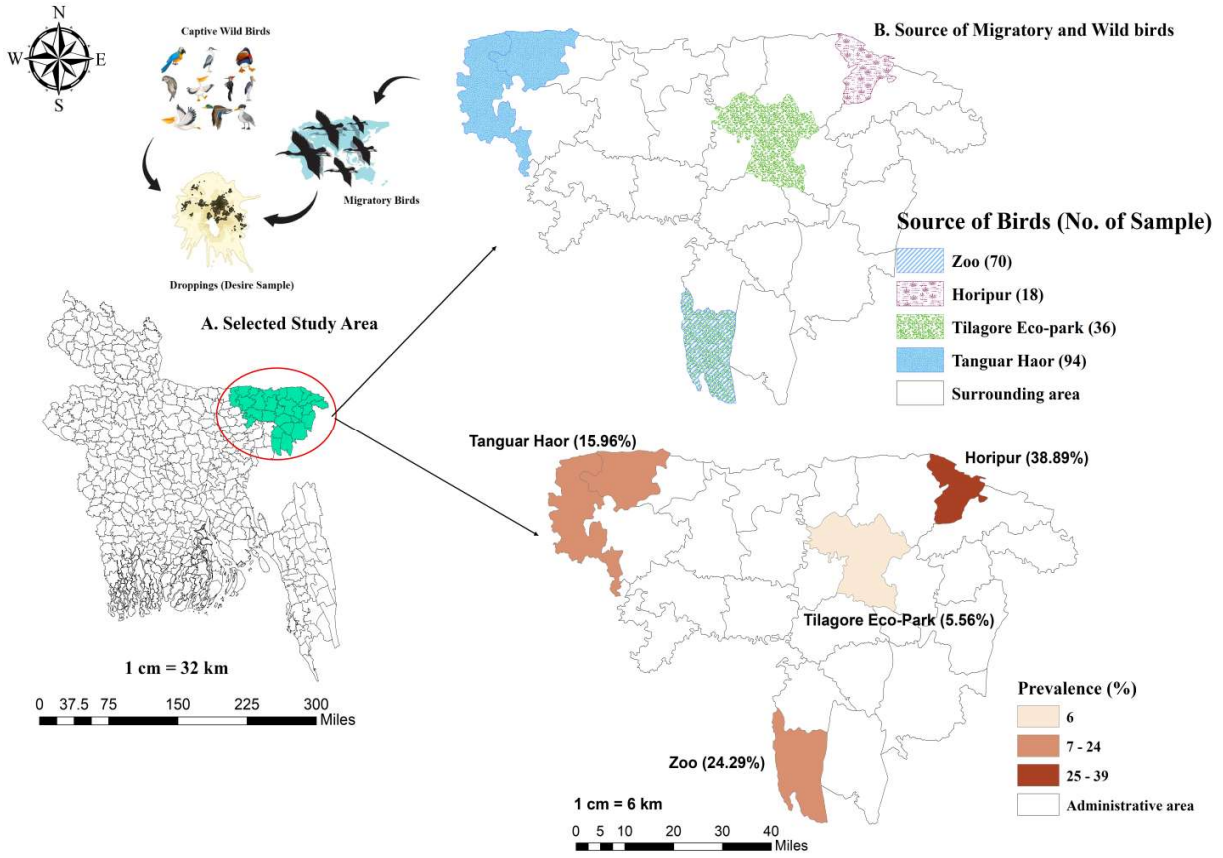

Fig. 1: Map of Bangladesh and Sylhet division, showing the study and sampling areas. The map was created using ArcMap 10.7.

Migratory bird species were prioritized due to their potential role in the long-distance dissemination of AMR bacteria. Captive wild birds were also included to provide a baseline for AMR prevalence in local avian populations. Species were selected based on prior studies indicating their exposure to anthropogenic influences in agricultural areas, wetlands or rural feeders. Sampling sites were chosen to represent a gradient of human impact (tourists, people living on boat in the haor areas), including agricultural and rural environments (e.g., sewage-contaminated wetlands and riverine areas). Data on livelihoods, antibiotic levels, GPS coordinates and habitat use were collected to assess AMR patterns.

Isolation and identification of *Pseudomonas* spp.: *Pseudomonas* species were isolated and identified in accordance with a previous study (GC Rodrigues *et al.*, 2021). In brief, for pre-enrichment samples were added with BPW and kept for twenty-four hours at 37°C. Following a streaking technique, pre-enriched samples were cultured aerobically for 24 hours at 37°C on *Pseudomonas* cetrimide agar (Hi-media, India). To reduce the potential for competitive inhibition, confluent growth plates were sub-cultured in order to be isolated. Putative *Pseudomonas* spp. colonies were sub-cultured, and isolated colonies were subjected to Gram staining and for biochemical confirmation, 2-3 single colonies from each sample were tested with triple sugar iron (TSI) agar, motility indole urea (MIU), and Methyl Red (MR) tubes were incubated at 37 ºC for 16–24 hours. Positive samples were further cultured on nutrient agar (NA) (Hi media, India) plates at an incubation temperature of 37 ºC for 16–24 hours and pure isolates were kept in brain heart infusion broth (BHI) (Hi media, India) containing 15% glycerol at -20 ºC for further use. Although biochemical tests (TSI, MIU, MR) were used for preliminary screening, the final confirmation of *P. aeruginosa* isolates was performed by PCR-based molecular detection.

Bacterial genomic DNA extraction: The AddPrep genomic DNA extraction kit was used to complete the DNA extraction in accordance with the manufacturer's instructions (AddBio Inc. Ltd., Korea). Briefly, 200 µL of cultivated cells were extracted overnight and centrifuged at 13,000 rpm for 30 seconds. The supernatant was disposed of and 200 µL of lysis solution was pipetted in and suspended. After adding 20 mg/mL of proteinase K solution, the mixture was incubated at 56°C to ensure full lysis. The mixture was thoroughly homogenized and incubated at 56°C for 10 minutes following centrifugation and the addition of binding solution. Following the addition of 100% ethanol, the lysate was cautiously moved to a spin column tube, centrifuged, and then cleaned with the use of cleaning agents. To eliminate any leftover ethanol, the spin column was dried using another centrifugation for one minute at 13,000 rpm. Add thirty microliters of elution buffer to the spin column and let it sit at room temperature for one minute. After centrifugation, the genomic DNA was eluted and kept at -80°C for further examination.

Molecular detection of Pseudomonas spp. and P. aeruginosa: PCR was used to validate the identification of *Pseudomonas* spp. and *P. aeruginosa* based on PA-GS and PA-SS primers, respectively. It was very simple, quick, and accurate PCR experiments based on these sequences enabled the separation of *P. aeruginosa* from other *Pseudomonas* species. Primers sequenced are presented in Table 2 (Spilker *et al.*, 2004). The targeted DNA was amplified in 25 µL reaction volumes that contained 12.5µL 2X master mix (AddBio Inc. Ltd., Korea), 2µL of primer mix and 5µL of DNA sample and 25 µL was adjusted by adding 5.5µL nuclease free water. In every reaction, 10 pmol of each primer (forward and reverse) was employed. Using a DLAB TC100-G apparatus (DLAB Scientific co., Ltd., USA), the PCR was carried out. Following a two-minute denaturation period at 95℃, 25 cycles of 20s at 94℃, 20s at the proper annealing temperature (Table 2), and 40s at 72℃ were accomplished. At 72℃, a last extension of one minute was used. The target DNA amplification process took this program a total of about 45 minutes (Spilker *et al.*, 2004). The PCR product was then identified by electrophoresis visualizing the specific band. A 100 bp ladder (AddBio Inc., Korea) was included to help with PCR product size estimation.

Antimicrobial Susceptibility Testing (AST): The commonly used antibiotics evaluated included ciprofloxacin, tetracycline, gentamicin, levofloxacin, meropenem, colistin sulfate, ceftriaxone, cefixime, ampicillin, sulfamethoxazole/trimethoprim, and azithromycin, eleven in total. The antibiotics used in AST were selected based on their relevance to both veterinary and human medicine in the country. This selection reflects antibiotics frequently used in clinical practice for treating bacterial infections in humans and animals, ensuring the interpretation of resistance patterns aligns with local antimicrobial usage trends. The Kirby-Bauer agar diffusion method was used to test the antimicrobial susceptibility following CLSI guidelines (CLSI, 2022). In 3 mL of normal saline, two to three fresh colonies were suspended, and the turbidity of the suspension was adjusted to maintain the 0.5 McFarland turbidity standard. Within 15 minutes, the antimicrobial discs were manually inserted onto the Mueller Hinton agar (MHA) plate after being wiped with this bacterial inoculum. After an incubation of 16–24 hours at 35–37℃, the results were determined. Using a manual millimeter scale, after measuring the diameter of the zone of inhibition encircling the discs, the findings were contrasted with the CLSI breakpoints.

Multiple antibiotic resistance (MAR) index and MDR: Using the formula MAR = (The number of antibiotics to which an isolate was resistant) / (The total number of antibiotics tested), the MAR index was computed in accordance with the parameters as previously established (Naser *et al.*, 2024). When an isolate showed resistance to at least three different classes of antibiotics, it was classified as multidrug resistant (MDR) (Magiorakos *et al.*, 2011). The MAR index ranging from 0 to 1, where values close to 1 indicated strong resistance and values around zero indicated high sensitivity. A significant level of resistance or high-risk bacterial contamination was indicated by an index of 0.20 or above.

PCR amplification for the detection of antimicrobial resistance genes (ARGs): *P. aeruginosa* was selected for ARG detection by PCR due to its clinical importance as a key multidrug-resistant opportunistic pathogen and its recognized role as a reservoir for transferable resistance genes. By using PCR, *P. aeruginosa* was examined for the presence of genes that are resistant to β-lactamases (*bla*TEM, *bla*SHV, *bla*OXA), tetracycline (*tetA*), sulfonamide (*sul1*), and streptomycin (*strA*). The selected ARGs (*bla*TEM, *bla*SHV, *bla*OXA, *tetA, sul1,* and *strA*) were targeted based on their frequent global occurrence in Gram-negative bacteria and their relevance to commonly used antibiotic classes in both human and veterinary settings. All the primers and annealing temperatures are listed in Table 3. All PCR tests were calibrated in a 25 µL reaction mixture that contained 5 µL of DNA template, 12.5 µL of 2x master mix (Add Bio Inc. Ltd., Daejeon, South Korea), 1 µL of forward and reverse primers (each 10 pmol/µL), and 5.5 µL of nuclease-free water. Denaturation at 94℃ for 10 min, 30 cycles of 94℃ for 40 sec, 60℃ for 40 sec, and 72^{0}C for 1 min, and a final elongation phase at 72^{0}C for 7 min were used to amplify the β-lactam gene using multiplex PCR. In order to find the sulfonamide resistant gene, uniplex PCR (u-PCR) was used. The process involved initial denaturation at 95 ^{0}C for 15 min, 30 cycles of 95℃ for 1 min, 1 min of annealing at 66℃ and 72℃, and a final elongation step at 72^{0}C for 10 min. Similar to this, tetracycline and streptomycin resistance genes were detected using uniplex PCR (which involved initial denaturation at 94℃ for 15 min; 30 cycles of 94℃ for 1 min; 1 min of annealing temperature at 63℃ and 72℃ for 1 min ; and a final elongation phase at 72℃ for 10 min. The table-2 below contains the PCR primers used in this study. After running amplicons at 100 V and 500 mA for 30 minutes in 1.5% agarose gel containing Red Safe TM Nucleic Acid Staining Solution, amplicons were visible. The size marker was a 100-bp DNA ladder from Thermo Scientific in the United States. Negative controls were included in all reactions to monitor contamination.

Statistical analysis: The data of this study were entered into Microsoft Excel 2013 (Los Angeles, CA, USA) and SPSS 26.0 (IBM SPSS Statistics version 26.0) (IBM SPSS-25.0, USA) was used for analysis. A statistically significant value was defined as a probability *p* value less than 0.05. We verified the accuracy of these estimations by computing a proportions' 95% confidence interval. Univariable logistic regression analysis was performed to identify significant risk factors which were subsequently included in a multivariate logistic regression using

Table 2: 16S rDNA primer sets used in this study (Spilker *et al.*, 2004).

Primer	Sequence (5'-3')	Target	Annealing Temp (℃)	Product size (bp)
PA-GS-F	GACGGGTGAGTAATGCCTA	*Pseudomonas species*	54	618
PA-GS-R	CACTGGTGTTCCTTCCTATA			
PA-SS-F	GGGGGATCTTCGGACCTCA	*P. aeruginosa*	58	956
PA-SS-R	TCCTTAGAGTGCCCACCCG			

backward stepwise selection. The final model retained biologically plausible variables, assessed for collinearity using Fisher's exact test, and evaluated for goodness-of-fit using the Hosmer-Lemeshow test. The dependent variable was dichotomized as PCR positive (1) and PCR negative (0).

Geospatial mapping and plotting: The "cor" function was used to calculate the Spearman correlation coefficient (r^2), and the "cor.test" function was used to determine the significance of the result. Following that, the "corrplot" package in R and RStudio (Version 4.2.1) was used to show the coefficient values. In an XY-coordinate map, latitude values were plotted on the X-axis and longitude values on the Y-axis to illustrate geographic data, such as the latitude and longitude coordinates of the sample cluster. With ArcMap 10.7, the map was finally created.

RESULTS

Prevalence of *Pseudomonas* spp: The overall prevalence of *Pseudomonas* spp. was 18.72% (95% CI: 13.78–24.53) as determined through molecular (PCR) methods (Figure 4A). Migratory birds exhibited a slightly higher prevalence of 19.64% (22/112) compared to captive wild birds at 17.76% (19/107), although the difference was not statistically significant in either univariate ($\chi2$=0.105, *P*=0.75) or multivariate analysis (OR = 2.69, 95% CI: 0.05–161.13, *P*=0.62) (Table 4).

Prevalence of P. aeruginosa: The prevalence of *P. aeruginosa* was estimated at 10.05% (22/219), with migratory birds showing a higher prevalence (11.61%; 13/112) compared to captive wild birds (8.41%; 9/107). Figure 4B displays the amplicons indicating the targeted genes of *P. aeruginosa* in the isolates. However, this difference was not statistically significant in both univariate ($\chi2$=0.62, *P*=0.50) and multivariate analysis (OR = 1.37, 95% CI: 0.27–6.92, *P*=0.71) (Table 5).

Prevalence by Location: Geographically, the prevalence of *Pseudomonas* spp. varied significantly ($\chi2$=10.77, *P*=0.01). The highest prevalence was observed in Haripur (38.89%, 7/18), followed by the Zoo (24.29%, 17/70), Sunamganj Haor region (15.96%, 15/94), and Sylhet Eco Park (5.56%, 2/36). Multivariate analysis revealed that the odds of testing positive were significantly higher in Haripur (OR = 4.19, 95% CI: 0.06–281.26) and in the Zoo (OR = 3.34, 95% CI: 1.12–10.02, *P*<0.05) compared to Sylhet Eco Park.

Table 3: Primers used for detection of antibiotic resistance genes

Target genes	Sequence (5'-3')	Product size (bp)	Annealing temperature(°C)	References
SulI	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG	433	66	(Kozak *et al.*, 2013)
tetA	F: GGCGGTCTTCTTCATCATGC R: CGGCAGGCAGAGCAAGTAGA	502	63	(Kozak *et al.*, 2013)
strA	F: ATGGTGGACCCTAAAACTCT R: CGTCTAGGATCGAGACAAAG	893	63	(Kozak *et al.*, 2013)
blaTEM	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	60	(Dallenne *et al.*, 2010)
blaSHV	F: AGCCGCTTGAGCAAATTAAAC R: ATCCCGCAGATAAATCACCAC	713	60	(Dallenne *et al.*, 2010)
blaOXA	F: GGCACCAGATTCAACTTTCAAG R: GACCCCAAGTTTCCTGTAAGTG	564	60	(Dallenne *et al.*, 2010)

Table 4: Univariate (χ^2) and multivariate analysis of PCR-positive *Pseudomonas* spp. in relation to bird categories

Variable (Factors)	Category	PCR (positive)	Sample Tested	Prevalence (%) (95% CI)	Univariate Analysis		Multivariate analysis		
					$\chi2$	*P*-value	OR	95% CI	*P*-value
Type of Birds					0.105	0.75			0.62
	Wild	19	107	17.76% (11.04-26.33)			Ref.		
	Migratory	22	112	19.64% (12.74-28.22)			2.69	0.05-161.13	
	Overall	41	219	18.72% (13.78-24.53)					
Location					10.77	0.01			0.01
	Ecopark, Sylhet	2	36	5.56% (0.68-18.66)			Ref.		
	Haripur	7	18	38.89% (17.30-64.25)			4.19	0.06-281.26	
	Sunamganj (Haor)	15	94	15.96% (9.22-24.95)			0.75	0.01-50.81	
	Zoo	17	70	24.29% (14.83-36.01)			3.34	1.12-10.02	
Species of Birds					30.58*	0.02			Not Tested#
	Black Crowned Night Heron	5	6	83.33% (53.51-99.58)					
	Dove	1	4	25.00% (0.63-67.43)					
	Eagle	0	6	N/A					
	Golden Pigeon	0	1	N/A					
	Gray Parrot	2	6	33.33% (4.33-77.72)					
	Gray Heron	0	8	N/A					
	Hornbill	3	5	60.00% (14.66-94.73)					
	Kalim	3	6	50.00% (11.81-88.19)					
	Kite	1	3	33.33% (0.84-90.57)					
	Macaw	0	1	N/A					
	Mathura	1	2	50.00% (1.26-98.74)					
	Moyna	2	8	25.00% (3.19-65.09)					
	Parrot	0	8	N/A					
	Peacock	0	5	N/A					
	Purple Heron	1	3	33.33% (0.84-90.57)					
	Red Jungle Fowl	0	4	N/A					
	Sun Conure	0	1	N/A					
	Vulture	0	3	N/A					

OR: Odds ratio, CI: Confidence Interval, #Superscript indicates the multinominal regression analysis was not performed, N/A: Not applicable, *superscript indicates Fisher exact test, Ref.: Reference Category.

For *P. aeruginosa*, Haripur again showed the highest prevalence (33.33%, 6/18), followed by Sunamganj Haor (15.96%, 7/94), the Zoo (9.86%, 7/71), and Sylhet Eco Park (5.56%, 2/36). Multivariate analysis indicated that Haripur had significantly higher odds of *P. aeruginosa* positivity (OR = 6.21, 95% CI: 1.79–21.61, *P*<0.001) compared to Sylhet Eco Park (Table 5).

Species-Specific Prevalence: Among bird species, the Black Crowned Night Heron exhibited the highest prevalence of *Pseudomonas* spp. at 83.33% (5/6, 95% CI: 53.51–99.58, *P*=0.02), followed by Hornbills (60.00%, 3/5), Kalim (50.00%, 3/6), and Grey Parrots (33.33%, 2/6). Several species, including Golden Pigeons, Gray Herons, and Vultures, tested negative for *Pseudomonas* spp. Due to insufficient data points, multivariate analysis was not performed for species-specific prevalence.

Antimicrobial Susceptibility Test: All the isolates had their susceptibility to 11 regularly used antibiotics from 8 different categories in the human and livestock sectors evaluated. Antimicrobial susceptibility testing revealed that antibiotics such as ciprofloxacin, tetracycline, gentamicin, levofloxacin, meropenem, colistin sulphate and cefixime exhibited complete (100%) susceptibility against *P. aeruginosa*. However, ampicillin demonstrated the highest resistance (73%) against *P. aeruginosa*, as shown in Table 6. The susceptibility pattern, indicated by the zone of inhibition, was visualized using a polar heat map (Fig. 2).

Table 6: Antimicrobial susceptibility profiling of *P. aeruginosa* isolates (n=22)

Organism	Sensitivity status	AMP	CIP	TE	CRO	CT	CN	LEV	MEM	SXT	CFM	AZM
P. aeruginosa (n=22)	Sensitive (%)	0.23	0	0	0.27	0	0	0	100	0.36	0	0.77
	Intermediate (%)	0.04	0	0	0.64	0	0	0	0	0.05	0	0
	Resistant (%)	0.73	0	0	0.09	0	0	0	0	0.59	0	0.23

*AMP=Ampicillin, CIP=Ciprofloxacin, TE=Tetracycline, CRO=Ceftriaxone CT= Colistin sulphate, CN=Gentamicin, LEV= Levofloxacin, MEM= Meropenem, SXT= Sulphamethoxazole/Trimethoprim CFM= Cefixime And AZM= Azithromycin.

Antibiotic resistance genes in *P. aeruginosa*: All the positive isolates (n=22) were tested for ARGs. The amplicons showing targeted genes in the isolates tested are illustrated in Fig. 4 (C-F). The *bla*TEM gene emerged as the most prevalent ESBL-resistant gene, with 20 out of 22 (90.90%) *P. aeruginosa* isolates showing resistance to it. Only one isolate tested positive for both *bla*TEM and *bla*SHV (Fig. 3B). Among antimicrobial-resistant genes, sulfonamide (*sul1*) resistance was the most prevalent at 22.73% (5/22) for *P. aeruginosa*, while the tetracycline-resistant gene (*tetA*) was the least prevalent. Only one isolate exhibited resistance to all three genes. Additionally, three isolates showed combined resistance to *sul1* and *strA* genes, and one isolate was resistant to *tetA* and *strA* (Fig. 3C). Genotypic correlations among the resistant genes were illustrated in Fig. 3A, showing a moderately strong positive correlation between ESBL-resistant genes (*bla*SHV, *bla*OXA) and selected antimicrobial-resistant genes (*strA, sul1*). Conversely, a weak negative correlation (r = -0.21) was observed between *bla*TEM and *strA, sul1* genes (Fig. 3A).

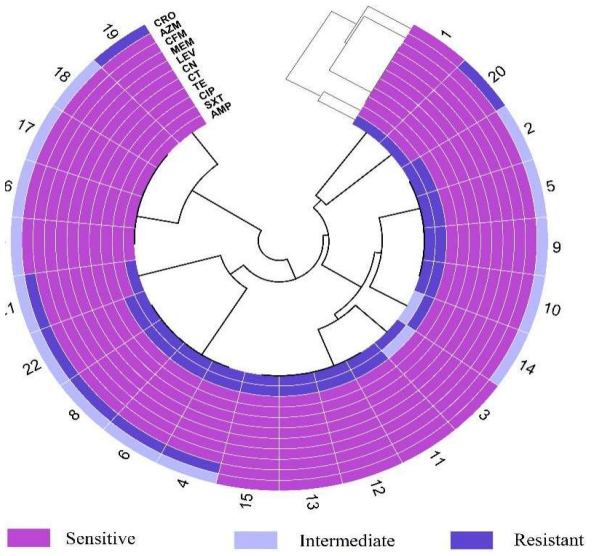

Fig. 2: Polar heat map showing the results of AST of isolated *P. aeruginosa* from captive wild and migratory birds in Sylhet, Bangladesh. [AMP=Ampicillin, CIP=Ciprofloxacin, TE=Tetracycline, CRO=Ceftriaxone CT= Colistin sulphate, CN=Gentamicin, LEV= Levofloxacin, MEM= Meropenem, SXT= Sulphamethoxazole/Trimethoprim CFM= Cefixime, AZM= Azithromycin]. The numerical values shown in figure represent the number of *P. aeruginosa* isolates corresponding to each susceptibility category.

The Venn diagrams illustrated the co-occurrence of Extended-Spectrum Beta-Lactamase (ESBL) genes and ARGs among the samples. In fig. 3B, the ESBL genes *bla*OXA, *bla*TEM, and *bla*SHV were represented. The results showed that *bla*TEM was the most prevalent gene, detected in 19 samples, while *bla*OXA and *bla*SHV were each found in only one sample. Notably, no co-occurrence of all three genes was observed in any sample, and no intersections were detected between *bla*TEM with either *bla*OXA or *bla*SHV.

Table 5: Prevalence of *P. aeruginosa* among migratory and captive wild birds

Variable (Factors)	Category	PCR (positive)	Sample Tested	Prevalence (%) (95% CI)	Univariate Analysis		Multivariate analysis		
					χ2	*P*-value	OR	95% CI	*P*-value
Type of Birds					0.62	0.50			0.71
	Captive wild birds	9	107	8.41% (3.92-15.37)			Ref.		
	Migratory	13	112	11.61% (6.33-19.03)			1.37	0.27-6.92	
	Total	22	219	10.05% (6.40-14.81)					
Location					12.31	0.008			<0.001
	Ecopark, Sylhet	2	36	5.56% (0.68-18.66)			Ref.		
	Haripur	6	18	33.33% (13.34-59.01)			6.21	1.79-21.61	
	Sunamganj	7	94	15.96% (2.14-12.75)			2.31	0.92-80.73	
	Zoo	7	71	9.86% (4.06-19.26)			1.86	0.37-9.45	

OR: Odds ratio, CI: Confidence Interval, #Superscript indicates the multinominal regression analysis was not performed, N/A: Not applicable, Ref.: Reference Category.

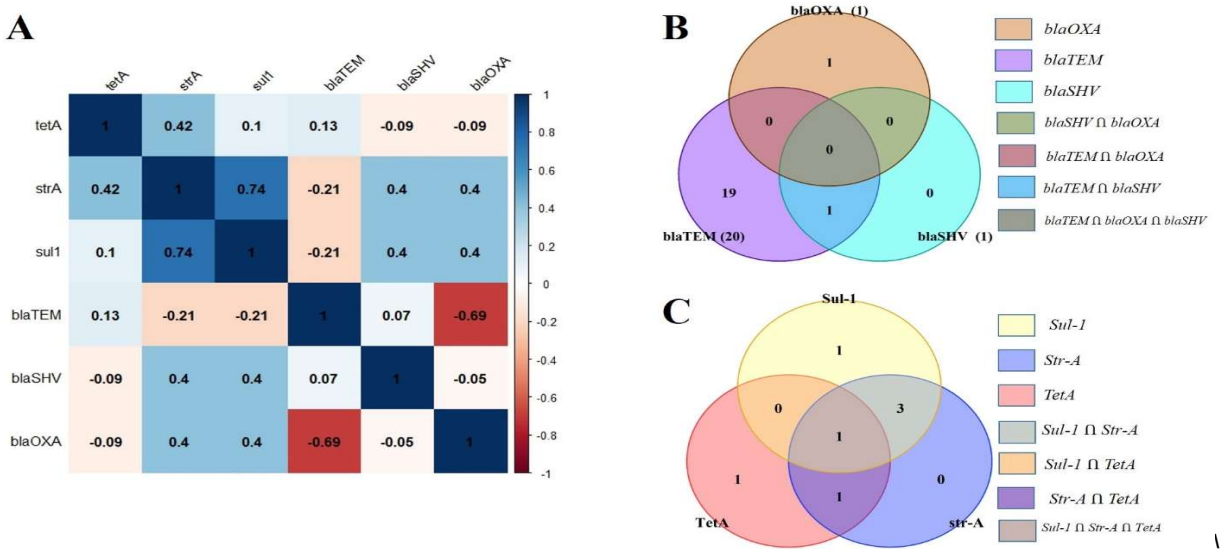

Fig. 3: Patterns of Antimicrobial Resistance Genes in *P. aeruginosa*. The genotypic correlation (Figure A) illustrates the relationship between ESBL-resistant genes and selected antimicrobial-resistant genes, (Figure B and C) the Venn diagram depicts the occurrence of resistant genes both independently and in combination. In Figure 3B (Venn diagram of β-lactamase genes), the intersection between *blaTEM* and *blaSHA* indicates 1 isolate carrying both genes, while no isolates were found in the blue intersection (*blaTEM* and *blaOXA*) or the gray intersection (*blaTEM*, *blaOXA*, and *blaSHV*), showing a lack of co-occurrence among all three genes. In Figure 3C (Venn diagram of *sul1*, *strA*, and *tetA*), the gray intersection represents 1 isolate carrying all three genes. The overlaps between *sul1* and *strA* (yellow-blue) indicates 3 isolates carry these two genes, *sul1* and *tetA* (yellow-red), and *strA* and *tetA* (blue-red) each indicate 1 isolate co-harboring the respective pairs of genes, highlighting the co-occurrence patterns of antimicrobial resistance genes.

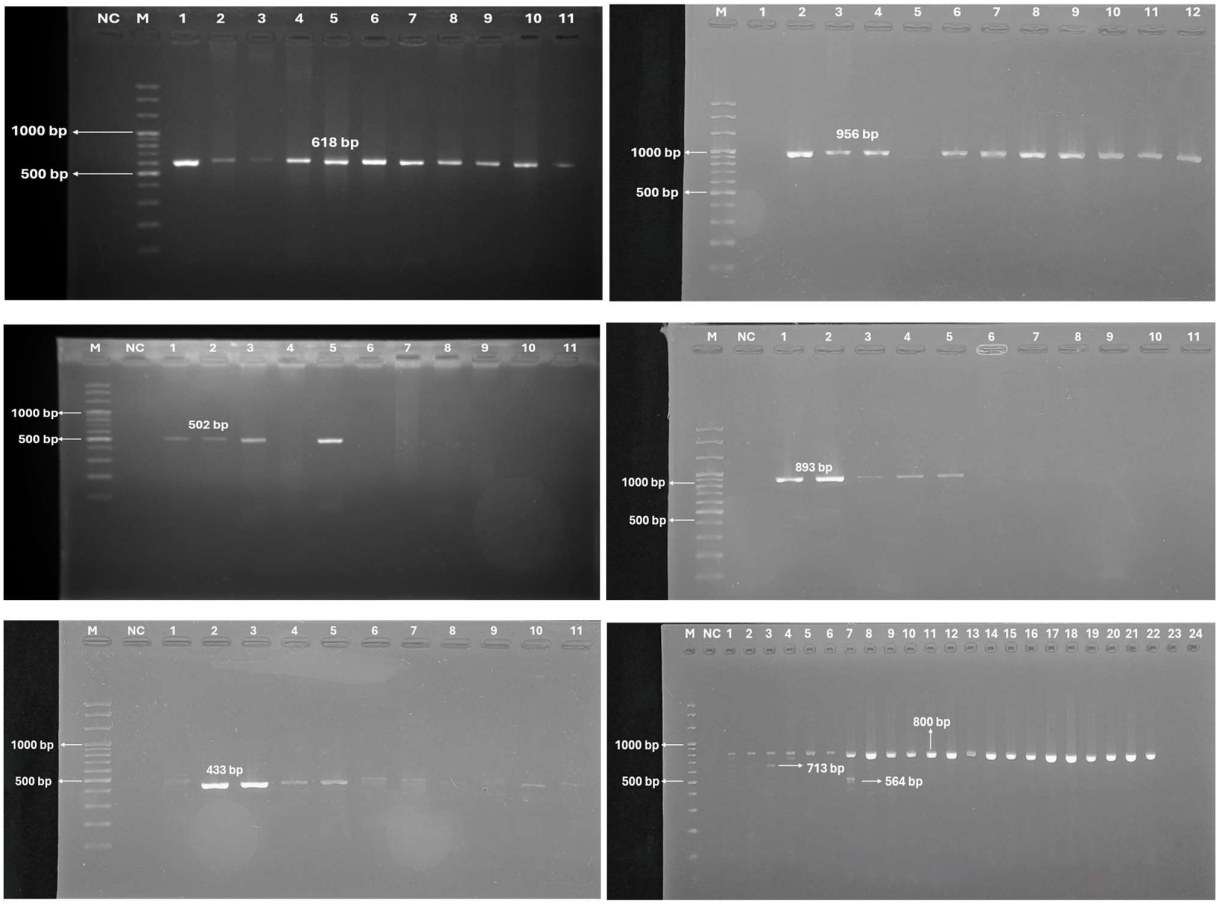

Fig. 4: PCR amplification of antimicrobial resistance genes. **(**A) Amplified DNA of Pseudomonas genus Positive DNA bands were shown at 618 bp. NC and M represent negative control and Marker respectively, 1-11: field isolates; (B) Amplified DNA of PA-SS gene of *P. aeruginosa* isolates at 956bp position. Lane M: 100bp DNA Ladder, Lane NC: -ve Control, Lane 1-12: field isolates; (C) Amplified DNA of *tetA* 502 bp gene. Lane M: 100bp DNA Ladder, Lane NC: -ve Control, Lane 1-5: field isolates; (D) Amplified DNA of *strA* 893 bp gene. Lane M: 100bp DNA Ladder, Lane NC: -ve Control, Lane 1-5: field isolates; (E) Amplified DNA of *sul1* 433 bp gene. Lane M: 100bp DNA Ladder, Lane NC: -ve Control, Lane 1-11: field isolates; (F) Amplified DNA of *bla*OXA (564 bp), *bla*SHV (713 bp), *bla*TEM (800 bp) gene. Lane M: 100bp DNA Ladder, Lane NC: -ve Control, Lane 1-22: field isolates.

In Fig. 3C, the antibiotic resistance genes *sul1, strA*, and *tetA* were analyzed. Each gene was found individually in one sample, while the co-occurrence of *sul1* and *tetA* was observed in another sample. Importantly, the simultaneous presence of all three genes (*sul1, strA*, and *tetA*) was detected in one sample (Fig. 4 C-E), indicating a multidrug-resistant profile.

Multiple antibiotic Resistance (MAR) and MDR: Tests for antibiotic sensitivity showed that most of the *P. aeruginosa* isolates had multiple antibiotic resistance (MAR) indices ranging from 0.18 to 0.36) (Table 7). Multidrug-resistant (MDR) isolates (63.64%) with MAR indices >0.2 may represent high-risk sources of contamination, while isolates with MAR indices ≤0.2 may represent low-risk sources of contamination (Woh *et al.*, 2023). An isolate may remain fully susceptible to certain potent agents while still being categorized as multidrug-resistant based on resistance to at least one agent in three or more antimicrobial classes.

Table 7: MAR index and MDR status of isolates

Antibiotics Resistance Profile	No. of agents	MAR Index	No. of MAR isolates (%)	Overall no. of MDR isolates (%)
AMP-CFM	2	0.18	2(11.76)	14(63.64)
CRO-CFM	2	0.18	1(5.88)	
AMP-SXT-CFM	3	0.27	8(47.06)	
AMP-SXT-CFM-AZM	4	0.36	6(35.30)	

*AMP=Ampicillin, CRO=Ceftriaxone, SXT= Sulphamethoxazole/ Trimethoprim CFM= Cefixime And AZM=Azithromycin

DISCUSSION

Migratory birds travel the world in large numbers every year, bringing resistant microbes with them. Given the limited understanding of multidrug-resistant bacteria in these avian populations, we analyzed fecal samples from captive wild and migratory birds in Sylhet, Bangladesh, to investigate the presence of multidrug-resistant *P. aeruginosa*. Migratory and domesticated wild birds can operate as reservoirs, mixing bowls, and important markers for understanding how human activity impacts the ecosystem, even when they don't directly interact with antimicrobial compounds (e.g. the inappropriate use of antimicrobials). Migratory birds carry antibiotic-resistant germs over large distances, according to numerous studies (Wang *et al.*, 2024). AMR monitoring studies should look into wildlife, particularly birds, as a study focus because of the possible risk to humans, animals, and the environment (Hernando-Amado *et al.*, 2019).

In the present study, 219 feces samples were taken from four different regions of Sylhet, Bangladesh. In this investigation, the frequency of *Pseudomonas* spp. was found to be 18.72% (n = 41/219; 95% CI, 13.78-24.53%), which is similar to a study in Cambridge, England reported 20.9% (n = 24/115; 95% CI, 12.6%–29.2%) (GC Rodrigues *et al.*, 2021). However, other studies reported that the prevalence varied from 2% to 10% (GC Rodrigues *et al.*, 2021). According to other research, high prevalence is typically associated with disease outbreaks or densely populated areas (Islam *et al.*, 2024). Additionally, the prevalence of *P. aeruginosa* in captive wild birds was found to be 8.41% (95% CI, 3.92-15.37), which is higher than the study by (GC Rodrigues *et al.*, 2021). In one investigation, *P. aeruginosa* was discovered in prey birds that exhibited oral lesions and systemic illness (GC Rodrigues *et al.*, 2021), however, in our study, the birds were clinically healthy. Our analysis revealed the prevalence of *P. aeruginosa* in migratory birds of 11.61% (95% CI, 6.33-19.03%). *P. aeruginosa* has been found in migratory birds in Egypt 45.2% and 18.3% cases, respectively (Ahmed *et al.*, 2019; Elshafiee *et al.*, 2022). This research demonstrates that birds may carry extremely harmful bacteria that can spread across the environment and infect humans directly or indirectly. It is crucial to take into account the possibility that migratory or captive wild birds could be a source of these infections. The univariate analysis revealed significant geographic variation in *Pseudomonas* spp. prevalence, with Haripur showing the highest rates (38.89%, P=0.01). Moreover, multivariate analysis identified Haripur as a significant high-risk area for *P. aeruginosa* (OR = 6.21, P<0.001), likely due to proximity to anthropogenic activities (Li *et al.*, 2024). These findings emphasize the role of environmental contamination in driving AMR hotspots.

The authors are aware scarcity data on AMRs and MDR profiles of *P. aeruginosa* either in wild birds or migratory birds outside of outbreak scenarios, despite the concern that more attention should be paid to the risk of transmission from and to other species, including humans. Birds serve as reservoirs and vectors, spreading AMR pathogens through fecal shedding, contaminating water sources, or directly interacting with humans and livestock (Mitchell, 2023). Wild birds frequenting urban areas or agricultural landscapes encounter environments rich in antimicrobial residues and resistant bacteria. These settings facilitate horizontal gene transfer (HGT) through plasmids, transposons, or integrons, enabling the acquisition of ESBL genes such as *bla*TEM. For example, urban birds have been shown to harbor up to three times more AMR genes compared to birds in remote habitats, underscoring the role of habitat contamination in shaping resistance patterns (Mourkas *et al.*, 2024; Munir *et al.*, 2025).

The current investigation shows that *Pseudomonas* is completely susceptible to ciprofloxacin, tetracycline, gentamicin, levofloxacin, meropenem, colistin sulphate, and cefixime. Besides, ampicillin showed the greatest resistance (73%) followed by sulphamethoxazole-trimethoprim (59%) and azithromycin (23%). The *bla*TEM gene is a significant contributor to ESBL production, hydrolyzing β-lactam antibiotics, including penicillins and cephalosporins. The presence of 90.90% prevalence in *P. aeruginosa* isolates exhibiting resistance indicates strong selective pressures from antimicrobial use in the environment. This high prevalence is commonly observed in other studies across diverse regions, including Asia and Europe (Gomes, 2024). Among other ARGs, *sul1* resistance was the most prevalent at 22.73% for *P. aeruginosa*, while *tetA* was the least prevalent. Only one isolate exhibited resistance to all three genes. Additionally, three isolates showed combined resistance to *sul1* and *strA* genes, and one isolate was resistant to *tetA* and *strA*. Genotypic correlations among the resistant genes, showing a moderately strong positive correlation between ESBL-resistant genes (*bla*SHV, *bla*OXA) and selected antimicrobial resistant genes (*strA, sul1*). Furthermore,

63.64% MDR isolates were recorded in our study. With a few minor variations, our genotypic result for the fraction of the ARGs is consistent with our phenotypic data. These findings emphasize the potential for wild and migratory birds to act as vectors for zoonotic disease transmission due to the presence of MDR *P. aeruginosa* and the public health risks associated with their fecal shedding into agricultural and urban environments (Mitchell, 2023).

However, some other study findings differ from our results. The fluoroquinolone (ciprofloxacin) which is included in the WHO's Essential Medicine List and is commonly used, was found 87.5% resistance in a study (Sharland *et al.*, 2018), whereas our result indicated completely sensitive (100%). Significantly, this investigation also revealed that 100% sensitivity was shown for meropenem, a vitally significant antibiotic belonging to the carbapenem class. To maintain its efficacy, meropenem is a last-resort antipseudomonal that is only used in human medicine. Because of the limited use of this group of antibiotics, animal studies frequently do not investigate or report susceptibility to them; the inclusion of carbapenem was deliberate to assess any unintended effects of using human antibiotics on animal *P. aeruginosa*. Nonetheless, no phenotypic resistance was discovered in wild birds in Singapore, indicating that regional variations exist in antibiotic resistance among migratory or wild birds (Aung *et al.*, 2019). The presence of many AGRs ensures genotypic resistance. These circumstances may lead to the development of MDR pathogens. According to a Russian study, wild birds have a high degree of resistance to crucial antimicrobials such as extended spectrum cephalosporins, fluoroquinolones, and colistin (Mukerji *et al.*, 2019). AMR genes may have entered the environment because of the establishment of resistant bacteria in wildlife. One important risk factor for the dissemination of resistant bacteria is the potential for birds' feces to contaminate water supplies or vice versa (Ahmed *et al.*, 2019).

Enhanced integration of wildlife into AMR surveillance programs will be critical for developing targeted interventions that address resistance dissemination at its ecological roots. Our findings align with evidence that wildlife, particularly birds, can serve as reservoirs and disseminators of resistant bacteria, including ESBL producers. This highlights the ecological pathways through which AMR genes circulate across ecosystems, wildlife, and human populations (Benavides *et al.*, 2024). National and regional initiatives, such as Canada's Pan-Canadian Action Plan and the National Antimicrobial Resistance Monitoring System (NARMS) in the U.S., provide examples of integrated AMR monitoring. These programs emphasize the inclusion of wildlife and environmental reservoirs in AMR surveillance to address gaps in understanding resistance dynamics (Franklin *et al.*, 2024). This study is limited by small geographic coverage, not use positive control in PCR, potential sampling bias, and the restriction to culture-based and selected molecular detection methods, which may not capture the full diversity of *P. aeruginosa* or its resistome. Future studies should include large-scale longitudinal surveillance, metagenomic approaches for comprehensive resistome profiling, and comparative studies across different ecological interfaces under a One Health framework.

Conclusions: This study demonstrated a comparatively high prevalence of *P. aeruginosa* in captive wild and migratory birds, raising concerns regarding its potential transmission to humans, livestock, and the environment. Notably, these avian isolates carried fewer antimicrobial resistance genes compared to reports from other countries, suggesting possible geographic or ecological variation in resistome profiles. However, the detection of multidrug-resistant (MDR) *P. aeruginosa* highlights the ongoing risk of antimicrobial resistance dissemination through wildlife reservoirs in Bangladesh. The findings underscore the importance of integrating wildlife surveillance into national AMR monitoring programs under a One Health framework. Strengthening coordination between veterinary, medical, and environmental sectors is essential for early detection and control of resistant pathogens. Future studies should expand sampling across diverse ecological zones, incorporate whole-genome sequencing for comprehensive resistome analysis, and investigate transmission dynamics between birds, livestock, and human populations to better inform targeted intervention strategies.

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Authors contribution: Milton Roy: Data curation, formal analysis, methodology, writing-original draft, writing review & editing. Ruhena Begum: Conceptualization, formal analysis, writing-original draft, writing review & editing. Muhammad Mujahidul Islam: Data curation, formal analysis, writing-original draft. Hemayet Hossain: Methodology, Formal analysis, software, writing-original draft, writing review & editing. Asikur Rahman: Data curation, formal analysis, writing-original draft. Md Bashir Uddin: Methodology, investigation, writing-original draft, writing review & editing. Md. Rafiqul Islam: Methodology, supervision, writing-original draft, writing review & editing. Md. Mahfujur Rahman: Methodology, supervision, writing-original draft, writing review & editing. Ho-Seong Cho: Methodology, investigation, supervision, writing-original draft, formal analysis, writing review & editing. Md. Mukter Hossain: Conceptualization, methodology, investigation, supervision, writing-original draft, formal analysis, writing review & editing.

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