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Spatiotemporal distribution of antimicrobial resistant organisms in different water environments in urban and rural settings of Bangladesh

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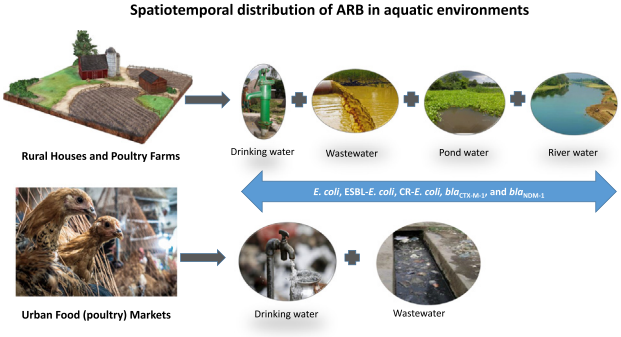
HIGHLIGHTS

- Antibiotic resistant bacteria (ARB) pose a major risk to water quality.
- ARB concentrations in aquatic environments in Bangladesh are high.
- Wastewater from urban markets and river water is highly contaminated with ARB.
- Seasonal differences in the concentration of major ARB were not significant.
- GIS based maps are useful for tracing environmental transmission of ARB.

GRAPHICAL ABSTRACT



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ABSTRACT

The spatial distribution of clinically important antibiotic resistant bacteria (ARB) and associated genes is important to identify the environmental distribution of contamination and 'hotspots' of antimicrobial resistance (AMR). We conducted an integrated survey of AMR in drinking water, wastewater and surface water (rivers and ponds) in three settings in Bangladesh: rural households, rural poultry farms, and urban food markets. Spatial mapping was conducted via geographic information system (GIS) using ArcGIS software. Samples (n = 397) were analyzed for the presence of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-Ec), carbapenem-resistant *E. coli* (CR-Ec) and resistance genes ($bla_{CTX\text{-}M\text{-}1}$, $bla_{NDM\text{-}1}$). In rural households, 5% of drinking water supply samples tested positive for ESBL-Ec, and a high proportion of wastewater, pond and river water samples were positive for ESBL-Ec (90%, 76%, and 85%, respectively). In poultry farms, 10% of drinking water samples tested positive for ESBL-Ec compared to a high prevalence in wastewater, pond and river water (90%, 68%, and 85%, respectively). CR-Ec prevalence in household wastewater and pond water was relatively low (8% and 5%, respectively) compared to river water (33%). In urban areas, 38% of drinking water samples and 98% of wastewater samples from food markets tested positive for ESBL-Ec while 30% of wastewater samples tested positive for CR-Ec. Wastewaters had the highest concentrations of ESBL-

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Ec, CR-Ec, bla_{CTXM-1} and bla_{NDM-1} and these were significantly higher in urban compared to rural samples ($p < 0.05$). ESBL-Ec is ubiquitous in drinking water, wastewater and surface water bodies in both rural and urban areas of Bangladesh. CR-Ec is less widespread but found at a high prevalence in wastewater discharged from urban food markets and in rural river samples. Surveillance and monitoring of antibiotic resistant organisms and genes in waterbodies is an important first step in addressing environmental dimensions of AMR.

1. Introduction

Antimicrobial resistance (AMR) is a global health problem affecting all humans, animals and environments (Lanyon et al., 2021). The role of the environment as an important reservoir for transmission of AMR to both humans and animals is increasingly recognized on a global scale with growing concerns regarding the public health threat (Fletcher, 2015). The 2017 United Nations Environment Programme report highlighted that the environment is key to AMR (Gaze and Depledge, 2017). Although AMR is a natural phenomenon of bacteria, the increased use of antimicrobial compounds in human, animal and agricultural sectors and their subsequent release into the environment has promoted the emergence of novel resistance and their dissemination in wider spaces (D'Costa et al., 2011; Holmes et al., 2016). Up to 90% of administered antibiotics are released in an active form through urine and feces from treated patients and animals (Singer et al., 2016), of which a significant proportion passes directly to the environment (Levison and Levison, 2009). Although the concentration of antibiotics in the environment is much less than the therapeutic doses, these are sufficient to induce antimicrobial resistance in environmental microbiota (Gullberg et al., 2011; Lundström et al., 2016; Kraupner et al., 2018). Compounding concerns are the release of many other compounds in the environment which can co-select resistance to antibiotics (Baker-Austin et al., 2006; Seiler and Berendonk, 2012; Wales and Davies, 2015). Consequently, antibiotic resistant organisms are ubiquitously found in environmental compartments (Holmes et al., 2016). Moreover, there is evidence that environmental bacteria share antibiotic-resistant genes (ARGs) with clinically important bacteria or pathogenic bacteria via horizontal gene transfer (Cantón et al., 2012; Ashbolt et al., 2013), and that humans with high exposure to contaminated environment are more likely to be colonized by antibiotic resistant organisms compared to people with less exposure (Leonard et al., 2018).

A significant proportion of deaths from AMR occur in low and middle-income countries (LMICs) where AMR rates are much higher than high-income countries (Murray et al., 2022). Community carriage of extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) is increasing at an estimated annual global growth rate of 1.5% with the highest prevalence reported from Southeast Asia (Bezabih et al., 2021). In our previous study we found that 74% of healthy infants in rural Bangladesh were colonized with ESBL- *Escherichia coli* (ESBL-Ec) (Islam et al., 2019). Factors that contribute to AMR carriage in LMICs include widespread availability and frequent use of antibiotics (Ghafur, 2010), high population density, and limited access to safe water and sanitation (Laxminarayan et al., 2013; Woerther et al., 2013). Globally, 1.8 billion people drink fecal-contaminated water, while Africa and Southeast Asia have the highest prevalences of water contamination (Bain et al., 2014). More than 80% of sewage or wastewater generated in LMICs flows into the environment untreated (UN Water, 2018; DeFrancis, 2011). In addition, pharmaceutical waste containing high concentrations of antibiotics; hospital effluent (Rozman et al., 2020; Zhang et al., 2020) and agricultural run-off from animal husbandry carrying antibiotic resistant organisms are directly released to the environment (Lübbert et al., 2017). All these wastes contain a cocktail of microorganisms of human, animal and environmental origin and residues of antibiotics/antimicrobial agents, which can select for AMR.

The aquatic environment is very important in the context of AMR, especially for a country like Bangladesh. Bangladesh has a landscape populated with numerous ponds and wetlands along with more than 250 rivers which are an integral part of human and animal subsistence (Islam, 2016). However, studies reporting contamination of surface waterbodies with antibiotic resistant bacteria (ARB) in Bangladesh are limited (Haque et al., 2014; Kamruzzaman et al., 2013) and none has investigated seasonal or spatial variation. A study conducted in urban Dhaka showed 71% and 62% of environmental water samples were positive for the ESBL gene, $bla_{CTX-M-15}$, and the carbapenemase gene, bla_{NDM-1}, respectively (Toleman et al., 2015). Similarly, wastewater samples in Dhaka city particularly at sites adjacent to hospitals were found to be contaminated with multi-drug resistant organisms including bla_{NDM-1} positive *E. coli* (Rabbani et al., 2017). While aquatic environments are likely to play a major role in the transmission of AMR, identification of major aquatic reservoirs to which humans and animals are exposed is the key to successful implementation of community-based intervention strategies for combating AMR (Singer et al., 2016). The aim of the current study was to adopt an integrated spatial approach to quantify the prevalence and abundance of AMR organisms and ARGs in different environmental compartments in both urban and rural areas of Bangladesh and visualize the results using geographic information system (GIS).

2. Materials and methods

2.1. Study design

Our primary objective was to adopt an integrated spatial approach to estimate the prevalence and abundance of antimicrobial resistant organisms and antimicrobial resistant genes in different environmental compartments that are likely to be impacted by intensive poultry production/selling practices in both urban and rural areas of Bangladesh. We investigated the point prevalence of ESBL-Ec and CR-Ec and associated resistant genes in water samples collected from drinking water supply, wastewater drains and surface water bodies in three different settings in Bangladesh. Sites included urban food markets in high-density residential areas in Dhaka city; rural small-scale commercial poultry farms, and rural households in Mirzapur sub-district (*upazila*) in Tangail district. In urban areas, we selected food markets as these are the largest outlets of commercial poultry where birds are slaughtered and processed on site with no regulated waste disposal system. Fecal wastes produced within urban markets are mostly disposed into the municipal drainage system through direct washout. Due to the complexity of the wastewater circulation and disposal system, we only sampled wastewater from markets at the points of discharge to the municipal drainage system. Although there are rivers surrounding Dhaka city, those are not in proximity of the selected markets and therefore were not sampled as there was no spatial connection.

In rural communities (households and farms), we sampled ground water (drinking water from deep tubewells) and the wastewater outlets adjacent to individual households or poultry farms. We then sampled pond water located within the same property, and river water at the closest point to the property. Ponds in rural areas are found on individual properties (homesteads) and are often artificially constructed (known as '*pukur*' in Bengali, ranging from 150 to 1000 square metres area) (Huq, 2017). The ponds collect run off from household drains, or drainage ditches as well as rainwater/surface water. They are used for a multitude of purposes, including waste disposal, bathing of people and animals, cooking and washing utensils. For the urban food markets in Dhaka, we sampled the municipal water supply and the wastewater directly discharged from market stalls consisting mainly of market run-off.

2.2. Study settings and duration

We collected environmental samples over two periods from February to April (which we considered as winter) (n = 197) and August to November

(summer) (n = 200). Average air temperatures in Bangladesh range from 17° − 20.6 °C in winter and 26.9° − 31.1 °C in summer (Shahid, 2010). Locally, winter is considered as the dry season due to low rainfall and limited navigability of water bodies and summer is considered as the wet season. Different households, farms and markets within the area were included in each sampling period to cover most of the aquatic environments in the study area.

For assessment of ARB, we investigated ESBL-Ec and CR-Ec, and for ARGs, we considered $bla_{\text{CTX-M-1}}$ and $bla_{\text{NDM-1}}$, which are the most common genes found in ESBL-Ec and CR-Ec, respectively (Bevan et al., 2017; Nordmann and Poirel, 2014). WHO has also recommended ESBL-Ec as an indicator organism for integrated One Health surveillance of AMR due to its clinical significance, ubiquitous presence in humans, animals and the environment, and its ability to be easily transmitted between these compartments (WHO, 2020a).

2.3. Sampling strategy and laboratory analysis

2.3.1. Sampling procedure

In the rural site, 40 households and 40 poultry farms were selected from different villages in Mirzapur sub-district which is surrounded by five rivers and their branches namely Bangshi, Lauhojong, Longla, Dhaleswari and Futjani. All the selected villages were in the vicinity of river branches. From each village (n = 20), the data collector selected 2 households with backyard poultry, which were 10 households apart from each other. For the selection of farms, 1 broiler poultry farm per village was selected using convenience sampling. For each household or farm, we collected samples from the drinking water supply to the individual household (tubewell water, 1 per household/farm, n = 80) located on the household/farm premises, wastewater (in the vicinity of the household premises or farm wastewater drains, 1 per household/farm, n = 80), water from the closest pond to the property (1 per household/farm, n = 80) and river water (at the closest point, approximately 0.5 to 1.0 km from the household/farm, n = 80).

In urban areas, we selected 40 different urban food markets within Dhaka North and South City Corporation following convenience sampling. In each market, the data collector selected the 11th eligible poultry stall starting from the center. Then, municipal water supply samples within the market (drinking water, n = 40, 1 per market) and wastewater drain samples containing market run-off (n = 40, 1 per market) were collected, both of which were near the poultry stall.

We collected samples during two different seasons to characterize the potential temporal variation in ABR and ARGs with changes in season temperature, pH, conductivity, and surface water volume. The first sampling took place in winter when surface water levels were low and the second sampling was conducted in summer.

2.3.2. Sample collection

In rural households and farms, we collected drinking water samples directly from tubewells and in urban markets, we collected water samples directly from taps for municipal water supply. For tubewell samples, we operated the hand pump of the tubewell continuously for 5 min, followed by heating the mouth of the pump by using a gas torch and then again pumping out several litres of water. At this stage, we collected water samples aseptically into a sterile bottle (Nalgene, Rochester, New York). For tap water samples, we first cleaned the outside nozzle of the tap and allowed the water to run to waste for 1 min. Using a gas torch, we heated the nozzle and allowed the water to run to waste for a few seconds. We collected water samples aseptically into a sterile bottle (Nalgene, Rochester, New York) and replaced the cap carefully. We collected approximately 150 ml of water samples.

We collected wastewater samples from three different locations of the drain proximal to rural households, farms and urban food markets. The total volume of the pooled sample was approximately 900 ml (300 ml from each location). In the case of ponds and rivers, we collected water samples by dipping a sterile bottle into the water approximately 30 cm below the water surface. We placed all water samples in a cold box (4 °C–8 °C) immediately after collection and transported to the laboratory within 8 h of collection. Samples were processed immediately upon arrival in the lab.

2.3.3. Temperature, pH and conductivity assessment of water samples

Temperature and electrical conductivity of water samples were assessed using a total dissolved solids (EC-TDS) meter (Hanna Instruments, UK; Model: HI 98311). Water pH was measured with an accuracy of ±0.1 using a portable pH meter (Hanna Instruments, UK; Model: HI 98127). The probe/electrode of both the EC-TDS meter and pH meter were submerged in the water samples at the point of collection. All readings were taken following manufacturer's instructions.

2.3.4. Culture of samples for E. coli resistant to third generation cephalosporin or carbapenem

For all water sample types, 3 × 100 ml of water was filtered through three separate 0.22 μm nitrocellulose membrane (Sartorius Stedim Biotech GmbH, Goettingen, Germany) using a Millipore manifold filtration system (EZ-Fit™ Manifold, Merck KGaA, Darmstadt, Germany) in which microorganisms were retained on the membrane surface. Membrane filters were placed in an upright position on three mTEC agar plates (BD Difco®); one without supplementation, one supplemented with cefotaxime (1 mg/l) and the other one with meropenem (0·5 mg/l) (Rousham et al., 2021). For each batch of sample, the same volume of autoclaved distilled water passed through nitrocellulose membranes plated on the same culture media was considered as lab blanks. Plates were incubated at 35 ± 0.5 °C for 2 h followed by further incubation at 44.5 ± 0.2 °C for approximately 22–24 h. After incubation, magenta colour colonies, typical of *E. coli*, were counted. At least two isolated colonies were extracted from each sample plate and stored in glycerol stocks at −80 °C.

For biochemical identification and confirmation, one *E. coli* isolate from each sample was tested using API20E kits (BioMérieux, France). For any sample where the first isolate tested negative for *E. coli*, the second isolate was tested to confirm *E. coli* identification. All isolates obtained from cefotaxime plates were tested for ESBL production by double disc synergy test (DDST) following the method described by CLSI (CLSI 2016). Isolates from meropenem plates were confirmed as resistant to carbapenem by doing susceptibility test against meropenem (10 μg), imipenem (10 μg) and ertapenem (10 μg).

2.3.5. Quantification of bla_{NDM-1} and $bla_{CTX-M-1}$ genes

For all types of water samples, 100 ml of sample was filtered through a 0.22 μm nitrocellulose membrane (Sartorius Stedim Biotech GmbH, Goettingen, Germany) using a Millipore manifold filtration system (EZ-Fit™ Manifold, Merck KGaA, Darmstadt, Germany). The filters were placed in 2-ml tubes with glass beads (GeneRite, North Brunswick, NJ) and DNA was extracted from the filters using the MO Bio Power Water DNA isolation Kit (MO BIO Laboratories Inc., USA) following user's instructions. DNA extraction was performed in sets of 10–20 filters at a time. For each extraction set, a process blank was created by passing through the same volume of autoclaved distilled water through nitrocellulose membrane and used for DNA extraction. Extraction process blanks were treated in the same way as the water samples, except that the extraction blank bead tube had no filter.

Gene amplification for $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-1}}$ was carried out in a Bio-Rad CFX96 real-time PCR platform using TaqMan technology. Details of primers, probes and PCR conditions are provided in Supplementary material 1. A recombinant plasmid DNA (pUCIDT-Kan^r) containing target gene sequences ($bla_{\text{CTX-M-1}}$ and $bla_{\text{NDM-1}}$) was commercially produced (IDT Inc.) and used to prepare a known concentration of DNA solution. The stock solution of plasmid DNA (40 ng/μl, 2.35×10^{10} copies/2 μl volume) was 10-fold serially diluted to make solutions containing 10^1–10^7 copies of plasmid DNA that were used for generating a standard curve. The amplification of standard DNA was linear over dilutions (r^2 = 0.999; slope = −3.66, *y*-int = 40.614, and E = 99.0%). The Cq value variation for $bla_{\text{CTX-M-1}}$ and $bla_{\text{NDM-1}}$ was 31.35–34.79 at the limit of detection (LOD).

Each sample was run in triplicate, and amplification was only considered as positive if all three technical replicates showed a positive result. Mean copy number of genes calculated from three replicates of each sample was used in subsequent analysis. Data were analyzed using Bio-Rad CFX Manager (version 3.1).

2.4. Geospatial mapping and geocoding

GPS (geographic positioning system) coordinates of all households, farms and markets and the downstream sample collection sites were recorded using a GPS device (Garmin, etrex 10). For household locations, GPS coordinates were recorded at the entrance to the kitchen, to represent the center of the homestead. Farm and urban market locations were recorded at the entrance of poultry sheds and the market stalls, respectively. GIS coordinates (northing and easting) were stored and downloaded to Microsoft Excel, recorded to three decimal places and finally converted to GIS shape file in ArcGIS software. Later, the GIS shape files were linked with microbiological and laboratory data. All the GIS coordinates related to water samples were geolocated on a base map using ArcMap® version 10.6.1 in ArcGIS (ESRI®, Redlands, CA). Each data point in the maps represents an environmental sample with the concentration of ESBL *E. coli* indicated by the size of the data point using three arbitrary cut-points for low ($\log_{10}$1.0- $\log_{10}$2.99 CFU/100 ml), middle ($\log_{10}$ 3.0- $\log_{10}$5.99 CFU/100 ml) and high ($\log_{10}$6.0- $\log_{10}$9.99 CFU/100 ml) concentrations. Black dots indicate a sample that was negative for ESBL-Ec.

2.5. Statistical analyses

The prevalence of ESBL-Ec and CR-Ec was assessed as the proportion of positive culture results obtained from the total number of samples for a given environmental compartment. As *E. coli* counts displayed a non-normal distribution, all counts were log transformed to $\log_{10}$ (1 + x), where x is the bacterial count. In addition, all zero values (negative for bacteria and quantitative gene counts) were assigned with a random generated number between zero and the limit of detection (LOD) for each sample type (drinking water, surface water and wastewater) (Canales et al., 2018). The copy number of $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-1}}$ genes was estimated using qPCR. While the number of ESBL-Ec or CR-Ec provides an estimation of the culturable ESBL-producing *E. coli* or carbapenem resistant *E. coli*, copies of $bla_{\text{CTX-M-1}}$ and $bla_{\text{NDM-1}}$ genes provide a presumptive estimation of the total number of ESBL-producing and carbapenem resistant organisms present in the sample.

ESBL-Ec and CR-Ec counts were expressed as a proportion of total culturable *E. coli* counts per sample according to the recommendation by the WHO Tricycle project guideline (Matheu et al., 2017). For each sample, we calculated the proportion of resistant bacteria by dividing the number of colonies on culture media (mTEC) with (cefotaxime) vs. without antibiotic supplementation. Only ESBL-Ec or CR-Ec positive samples were used for calculating the proportions and for each sample type, median proportion and interquartile range (IQR) were enumerated. Frequencies of ARGs were expressed as a proportion of all samples that were positive for ESBL-Ec isolates.

We used IBM SPSS (version 23.0) and Stata (version 13.0) software for data analysis. ARB and ARG counts were tested for symmetric distribution using Shapiro-Wilk test. Most of the variables showed non-normal distributions for which non-parametric statistical tests such as Wilcoxon test or Kruskal Wallis test were executed. The percent prevalence for ARB and ARGs was compared using Chi-square test. Binary logistic regression was used to identify the potential risk factors for ESBL-*E. coli* contamination of wastewater, and pond water. The *p*-value for the Pearson correlation coefficient was calculated to find out if contamination of surface water is associated with physicochemical properties of water including pH, conductivity, and temperature in different seasons. For multiple comparisons among different sample types, Tukey's post hoc test was employed. Statistical significance was defined by 95% confidence intervals at $p < 0.05$.

Table 1
Prevalence of ESBL-Ec, CR-Ec, $bla_{\text{CTX-M-1}}$, and $bla_{\text{NDM-1}}$ in water samples from rural households, poultry farms and urban food markets.

Locations	Organisms	Drinking water n (%)	Wastewater n (%)	Pond water n (%)	River water n (%)
Rural households	ESBL-Ec	2 (5)	36 (90)	28 (76)	34 (85)
	CR-Ec	0 (0)	3 (8)	2 (5)	13 (33)
	$bla_{\text{CTX-M-1}}$	3 (8)	24 (60)	5 (14)	11 (28)
	$bla_{\text{NDM-1}}$	2 (5)	14 (35)	3 (8)	8 (20)
Poultry farms	ESBL-Ec	4 (10)	36 (90)	27 (68)	34 (85)
	CR-Ec	0 (0)	2 (5)	3 (8)	5 (13)
	$bla_{\text{CTX-M-1}}$	0 (0)	17 (43)	5 (13)	3 (7)
	$bla_{\text{NDM-1}}$	0 (0)	13 (33)	2 (5)	6 (15)
Urban food markets	ESBL-Ec	15 (38)	39 (98)	NA	NA
	CR-Ec	0 (0)	12 (30)	NA	NA
	$bla_{\text{CTX-M-1}}$	0 (0)	33 (83)	NA	NA
	$bla_{\text{NDM-1}}$	0 (0)	23 (58)	NA	NA

ESBL-Ec = extended-spectrum β-lactamase-producing *E. coli*.
CR-Ec = carbapenem-resistant *E. coli*.
NA = no surface water samples were collected in urban setting.

3. Results

3.1. Prevalence of ESBL-Ec and CR-Ec in aquatic environmental samples

In rural households, 5% of tubewell water (drinking water) samples tested positive for ESBL-Ec by direct culture of samples, while 90% of wastewater, 76% of pond water and 85% of river water samples were positive for ESBL-Ec. Similarly, 10% of drinking water samples from poultry farms tested positive for ESBL-Ec while a higher prevalence was observed in wastewater, pond and river water samples (90%, 68%, and 85%, respectively) (Table 1, Fig. 1). In urban food markets, 38% of drinking water samples and 98% of wastewater samples were positive for ESBL-Ec.

CR-Ec was less common than ESBL-Ec and no drinking water samples from any of the three sites were positive for CR-Ec. However, in urban food markets, 30% of wastewater samples were positive for CR-Ec, which is significantly higher than wastewater samples from rural households and farms (8% and 5%, respectively, $p < 0.05$, ANOVA) (Table 1). Around 33% of river water samples adjacent to rural households were positive for CR-Ec while only 13% of river water samples adjacent to farms were positive ($p < 0.05$, Chi-square test) (Table 1). In pond water, the difference in prevalence of CR-Ec between household ponds and farm ponds was not statistically significant (5% versus 8% respectively, $p = 0.470$, Chi-square test) (Table 1).

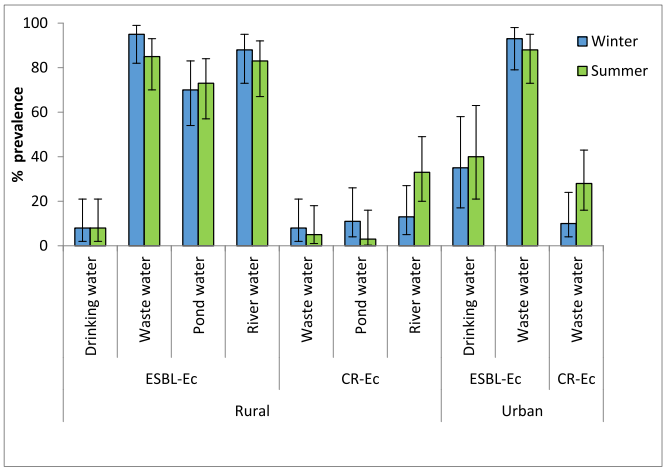

Fig. 1. Winter and summer prevalence of ESBL-Ec and CR-Ec in differnt aquatic environments *CR-Ec = carbapenem resistant *E. coli*; ESBL-Ec = extended-spectrum β-lactamase-producing *E. coli*.

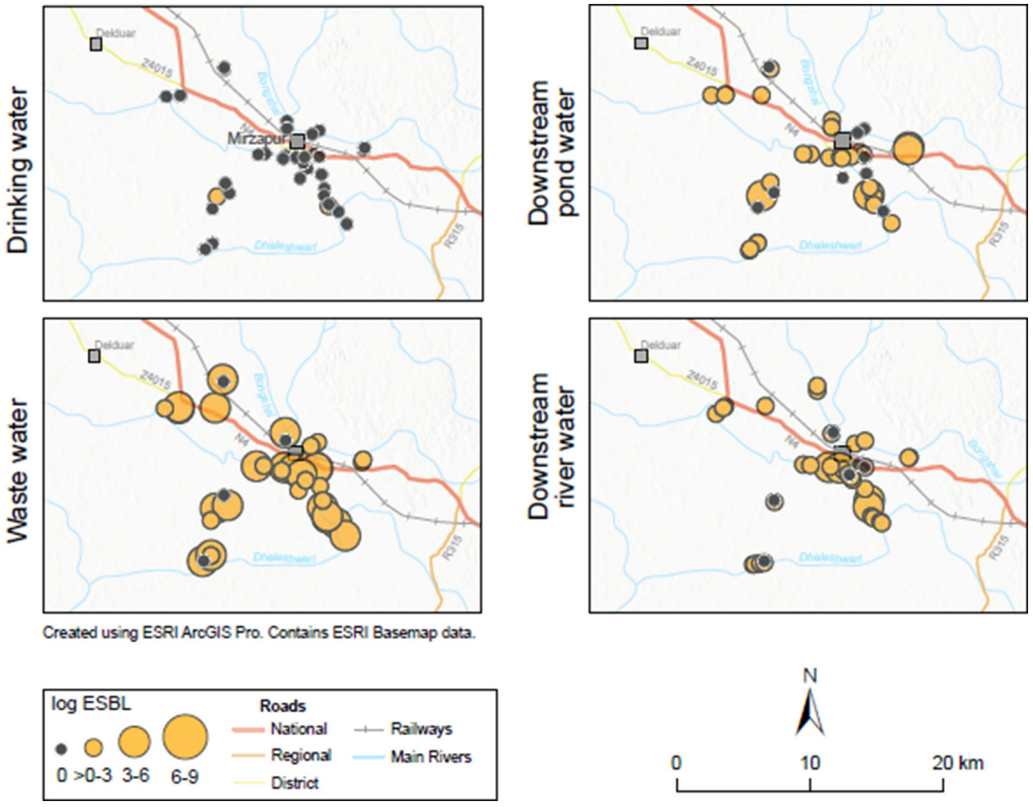

Fig. 2. Concentrations of ESBL-Ec (log_{10} CFU/ml) in household drinking water supply, household wastewater, household pond water and downstream river water adjacent to each household for 40 rural households in Mirzapur*. *Each data point represents an environmental sample with the concentration of log_{10} ESBL-Ec indicated by the size of the data point using three arbitrary cut-points for low (log_{10}1.0- log_{10}2.99), middle (log_{10} 3.0- log_{10}5.99) and high (log_{10}6.0- log_{10}9.99) concentrations. Black dots indicate a sample that was negative for ESBL-Ec.

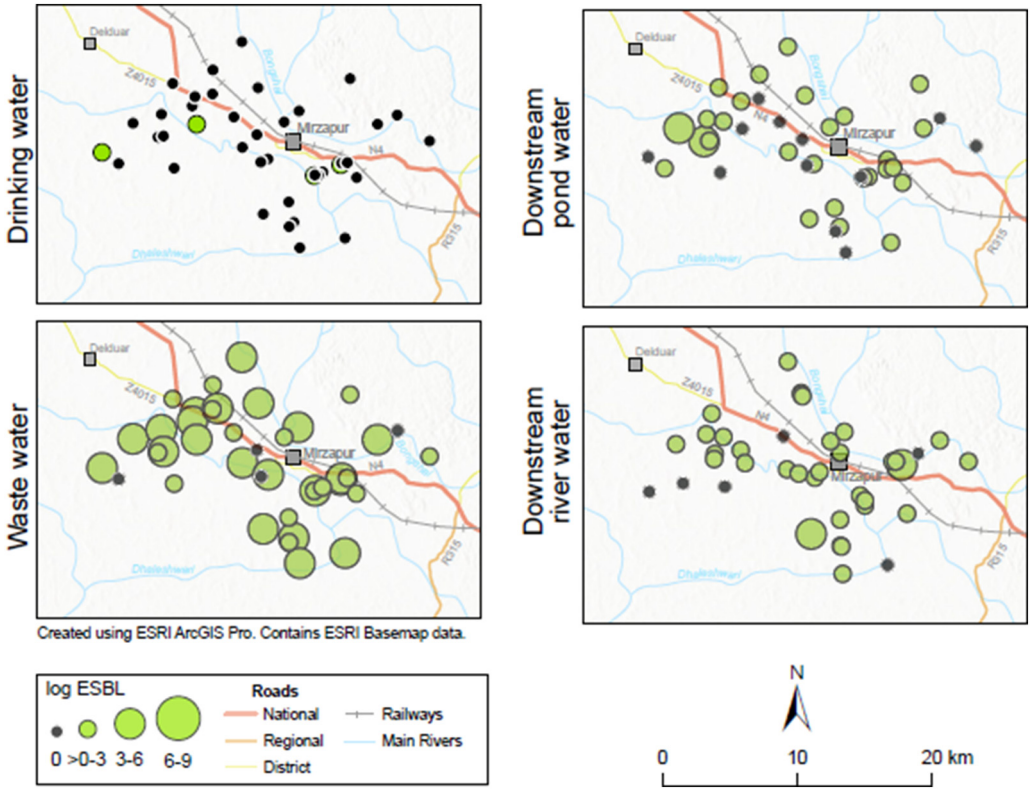

Fig. 3. Concentrations of ESBL-Ec (log_{10} CFU/ml) in drinking water supply, farm wastewater, farm pond water and downstream river water adjacent to each farm for 40 poultry farms in Mirzapur*. *Each data point represents an environmental sample with the concentration of log_{10} ESBL-Ec indicated by the size of the data point using three arbitrary cut-points for low (log_{10}1.0- log_{10}2.99), middle (log_{10} 3.0- log_{10}5.99) and high (log_{10}6.0- log_{10}9.99) concentrations. Black dots indicate a sample that was negative for ESBL-Ec.

3.2. Spatial distribution of ESBL-Ec and CR-Ec in aquatic environments

The spatial distribution of ESBL-Ec in aquatic samples is presented for rural households (Fig. 2), poultry farms (Fig. 3) and urban food markets in Dhaka city (Fig. 4). Spatial distribution clearly demonstrates the relatively lower concentration of ARB in ground water or water supply compared to wastewater. In rural households and farms, drinking water samples were mostly negative for ESBL-Ec while the majority of wastewater, pond and river water samples were positive for these organisms. In urban markets, a higher proportion of drinking water samples were positive for ESBL-Ec compared to the samples from rural areas (Fig. 4).

We found a significantly higher concentration of ESBL-Ec and CR-Ec in wastewater compared to pond and river water samples ($p < 0.001$, Kruskal Wallis test) except for CR-Ec in poultry farm wastewater (Table 2; Figs. 2 and 3). The mean ESBL-Ec counts ($\log_{10}$ CFU/100 ml) in wastewater adjacent to rural households and farms were 2.91 ± 1.32 SD and 3.06 ± 1.55 SD, respectively (Table 2). The pond and river water adjacent to rural households had mean ESBL-Ec counts ($\log_{10}$ CFU/100 ml) of 1.49 ± 1.10 SD and 1.66 ± 1.08 SD, respectively. In pond and river water adjacent to farms, the mean ESBL-Ec counts ($\log_{10}$ CFU/100 ml) were 1.16 ± 0.95 SD and 1.38 ± 0.82 SD, respectively. River water samples appeared to have a significantly higher proportion of ESBL-Ec over total *E. coli* in the sample compared to pond and wastewater samples in rural settings (Table 2). We also found a relatively high concentration of CR-Ec in the wastewater from rural households and farms with a mean count of 2.07 ± 1.19 SD and 1.70 ± 0.38 SD $\log_{10}$ CFU/100 ml, respectively. The concentration of CR-Ec in pond water samples adjacent to rural households and farms were higher (1.13 ± 0.40 SD, 0.69 ± 0.37 SD, respectively) than the river water samples adjacent to rural households and farms (0.85 ± 0.44 SD, 0.55 ± 0.22 SD, respectively). Overall, river water samples had a higher concentration of ESBL-Ec than pond water samples but in the case of CR-Ec this association was reversed.

In urban food markets, wastewater had a significantly higher concentration of ESBL-Ec and CR-Ec compared to drinking water samples ($p < 0.001$, Kruskal Wallis test) (Table 2; Fig. 4). The mean ESBL-Ec counts ($\log_{10}$ CFU/100 ml) in wastewater samples and drinking water samples were 5.59 ± 2.85 SD and 0.53 ± 0.34 SD respectively. The abundance of CR-Ec was also very high in market wastewater compared to all other samples with a mean CR-Ec of 3.48 ± 1.61 SD $\log_{10}$ CFU/100 ml. The ratio of ESBL-Ec to total number of *E. coli* colony forming units in drinking water samples from urban food markets were significantly higher than that of wastewater (20.79% vs. 6.67%) ($p < 0.001$, Kruskal Wallis test).

We also compared the concentration of ARB and ARGs in rural and urban settings (Table 3) which showed greater contamination in urban environments. The mean ESBL-Ec counts ($\log_{10}$ CFU/100 ml) in wastewater adjacent to urban settings (5.59 ± 2.85 SD) were significantly higher than those counts in rural settings (2.99 ± 2.39 SD) ($p < 0.001$, Wilcoxon Rank Sum test) (Table 3). Similarly, drinking water adjacent to rural and urban settings had mean ESBL-Ec counts ($\log_{10}$ CFU/100 ml) of 0.23 ± 0.16 SD and 0.53 ± 0.34 SD, respectively ($p < 0.05$, Wilcoxon Rank Sum test). The mean total *E. coli* counts ($\log_{10}$ CFU/100 ml) was significantly higher in urban wastewater (6.91 ± 1.25 SD) and rural drinking water (0.93 ± 0.99 SD) ($p < 0.001$, Wilcoxon Rank Sum test) which is consistent with the result of the median proportion of ESBL-Ec in total *E. coli* (Table 3). CR-Ec mean counts ($\log_{10}$ CFU/100 ml), concentration of $bla_{\text{CTX-M-1}}$, and $bla_{\text{NDM-1}}$ genes ($\log_{10}$ gene copy number/100 ml) in wastewater were significantly higher in the urban markets compared to those from rural areas ($p < 0.05$, Wilcoxon Rank Sum test).

We investigated the risk factors associated with AMR contamination, particularly in relation to waste disposal practices in poultry farms and rural households (Tables S1 & S2). To assess the risk of ESBL-Ec contamination of wastewater and pond water in rural settings, we selected several variables such as number of poultry, types of waste disposed, distance of disposal sites and pond from households or farms, likelihood of pond water contamination with ESBL-Ec if wastewater of adjacent households or farms is positive for ESBL-Ec. However, we did not find any statistically significant risk factors associated with ESBL-Ec contamination of wastewater and pond water in rural settings.

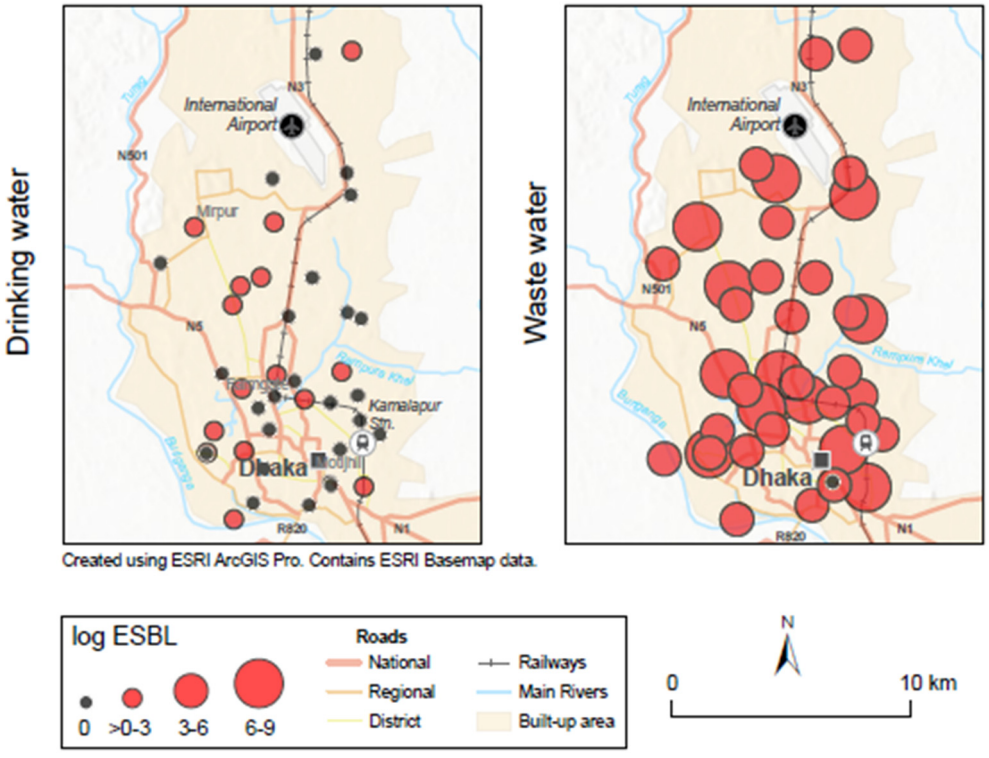

Fig. 4. Concentrations of ESBL-Ec ($\log_{10}$ CFU/ml) in market drinking water supplies and wastewater from 40 urban food markets within Dhaka city*. *Each data point represents an environmental sample with the concentration of $\log_{10}$ ESBL-Ec indicated by the size of the data point using three arbitrary cut-points for low ($\log_{10}$1.0- $\log_{10}$2.99), middle ($\log_{10}$ 3.0- $\log_{10}$5.99) and high ($\log_{10}$6.0- $\log_{10}$9.99) concentrations. Black dots indicate a sample that was negative for ESBL-Ec.

Table 2
Comparison of the abundance of ESBL-Ec, total *E. coli*, ESBL gene ($bla_{CTX-M-1}$), CR-Ec and CR gene (bla_{NDM-1}) in aquatic samples from rural households, poultry farms and urban food markets.

Attributes	Rural households				Poultry farms				Urban food markets		
	Wastewater	Ponds	River	p^b	Wastewater	Ponds	River	p^b	Drinking water	Wastewater	p^b
	(N = 40)	(N = 37)	(N = 40)		(N = 40)	(N = 40)	(N = 40)		(N = 40)	(N = 40)	
	Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
ESBL-*E. coli* counts (log_{10} CFU/100 ml)	2.91 ± 1.32[a]	1.49 ± 1.10[a]	1.66 ± 1.08[a]	<0.001	3.06 ± 1.55[a]	1.16 ± 0.95[a]	1.38 ± 0.82[a]	<0.001	0.53 ± 0.34	5.59 ± 2.85	<0.001
Total *E. coli* counts (log_{10} CFU/100 ml)	4.76 ± 0.93[a]	3.07 ± 1.22[a]	2.94 ± 1.02[a]	<0.001	5.31 ± 1.38	2.94 ± 1.14[a]	2.70 ± 0.97	<0.001	0.93 ± 0.99	6.91 ± 1.25	<0.001
Median proportion (IQR) of ESBL-Ec in total Ec (%)	3.10 (17.93)	4.07 (10.34)	6.38 (22.34)	0.036	0.95 (5.12)	2.86 (6.05)	8.05 (20.93)	0.005	20.79 (29.01)	6.67 (20.97)	<0.001
Carbapenem-resistant *E. coli* (log_{10} CFU/100 ml)	2.07 ± 1.19	1.13 ± 0.40	0.85 ± 0.44	<0.001	1.70 ± 0.38	0.69 ± 0.37	0.55 ± 0.22	0.508	–	3.48 ± 1.61	–
Median proportion (IQR) of Carbapenem Ec in total Ec (%)	0.02 (0.96)	0.02 (0.02)	0.70 (3.13)	0.038	0.01 (0.004)	0.29 (2.12)	0.91 (0.90)	0.235	–	0.02 (0.41)	–
$bla_{CTX-M-1}$ (log_{10} gene copy number/100 ml)	4.35 ± 1.48[a]	0.75 ± 1.93[a]	1.38 ± 2.47[a]	<0.001	4.04 ± 1.61[a]	0.68 ± 1.87[a]	0.41 ± 1.51[a]	<0.001	2.81 ± 0.31	6.01 ± 1.85	<0.001
bla_{NDM-1} (log_{10} gene copy number/100 ml)	2.10 ± 3.61[a]	0.42 ± 1.46[a]	1.14 ± 2.71[a]	0.110	1.86 ± 2.83[a]	0.27 ± 1.16[a]	0.90 ± 2.69[a]	0.082	–	3.33 ± 3.21	–

Using Tukey post-hoc comparisons [a] denotes pairs of groups significantly different at $p < 0.001$; [b] denotes *p*-values generated using ANOVA/Kruskal Wallis test.

3.3. Distribution of $bla_{CTX-M-1}$ and bla_{NDM-1} genes in aquatic environments

The mean concentration of $bla_{CTX-M-1}$ in drinking water samples from rural households was 2.81 ± 0.31 SD log_{10} gene copy number/100 ml. The concentration of $bla_{CTX-M-1}$ genes in wastewater from rural households and farms was similar with a mean of 4.35 ± 1.48 and 4.04 ± 1.61 log_{10} copy number/100 ml of water, respectively. The average number of $bla_{CTX-M-1}$ genes in pond water samples from rural households and farms was 0.75 ± 1.93 SD and 0.68 ± 1.87 SD, respectively, while the number was relatively higher in river water samples (1.38 ± 2.47 SD rural households vs 0.41 ± 1.51 SD farms). The drinking water samples from the study sites were mostly negative for bla_{NDM-1} genes except two samples from rural households with a mean count of 1.74 ± 1.09 SD log_{10} gene copy number/100 ml. Wastewater samples from all three sites were positive for bla_{NDM-1} with a higher concentration in urban markets (mean 3.33 ± 3.21 SD log_{10} gene copy number/100 ml) compared to rural households (mean 2.10 ± 3.61 SD log_{10} gene copy number/100 ml) and farms samples (mean 1.86 ± 2.83 SD log_{10} gene copy number/100 ml) with a non-significant statistical difference ($p = 0.082$, ANOVA test) (Table 2). However, the concentration of bla_{NDM-1} genes in river water samples adjacent to rural households and farms were higher than that of pond water samples but the difference was not statistically significant (Table 2).

3.4. Seasonal variation in physiochemical properties and prevalence of ESBL-Ec and CR-Ec in the aquatic environments

The average temperature of surface water bodies varied between the winter and summer sampling periods. In pond water, the mean temperature increased from 24.9 °C ± 1.80 SD in winter to 28.7 °C ± 2.40 SD in summer ($p < 0.001$, *t*-test). River water temperature also increased significantly from 25.4 °C ± 0.23 SD in winter to 29.0 °C ± 2.06 SD in summer ($p < 0.001$, *t*-test).

We also observed significant variations in water conductivity between seasons. The average conductivity of pond water in winter was 241.14 μS/cm ± 270.73 SD which was significantly higher than in summer, coinciding with monsoon (122.58 μS/cm ± 56.11 SD) ($p < 0.05$, *t*-test). Similarly, pond water pH was 7.80 ± 0.38 SD in winter, significantly higher than that in summer (7.52 ± 0.36 SD), ($p < 0.005$, *t*-test). In river water, conductivity was significantly higher in winter (245.80 ± 79.08 SD) than in summer (100.75 ± 38.40 SD) ($p < 0.001$, *t*-test) though no significant change in pH (7.6 in both seasons) was observed. In the case of wastewater, conductivity was also higher in winter (782.54 ± 128.21 SD) than in summer (704.95 ± 94.71 SD) but the difference was not statistically significant.

There was a positive correlation between ESBL-Ec count (log_{10} CFU/100 ml) and conductivity of wastewater, pond and river water samples ($r = 0.31$; $r = 0.49$; $r = 0.25$ respectively, all $p < 0.05$, Pearson correlation). However, no correlation was observed between ESBL-Ec counts and water temperature or pH. The prevalence of ESBL-Ec in wastewater samples did not vary by season (all sites combined: 95% in winter and 90% in summer, unadjusted OR 0.47, 95% CI 0.11, 1.99). The same was observed for CR-Ec in wastewater (all sites 11.7% vs 16.7% in winter and summer respectively, unadjusted OR 1.51, 95% CI 0.53, 4.29). Similarly, we did not find any seasonal variations in the prevalence of ESBL-Ec in pond water samples (unadjusted OR 1.11, 95% CI 0.41, 3.00) or river water samples (unadjusted OR 0.67, 95% CI 0.19, 2.33), even after adjusting for study sites (Fig. 1). We observed the same for CR-Ec in pond water. However, the prevalence of CR-Ec in river water samples was significantly lower in winter compared to summer (12.5% vs. 32.5%, Chi-square, $p < 0.05$ for household and farm river samples combined). The concentrations of total *E. coli* and ESBL-Ec (CFU/100 ml) in each study setting according to season are presented in Fig. 5 for rural households, farms and urban food markets. In rural households, the concentration of *E. coli* and ESBL-Ec was highest in wastewater and lowest in drinking water in both seasons. This trend was similar for all three settings. The level of contamination with *E. coli* and ESBL-Ec in pond and river water samples adjacent to poultry farms was

Table 3
Comparison of the abundance of ESBL-Ec, total *E. coli*, ESBL gene ($bla_{CTX\text{-}M\text{-}1}$), CR-Ec and CR gene ($bla_{NDM\text{-}1}$) in aquatic samples from rural and urban settings.

Attributes	Wastewater			Drinking water		
	Rural households + rural farms	Urban food markets	p^b	Rural households + rural farms	Urban food markets	p^b
	(N = 80)	(N = 40)		(N = 80)	(N = 40)	
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
ESBL-*E. coli* counts (log_{10} CFU/100 ml)	2.99 ± 2.39[a]	5.59 ± 2.85	<0.001	0.23 ± 0.16	0.53 ± 0.34	<0.05
Total *E. coli* counts (log_{10} CFU/100 ml)	5.03 ± 1.21[a]	6.91 ± 1.25	<0.001	0.93 ± 0.99	0.62 ± 0.65	<0.05
Median proportion (IQR) of ESBL-Ec in total Ec (%)*	2.01 (10.27)	6.67 (20.97)	<0.05	33.73 (52.02)	20.79 (29.01)	0.410
Carbapenem-resistant *E. coli* (log_{10} CFU/100 ml)	1.91 ± 1.23	3.48 ± 1.61	<0.05	–	–	
Median proportion (IQR) of Carbapenem Ec in total Ec (%)*	0.01 (0.01)	0.02 (0.41)	0.916	–	–	
$bla_{CTX\text{-}M\text{-}1}$ (log_{10} gene copy number/100 ml)	2.89 ± 1.25	6.01 ± 1.85	<0.001	4.12 ± 1.66	2.81 ± 0.31	0.697
$bla_{NDM\text{-}1}$ (log_{10} gene copy number/100 ml)	1.98 ± 3.18	3.33 ± 3.21	<0.05	–	–	

[a] denotes pairs of groups significantly different at $p < 0.001$; *based on only positive isolates; [b] denotes *p*-values generated using Wilcoxon Rank Sum test.

higher in winter season. In drinking water samples from urban food markets, the mean concentrations of both total *E. coli* and ESBL-Ec were the same but in wastewater, the mean concentration of total *E. coli* was higher than ESBL-Ec. However, the concentration of $bla_{CTX\text{-}M\text{-}1}$ and $bla_{NDM\text{-}1}$ in water samples did not vary significantly between seasons (Supplementary materials 2).

4. Discussion

Our study presents the spatial distribution of ARB and ARGs in drinking water, wastewater and surface water in rural and urban settings in Bangladesh. The high abundance of ESBL-Ec in wastewater within all settings, and drinking water in the urban area, raise important public health concerns. These findings also help to fill highlighted gaps in research on the environmental dimensions of AMR (Larsson et al., 2018). Despite a greater awareness of environmental AMR, this has not yet been prioritized within environmental health policies or country-level national action plans on AMR, especially in LMICs (Rousham et al., 2018; WHO, 2020b). International regulatory documents on water quality, such as WHO guidelines for drinking-water quality and WHO guidelines for the safe use of wastewater, excreta and greywater, do not provide guidelines on acceptable limits for antibiotic residues or clinically significant MDR bacteria such as ESBL-Ec or CR-Ec in water bodies (WHO, 2017; WHO, 2006). This contributes to the lack of imperative for action on high AMR contamination as observed in this study. The visualization of concentration gradients through spatial mapping of resistant organisms from drinking water sources to wastewater run-off (urban areas) and to surface water bodies (rural areas) highlights environmental hotspots for AMR and the critical role of the aquatic environment as reservoirs for ARB and ARGs. These are likely to pose risks for onward transmission of AMR to humans and animals through direct and indirect exposures. These findings also reinforce the need to evaluate poultry farming practices in Bangladesh which play a significant role in the emergence of AMR (Rousham et al., 2018).

Bangladesh is a densely populated country. Limited water treatment facilities mean that only 17% of wastewater is treated, mostly in urban areas (Connor et al., 2017). In rural areas, wastewater often directly drains into the aquatic ecosystem via open channels into nearby ponds and rivers. We have demonstrated that wastewaters discharged from urban markets flow into surrounding drains and ultimately the storm water carries a high abundance of ESBL-Ec, CR-Ec and associated resistance genes. In rural households and urban food markets in Bangladesh, wastewater generally carries a combination of human, animal and general waste with the likelihood of both human and animal fecal contamination (Pickering et al., 2018; Huda et al., 2018; Harris et al., 2018; Harris et al., 2016) along with low concentrations of antibiotic residues (Karkman et al., 2017). The high prevalence of ESBL-Ec in wastewater discharged from the urban food markets, with more than 95% of samples positive, and a high mean concentration of ESBL-Ec (5.59 log_{10} CFU/100 ml) is notable. Of even greater concern is the high prevalence (30%) of CR-Ec in wastewater samples with a mean concentration of 3.48 log_{10} CFU/100 ml which is higher than the concentrations of CR-Ec in community wastewater reported by other studies in Bangladesh and Thailand (Islam et al., 2017; Thamlikitkul et al., 2019). Previous studies reported that hospital waste is a major source of CR-Ec in the environment, especially where hospital waste is disposed directly to the environment without treatment (King et al., 2020; Lamba et al., 2017; Al Salah et al., 2020; Daoud et al., 2018; Park et al., 2020). However, evidence related to wastewater discharged from urban wet markets as a potential source of CR-Ec has not been widely reported. Therefore, findings of this study provide important evidence on the contribution of urban food market waste to the environmental spread of AMR. More recently, the WHO has recommended the inclusion of wastewater discharge from urban food markets in LMICs as part of integrated One health surveillance systems for AMR (Matheu et al., 2017).

The prevalence of ESBL-Ec in drinking water sampled directly from groundwater in rural households and farms was relatively low (5–10%), corresponding to other studies conducted in Bangladesh and India (Mahmud et al., 2020; Mahmud et al., 2019; Varghese and Roymon, 2013). Conversely, the high prevalence of ESBL-Ec in municipal supply water samples in urban markets (38%) is alarming as this same water supply also serves residential areas in the city. We found that the estimated proportion of ESBL-Ec in total *E. coli* in municipal supply water samples was significantly higher than that of wastewater. This might be due to presence of lower number of *E. coli* in municipal supply water compared to the wastewater which harbour *E. coli* from all different sources including both fecal and non-fecal origins. This also indicates that people are directly exposed to a substantial number of ESBL-producing organisms by drinking contaminated water on a regular basis and are thus likely to be colonized by these organisms. A previous study showed that around 80% of the municipal supply water in Dhaka contain coliform bacteria, 63% of water samples were contaminated with *E. coli* and almost half of these *E. coli* (49%) were MDR pathogens with 9% ESBL-Ec (Talukdar et al., 2013). The increasing prevalence of ESBL-Ec in the municipal supply water in Dhaka city over the last decade is a serious concern that requires rapid attention by the respective authorities.

The occurrence of MDR organisms, resistance genes, and antibiotic residues in surface water bodies is well established outside Bangladesh, among similar settings in Asia (O'Flaherty and Cummins, 2017; Qiao et al., 2018; Waseem et al., 2018; Zhang et al., 2019; Singh et al., 2019; Reddy and Dubey, 2019; Ahammad et al., 2014). Natural flow of untreated wastewater into surface water bodies including rivers, lakes or canals is commonly observed in many LMICs and is likely a key driver of environmental transmission of clinically important antibiotic resistant organisms (Rabbani et al., 2017; Karkman et al., 2017; Islam et al., 2017). Though we did not collect any surface water samples adjacent to urban food markets, previous studies reported a high prevalence of ESBL-Ec in the lakes (70% of the isolates and all containing CTX-M genes) and rivers (50% of the isolates and all containing CTX-M-15 genes) around Dhaka city (Haque et al., 2014; Rashid et al., 2015).

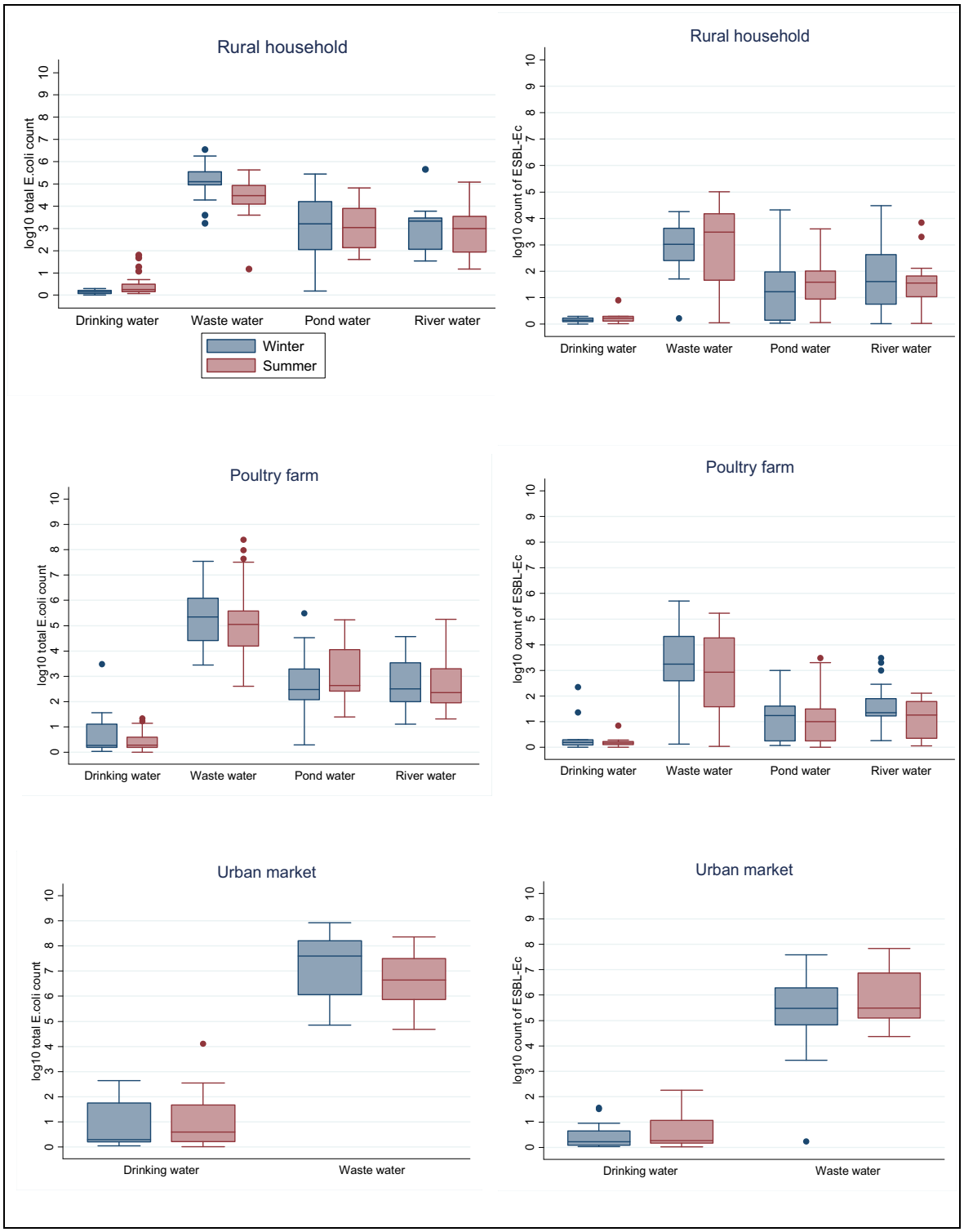

Fig. 5. Seasonal distribution of the mean count (log_{10} CFU/ml) of total *E.coli* and ESBL-Ec in different aquatic environments.

In the context of risk factor analysis for ARB and ARGs, we observed multiple practices that are likely to contribute to contamination of the aquatic environment, mostly related to biosecurity and waste disposal such as the disposal of wastewater from farms into adjacent ponds and direct disposal of household liquid waste into the nearby ditches. We also observed high antibiotic use in rural poultry farms and abundance of ESBL-Ec in the adjacent wastewater samples. Nevertheless, these associations were not significant due to small sample size although the effect of antibiotic use in farm animals on nearby waterbodies has been previously established (Landers et al., 2012).

One limitation of our study is that sampling was confined to one urban and one rural locality. However, the sampling across 2 seasons of 40 households from 20 villages; 40 farms from 35 villages and 40 urban markets in Dhaka provides good representation of the distribution of aquatic environments typical of many rural and urban localities in Bangladesh.

In our study, seasonal variation was only evident in the case of CR-Ec in river water which had a higher prevalence and concentration in summer compared to winter. We did not find seasonal variation in the prevalence and concentration of ESBL-Ec or CR-Ec in any other water systems in the urban or rural settings, though CR-Ec prevalence was low. We also investigated a small number of physicochemical parameters such as temperature, pH and electrical conductivity of selected water bodies to explore potential associations with ARB concentration across seasons. The increase in both pH and electrical conductivity in winter indicates a higher concentration of metals, minerals and other dissolved solutes that may be derived from waste (Kumar and Prabhahar, 2012; Rashid and Romshoo, 2013). We observed a significant increase in surface water temperature in the summer compared to winter, which might be a potential effect modifier to the changes in conductivity, along with increased rainfall. The widespread prevalence of antibiotic resistant organisms across aquatic environments due to continuous shedding of antibiotic residues from nearby hospitals, unregulated farm practices along with repeated human anthropogenic activities could be the possible reasons for not observing any seasonal effects on prevalence and abundance of AMR in aquatic environments.

5. Conclusion

AMR is a major threat to planetary health, which requires an ecosystem approach and demands One Health solutions. Our study findings provide an insight into the waterborne transmission of AMR by mapping and analyzing the spatiotemporal distribution of ARB and ARGs in different aquatic environments in Bangladesh. Discharge of untreated wastewater from rural households, poultry farms and urban markets to the open environment is a significant contributor to pollution of surface water bodies with antibiotic-resistant bacteria and genes. Future interventions are needed to reduce the burden of AMR in the environment. Such interventions could include improved biosecurity practices, particularly waste disposal systems, along with improved water, sanitation and hygiene infrastructure in rural and in urban areas, as well as improved practices for intensive poultry farming. GIS based mapping of AMR using quantitative microbiological data from connected environmental compartments can provide valuable insights into the dynamics of AMR in an ecological setting and this could be an important step in addressing environmental dimensions of AMR through tailored mitigation measures.

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CRediT authorship contribution statement

Muhammad Asaduzzaman: Writing original draft, conceptualization of manuscript idea, method development, data collection and analysis, expertise in One Health. **Emily Rousham:** Conceptualization of the project, funding acquisition, method development, data analysis, supervision of M.A and M.R.I, expertise in global health, review & editing. **Leanne Unicomb:** Conceptualization of the project, data analysis and discussion, expertise in WASH research, review & editing. **Md. Rayhanul Islam:** Data analysis and discussion, expertise in statistics, review & editing. **Mohammed Badrul Amin:** Laboratory analysis, expertise in Microbiology, review & editing. **Mahdia Rahman:** Laboratory analysis, expertise in microbiology, review & editing. **Muhammed Iqbal Hossain:** Laboratory analysis, expertise in microbiology, review & editing. **Zahid Hayat Mahmud:** Laboratory analysis, expertise in microbiology, review & editing. **Mark Szegner:** Data analysis and discussion, expertise in GIS mapping, review & editing. **Paul Wood:** Conceptualization of the research, method development, data analysis and discussion, expertise in GIS, review & editing. **Mohammad Aminul Islam:** Conceptualization of the project, funding acquisition, method development, data analysis, supervision of M.A, M.B.A, M.R, M.I.H and M.R.I, expertise in antimicrobial resistance, review & editing.

Ethical statement

Ethical approval for the study was obtained from the Institutional Review Board of icddr,b, Bangladesh (PR-16071) and the ethics committee of Loughborough University, UK (R17-P037). Written informed consent was given by participants to collect water, wastewater and surface water samples on their premises after receiving written and verbal information in Bangla about the study. Participants were informed of their right to withdraw from the study. Community stakeholders (the local government veterinary office and government community centres in Mirzapur) were also informed about the study as were market authorities.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Role of the funding source

The funding agency had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

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