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Hotspots of Resistance: Screening of Antimicrobial Resistant Genes In Poultry-Related Water Sources and Wastewater Treatment Plants by A Culture-Independent Technique

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Abstract

ANTIMICROBIAL resistance genes (ARGs) are emerging micropollutants that spread widely yet remain difficult to control. Wastewater treatment plants (WWTPs) are central to contaminant removal, but their role in ARG dissemination remains limited, especially in Egypt under a One Health framework. This study investigated ARG prevalence in WWTPs and poultry-associated waters (n=33) within the Greater Cairo Metropolitan Area (GCMA). Twenty-five clinically and environmentally relevant ARGs were screened using direct resistome PCR across three WWTPs (Zenin, Belqas, and El-Berka) and adjacent waters, alongside water quality analyses. Zenin WWTP (Giza) showed the highest ARG richness (mean=12.8, ±1.3 SE) across treatment steps. Activated sludge (66.6%) emerged as a hotspot for ARG persistence, whereas clarifiers showed lower counts. Shared ARG profiles across influent, sludge, and effluent were recorded: Belqas, Zenin, and El-Berka harbored 8, 6, and 1 shared genes, respectively. Several water quality parameters, including temperature, TSS, TP, TN, COD, MLSS, SRT, and HRT were positively correlated with ARG richness and counts in sludge and effluents. All environmental samples amplified at least one ARG: agricultural and fecal waters contained 19/25 genes (76%), while drinking water harbored 10/25 (40%). Geographically, ARGs were most frequent in Giza (16), followed by Cairo (10) and Kalyoubia (4). To the best of our knowledge, our study is the first survey in Egypt to provide a snapshot of 25 ARGs across WWTPs and poultry-associated waters using direct PCR. It highlights treatment processes and environmental interfaces as drivers of ARG spread in the GCMA and calls for One Health strategies to mitigate AMR risks.

Keywords: Antimicrobial resistant genes, Poultry farms, Resistome PCR based method, Sludge, Wastewater, Water.

Introduction

Antimicrobials have historically been regarded as the "silver bullets" that revolutionized modern medicine, saving countless lives in the twentieth century [1, 2]. Today, antimicrobial resistance (AMR) has emerged as a silent, manmade pandemic, driven by the widespread abuse and misuse of these life-saving medications across human, veterinary, and agricultural settings, ironically leading to 700,000 deaths annually [3,4,5]. Wastewater treatment plants (WWTPs) are key mixing hubs influenced by human misuse of antibiotics as they collect water from diverse settings, including domestic, industrial, and agricultural sources, ultimately turning WWTPs into anthropogenic reservoirs for antibiotics, antibiotic-resistant bacteria (ARBs), and antibiotic-resistant genes (ARGs) [3,6]. Additionally, biological treatment in these WWTPs expose microbes to low levels of antibiotics, metals, and other pollutants, creating selective pressure that promotes horizontal gene transfer [3,7]. As a result, WWTPs act as hotspots, recipients, and sources of antimicrobial resistance, influencing both upstream inputs and downstream environmental release [8,9].

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Water bodies in poultry-dense areas, such as drinking water, fecal-contaminated runoff, and nearby agricultural water, are among these inputs and interfaces that serve as environmental sources of antibiotic-resistant bacteria (ARBs) [10, 11, 12]**.** Poultry farming often uses antibiotics intensively, and water associated with farm operations carries large loads of antibiotics, ARBs, and mobile genetic elements (MGEs) [13, 14]. This consequently, results in antibiotic resistance genes (ARGs) entering wastewater treatment plants and contributing to the ongoing cycle of resistance [15, 16]**.**

Despite several studies reporting the presence of antibiotic resistance genes (ARGs) in wastewater treatment plants and poultry farm water sources [3, 7, 10, 11], ARGs surveillance remains limited, especially in low- and middle-income countries (LMICs**)** [17]. Moreover, data regarding the fate of ARGs during various stages of wastewater treatment and their downstream impacts on the environment is scarce and fragmented [7, 18]**.** Most studies focus solely on specific bacterial species or indicator organisms within farms and WWTPs [19, 20], with few combining data across environmental compartments.

Culture-independent methods, such as PCR-based techniques, quantitative PCR (qPCR), and metagenomics, offer a feasible and quick tool for surveillance that can overcome these challenges [21]**.** In our study**,** we examined ARG prevalence in three WWTPs and their surrounding water sources associated with poultry farming in the Greater Cairo Metropolitan Area (GCMA) in Egypt. Our aims were (1) to determine the prevalence of ARGs within wastewater treatment plants (WWTPs**),** and (2) to detect ARG hotspots across different stages of WWTP treatment processes in urban populated areas mainly influenced by human activities in Egypt. Furthermore, (3) assess the occurrence of ARGs in water sources within and around poultry farms. Finally, (4) explore shared ARGs profiles and links between WWTPs and poultry farm water sources. Our study is first in Egypt to provide a comprehensive snapshot of ARGs' dynamics across interconnected water systems. This approach allows a better understanding of environmental dimensions of AMR transmission.

Material and Methods

Study Area

This study screened several water bodies across the Greater Cairo Metropolitan Area (GCMA), Egypt's most densely populated region [22]**.** A total of 33 samples (wastewater, drinking water and agricultural water runoff) were collected from three WWTPs and from poultry-associated environmental sources across Cairo, Kalyoubia, and Giza governorates during the summer of 2024 (June to September).

WWTP sampling

Three wastewater treatment plants (WWTPs) were selected within GCMA: Belqas WWTP (Kalyoubia Governorate, coded S1), Zenin WWTP (Giza Governorate, coded S2), and El-Berka WWTP (Cairo Governorate, coded S3). From each WWTP, six grab samples were collected representing key treatment stages: influent, primary clarifier, aeration tank, activated sludge, secondary clarifier, and disinfected final effluent, yielding 18 WWTP water samples in total. For each stage, 500 mL of water was collected from both sides of the outlet and combined into a 1-liter integrated sample using sterile, labeled polyethylene bottles. Sampling points within the WWTPs are shown in (Fig. 1). However**,** details of the WWTPs and their site characteristics are provided in (Table 1). Additionally, Supplementary File 1 provides the questionnaire form and specific questions employed to gather metadata about the WWTPs and the samples collected.

Environmental and poultry farm sampling

In addition to WWTP samples, 15 environmental water samples were collected in sterile 500 mL containers from areas surrounding poultry operations within the same governorates. These included 10 samples from five broiler farms; comprising of five drinking water samples (DW1-DW5) collected from tanks or drinkers inside poultry houses, and five farm wastewater samples (FW1-FW5) obtained from outlet pipes, leaks or accumulation pits. Additionally, five surface water samples (AW1-AW5) were collected from irrigation and drainage canals near agricultural or urban areas that receive WWTP discharge or farm runoff. Details on sampling sites, farm or water characteristics, and water additives of environmental samples are provided in (Table 2). All samples were collected according to the American Public Health Association recommendations [23], stored on ice, and transported immediately to the laboratory for further water and molecular analysis.

Metagenomic DNA Extraction from Water Samples

Microbial DNA from all 33 water samples**,** was extracted from 0.22 µm membrane filters using a modified CTAB protocol [24]. Each filter was placed in a sterile 15 ml falcon tube with 5 ml of extraction lysis buffer (1%, w/v cetyltrimethylammonium bromide (CTAB), 3%, w/v sodium dodecyl sulfate (SDS), 100 mM Tris–HCl, 100 mM NaEDTA, 1.5 M NaCl, pH 8.0). Falcon tubes were incubated in a water bath at 70 °C for 1 hour with intermittent vortexing. After centrifugation at 4500 xg for 15 min, the supernatant was transferred to a new sterile falcon tube. Isopropanol (4 mL) was used to precipitate DNA by overlaying it onto the supernatant and incubating on ice for 20 minutes. The mixture was then centrifuged at 4,500 xg at 4 °C for 15 min. The supernatant was carefully discarded

without disturbing the pellet. The pellet was washed with 200 µL of 70% ethanol and transferred to a 1.5ml sterile Eppendorf tube. Final centrifugation was performed at 12,000 xg at 4 °C for 10 min, then the supernatant was aspirated and discarded carefully. The DNA pellet was air-dried and resuspended in 100 µL DNase-free water, then stored at -20 °C till further use.

ARGs screening and PCR conditions via targeted resistome PCR based method

A total of 25 primers targeting genes conferring resistance to six clinically relevant antimicrobial classes, along with the class 1 integron integrase gene (intl1 gene), were screened in all 33 samples (WWTP + environmental). These included 10 Beta-lactamase genes [*bla*NDM, *bla*KPC, *bla*OXA-48, *bla*VIM, *bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX-M, *bla*CMY-2, and *mec*A]; 4 quinolone resistant genes [*qnr*A, *qnr*B, *qnr*S, and *par*C]; 3 tetracycline resistant genes [*tet*A, *tet*B, and *tet*M]; 3 sulfonamide resistant genes [*sul*1, *sul*2 and *sul*3]; 2 aminoglycoside resistant genes [*aac*(3)-Ia, and *arm*A] and 2 glycopeptide resistant genes [*van*A and *van*B], and one mobile gene *[intl*1 gene]. All primers were obtained from previously published studies [25-36] as shown in Table in 3, except sulphonamide primers, which were designed using Invitrogen Oligo Perfect primer design software based on consensus sequences retrieved from NCBI. Moreover, (Table 3) shows primer sequences and annealing temperatures.

All PCR reactions were carried out in 25 µL volumes containing: 12.5 µl of 2X DreamTaq Green PCR Master Mix (Vilnius, Lithuania, Europe), 3 µl of DNA template, 1 µl of each primer (10 mM), and Nuclease free water. Primers were grouped into sets based on amplicon size and annealing temperature to reduce the number of separate PCR reactions. Groupings and thermocycling conditions are provided in (Table 4).

Water quality analysis

For all samples (n=33) (WWTP & environmental), pH and temperature were measured on site during sample collection using a Digital Water Tester (model: EZ9908, China). Only for WWTPs samples, the following parameters were measured: Total suspended solids (TSS), Total Phosphorus (TP), Total Nitrogen (TN), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) in the laboratory according to the American Public Health Association [23]. Additional data including heavy metal concentration, residual chlorine levels, and sludge characteristics (Mixed Liquor Suspended Solids (MLSS), TSS, and Dissolved Oxygen (DO) were obtained from WWTP's routine monitoring reports using the same sampling batches.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA) with a significance level of $p < 0.05$ with 95% confidence intervals. Comparisons of ARG prevalence across different treatment steps within each wastewater treatment plant (WWTP), as well as between WWTPs, were conducted using two-way ANOVA. One-way ANOVA was applied to compare the average number of detected ARGs between WWTPs. In addition, the abundance of each ARG per environmental samples was analyzed using one-way ANOVA. To explore associations between water quality parameters and the average number of ARGs, Spearman's rank correlation analysis was performed separately for WWTP influent, sludge, effluent, and environmental samples.

Results

Sample overview and site characteristics

A total of 33 water samples were collected: 18 wastewater samples from three municipal wastewater treatment plants (S1, S2, and S3) and 15 environmental water samples. A detailed description of sample types, locations, and site characteristics (WWTPs or Farms) are summarized in (Table 1) and (Table 2). All 3 WWTPs used aerobic activated sludge as the sole biological treatment method. Among them, Zenin WWTP (S2) had the smallest design capacity (400, 000 m3/day) and the lowest average daily flow (330,000 m^3/day), while Belqas WWTP (S1) and El-Berka WWTP (S3) exhibited similar capacities (600,000 m^3/day) and comparable daily flows (WWTP-S1: 410,000 m^3/day; WWTP-S3: 400,000 m^3/day). Of note, Zenin WWTP (S2) is located in a densely populated area and receives hospital waste; also no official catchment data were available, so hospitals within a 1 km radius (n=4) were used as a proxy for healthcare-associated waste input. Additional comparable metadata between the three WWTPs, including the number of inhabitants, catchment cities, hospitals, and wastewater types, are presented in (Table 1). As for the environmental water samples (n=15), 10 samples (Drinking water and wastewater) were from broiler farms (n=5). These five broiler farms reared flocks ranging from 4000-80000 birds and all farms (100%) reported the use of at least 3 different antimicrobial agents per production cycle (Table 2). Tetracycline (4/5) and amoxicillin (3/5) were the most frequently used drugs in these farms. Other water additives reported were antitoxins (2/5) and diuretics (1/5) (Table 2). Moreover, surface water samples (n=5) from drainage canals or agricultural streams reported pesticide application in 3 out of 5 sites. Geographically, each WWTP represented a different governorate, while most environmental samples were collected from Giza and Kalyoubia (6/15), followed by Cairo (3/15). These samples were selected for comparative analysis with WWTP discharge and farm-related sources in 3 governorates.

ARGs prevalence in WWTPs

A total of 25 ARGs were screened across the metagenomic DNA of wastewater treatment plant samples. The distribution of ARGs varied by treatment step and plant. Overall, Zenin WWTP (S2) exhibited significantly higher ARG counts across all treatment steps compared to Belqas WWTP (S1) and El-Berka WWTP (S3) (Fig. 2) (two-way ANOVA, $p < 0.05$). The average ARG counts in WWTP S1, WWTP S2, and WWTP S3 were 7.8, 12.8, and 5, respectively, indicating that WWTP S2 harbored significantly higher ARG richness among all 3 plants (one-way ANOVA, $p < 0.05$) (Fig. 3). Across treatment steps, sludge samples showed the highest ARG richness, with two of the three plants containing significantly more ARGs in sludge compared to their respective influent or effluent samples (two-way ANOVA, $p < 0.05$. For example, the sludge sample from WWTP S2 contained 18 ARGs, whereas the influent and effluent samples contained 14 and 9 ARGs, respectively as shown in (Fig. 4). In contrast, at least one clarifier stage (primary or secondary) in each WWTP exhibited a significant decrease in ARG counts compared to influent (two- way ANOVA; $p < 0.05$).

For instance, in WWTP S1, ARG numbers significantly declined from 11 in influent to 2 and 7 in the primary and secondary clarifiers, respectively (Fig. 2). Interestingly, statistical analysis showed no significant decrease in ARG richness between influent and effluent samples in WWTP S1 and WWTP S3, despite both plants harboring fewer ARGs overall compared to WWTP S2 (two-way ANOVA, $p > 0.05$). Analysis of shared genes revealed a variable degree of overlap between influent, sludge, and effluent. Specifically, WWTP S1 had six shared genes (*qnr*B, *qnr*S, *par*C, *sul*2, *tet*M, and *intI*-1), while WWTP S2 possessed eight shared genes, including the six found in WWTP S1 plus *bla*TEM and *sul*3. In contrast, WWTP S3 showed only a single shared gene (*qnr*S) (Fig. 4).

ARGs prevalence in environmental samples

All environmental water samples were positive for at least one ARG. Agricultural and fecal waters each had 19/25 positive genes (76%), while drinking water had 10/25 (40%) positive genes. At the class level, β-lactam (86.6%), sulfonamide (80%) resistance genes, tetracycline resistance genes, and mobile gene (*intl*-1) (73.3%), were the most common, whereas glycopeptide and aminoglycoside genes (40%) were the least frequent (Fig. 5). The most prevalent individual genes (> 50% of samples) were *bla*TEM (93.3%), *sul*1/*sul*2 (80%), *intl*-1 (73.3%), and tetA/tetM (66.6%) (Fig. 6). In drinking water, blaTEM and sul2 were amplified in 80% of samples while, *tet*A, *sul*1, and *int*l-1 were present in 60% of samples. Agricultural waters showed *bla*TEM, *tet*A, *tet*M, *par*C, and *intl*-1 in 100% of samples, while fecal waters had *bla*TEM and *sul*1 in 100%, while other genes were present in varying degrees within samples (Fig. 7 A, B, C). Interestingly, all 4 carbapenem-resistant genes were absent in drinking waters, while only blaOXA-48 was detected in fecal waste samples, but all 4 genes were present in agricultural samples ((*bla*OXA-48 (60%); *bla*KPC, *bla*NDM, and *bla*VIM (40%)).

Water quality parameters and correlations with ARG profile

All measured effluent parameters (TSS, TP, TN, BOD, COD, Temperature, and pH) from 3 WWTPs were within standard ranges set by Egyptian law no.48, (1982) [37] (Table 5). Although figures were within guided limits, WWTP S2 had slightly higher values compared to WWTP S1 and WWTP S3 (Table 5). Correlation analysis revealed that the average ARG richness in effluent samples was positively associated with all parameters. Among these, temperature showed the strongest correlation (Spearman, r=0.88; $p < 0.05$), while TSS, TP, TN, and COD showed moderate associations, and BOD and pH were weakly correlated (Table 5). Significant correlations were found for all variables except BOD and pH (Spearman; $p > 0.05$).

When sludge-specific parameters and operational indicators were examined, average ARG richness, sludge and effluent ARGs counts were all positively correlated with MLSS, SRT, and HRT. On the other hand, negative correlations were observed with DO and residual chlorine (Table 6). However, none of these relations were significant, likely due to the limited number of plants (n=3) (Spearman; $p > 0.05$).

As for environmental samples only, temperature showed significant strong positive correlation with mean ARG in environmental samples (mean ARGs= ±8.533, 1.245 SE (Spearman, r=0.9964; $p < 0.05$), while pH had no significance (Spearman; $p > 0.05$), and average temperature and pH were 30.39, ±0.2744 SE, and 7.465, ±0.093 SE, respectively.

Regional distribution and shared profiles between WWTPs and environmental samples

ARG occurrence of environmental samples and WWTP samples varied by region, with the highest frequency in Giza (23/ 25ARGs), followed by Kalyoubia (22/25) and Cairo (15/25). In Giza, *sul*2 (100%), *bla*TEM (91.6%), *intl*-1 (83.3%), and *sul*1 (75%) were predominant (n=12, environmental + WWTP samples). Kalyoubia (n=12) was dominated by *intl*-1(91.7%), *bla*TEM/*tet*M (83.3%), and sul2 (75%) genes. In Cairo (n=9), the most frequent genes were *tet*M and *intl*-1(66.6%), followed by *sul*2, *bla*CMY-2, and *bla*TEM (55.5%). Carbapenem resistance genes (*bla*OXA-48, *bla*NDM, *bla*KPC) were prevalent across regions except in Cairo, where only *bla*OXA-48 was amplified (Fig. 8 A, B, C).

Shared gene analysis revealed overlaps between WWTP effluents and surrounding water bodies, highlighting possible dissemination of β-lactam, sulphonamide, and quinolones resistant genes (Fig. 9). In Cairo, 10 ARGs were shared in both sources, suggesting strong connectivity between WWTPs and local environment. In Kalyoubia, only 4 ARGs were shared, while one was unique to WWTP and 7 were unique to environmental waters, indicating greater environmental inputs. In Giza, the overlap was the highest with 16 shared genes, 4 unique to the environmental samples, and 2 unique to WWTPs (Fig. 9).

Discussion

Antimicrobial resistance genes (ARGs) are invisible micropollutants that evade routine water quality testing and persist in both free and cellular forms, thus considered a significant environmental risk [38]**.** Hence, a comprehensive interconnected surveillance system is crucial for monitoring ARGs in WWTPs and their upstream/downstream water bodies to capture ARG dynamics and environmental impact [39, 40]**.** To the best of our knowledge, our study is the first in Egypt to assess the prevalence of ARGs in 3 WWTPs and their associated water sources using direct PCR screening. Across three plants, Zenin WWTP (S2) exhibited significantly higher ARG counts across all treatment steps, with a mean of 12.8, ± 1.3 SE ARG richness. The elevated ARG counts observed in plant S2 may be linked to multiple factors. First, the plant is located in a densely populated area of Giza characterized by poor sanitation and hygiene. Likewise, several studies have highlighted high population density and inadequate sanitation as key drivers of increased antibiotics and antibiotic-resistant bacteria (ARB) in receiving waters [41, 42]**.** Second, our data revealed that the plant's catchments included 4 hospitals within a 1Km radius, with Boulaq El-Dakrour General Hospital only 350 m away. This aligns with reports stating hospitals as reservoirs for ARGs and multidrug-resistant organisms (MDR) [42, 43], emphasizing the need for hospital-level control strategies and socio-demographic awareness to mitigate ARGs in the environment.

In Plant 2, two out of three sludge samples showed the highest ARG counts compared to other treatment samples within the same WWTP. This pattern is consistent with the role of sludge as a concentration site for bacteria, viruses, bacteriophages, DNA, and mobile genetic material (plasmids, transposons, integrons**)** [44, 45]. Supporting our findings, a metagenomic analysis identified 578 ARG subtypes in sludge, reflecting the wide diversity of resistance mechanisms present within sludge matrices [46]. Also, according to Calero-Cáceres et al. (2014) [44], several genes, including *bla*TEM and *sul*1, reached 5–8 $\log_{10}$ gene copies per gram of bacterial DNA within sludge samples. By contrast, clarifier samples had the lowest ARG levels, which aligns with the removal of suspended particles during this sedimentation stage [47, 48]. Similarly, several studies as in South Korea, Canada, and China, have reported significant log decreases in ARGs across clarifying processes, resulting in effluent with distinctly lower concentrations than sludge [48, 49]**.** Moreover, our shared ARGs analysis revealed variable overlap between influent, sludge, and effluent, likely shaped by influent composition, treatment efficiency, and socio-environmental factors [41, 42, 50]**.** This is supported by our previous finding, where S2, characterized by higher organic load and poorer hygiene, showed a markedly greater overlap of ARGs across influent, sludge, and effluent (n = 8), highlighting ARG persistence throughout WWTP S2. In contrast, WWTP S3, with lower load and better hygiene, corresponded with only one shared gene (*qnr*S). The persistence of *qnr*S aligns with previous reports showing that quinolone resistance genes are often difficult to remove during treatment and can spread widely through mobile plasmids [51, 52]**.** However, some studies have reported significant removal of *qnr*S by trickling filters, achieving reductions of 28–75% [51, 52]**.**

ARG richness was strongly and positively correlated with several water quality parameters, including temperature, TSS, TP, TN, BOD, and COD (Spearman; $p > 0.05$)**.** Specifically, positive correlations have been observed between ARGs and nutrients such as TP, TN, and COD, suggesting that nutrient-rich and organic-loaded environments promote microbial growth and biofilm formation, which in turn support ARG persistence and facilitate horizontal gene transfer [53, 54]**.** However, the association of ARGs with temperature is more debatable: some studies reported a negative correlation between temperature and ARG abundance, indicating that lower temperatures may favor ARG persistence [53], while others reported the absolute abundance of microbial DNA and ARG such as *intI*1, *sul*1, *sul*2, *tet*A, *tet*M, *and bla*TEM, similarly to our findings [55]. Additionally, sludge retention time (SRT) and hydraulic retention time (HRT) also correlated positively with ARG count. Longer retention time increases the contact time of microbes to antibiotics, thereby increasing antibiotic-resistant bacteria (ARB) [56]. Another explanation is that prolonged HRT and SRT favor the growth of slow-growing microorganisms [56]**.** These slow growers enhance ARG persistence by forming biofilms, maintaining prolonged cell to cell contact, acting as long-term reservoirs of ARGs, and sustaining activity in nutrient-rich and complex environments where horizontal transfer is favored [57, 58, 59]**.** Zenin WWTP (S2) reported the highest values for these parameters (Table 5), and also exhibited the highest ARG richness (12.8, ± 1.3 SE), providing further support for these findings.

Conversely, chlorine and dissolved oxygen were negatively associated with ARG richness (Spearman; $p > 0.05$). This can be explained by the role of chlorination in reducing ARG abundance through bacterial inactivation and extracellular DNA degradation. Moreover, its efficiency is amplified under higher DO conditions, where reactive oxygen species further intensify cellular and plasmid damage, thereby promoting more effective removal of resistant bacteria and extracellular genes [60, 61].

ARG prevalence varied across environmental water sources. At the class level, resistance genes against β-lactams (96.6%), sulphonamides (80%), and tetracyclines (73.3%), along with mobile genetic elements such as intI1 (73.3%), were the most prevalent. This pattern highlights the dominance of broad-spectrum antimicrobials that have been extensively used over time, and also reflects the contribution of horizontal gene transfer to their spread [62, 63]. At the gene level, *bla*TEM, *sul*1, *sul*2, *intI*1, *tet*A, and *tet*M were mostly frequent, being detected in 93–66% of samples. These genes are common resistance determinants in both poultry production systems and aquatic environments [63, 64]. Similar findings have been reported in surface waters from North Sea and English Channel for *tet*A, *bla*TEM, *sul*1, and *int*I1 with a 63-88% prevalence rate [66, 67], wastewater from WWTPs [56], and groundwater [68, 69], with similar high detection rates in regions such as Asia [69], Europe [66, 68], and Africa [70]. Upon stratification by source, drinking water harbored the lowest ARG diversity (10 positive out of 25 genes), including many clinically important ARGs such as (*bla*TEM, cmy-2, *sul*1, *sul*2, *tet*A, *intI*1, *tet*M, *qnr*A, *aac* (3*)-1a*, and *van*A). This indicates that treatment barriers may reduce but eliminate ARG contamination, consistent with previous reports of residual ARGs conferring resistance to sulfonamides, tetracyclines, beta-lactams, and multidrug resistance found in drinking water sources [71]. In contrast, agricultural wastewater harbored the richest ARG profile, with 19/25 positive genes, including all four carbapenemase genes. This diversity is likely attributable to the intensive antibiotic selection pressure typical of agricultural settings, together with favorable conditions, such as nutrient-rich wastewater that promote microbial growth and facilitate horizontal gene transfer [72]. Moreover, 60% of agricultural water samples in our study reported the use of pesticides. Pesticides at field level concentrations have been shown to facilitate plasmid-mediated ARG transfer by altering bacterial cell properties and, therefore adding to the ARG diversity in agricultural samples [73]. The detection of carbapenemase genes in particular is alarming, as carbapenems are last-resort drugs in human medicine [74]. Previous studies in Egypt and Spain have similarly linked agricultural runoff and wastewater to the emergence of carbapenem-resistant bacteria and resistance genes [75, 76]. For example, Nasser-Ali et al. (2024) [76] isolated carbapenemase-producing Gram-negative bacteria from treated effluent samples from a Spanish WWTP. Likewise, *bla*NDM and *bla*OXA-48 were detected in carbapenem-resistant *Klebsiella pneumoniae* isolates from irrigation water, fresh crops, and farm workers in Egypt [75]. Poultry Farm wastewater also contained 19/25 genes, but only *oxa-48* was detected among carbapenemases. A systematic review reported that Class D carbapenemases, especially blaOXA-48, are the most frequent carbapenemases among *Enterobacteriaceae* isolates in Africa, especially in Northern African countries, and further highlighted the widespread circulation of OXA-48 producers in the Middle East, suggesting it may now be endemic in region [77].

Across all sample types, aminoglycoside (except aac(3)-la), and glycopeptide resistance genes were uniformly low (40%). This observation is consistent with their limited use in poultry production, as well as the restricted veterinary application of glycopeptides such as vancomycin, which are primarily reserved for human medicine [78]. In addition, aminoglycosides are not approved as growth promoters in poultry, unlike tetracyclines or sulphonamides [79]. Shared gene analysis revealed overlaps between WWTP effluents and surrounding water bodies in Giza (16 shared genes), Kalyoubia (n=4), and Cairo (n=10). The ARG overlap was more pronounced in regions with urbanization and hospital density such as Giza, reinforming the influence of sociodemographic and antibiotic use on environmental resistomes [41, 42]. Taken together, these findings highlight the widespread persistence of clinically relevant ARGs, such as *bla*TEM, *sul*1, *sul*2, *intI*1, *tet*A, and *tet*M across various environmental water sources and WWTPs in GCMA, Egypt. These sites act as hotspots for resistance, directly influenced by water quality, environmental factors, and socio-demographic behaviors, underscoring the urgent need for integrated, multi-level surveillance systems.

Conclusion

Overall, our findings demonstrate that ARG distribution in WWTPs and environmental waters is strongly influenced by anthropogenic activities, nutrient-rich conditions, and treatment processes. Specifically, β-lactam, sulfonamide, tetracycline resistance genes, and mobile elements (intl1) were dominant. This underscores both the persistence and mobility of ARGs within WWTP systems and their surrounding environmental and poultry-associated areas in Giza, Kalyoubia, and Cairo governorates. Moreover, stratification by source revealed that agricultural and poultry farm wastewater act as major reservoirs for ARGs, whereas poultry drinking water exhibited a more limited, yet concerning, ARG profile. In addition, Environmental and water quality factors such as organic load, temperature, retention

times, pesticide use, and disinfection efficiency were significantly associated with ARG increase and diversity. Notably, the application of PCR-based methods provided a rapid, sensitive, and cost-effective approach for simultaneously screening multiple ARGs from various sources, thereby providing a timely and targeted approach for ARGs monitoring. Collectively, these results highlight the urgent need for integrated surveillance and the implementation of improved management practices to mitigate the environmental dissemination of antimicrobial resistance.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The Sanitary Drainage Authority granted permission for wastewater sample collection from WWTPs for Greater Cairo under approval no. 887, dated 23/5/2024.

TABLE 1. Location and characteristics of 3 WWTPs in GCMA used in our study

Item of comparison	Waste water treatment plants (WWTP)		
	Belqas WWTP	**Zenin WWTP**	**El-Berka WWTP**
1. Governorate	Kalyoubia	Giza	Cairo
2. Main cities served	All Shubra El-Kheima City/ Bahtim/ Mostorod / El-Warraq Island / Om bayoumi village	Boulaq El-Dakrour/Saft El-Laban/Faisal Omraneya/El-Moneib/Bein El-Sarayat	Heliopolis/El-Zeitoun/El-Matariya/ Ain Shams/Nasr City/El-Marg/ El-Amiriya/El-Salam City/El-Nahda City
3. Site characteristics	Moderate condensed area with moderate hygiene influenced by both urban and agriculture activities	Highly condensed urban area with poor hygiene	Vast new urban area with good hygiene
4. Type of sewage	Mainly domestic + some industrial pre-treated waste	Mainly domestic waste	Mainly domestic waste
5. No. of inhabitants served	3 million	2 million	3 million
6. Average daily flow (m3/day)	410, 000 m3/day	330, 000 m3/day	400,000 m3/day
7. Maximum daily capacity (m3/day)	600, 000 m3/day	400, 000 m3/day	600, 000 m3/day
8. Catchment Hospitals (1 km Radius)	3	4	2
9. Type of biological treatment	Activated sludge process	Activated sludge process	Activated sludge process
10. Disinfection	Chlorination	Chlorination	Chlorination

TABLE 2. Summary of 15 environmental water samples: governorates, sources, sample Types, collection sites, and antimicrobials/additives used

Sample ID	Governorate	Source of water sample	Type of water	Sampling site	Antimicrobials used	Ex. on antimicrobials (in sample/ production cycle)	Additives in water/used in farm
DW1	Cairo	local broiler farm (4000 birds)	Farm drinking water	Farm water tank	No	None	Diuretics
DW2	Giza	white broiler farm (9000 birds)	Farm drinking water	Farm water tank	Yes	Amoxicillin Enrofloxacin Tylosin	Antibiotics Antitoxins
DW3	Kalyoubia	white broiler farm (16000 birds)	Farm drinking water	Farm water drinkers	No	None	None
DW4	Giza	white broiler farm (80000 birds)	Farm drinking water	Farm water tank	No	None	None
DW5	Kalyoubia	white broiler farm (5000 birds)	Farm drinking water	Farm water drinkers	Yes	Amoxicillin tetracycline	Antibiotics
FWW1	Cairo	local broiler farm (4000 birds)	Farm waste water	Farm washing water	NG	Streptomycin Tetracycline Enrofloxacin Amoxicillin	Disinfectant (chlorine) Diuretics Antibiotics

FWW2	Giza	white broiler farm (9000 birds)	Farm waste water	Farm sewer pipe	NG	Amoxicillin Enrofloxacin Tylosin	Antibiotics Antitoxins
FWW3	Kalyoubia	white broiler farm (16000 birds)	Farm waste water	Farm sewer pipe	NG	Cephalosporin Vancomycin Tetracycline	Antibiotics
FWW4	Giza	white broiler farm (80000 birds)	Farm waste water	Farm washing water	NG	Cephotax Sulphax Tetracycline Enrofloxacin	Disinfectant (chlorine) Antibiotics
FWW5	Kalyoubia	white broiler farm (5000 birds)	Farm waste water	Farm sewer pipe	NG	Tetracycline Sulphax Amoxicillin Enrofloxacin	Disinfectant Antibiotics Antitoxins
AW1	Kalyoubia	urban land	Drainage canal	Drainage canal	NG	NG	NG
AW2	Kalyoubia	agricultural land	Agricult. water	Agricult. river	NG	NG	Pesticides
AW3	Cairo	urban land	Drainage canal	Drainage canal	NG	NG	NG
AW4	Giza	agricultural land	Agricultural water	Agricultural river	NG	NG	Pesticides
AW5	Giza	agricultural land	Agricult. water	Agricult. river	NG	NG	pesticides

TABLE 3. List of primers used for detection of antibiotic resistance genes in water samples

Antimicrobial class	Gene name	Gene sequence (5-3) direction	Band size (bp)	Annealing temperature (°C)	Ref.
1. Beta-lactam					
a. Extended-spectrum beta-lactamase (ESBL) resistance genes	***bla*$_{TEM}$**	F: CGCCGCATACACTATTCTCAG AATGA R: ACGCTCACCGGCTCCAGATTTAT	445	62	
	***bla*$_{SHV}$**	F: CTTTATCGGCCCTCACTCAA R: AGGTGCTCATCATGGGAAAG	237	62	
	***bla*$_{CTX-M}$**	F: ATGTGCAGYACCAGTAARGTKATGGC R:TGGGTRAARTARGTSACCAGAAYCAGCGG	593	62	
	***bla*$_{OXA-1}$**	F: ACACAATACATATC AACTTCGC R: AGTGTGTTTAGAATGGTGATC	813	62	
	***bla*$_{KPC}$**	F: ATGTCACTGTATCGC CGTCT R: TTTTCAGAGCCTTACTGCCC	882	55	[25]
b. Carbapenem resistance genes	***bla*$_{OXA-48}$**	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTTGTGAT GGC	743	55	
	***bla*$_{NDM}$**	F: GGTTTGGCGATC TGG TTTTC R: CGGAATGGCTCATCACGATC	621	55	
	***bla*$_{VIM}$**	F: GGTCTCATTGTCCGTGATGGTGAT GAG R: CTCGATGAGAGTCCTTCTAGAG	261	55	
c. Amp C beta-lactamase resistance genes	***bla*$_{CMY-2}$**	F: AGCGATCCGGTCACGAAATA R: CCCGTTTTATG CACCCATGA	695	61	
d. Methicillin resistance genes	***mec*A**	F: TGGCTCAGGTACTGCTATCCAC R: AGTTCTGCAGTACCGGATTTGC	776	60	[26]
2. Tetracycline resistance genes	***tet*A**	F: GGCCTCAATTTCCTGACG R: AAGCAGGATGTAGCCTGTG	372	55*	[27]
	***tet*B**	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	634	60*	[28]
	***tet*M**	F: ACAGAAAGCTTATTATATAAC R: TGGCGTGTCTATGATGTTCAC	171	47*	[28]
3. Sulphonamide resistance genes	***sul*1**	F: CGCACCGGAAACATCGCTGC R: TGAAGTTCCGCCGCAAGGCT	162	55	Designed in this study
	***sul*2**	F: TCCGATGGAGGCCGGTATCTGG R: CGGGAATGCCATCTGCCTTGAG	190	68	Designed in this study
	***sul*3**	F: AGTAGCTGCACCAATACGCT R: CAACTGAAGTGGGCGTTGTG	248	58	Designed in this study
4. Aminoglycoside resistance genes	***arm*A**	F: AGGTTGTTTCCATTTCTGAG R: TCTCTTCCATTCCCTTCTCC	776	50*	[29]
	***aac* (3)-Ia**	F: ATGGGCATC ATTCGCA R: TCTCGGCTTGAACGAATTGT	484	57*	[30]
5. Quinolones	***qnr*S**	F: GACGTGCTAACTTGCGTGAT R: TGGCATTGTTGGAAACTTG	118	50*	[31]
a. Quinolone resistance genes	***qnr*A**	F: TCAGCAAGAGGATTTCTCA R: GGCAGCACTATTACTCCCA	661	56*	[32]

	***qnr*B**	F: TCCGCTGTCAGTTCTATGATCG R: TCCATGAGCAACGATGCCT	495	52*	[33]
b. Fluroquinolone resistance genes	***par*C**	F: GCCTTGCGCTACATGAATTT R: ACCATCAACCAGCGGATAAC	287	47*	[34]
6. Glycopeptide (Vancomycin resistance genes)	***van*A**	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	732	55*	[35]
	***van*B**	F: AAGCTATGCAAGAAGCCATG R: CCGACAATCAAATCATCCTC	536	47*	
7. MGEs (class 1 integron integrase)	***intl*1**	F: GGCTTCGTGATGCCTGCTT R: CATTCCTGGCCGTGGTTCT	146	57*	[36]

Asterisk (*) indicate that these temperatures were modified from the original reference to optimize amplification conditions within groups in this study

TABLE 4. Grouping of antibiotic resistance genes with their respective amplification conditions used in water samples

NO.	Name of gene	Band size (bp)	Conditions	Type of PCR and annealing temperature of group
Gp 1	*bla* TEM *bla* SHV *bla* CTXM *bla* OXA	445 bp 237 bp 593 bp 813 bp	Initial denaturation at 95C/5min, Denaturation at 95C/30sec, Annealing at 62C/90sec, Extension at 72C/60sec, Final extension at 72 C/10min For 30 cycles	Multiplex (62 °C)
Gp 2	*bla* KPC *bla* OXA-48 *bla* NDM *bla* VIM	882 bp 743 bp 621 bp 261 bp	Initial denaturation at 94C/5min, Denaturation at 94C/1min, Annealing at 55C/1min, Extension at 72C/1min, Final extension...72/10min For 35cycles	Multiplex (55 °C)
Gp 3	*bla*CMY-2	695 bp	Initial denaturation at 94C/5min, Denaturation at 94C/1min, Annealing at 61C/1min, Extension at 72C/1min, Final extension.at 72/7min For 30cycles	Uniplex (61 °C)
Gp4	*mec*A *tet*B	776 bp 634 bp	Initial denaturation at 95C/5min, Denaturation at 95C/45sec, Annealing at 60C/45sec, Extension at 72C/1.30min, Final extension at 72C/10min For 30cycles	Duplex (60 °C)
Gp5	*sul*1 *tet*A	162 bp 372 bp	Initial denaturation at 94C/5min, Denaturation at 94C/1min, Annealing at 55C/1min, Extension at 72C/1min, Final extension at 72/7min For 35cycles	Duplex (55 °C)
Gp6	*sul2*	190 bp	Initial denaturation at 95C/5min, Denaturation at 95C/30 sec, Annealing at 68C/45 sec, Extension at 72C/1min, Final extension.at 72/7min For 30cycles	Uniplex (68 °C)
Gp7	*sul3*	248 bp	Initial denaturation at 94C/5min, Denaturation at 94/1min, Annealing at 58C/1min, Extension at 72/1min, Final extension at 72/7min For 30 cycles	Uniplex (58 °C)
Gp8	*arm*A	776 bp	Initial denaturation at 94C/5min, Denaturation at 94C/1min, Annealing at 50C/1min, Extension at 72C/1min, Final extension at 72C/7min For 35cycles	Uniplex (50 °C)
Gp9	*aac* (3)-la *int*1	484 bp 146 bp	Initial denaturation at 94C/5min, Denaturation at 94C/1min, Annealing at 57C/1;15min, Extension at 72C/1:30min, Final extension at 72/7min For 30cycles	Duplex (57 °C)
Gp10	*qnr*S	118 bp	Initial denaturation at 94C/5min, Denaturation at 94C/1min, Annealing at 50C/1min, Extension at 72C/1min, Final extension at 72/7min For 35cycles	Uniplex (50 °C)
Gp11	*qnr*A	661 bp	Initial denaturation at 94C/5min, Denaturation at 94C/45 sec, Annealing at 56C/1min, Extension at 72C/1.30min, Final extension at 72/10min For 35cycles	Uniplex (56 °C)
Gp12	*qnr*B	495 bp	Initial denaturation at 94C/5min, Denaturation at 94C/45 sec, Annealing at 52C/1min, Extension at 72C/1.30min, Final extension at 72/10min For 35cycles	Uniplex (52 °C)
Gp 13	*tet*M *par*C *van*B	171 bp 287 bp 536 bp	Initial denaturation at 95C/5min, Denaturation at 95C/45 sec, Annealing at 47C/1.30min, Extension at 72C/1min, Final extension at 72/10min For 35cycles	Triplex (47 °C)
Gp14	*van*A	732 bp	Initial denaturation at 95C/5min, Denaturation at 95C/45 sec, Annealing at 55C/1.30min, Extension at 72C/1min, Final extension at 72/10min For 35cycles	Uniplex (55 °C)

TABLE 5: Physiochemical characteristics of wastewater samples collected from various stages of treatment in WWTPs and Risk factors associated with ARGs per sample

Sample ID	WWT Plant	Step	TSS* (mg/L)	Total P* (mg/L)	Total N * (mg/L)	BOD (mg/L)	COD* (mg/l)	Temp* (C)	pH
S1A	Belqas	Influent	300	12	32	280	610	31.1	7.57
S1B	Belqas	Primary Sedimentation	180	5	16	140	340	30.3	6.57
S1C	Belqas	Aeration tank	3000	4	10	60	120	30.9	7.54
S1D	Belqas	Activated sludge	20,000	200	500	5,000	10,000	34.2	6.89
S1E	Belqas	Secondary Sedimentation	39	2	5	26	87	30.9	7.24

SIF	Belqas	Effluent	28	1.5	0.8	20	70	30.6	7.27
S2A	Zenin	Influent	408	11	50	343.34	683	32.9	6.88
S2B	Zenin	Primary Sedimentation	226	7	38	210	431	30.8	7.58
S2C	Zenin	Aeration tank	3650	5	30	70	150	32.6	7.28
S2D	Zenin	Activated sludge	35,000	500	650	8000	19000	33.9	7
S2E	Zenin	Secondary Sedimentation	48	4	16	35	92	32.7	7.45
S2F	Zenin	Effluent	35	2	1.5	24	80	32.5	7.27
S4A	El-Berka	Influent	228	11	30	216	432	30.4	7.4
S4B	El-Berka	Primary Sedimentation	112	5	13	124	249	30.1	7.3
S4C	El-Berka	Aeration tank	2000	2.5	7	68	149	30	7.4
S4D	El-Berka	Activated sludge	19,000	190	600	3500	8000	30.8	7.1
S4E	El-Berka	Secondary Sedimentation	28	2	4	26	52	29.8	7.8
S4F	El-Berka	Effluent	19	1	0.6	19	45	29.9	7.7
Statical analysis (Spearman correlation) P value < 0.05 (CI=95%)			r= 0.4920 p=0.0381*	r=0.5167 p=0.028*	r=0.6116 p=0.007*	r=0.4504 p=0.06	r=0.4886 p=0.0396*	r= 0.8801 p<0.0001*	r=0.2409 p=0.3355
Type of correlation			Positive						
Correlated metric			Total ARGs per sample (ARG richness) =range= 2-18						

TABLE 6. Specific activated sludge and treatment performance parameters (Metals, Pesticides, and Residual chlorine) recorded in wastewater treatment plants reports and statical analysis in relation to ARG presence

WWTPs	**Specific activated sludge parameters**			**Treatment performance parameters**			
	MLSS (mg/L)	**DO (mg/L)**	**SRT (days)**	**HRT (hours)**	**Residual Chlorine (mg/L)**	**metals**	**pesticides**
1. Belqas WWTP (S1)	3000	2.5	12-15	6-12	0.5	WSR	NG
2. Zenin WWTP (S2)	3500	1.5	14	12	0.3	NG	NG
3. El-Berka WWTP (S3)	2000	3	7-10	4-6	0.7	WSR	ND
Statical analysis (Spearman correlation) P value < 0.05 (CI=95%)	r^a= 1 p^a= 0.33 r^b= 0.86 pb= 0.67 r^c= 1 p^c= 0.33	r^a= -1 p^a= 0.33 r^b= -0.86 pb=0.67 r^c= -1 pc=0.33	r^a= 1 pa= 0.33 r^b= 0.86 p^b=0.67	r^a= 1 pa= 0.33 r^b=0.86 p^b=0.67	r^a= -1 pa= 0.33 r^b= -0.86 p^b= 0.67	ND	ND
Type of correlation	Positive	Negative	Positive	Positive	Negative	ND	ND
Correlated metric	**(a)** Average ARG in WWTPS1, WWTPS2, WWTPS3 = 7.833, 12.83, 5 respectively **(b)** No. of ARGs in effluent of WWTPS1, WWTPS2, WWTPS3 = 9, 9, 4 respectively **(c)** No. of ARGs in sludge of WWTPS1, WWTPS2, WWTPS3 = 10, 18, 9 respectively						

MLSS: Mixed Liquor Suspended Solids, **DO:** Dissolved Oxygen, **SRT:** Sludge Retention Time, **HRT:** Hydraulic Retention Time, **WSR**: Within standard range according to Egyptian law 2013, **NG:** Not given, **ND:** Not detected

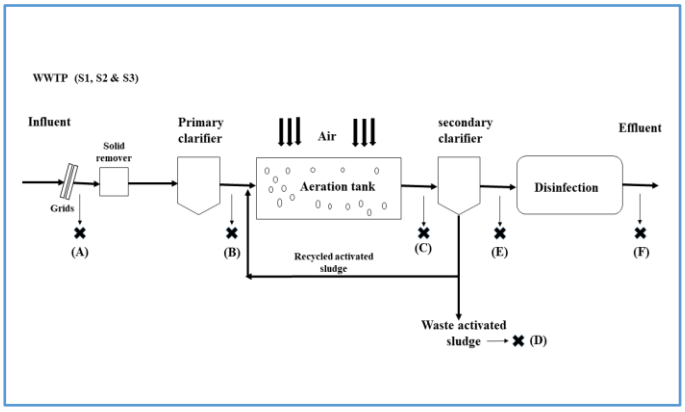

Fig. 1. Sampling points and flow chart of wastewater treatment plants ((Belqas WTTP (S1), Zenin WWTP (S2), & El-Berka WWTP (S3)); A: Influent after primary treatment, B: Primary clarifier outlet, C: Aeration tank outlet, D: Activated sludge, E: Secondary clarifier outlet, and F: Disinfected final effluent.

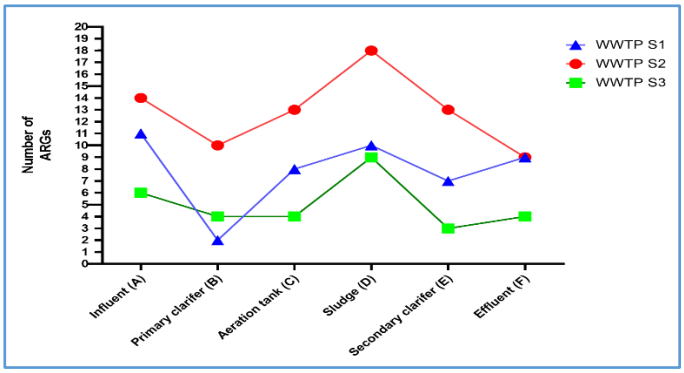

Fig. 2. ARGs counts across all treatment processes in 3 surveyed WWTPs

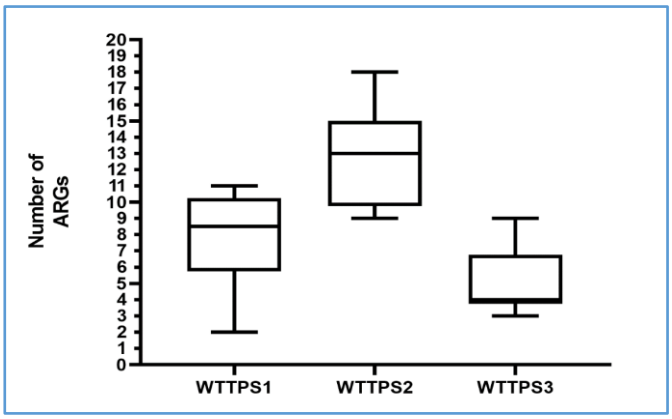

Fig. 3. Box plot showing mean ARGs counts across WWTPs

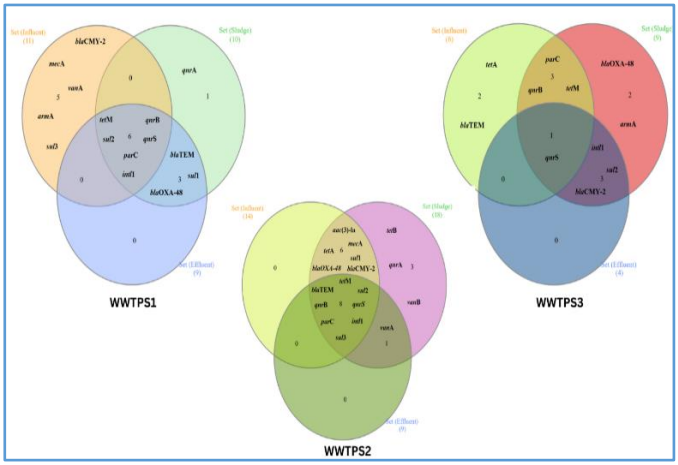

Fig. 4. Venn diagram showing shared ARG profiles in influent, sludge and effluent samples in 3 WWTPs.

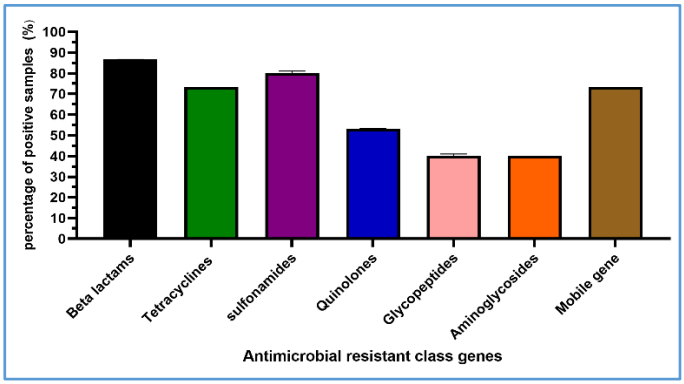

Fig. 5. Percentage of positive samples per antimicrobial resistance class across environmental samples (n =15)

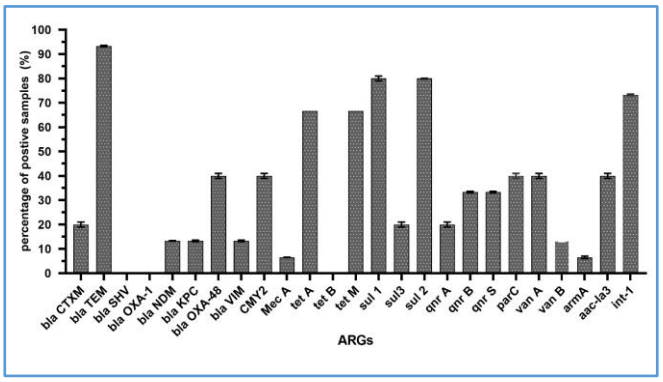

Fig. 6. Abundance of ARGs across environmental water samples (n =15)

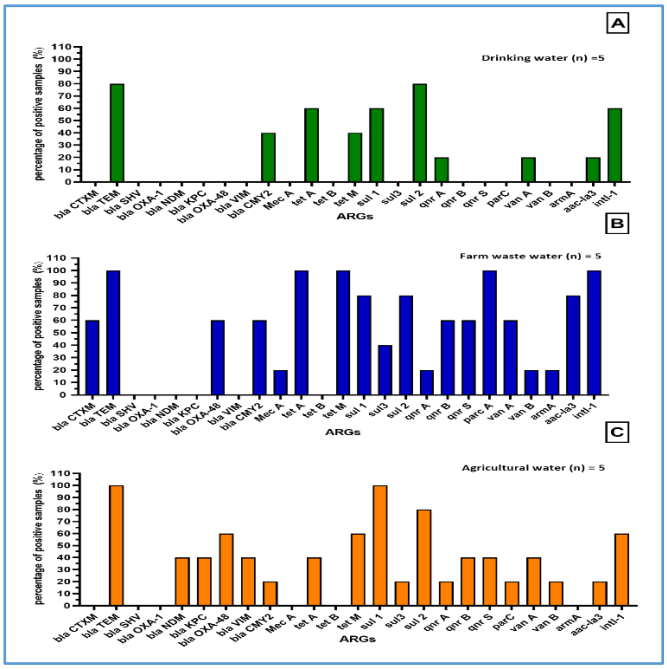

Fig. 7. Abundance of ARGs across each environmental water sample; A: percentage of positive samples per ARG in drinking water samples n=5; B: percentage of positive samples per ARG in farm waste water samples n=5; C= percentage of positive samples per ARG in Agricultural water samples n=5.

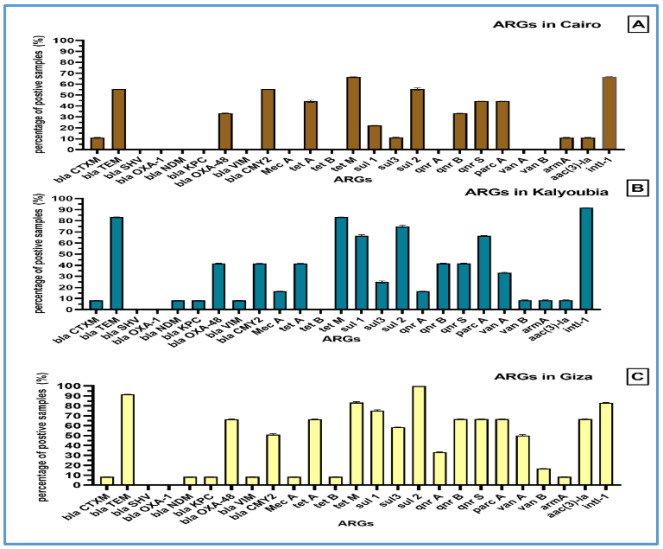

Fig. 8. Frequency of ARG in regions, A: positive samples per ARG in Cairo n= 9; B: percentage of positive samples in Kalyoubia n=12; C= percentage of positive samples in Giza n=12.

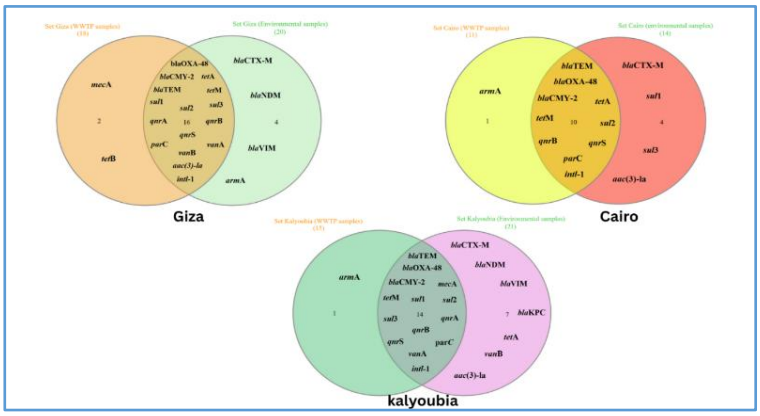

Fig. 9. Venn diagram showing shared ARG profiles in regions

References

1. Wright, G.D. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature reviews microbiology*, **5**(3), 175-186 (2007).
2. Zhuang, M., Achmon, Y., Cao, Y., Liang, X., Chen, L., Wang, H., Siame, B.A. and Leung, K.Y. Distribution of antibiotic resistance genes in the environment. *Environmental Pollution*, **285**, 117402 (2021).
3. Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M.C., Michael, I. and Fatta-Kassinos, D. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the Total environment*, **447**, 345-60 (2013).
4. Paneri, M. and Sevta, P. Overview of Antimicrobial Resistance: An Emerging Silent Pandemic. *Global Journal of Medical Pharmaceutical & Biomedical Update*, **18**, (2023).
5. Al-Tawfiq, J.A., S.H. Ebrahim, and Z.A. Memish, Preventing Antimicrobial Resistance Together: Reflections on AMR Week 2023. *J Epidemiol Glob Health*, **14**(2), 249-251 (2024).
6. Lupo, A., Coyne, S. and Berendonk, T.U. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. *Frontiers in Microbiology*, **3**, 18 (2012).
7. Auerbach, E.A., Seyfried, E.E. and McMahon, K.D. Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water Research*, **41**(5), 1143-51(2007).
8. Liu, Z., Klümpe,r U., Liu, Y., Yang, Y., Wei, Q., Lin, J.G., Gu, J.D. and Li, M. Metagenomic and metatranscriptomic analyses reveal activity and hosts of antibiotic resistance genes in activated sludge. *Environment International*, **129,** 208-220 (2019).
9. Amarasiri, M., Sano, D. and Suzuki, S. Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Current knowledge and questions to be answered. *Critical Reviews in Environmental Science and Technology*, **50**(19), 2016-2059 (2020).
10. Lopes, E.S., Parente, C.E., Picão, R.C. and Seldin, L. Irrigation ponds as sources of antimicrobial-resistant bacteria in agricultural areas with intensive use of poultry litter. *Antibiotics*, **11**(11), 1650 (2022).
11. Piccirillo, A., Tolosi, R., Mughini-Gras, L., Kers, J.G. and Laconi, A. Drinking water and biofilm as sources of antimicrobial resistance in free-range organic broiler farms. *Antibiotics*, **13**(9), 808 (2024).
12. Rui, Y. and Qiu, G.. Analysis of antibiotic resistance genes in water reservoirs and related wastewater from animal farms in central China. *Microorganisms*, **12** (2), 396 (2024).
13. Mottet, A. and Tempio, G. Global poultry production: current state and future outlook and challenges. *World's Poultry Science Journal*, **73** (2), 245-256 (2017).
14. Hedman, H.D., Vasco, K.A. and Zhang, L.A. review of antimicrobial resistance in poultry farming within low-resource settings. *Animals.***10** (8), 1264 (2020).
15. Krzemiński, P., Markiewicz, Z. and Popowska, M. Entry routes of antibiotics and antimicrobial resistance in the environment. InAntibiotics and Antimicrobial Resistance Genes: *Environmental Occurrence and Treatment Technologies*, 1-26 (2020).
16. Chen, Y., Liu, Y., Zhao, C., Ma, J. and Guo, J. Antibiotic resistance gene pollution in poultry farming environments and approaches for mitigation: A system review. *Poultry Science*, 104858 (2025).
17. Gudda, F., Muloi, D., Nganga, F., Nolari, C., Gao, Y. and Moodley, A. Antibiotic ecotoxicity and resistance risks in resource-constrained chicken and pig farming environments. *npj Antimicrobials and Resistance*, **2**(1), 51(2024).
18. Agga, G.E., Arthur, T.M., Durso, L.M., Harhay, D.M. and Schmidt, J.W. Antimicrobial-resistant bacterial populations and antimicrobial resistance genes obtained from environments impacted by livestock and municipal waste. *PloS one*. **10**(7), e0132586 (2015).
19. Richiardi, L., Pignata, C., Fea, E., Bonetta, S. and Carraro, E. Are indicator microorganisms predictive of pathogens in water? *Water*, **15**(16), 2964 (2023).

20. Rueanghiran, C., Dawanpa, A., Pinneum, N., Sanguankiat, A., Chiemchaisri, C., Chiemchaisri, W., Sritumpawa, W., Kijpreedaborisuthi, O., Jeon, B. and Tulayakul, P. Environmental risk quotient of the antibiotic, phenotypic, and genotypic profiles for antibiotic resistance of Escherichia coli collected from manure and wastewater at swine farms in Prachinburi Province, Thailand. *Emerging Contaminants*, **8**, 340-50 (2022)

21. Ferreira, C., Otani, S., Aarestrup, F.M. and Manaia, C.M. Quantitative PCR versus metagenomics for monitoring antibiotic resistance genes: balancing high sensitivity and broad coverage. *FEMS Microbes*, **4**, xtad008 (2024).

22. Rasslan, A.H. and Sameh, A.E. The population density map of the greater Cairo Region Comparison of 2017 choropleth map and dasymetric map. *Asian J. Geograph. Res.*, **5**(1), 37-46 (2022).

23. American Public Health Association (APHA). Standard methods for the examination of water and wastewater. *American Public Health Association*, **6,** (1926).

24. Hassan, M., Essam, T. and Megahed, S. Illumina sequencing and assessment of new cost-efficient protocol for metagenomic-DNA extraction from environmental water samples. *Brazilian Journal of Microbiology*, **49,** 1-8 (2018).

25. Abdel-Kader, F., Hamza, E., Abdel-Moein, K.A. and Sabry, M.A. Retail chicken giblets contaminated with extended-spectrum cephalosporin-and carbapenem-resistant Salmonella enterica carrying blaCMY-2. *Veterinary World*, **15** (5), 1297 (2022).

26. Attia, A.R., Abdel-Moein, K.A., Zaher, H.M. and Samir, A. The burden and antibiogram of methicillin-resistant Staphylococcus Aureus among companion animals with respiratory illness. *Adv. Anim. Vet. Sci.*, **9**(10), 1655-1659 (2021).

27. Guillaume, G., Verbrugge, D., Chasseur-Libotte, M.L., Moens, W. and Collard, J.M. PCR typing of tetracycline resistance determinants (Tet A–E) in Salmonella enterica serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. FEMS *Microbiology Ecology*,. **32**(1), 77-85 (2000).

28. Aminov, R.I., Garrigues-Jeanjean, N. and Mackie, R. Molecular ecology of tetracycline resistance: development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. *Applied and Environmental Mcrobiology*, **67**(1), 22-32 (2001).

29. Kim, M.H., Sung, J.Y., Park, J.W., Kwon, G.C. and Koo, S.H. Coproduction of qnrB and armA from extended-spectrum beta-lactamase-producing Klebsiella pneumoniae. *Korean J Lab Med.*, **27**(6), 428-436 (2007).

30. Samadi, N., Pakzad, I., Sefidan, A.M., Hosainzadegan, H., Tanomand, A. Study of aminoglycoside resistance genes in enterococcus and salmonella strains isolated from ilam and milad hospitals, Iran. *Jundishapur Journal of Microbiology*, **8**(4), e18102 (2015).

31. Marti, E. and Balcázar, J.L. Real-time PCR assays for quantification of qnr genes in environmental water samples and chicken feces. *Applied and Environmental Microbiology*, **79**(5), 1743-1745 (2013).

32. Cambau, E., Lascols, C., Sougakoff, W., Bebear, C., Bonnet, R., Cavallo, J.D., Gutmann, L., Ploy, M.C., Jarlier, V., Soussy, C.J. and Robert, J. Occurrence of qnrA-positive clinical isolates in French teaching hospitals during 2002–2005. *Clinical Microbiology and Infection*, **12**(10), 1013-1020 (2006).

33. Kehrenberg, C., Friederichs, S., de Jong, A., Michael, G.B. and Schwarz, S. Identification of the plasmid-borne quinolone resistance gene qnrS in Salmonella enterica serovar Infantis. *Journal of Antimicrobial Chemotherapy*, **58**(1), 18-22 (2006).

34. Johnning, A., Kristiansson, E., Fick, J., Weijdegård, B. and Larsson, D.J. Resistance mutations in gyrA and parC are common in Escherichia communities of both fluoroquinolone-polluted and uncontaminated aquatic environments. *Frontiers in Microbiology*, **6**, 1355 (2015).

35. Seker, E., Ozenc, E. and Yilmaz, M. Investigation of vancomycin resistance genes in Enterococcus species isolated from bovine mastitis. *Veterinarski Arhiv.*, ***93***(3), 287-98 (2023).

36. Yang, F., Han, B., Gu, Y. and Zhang, K. Swine liquid manure: a hotspot of mobile genetic elements and antibiotic resistance genes. *Scientific Reports*, **10** (1), 15037 (2020).

37. Egyptian Law 48. The Implementer Regulations for Law 48/1982 regarding the Protection of the River Nile and Water Ways from Pollution. *Periodical Bulletin*, 12-35 (1982)

38. Di, Cesare. A., Fontaneto, D., Doppelbauer, J. and Corno, G. Fitness and recovery of bacterial communities and antibiotic resistance genes in urban wastewaters exposed to classical disinfection treatments. *Environmental Science & Technology*, **50**(18), 10153-10161 (2016).

39. An, X.L., Su, J.Q., Li, B., Ouyang, W.Y., Zhao, Y., Chen, Q.L., Cui, L., Chen, H., Gillings, M.R., Zhang, T. and Zhu, Y.G. Tracking antibiotic resistome during wastewater treatment using high throughput quantitative PCR. *Environment International*, **117,** 146-53 (2018).

40. Freeman, C.N., Scriver, L., Neudorf, K.D., Truelstrup, Hansen. L., Jamieson, R.C. and Yost, C.K. Antimicrobial resistance gene surveillance in the receiving waters of an upgraded wastewater treatment plant. *Facets*, **3**(1), 128-38 (2018).

41. Elder, F.C., Proctor, K., Barden, R., Gaze, W.H., Snape, J., Feil, E.J. and Kasprzyk-Hordern, B. Spatiotemporal profiling of antibiotics and resistance genes in a river catchment: human population as the main driver of antibiotic and antibiotic resistance gene presence in the environment. *Water Research*, **203**,117533 (2021).

42. Silvester, R., Perry, W.B., Webster, G., Rushton, L., Baldwin, A., Pass, D.A., Healey, N., Craine, N., Cross, G., Kille, P. and Weightman, A.J. Metagenomics unveils the role of hospitals and wastewater treatment plants on the environmental burden of antibiotic resistance genes and opportunistic pathogens. *Science of the Total Environment*, **961**,178403 (2025).

43. Zhang, S., Huang, J., Zhao, Z., Cao, Y. and Li, B. Hospital wastewater as a reservoir for antibiotic resistance genes: a meta-analysis. *Frontiers in Public Health*, **8**, 574968 (2020).

44. Calero-Cáceres, W., Melgarejo, A., Colomer-Lluch, M., Stoll, C., Lucena, F., Jofre, J. and Muniesa, M. Sludge as a potential important source of antibiotic resistance genes in both the bacterial and bacteriophage fractions. *Environmental Science & Technology*, **48**(13), 7602-7611 (2014).

45. Han, I. and Yoo, K. Metagenomic profiles of antibiotic resistance genes in activated sludge, dewatered sludge and bioaerosols. *Water*, **12**(6), 1516 (2020).

46. Ali, O.S., Hozayen, W.G., Almutairi, A.S., Edris, S.A, Abulfaraj, A.A., Ouf, A.A. and Mahmoud, H.M. Metagenomic analysis reveals the fate of antibiotic resistance genes in a full-scale wastewater treatment plant in Egypt. *Sustainability*, **13** (20), 11131 (2021).

47. Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J. and Kim, H. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere*, **150**, 702-14 (2016).

48. Pourrostami, Niavol. K. and Bordoloi, A. Suri, R.. An overview of the occurrence, impact of process parameters, and the fate of antibiotic resistance genes during anaerobic digestion processes. *Environmental Science and Pollution Research*, **31**(29), 41745-41774 (2024).

49. Lee, J., Jeon, J.H., Shin, J., Jang, H.M., Kim, S., Song, M.S. and Kim, Y.M. Quantitative and qualitative changes in antibiotic resistance genes after passing through treatment processes in municipal wastewater treatment plants. *Science of the Total Environment*, **605**, 906-914 (2017).

50. Azli, B., Razak, M.N., Omar, A.R., Mohd-Zain, N.A., Abdul Razak, F. and Nurulfiza I. Metagenomics insights into the microbial diversity and microbiome network analysis on the heterogeneity of influent to effluent water. *Frontiers in Microbiology*, **13**, 779196 (2022).

51. Castrignanò, E., Kannan, A.M., Proctor, K., Petrie, B., Hodgen, S., Feil, E.J., Lewis, S.E., Lopardo, L., Camacho-Muñoz, D., Rice, J. and Cartwright, N. (Fluoro) quinolones and quinolone resistance genes in the aquatic environment: a river catchment perspective. *Water Research*, **182**, 116015 (2020).

52. Oliveira, M., Nunes, M., Crespo, M.T. and Silva, A.F. The environmental contribution to the dissemination of carbapenem and (fluoro) quinolone resistance genes by discharged and reused wastewater effluents: The role of cellular and extracellular DNA. *Water Research*, **182**, 116011 (2020).

53. Sabri, N.A., Schmitt, H., Van Der, Zaan. B.M., Gerritsen, H.W., Rijnaarts, H.H and Langenhoff. A.A.. Performance of full scale constructed wetlands in removing antibiotics and antibiotic resistance genes. *Science of the Total Environment*, **786**, 147368 (2021).

54. Ren, Z., Guo, H., Jin, H., Wang, Y., Zhang, G., Zhou, J., Qu, G., Sun, Q. and Wang, T.P.N. C-related functional genes in SBR system promoted antibiotics resistance gene transmission under polystyrene microplastics stress. *Water Research*, **235**,119884 (2023).

55. Yang, J., Wang, H., Roberts, D.J., Du, H.N., Yu, X.F., Zhu, N.Z. and Meng, X.Z. Persistence of antibiotic resistance genes from river water to tap water in the Yangtze River Delta. *Science of the Total Environment*, **742**, 140592 (2020).

56. Nnadozie, C.F., Kumari, S. and Bux, F.. Status of pathogens, antibiotic resistance genes and antibiotic residues in wastewater treatment systems. *Reviews in Environmental Science and Bio Technology*, **16** (3), 491-515 (2017).

57. Sun, C., Zhang, B., Chen, Z., Qin, W. and Wen, X. Sludge retention time affects the microbial community structure: A large-scale sampling of aeration tanks throughout China. *Environmental Pollution*, **261**, 114140 (2020).

58. Trebuch, L.M., Oyserman, B.O., Janssen, M., Wijffels, R.H., Vet, L.E. and Fernandes, T.V. Impact of hydraulic retention time on community assembly and function of photogranules for wastewater treatment. *Water Research*, **173**, 115506 (2020).

59. Sun, S., Chen, Z., Wang, X., Wang, S., Liu, L., Yan, P., Chen, Y., Fang, F. and Guo, J. Effect of different feeding strategies on performance of aerobic granular sludge: From perspective of extracellular polymeric substances and microorganisms. Journal of *Environmental Chemical Engineering*, **12**(1), 111688(2024).

60. Stange, C., Sidhu, J.P., Toze, S. and Tiehm, A. Comparative removal of antibiotic resistance genes during chlorination, ozonation, and UV treatment. *International Journal of Hygiene and Environmental Health*, **222**(3), 541-548 (2019).

61. Zhang, S., Yao, Z., Wang, S., Zhan,g Y., Liu, T. and Zuo, X. Dissolved oxygen facilitates efficiency of chlorine disinfection for antibiotic resistance. *Science of the Total Environment*, **934**,173210 (2024).

62. Amarasiri, M., Takezawa, T., Malla, B., Furukawa, T., Sherchand, J.B., Haramoto, E. and Sei, K. Prevalence of antibiotic resistance genes in drinking and environmental water sources of the Kathmandu Valley, Nepal. *Frontiers in Microbiology*, **13**, 894014 (2022).

63. Srathongneam, T., Sresung, M., Paisantham, P., Ruksakul, P., Singer, A.C., Sukchawalit, R., Satayavivad, J., Mongkolsuk, S. and Sirikanchana, K. High throughput qPCR unveils shared antibiotic resistance genes in tropical wastewater and river water. *Science of the Total Environment*, **908**, 167867 (2024).

64. Vuthy, Y., Lay, K.S., Seiha, H., Kerleguer, A. and Aidara-Kane, A. Antibiotic susceptibility and molecular characterization of resistance genes among Escherichia coli and among Salmonella subsp. in chicken food chains. *Asian Pacific Journal of Tropical Biomedicine*, **7**(7), 670-674 (2017).

65. Cheng, X., Lu, Y., Song, Y., Zhang, R., ShangGuan, X., Xu, H., Liu, C. and Liu, H. Analysis of antibiotic resistance genes, environmental factors, and microbial community from aquaculture farms in five provinces, China. *Frontiers in Microbiology*, **12**, 679805 (2021).

66. Bourdonnais, E., Le, Bris. C., Brauge, T. and Midelet, G. Monitoring indicator genes to assess antimicrobial resistance contamination in phytoplankton and zooplankton communities from the English Channel and the North Sea. *Frontiers in Microbiology*, **15**, 1313056 (2024).

67. Wolf-Baca, M. and Siedlecka, A. Seasonal and spatial variations of antibiotic resistance genes and bacterial biodiversity in biofilms covering the equipment at successive stages of drinking water purification. *Journal of Hazardous Materials*, **456**, 131660 (2023).

68. Andrade, L., Ryan, M.P., Burke, L.P., Hynds, P. and Weatherill, J., O'Dwyer, J. Assessing antimicrobial and metal resistance genes in Escherichia coli from domestic groundwater supplies in rural Ireland. *Environmental Pollution*, **333**, 121970 (2023).

69. Yuan, W., Liu, Y., Liu, R., Li, L., Deng, P., Fu, S., Riaz, L., Lu, J., Li, G. and Yang, Z. Unveiling the overlooked threat: antibiotic resistance in groundwater near an abandoned sulfuric acid plant in Xingyang, China. *Environmental Geochemistry and Health*, **46**(9), 309 (2024).

70. Kayode, A.J. and Okoh, A.I. Assessment of multidrug-resistant Listeria monocytogenes in milk and milk product and One Health perspective. *PLoS One*, **17**(7), e0270993 (2022).

71. Tsholo, K., Molale-Tom, L.G., Horn, S. and Bezuidenhout, C.C. Distribution of antibiotic resistance genes and antibiotic residues in drinking water production facilities: Links to bacterial community. *Plos One*, **19(**5), e0299247 (2024).

72. Ibekwe, A.M., Bhattacharjee, A.S., Phan, D., Ashworth, D., Schmidt, M.P., Murinda, S.E., Obayiuwana, A., Murry, M.A., Schwartz, G., Lundquist T and Ma J. Potential reservoirs of antimicrobial resistance in livestock waste and treated wastewater that can be disseminated to agricultural land. *Science of the Total Environment*, **872**, 162194 (2023).

73. Zhu, S., Yang, B., Yu, F., Zhang, J., Wang, Z. and Liu, Y. Investigation of the impact of widely used pesticides on conjugative transfer of multidrug resistance plasmids. *Journal of Hazardous Materials*, **478**, 135436 (2024).

74. Gupta, K., Gupta, A. and Shrivastava, D. The last resort antibiotics: carbapenems. *Int. J. Adv. Res.*, **5**(4), 1410-1413(2017).

75. Elshafiee, E.A., Kadry, M., Nader, S.M. and Ahmed, Z.S. Extended-spectrum-beta-lactamases and carbapenemase-producing Klebsiella pneumoniae isolated from fresh produce farms in different governorates of Egypt. *Veterinary World*, **15**(5), 1191 (2022).

76. Ali, M.N. Emergence of Carbapenemase Genes in Gram-Negative Bacteria Isolated from the Wastewater Treatment Plant in A Coruña, Spain. *(Doctoral Dissertation, Universidade da Coruña)* (2024).

77. Manenzhe, R.I., Za,r H.J., Nicol, M.P. and Kaba, M. The spread of carbapenemase-producing bacteria in Africa: a systematic review. *Journal of Antimicrobial Chemotherapy*, **70**(1), 23-40 (2015).

78. Aqib, A.I. and Alsayeqh, A.F. Vancomycin drug resistance, an emerging threat to animal and public health. *Frontiers in Veterinary Sience*, **9**, 1010728 (2022).

79. Rahman, M.R., Fliss, I. and Biron, E. Insights in the development and uses of alternatives to antibiotic growth promoters in poultry and swine production. *Antibiotics*, **11**(6), 766 (2022).

بؤر المقاومة: فحص الجينات المقاومة للمضادات الحيوية في مصادر المياه المرتبطة بالدواجن ومحطات معالجة مياه الصرف الصحي باستخدام تقنية غير معتمدة على الزراعة

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الملخص

جينات مقاومة المضادات الحيوية (ARGs) تعد من الملوثات الدقيقة الناشئة التي تنتشر على نطاق واسع ويصعب السيطرة عليها. وتُعتبر محطات معالجة مياه الصرف الصحي (WWTPs) محورية في إزالة الملوثات، إلا أن دورها في انتشار جينات المقاومة ما زال محدود الفهم، خاصة في مصر ضمن إطار "صحة واحدة .(One Health) "هدفت هذه الدراسة إلى استقصاء مدى انتشار جينات المقاومة في محطات معالجة مياه الصرف الصحي والمياه المرتبطة بمزارع الدواجن (عدد العينات = 33) في منطقة القاهرة الكبرى .(GCMA) تم فحص 25 جيناً ذا أهمية اكلينيكية وبيئية باستخدام تقنية **المقاومة المباشرة PCR** عبر ثلاث محطات (زينين، بلقاس، البركة) والمياه المجاورة لها، بالإضافة إلى تحليل جودة المياه. أظهرت محطة زنين (الجيزة) أعلى ثراء جيني متوسط = 12.8 ± 1.3 (SE عبر مراحل المعالجة. وبرز الحمأة المنشطة (66.6%) كنقطة ساخنة لاستمرار جينات المقاومة، في حين سجلت المراحل الترسيبية (clarifiers) أعداداً أقل. كما تم رصد أنماط جينات مشتركة بين المياه الداخلة والحمأة والمياه الخارجة: حيث احتوت محطة بلقاس على 8 جينات مشتركة، وزنين على 6، والبركة على جين واحد فقط. وارتبطت عدة مؤشرات نوعية للمياه – بما في ذلك درجة الحرارة، المواد الصلبة العالقة (TSS) ، الفوسفور الكلي (TP) ، النيتروجين الكلي(TN) ، الطلب الكيميائي للأوكسجين(COD) ، المواد الصلبة العالقة في الحمأة المختلطة (MLSS) ، عمر الحمأة (SRT) ، وزمن المكوث الهيدروليكي – (HRT) إيجابياً بثراء وعدد جينات المقاومة في الحمأة والمياه الخارجة. جميع العينات البيئية احتوت على جين مقاومة واحد على الأقل: حيث سجلت مياه الري والبراز 25/19 جيناً (76%)، في حين احتوت مياه الشرب على 25/10 (40%). جغرافياً، كانت الجينات أكثر تكراراً في الجيزة (16)، تلتها القاهرة (10) ثم القليوبية (4). ولعلمنا، تُعد هذه أول دراسة في مصر تقدّم لمحة عن 25 جين مقاومة عبر محطات معالجة مياه الصرف والمياه المرتبطة بالدواجن باستخدام تقنية PCR المباشر. وتبرز النتائج دور عمليات المعالجة والواجهات البيئية في نشر جينات المقاومة في منطقة القاهرة الكبرى، وتدعو إلى تطبيق استراتيجيات "صحة واحدة" للحد من مخاطر مقاومة المضادات الحيوية.

الكلمات الدالة: جينات مقاومة المضادات الحيوية، مزارع الدواجن، تقنية PCR المباشرة لتحليل المقاومة الميكروبية، الحمأة، مياه الصرف الصحي، المياه.