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# Open Waste Canals as Potential Sources of Antimicrobial Resistance Genes in Aerosols in Urban Kanpur, India

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*Abstract.* Understanding the movement of antimicrobial resistance genes (ARGs) in the environment is critical to managing their spread. To assess potential ARG transport through the air via urban bioaerosols in cities with poor sanitation, we quantified ARGs and a mobile integron (MI) in ambient air over periods spanning rainy and dry seasons in Kanpur, India ($n$ = 53), where open wastewater canals (OCWs) are prevalent. Gene targets represented major antibiotic groups—tetracyclines (*tetA*), fluoroquinolines (*qnrB*), and beta-lactams ($bla_{TEM}$)—and a class 1 mobile integron (*intI1*). Over half of air samples located near, and up to 1 km from OCWs with fecal contamination ($n$ = 45) in Kanpur had detectable targets above the experimentally determined limits of detection (LOD): most commonly *intI1* and *tetA* (56% and 51% of samples, respectively), followed by $bla_{TEM}$ (8.9%) and *qnrB* (0%). ARG and MI densities in these positive air samples ranged from $6.9 \times 10^1$ to $5.2 \times 10^3$ gene copies/m$^3$ air. Most (7/8) control samples collected 1 km away from OCWs were negative for any targets. In comparing experimental samples with control samples, we found that *intI1* and *tetA* densities in air are significantly higher ($P$ = 0.04 and $P$ = 0.01, respectively, alpha = 0.05) near laboratory-confirmed fecal contaminated waters than at the control site. These data suggest increased densities of ARGs and MIs in bioaerosols in urban environments with inadequate sanitation. In such settings, aerosols may play a role in the spread of AR.

## INTRODUCTION

Antibiotic resistance (AR) is a growing threat to global public health driven by poor antibiotic stewardship and rapid development and dissemination of resistance in microbial communities as bacteria respond to selective pressure.[1–4] For example, in 2010, India was the largest consumer of antibiotics and specifically has some of the highest rates of nonprescription use of antibiotics such as carbapenems in addition to minimally funded healthcare and poor sanitation conditions.[5,6] As a result, studies are finding high prevalence of resistance to carbapenems and other closely related β-lactam antibiotics.[7,8] This selective pressure coupled with optimal conditions for bacterial dissemination in the environment may lead to bacterial development of resistance to antimicrobials with genes evolved through random mutations or genes acquired by horizontal gene transfer through the processes of transformation, transduction, or conjugation.[9] Genes that confer resistance to antimicrobials are known as AR genes (ARGs).

Although most studies have focused on AR development and dissemination in clinical settings,[10,11] understanding the fate and transport of antimicrobial resistance genes (ARGs) in other settings, including in the ambient environment, is crucial to controlling AR. Studies of ARGs and mobile integrons (MIs) that indicate that genetic transfer has happened or could potentially happen in the environment—including water runoff from animal feedlots; air, soils, and groundwater surrounding wastewater treatment plants; air surrounding poultry farms and markets; and urban environments suffering from severe smog events—indicate prevalent ARGs and high potential for mobility in these environments.[2,12–22] Some studies have characterized ARGs in ambient urban air where sources are not obvious or widespread fecal contamination is not present. Li et al.[20] described the global prevalence and relative abundances of 30 different ARG and MI targets in ambient urban air across 19 cities in 13 countries, suggesting widespread presence of ARGs associated with a range of bacterial taxa in cities. However, sources of ARGs in ambient aerosols, absolute densities of specific ARGs of concern, and transport through this pathway as it relates to human exposure remain uncharacterized. Of particular concern are highly contaminated urban areas in low- and middle-income countries (LMICs), where conditions favor AR emergence and transfer; such settings may have widespread environmental contamination, including uncontained fecal waste from people and animals, poor antibiotic stewardship, and high prevalence of pathogens that may acquire resistance to antibiotics.[22–24] ARG diversity and AR protein concentrations may be effectively reduced when sanitation systems are operating in LMICs, suggesting that fecal waste streams in particular may play an important role in the development and airborne dissemination of resistance.[25]

Because antibiotic-resistant bacteria and ARGs have been measured in bioaerosols near to wastewater treatment plants,[13,22,26] composting facilities,[27] and other potential sources where concentrated fecal waste exists,[12,15,17,18,28,29] we hypothesized that airborne ARGs would be present near uncontained wastewater flows in urban ambient environments where sanitation is poor. We further hypothesized that densities of ARGs in aerosols would be elevated in the dry season when ambient aerosols are relatively higher compared with the rainy season.[30]

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## METHODS

**Study sites and sample collection.** We conducted daily aerosol sampling in Kanpur, India, from May to July 2017. Kanpur is densely populated (Nagar district: 4.6 million people, population density of 1,500 persons/km$^2$),[31] with a majority of untreated industrial, agricultural, and raw sewage waste conveyed via a system of uncovered and uncontained canals open wastewater canals (OWCs) discharging to the Ganges River.[32,33]

Following mapping OWCs across preselected areas in the city, we identified sites meeting the following criteria: 1) proximity to known OWCs ranging from adjacent to up to a 1-km distance, 2) easily accessible, and 3) nonintrusive to residents. We selected a control site greater than 1 km away from known OWCs and located on IIT-Kanpur's campus. The campus is a controlled private area with limited access to nonstudents and nonfaculty, less densely populated, has underground piped sewerage, and has a much lower animal presence (Figure 1). Kanpur has distinct dry (October to June) and rainy (July to September) seasons. To capture potential seasonal effects, we sampled from May to July to capture periods before and after arrival of the monsoon.

We collected air samples over a period of approximately four hours with the ACD-200 BobCat Dry Filter Air Sampler (InnovaPrep, Drexel, MO, recovery efficiency for 1 μm particles is 73%[34]) with 52 mm electret filters and a flow rate of 150–200 L per minute, to yield a total sample volume of 36–48 m$^3$ of air per sample. We recorded time of day of sampling (grouped into morning and afternoon) to assess potential diurnal variation of target densities in bioaerosols. To assess OWCs as a potential source, we collected one 45-mL grab sample of wastewater concurrent with aerosol sampling at each of the sites where aerosol samples were taken adjacent to OWCs. Following collection, we transported filters and OWC wastewater samples on ice to the laboratory. We used a single-use wet foam elution kit (InnovaPrep) to elute filters, yielding approximately 6 mL of liquid eluate for analysis.[35] In Supplemental Table S1, we include a detailed breakdown of sampling sites, their proximity to OWCs, and how many bioaerosol samples were taken at each in the dry and rainy seasons. In addition, we include at which sites we collected wastewater samples.

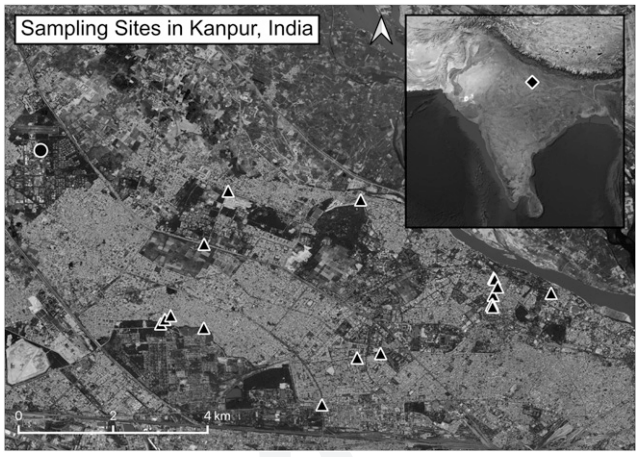


FIGURE 1. Location of sites < 1 km from open wastewater canals (OWCs) (triangles) and control site > 1 km from OWCs (circle). This figure appears in color at www.ajtmh.org.

**Sample extraction, culture, and analysis.** We cultured all aerosol samples to determine the presence of viable *Escherichia coli* as an indicator of aerosolized fecal waste. Immediately following arrival at the laboratory, we analyzed undiluted air sample eluate and air sample eluate diluted 1:10, and 1:100 via Compact Dry™ EC plates (Hardy Diagnostics, Santa Maria, CA).[36] We incubated plates overnight at 37°C. The limit of detection (LOD) for the culture analysis was determined by dividing one colony-forming unit (CFU, the minimum amount that can be detected on a plate) by the volume of air sampled.

For subsequent molecular analysis, we treated filter eluate and wastewater samples with a guanidine thiocyanate–based universal extraction (UNEX; Microbiologics, St. Cloud, MN) lysis buffer in a 1:1 ratio, stored in SK38 bead tubes (Bertin Corp, MD), and stored all samples at –80°C until extraction. We used 300 μL of the sample and UNEX mixture for extraction. After DNA extraction,[37] we stored extracted nucleic acids in 50–75 μL of 10 mM Tris-1 mM EDTA (pH 8) in a –80°C freezer until further analysis. We estimated DNA yield in samples via Qubit™ dsDNA HS (Thermo Scientific™) and NanoDrop spectrophotometer™ (Thermo Scientific).[38,39]

**Antibiotic resistance gene and MI quantification.** We conducted absolute quantification of ARGs via droplet digital PCR (ddPCR™, Bio-Rad, Hercules, CA). Reaction mixes were set to a total volume of 20 μL, containing a primer concentration of 900 nM, probe concentration of 250 nM, and 1X Supermix for Bio-Rad's QX200™ ddPCR system (Bio-Rad, Hercules). We used the ddPCR™ Supermix for probes for all targets except bla$_{TEM}$, for which we used ddPCR™ Supermix for Residual DNA Quantification because of the known presence of residual sequence in less purified commercial supermixes.[40] On each ddPCR™ plate for all assays, we included two gBlock™ (IDT, Coralville, Iowa) positive control wells diluted to approximately 10$^3$ gene copies (gc)/μL of the reaction mixture (Table 1). The positive control sequence is also included in a Supplemental File. In addition, we included at least two no template controls using molecular water to control for contamination via human or other error. For two replicates of each sample extract, we quantified gene copies of each target in the ddPCR reaction mixture (2 μL extract, 21 μL of ddPCR reagents) and averaged the results together.

Antibiotic resistance gene targets spanned three major antibiotic groups commonly used in low-income settings and whose ARGs have been detected previously in environmental samples—tetracyclines (*tetA*),[41] fluoroquinolones (*qnrB*),[42] and β-lactams (*bla*$_{TEM}$).[43] We also quantified a MI (*intI1*)[44] also previously detected in environmental media. Although chosen a priori, a literature review has demonstrated their relevance. Studies have shown that fluoroquinolone resistance has increased in Enterobacteriacea by 7–20% in just 5 years.[6] In a pilot study conducted in Vellore, India, 25% of *E. coli* isolated from urban hospitals ($n$ = 1,075) were resistant to *tetA*.[45] The *bla*$_{TEM}$ assay used in our analysis represents 135 variants of β-lactam resistance, including resistance to antibiotics commonly used in the study area such as penicillins, cephalosporins, carbapenems, and other antibiotics that have a β-lactic ring in their structure.[87] These ARGs therefore capture a wide range of resistance mechanisms and target drugs that are commonly used in sampling locations.[43] We processed all samples in duplicate and report means. Reaction mixes,

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TABLE 1

Primers, probe positive control sequence, and experimentally determined limit of detection for each assay used in this study to analyze the presence of antimicrobial resistance genes

| Gene target | Primers | Probes | Limit of detection (gc/μL ddPCR reaction mix) | Cycling conditions |
|---|---|---|---|---|
| *tetA* | F: CCGCGCTTTGGGTCATT | FAM-TCGGCGAGGATCG-BHQ1 | 0.19 | 95°C for 10 minutes<br>45 cycles of 95°C for 30 seconds and 56°C for 1 minute |
| | R: TGGTCGCGTCCCAGTGA | | | 98°C for 10 minutes |
| Fluoroquinolines | F: CAGATTTYCGCGGCGCAAG | FAM-CGCACCTGGTTTTGYAG | 0.24 | 95°C for 10 minutes<br>45 cycles of 95°C for 30 seconds and 56°C for 1 minute |
| | R: TTCCCACAGCTCRCAYTTTTC | YGCMTATATCAC-BHQ1 | | 98°C for 10 minutes |
| $bla_{TEM}$ | F: CACTATTCTCAGAATGACTTGGT | FAM-CCAGTCACAGAAAAGCATCTTA | 0.12 | 95°C for 10 minutes<br>45 cycles of 95°C for 30 seconds and 56°C for 1 minute |
| | R: TGCATAATTCTCTTACTGTCATG | CGG-BHQ1 | | 98°C for 10 minutes |
| *intI1* | F: GCCTTGATGTTACCCGAGAG | 6HEX-ATTCCTGGCCGTGGTTCTGGG | 0.10 | 95°C for 10 minutes<br>45 cycles of 95°C for 30 seconds and 57°C for 1 minute |
| | R: GATCGGTCGAATGCGTGT | TTTT-BHQ1 | | 98°C for 10 minutes |
| *Positive control* | ACTTGTCGGACAGGTGCCGGCCGCGCTTTGGGTCATTTTCGGCGAGGATCGCTTTCACTGGGAC<br>GCGACCACGATCGGCATTTCGCTTGCCGAAATCCTTCTTGGGCGCCACCGTTGGCCTTCCTGTAA<br>AGGATCTGGGTCCAGCGAGCCTTGCGGCGGAACTTCACGCGATCGGCAATGGCGCTGACTACGT<br>CCGCATGGGCACCCATCCAACGGTTTTCCCACAGCTCACACTTTTCCAACACGACTTTCGAAAAA<br>TTGGCGTAGCTTAGATTGGTATTCGTGATATATGCGCTACAAAACCAGGTGCGCGTGGTGATCAT<br>ATTCATAAAGCTTGCGCCGCGGAAATCTGCGCCTTGTGCGCGGCAGTGGAGCAACTCGGTCGCC<br>GCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGAT<br>GGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATCGGCGAGTTCTTGGGA<br>TGGCAGGCGATATTCATTACTTTGGCTATACTGGCGATGCTCGCACTCCTAAATGCGGGTTTCAG<br>GTGGCACGAAACCCGCCCTCTGGATCAAGTCAAGACGCGCCGATCTGTCTTGCCGATCTTCGCG<br>AGTCCGGCTTTTTGGGTTTACACTGTCGGCTTTAGCGCCGGTATGGGCACCTTCTTCGTCTTCTTC<br>TCGACGGCTCCCCGTGTGCTCATACGCACGACACCGCTCCGTGGATCGGTCGAATGCGTGTGCTG<br>CGCAAAAACCCAGAACCACGGCCAGGAATGCCCGGCGCGCGGATACTTCCGCTCAAGGGCGTCG<br>GGAAGCGCAACGCCGCTGCGGCCCTCGGCCTGGTCCTTCAGCCACCATGCCCGTGCACGCGACAGC<br>TGCTCGCGCAGGCTGGGTGCCAAGCTCTCGGGTAACATCAAGGCCCGATCCTTGGAGCCCTTGC | | | |

conditions, ARG and MI target sequences, and experimentally determined limits of detection (LODs) for each target are described in Table 1.

We experimentally determined 95% LODs for each assay using a probit analysis outlined by Stokdyk et al.[46,47] The 95% LOD represents the concentration for which the probability of a single ddPCR reaction being positive is 95%. We calculated associations between ARG and MI densities detected in control samples and samples near uncontained fecal waste as well as associations between season and ARG and MI density using nonparametric Wilcoxon tests for non-equal variance based on 95% confidence (alpha = 0.05). We conducted all statistical analysis in *R* version 1.1.383 (R Foundation for Statistical Computing, Vienna, Austria).[48]

## RESULTS AND DISCUSSION

***E. coli* in aerosols.** Because fecal indicator bacteria can be enriched in aerosols near uncontained fecal waste sites,[49–51] we hypothesized that culturable *E. coli* would be present in aerosols near OWCs. Of the 45 air samples within the city center of Kanpur and in close proximity to OWCs (< 1 km), 61% had detectable culturable *E. coli*, with an average density of 0.9 CFU/$m^3$. Control samples taken > 1 km away from OWCs were negative for culturable *E. coli* (Figure 2, right). F2 Sampling conditions in the Bobcat may have led to an underestimation of *E. coli*: the dry filter and high relative flow rate can limit bacterial survival on capture. We cannot directly link the presence of *E. coli* with ARGs in the present analysis, as ARGs can be carried by a range of bacteria. However, the presence of airborne culturable *E. coli* does confirm aerosolized fecal waste and may indicate the potential presence of resistant phenotypes as observed in a study we conducted with partners in Bolivia.[52] The finding that *E. coli* is enriched in aerosol samples near OWCs suggests, but cannot unambiguously confirm, that OWC waste may be aerosolized in this setting.

**Antibiotic resistance genes and MIs in aerosols.** We analyzed 53 air samples for the presence of ARG and MI targets. Seven of eight samples from the control site were negative for ARGs. We analyzed the presence of ARGs and MIs in all samples before and after the 95% LOD was applied. Targets detected above the LOD represent true positives with 95% confidence (Table 2). T2

For two replicates of each sample extract, we quantified gene copies of each target in the ddPCR reaction mixture (2 μL sample extract, 21 μL of ddPCR reagents). Figure 2 (left) shows the mean densities and standard error for each target in samples from each site and the LOD for each target.

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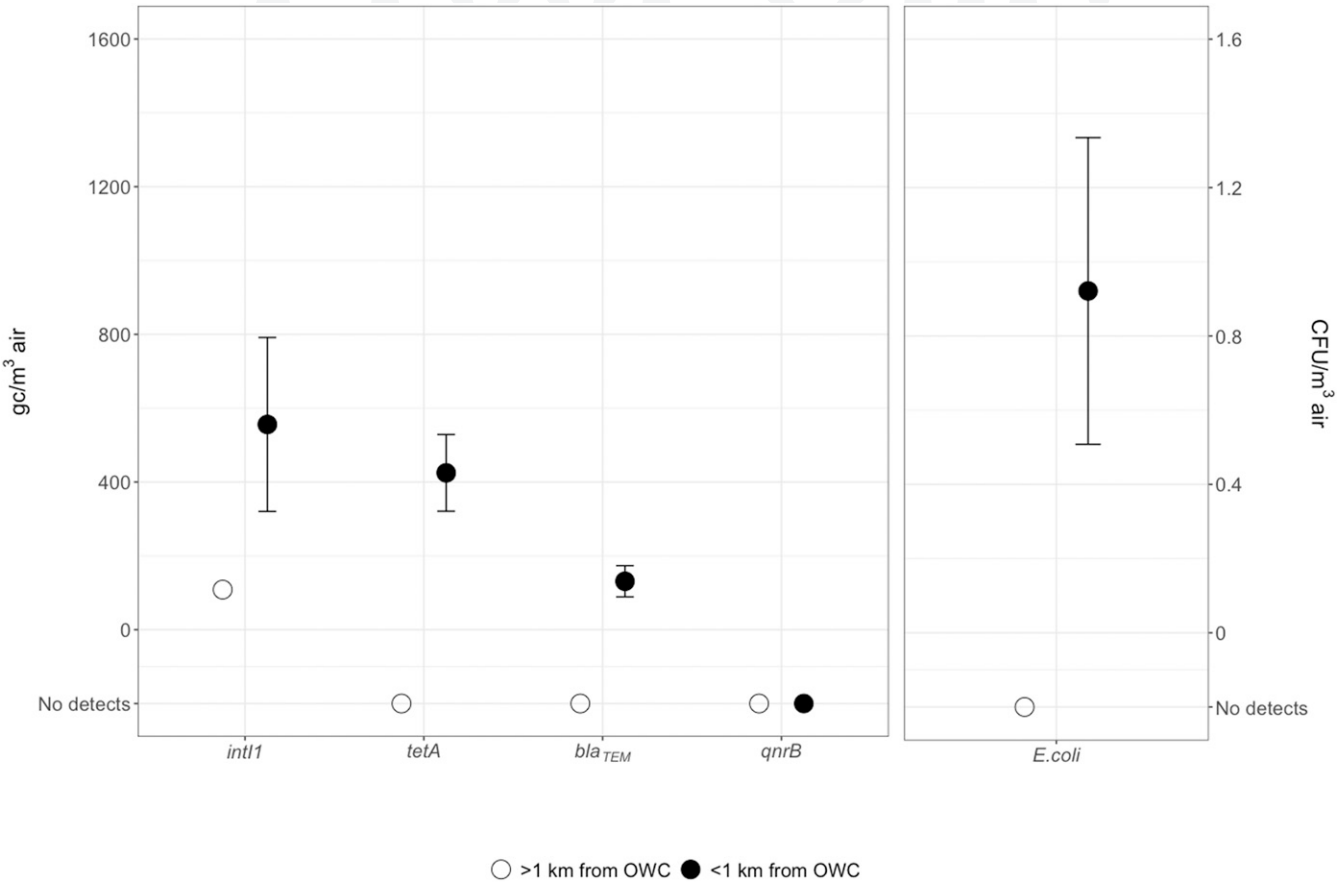


FIGURE 2. Mean antimicrobial resistance gene and mobile integron densities with mean standard error bars for the distribution in gene copies per cubic meter of air, where targets were detected at levels equal to or above the limits of detection (left). Estimated mean culturable *E. coli* as colony-forming unit per cubic meter of air with mean standard error bars for the distribution (right).

We did not detect *qnrB* in any samples above the LOD at any sites. However, we detected genes encoding resistance to tetracycline (*tetA*), genes encoding resistance to β-lactams ($bla_{TEM}$), and genes encoding genetic transfer mobility (*intI1*) above the LODs at all sites < 1 km from OWCs. One control sample was positive for *intI1* above the LOD at a concentration of 108.6 gc/m$^3$. This sample was taken at a height of approximately 10 m as opposed to the others that were taken at ground level, which may be due to a lack of obstacles at the higher elevated control location. We observed a statistically significant increase in MI and ARG densities in samples < 1 km away from OWCs compared with the samples > 1 km from OWCs for *intI1* (*P*-value = 0.038) and tetA (*P*-value = 0.012.) Although we observed an increase in $bla_{TEM}$ density in the samples < 1 km away from OWCs compared with the samples > 1 km away, this increase was not significant (*P*-value = 0.4). Other studies in high- and-middle-income countries have reported comparable ARG absolute densities to our ambient outdoor urban samples for specific contaminated sites (e.g., homeless shelters, composting sites, and concentrated animal feeding operations), but urban OCWs are not prevalent where sanitation infrastructure is adequate.[27,53] Studies reporting relative abundances of ARGs in outdoor ambient air, such as Li et al.,[20] show that a wide range of ARG and MI subtypes may be detected and that urban air may be enriched with these genes, although sources remain mostly uncharacterized.

TABLE 2

Summary of positive detections through ddPCR in all air samples < 1 km from open wastewater canals before and after leaving the data censored using the 95% LOD as a conservative threshold for positivity

| | ddPCR detections in bioaerosol samples (%, *n* = 45) | ddPCR detections in bioaerosol samples above the experimentally determined LOD (%, *n* = 45) |
|---|---|---|
| intI1 | 73 | 53 |
| qnrB | 2.0 | 0.0 |
| *tetA* | 71 | 5.0 |
| $bla_{TEM}$ | 24 | 9.0 |

LOD = limits of detection.

In an analysis of the associations between ARG and MI density and season (rainy and dry) (Figure 3), we calculated average densities for each target above the LOD by season. We observed no significant difference between rainy and dry seasons for any target, although we cannot rule out that such differences may exist. *IntI1* and *tetA* appeared to have increased marginally in the rainy season, and $bla_{TEM}$ decreased similarly in the rainy season. Rainfall may decrease the amounts of larger aerosols in the atmosphere,[54] but our results indicate no strong apparent seasonal trends in this regard related to our targets of interest in this setting.

**Open wastewater canal samples.** Mean densities of ARG and MI targets in OWC samples (*n* = 11) were highest for *intI1*

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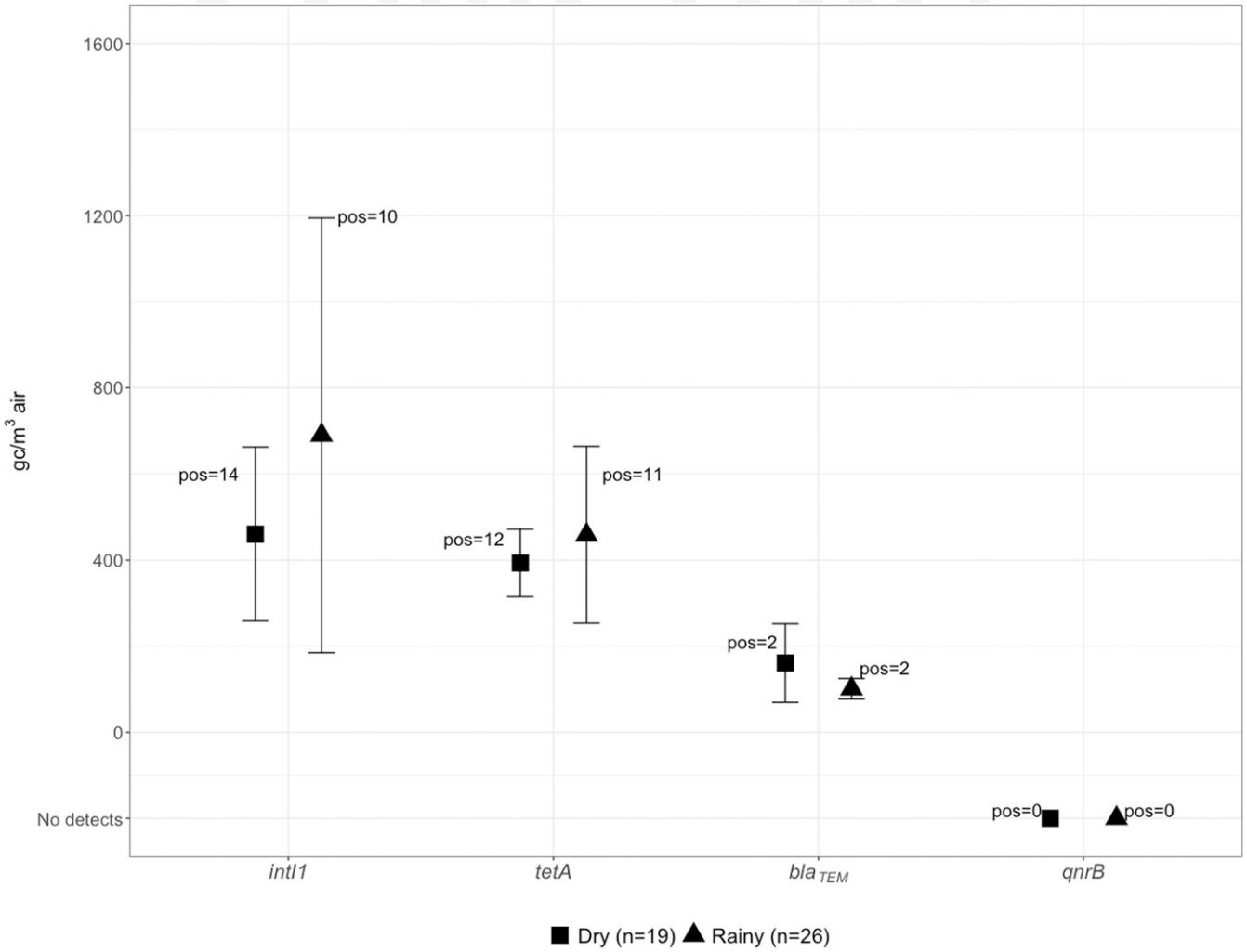


FIGURE 3. Average density of antimicrobial resistance gene and mobile integron targets in gene copies per meter cubed of air sampled. In total, 19 samples were analyzed from the dry season and 26 samples were analyzed from the rainy season. Above each data set for each target, the number of data points that were equal to or above the limits of detection and averaged together for this analysis is displayed.

and *tetA* ($1.8 \times 10^8$ gc/100 mL for both), followed by $bla_{TEM}$ ($2.8 \times 10^6$ gc/100 mL) and *qnrB* ($1.0 \times 10^6$ gc/100 mL). This distribution averaged across OWC samples is consistent with the distribution of average target magnitudes across all aerosol samples, potentially indicating a relationship between the two. Although we collected each OWC sample during a paired aerosol sample, some of the aerosol samples in the pairs were not included in this analysis for a variety of reasons, including instrument malfunction and interference from surroundings. Nevertheless, 6/11 OWC samples have a paired air sample, and a comparison between samples can be found in Supplemental Figure S1.

Open wastewater canal target densities were higher than abundances reported in Chinese surface waters (*tetA*, e.g., ranging from $1.0 \times 10^5$ to $5.0 \times 10^5$ gc *tetA*/100 mL) but are lower than abundances reported in raw sewage ($1.0 \times 10^{10}$ gc *tetA*/100 mL).[53,55] Our results are consistent with previous work implicating wastewaters as sites of concern in the development and dissemination of AR.[6,56–59] For cities with OCWs, direct exposure to this material is possible via flooding or other contact.[55] Class 1 integrons (*intI1*) are associated with multiple AR and increased ARG mobility and typically are present in high densities in environments associated with fecal waste.[53]

**Open wastewater canals as potential sources.** We observed elevated ARG and MI densities at sites near OWCs. With high densities of fecal matter and ARGs in wastewater samples, aerosolization of fecal microorganisms and ARGs is possible,[50,60] including via mechanical actions such as bubble bursting,[60,61] rain droplet impacts,[54] and other phenomena. We further observed that both *tetA* and *intI1*, which were detected in OWC samples at substantially higher densities than the other gene targets, were also detected at higher concentrations in the air as well, suggesting a potential quantitative association between density of targets in the OWCs and in nearby air. Although our data strongly suggest that OWCs are a contributing source to aerosolized ARGs in this setting, we cannot rule out other sources that may contribute to elevated ARGs in both matrices. With many possible sources, ARGs could originate from sources like fecally contaminated soils,[62] animal waste,[63] and other human activities including composting or solid waste disposal. Future studies could help link aerosolized ARGs and specific sources using additional tools of microbial source tracking, aerosol source apportionment,

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or metagenomics to assess similarity of microbial populations between putative sources and bioaerosols.

Interpretation of our results requires an acknowledgment of the study's fundamental limitations. The number of samples taken prevented inclusion of potentially important covariates, such as time of day of sampling, UV measurements, and other environmental atmospheric data in regression models. In addition, our control site was limited to one location, which may or may not be representative of other areas with similar characteristics. We identified a limited number of ARGs to assess a priori, and despite being of widespread interest in the field, these may or may not have been the most relevant targets for the study setting or for exposure relevance more generally. A wide range of ARGs may be present in ambient urban air,[20] and they may be attributable to multiple sources and mechanisms of aerosolization, requiring further work to characterize. In addition, although quantification of low gene target densities via ddPCR is improved when compared with quantitative PCR,[42] concerns of false-positive identification remain and can be a limitation, like any molecular detection method.[64]

Despite these limitations, our results further support the observation that ARGs are present in outdoor ambient air at detectable and quantifiable levels, and that likely sources in our setting of interest are OWCs. To our knowledge, our data are the first to report absolute rather than relative quantification of sanitation-related ARGs in urban aerosols. Density data for ARGs and mobile genetic elements are a necessary first step in the development of risk assessment models that can interrogate their public health relevance, if any, through this pathway. Further work is needed to determine source apportionment and the potential role that aerosols may play in fate and transport of ARGs, and ultimately whether airborne transport is a meaningful pathway in the development and dissemination of AR in highly contaminated environments.

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The following are supplemental materials and will be published online only

**SUPPLEMENTARY MATERIAL**

***Table S1****. Summary of high volume aerosol samples and wastewater samples taken throughout the sampling period at each site.*

| **Sampling Site Specifications** | | | | | | | | | | | | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Distance from OWC** | >1 km | <10 m | | | | | | | | | | | | 10-100 m | | | 100-1000 m | | | ____ |
| **Site ID** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **15** | **17** | **14** | **16** | **18** | ____ |
| **Dry Season Bioaerosol Samples** | 4 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | **23** |
| **Rainy Season Bioaerosol Samples** | 4 | 4 | 1 | 2 | 0 | 2 | 2 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | **30** |
| **Total Bioaerosol Samples** | 8 | 6 | 2 | 3 | 1 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | **53** |
| **Wastewater sample collected** | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | **11** |

In a subset analysis, we assessed each individual aerosol sample that was collected at the same time as the OWC sample in Figure S1. The comparison reveals that *intI1* and *tetA* have the highest magnitudes for both OWC and aerosol samples., In comparing within each pair, *tetA* is detected most often in both OWC and aerosol samples, followed by *intI1*.

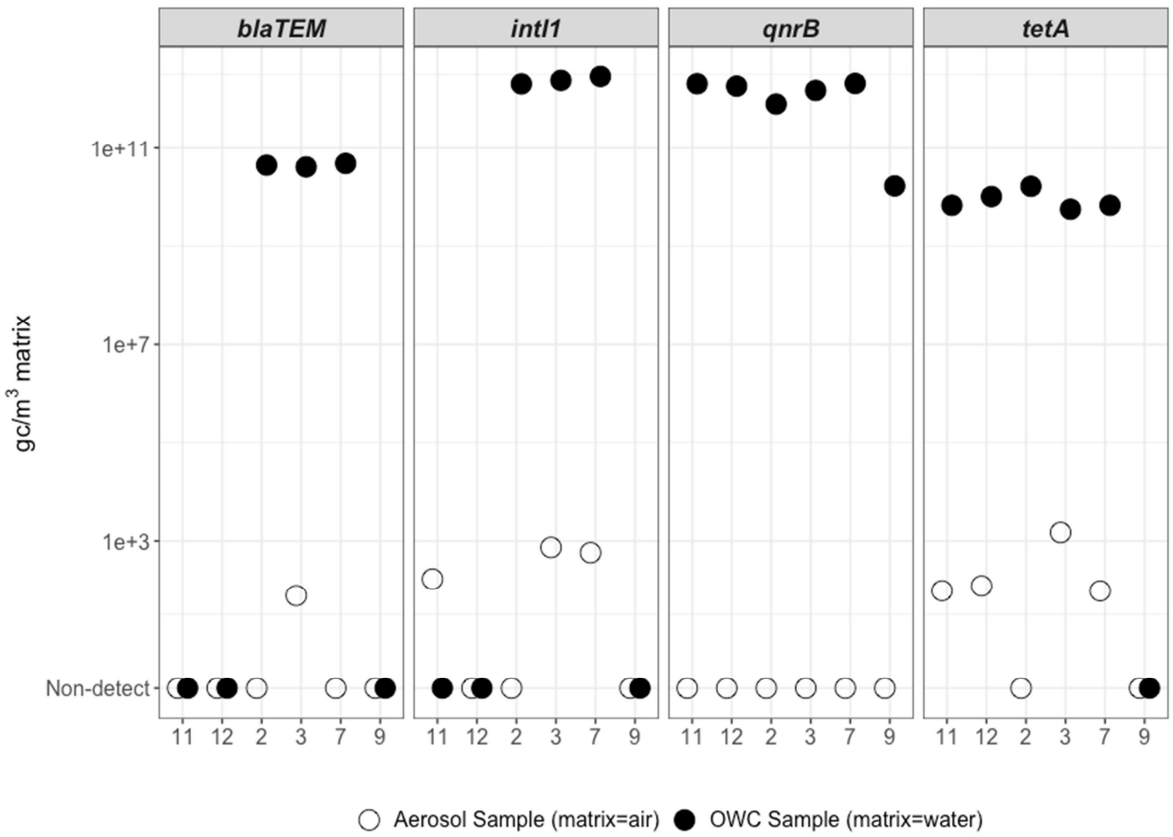


***Figure S1****: Six OWC samples have a paired aerosol sample in this analysis. The figure displays the target density for each paired OWC and air sample.*