Carbapenem Resistance Exposures via Wastewaters across New Delhi

Manisha Lamba[1], Sonia Gupta[1], Rishabh Shukla[1], David W. Graham[2], T.R. Sreekrishnan[1], S. Z. Ahammad[1]*

1. Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India
2. School of Engineering, Newcastle University, Newcastle upon Tyne, United Kingdom

***Corresponding Author:** Dr. S. Z. Ahammad
Address: I-135, Dept. of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi -110016, India
Phone: +91 11 2659 1006
Fax: +91 11 2658 2282
E-mail: zia@iitd.ac.in

Abstract

Antimicrobial resistance (AMR) is a major global concern, especially in India where the burden of infectious diseases is high and health care spending is low. Here we quantified total coliform, faecal coliforms (FC), carbapenem-resistant enteric bacteria (CRE), *bla*$_{NDM-1}$, and three integron genes in samples collected from wastewater effluent of 12 hospitals, 12 sewage treatment plants (STPs), 20 sewer drains, and five locations along the Yamuna River in New Delhi over two seasons. Significant correlations were found between FC levels, CRE ($r = 0.903$, $p = 0.004$, $n = 49$) and *bla*$_{NDM-1}$ ($r = 0.787$, $p = 0.003$, $n = 49$) concentrations across all samples. Concentrations of coliforms, CRE, *bla*$_{NDM-1}$, *int1*, and *int3* were highest in hospital effluents compared to other locations in both seasons. Although absolute concentration data indicate greater abundances of CRE and *bla*$_{NDM-1}$ in the winter, normalised data indicates greater carriage of *bla*$_{NDM-1}$ per cell in summer samples. In general, observed CRE levels were highest in surface water downstream of areas with higher population densities. Among CRE isolates (n = 4077), 82%, 75%, 71% and 43% of the strains from hospitals, sewer drains, river samples, and STPs, respectively, contained *bla*$_{NDM-1}$, implying STPs have relatively fewer *bla*$_{NDM-1}$ positive CRE in their effluents. The most common CRE isolates in the drains were *Pseudomonas putida* (39%) followed by *Acinetobacter baumanni* (20%) and *Pseudomonas montelli* (19%). The present scenario in New Delhi highlights the urgent need for increased coverage of appropriate waste treatment facilities across the city to reduce CRE exposures from polluted surface waters.

Keywords: antibiotic resistance; β-lactams; carbapenem resistant enteric bacteria; human wastes

1. Introduction

Increasing antimicrobial resistance (AMR) is a global concern in human and veterinary healthcare systems. AMR problems are greatest in emerging countries like India where infectious disease levels are high and per capita healthcare spending is low (Public Health Foundation of India, 2011). Although AMR is intrinsic in nature (D'Costa *et al*., 2011), continued use of antibiotics, pollution, and other factors have selected and mobilised AMR genes (ARGs) across the microbiome, including the acquisition of antibiotic resistance genes (ARGs) in strains of health importance. The mobilisation is largely fuelled by promiscuous mobile genetic elements (MGEs), including plasmids, transposons, and integrons (Mazel, 2006), which mediate AMR spread via horizontal gene transfer (HGT), creating resistant and multi-resistant phenotypes in pathogenic and non-pathogenic strains. Until fairly recently, AMR evolution and transmission was assumed to be a consequence of medical and veterinary antimicrobial use (Jin *et al*., 2017). However, it is now apparent that consequential AMR dissemination and exposure also occur away from places of antibiotic use through environmental pathways (Arcilla *et al*., 2016), such as via wastewater releases, and associated contaminated water and food (Christgen *et al*., 2015).

One place where AMR spread in the environment is most evident in New Delhi, which is where New Delhi metallo-β-lactamase protein (i.e., NDM-1) was first detected in 2008 in a carbapenem-resistant *Klebsiella pneumonia* isolate from a hospital patient (Yong *et al.*, 2009). NDM-1 confers multi-resistance to many bacteria, including therapeutically critical carbapenems (Papp-Wallace *et al*., 2011). Unfortunately, $bla_{\text{NDM-1}}$ (gene that codes for NDM-1) was detected soon thereafter in New Delhi surface waters and seeps in 2010, presumably associated with faecal releases (Walsh *et al.*, 2014), and $bla_{\text{NDM-1}}$ and variants are now found in patients and the environment in over 100 countries (Kumarasamy et al., 2010; Nordmann et al., 2011a, 2011c; Wilson and Chen, 2012).

Phenotypes include strains functionally resistant to all antibiotics, including colistin and tigecycline (Kumarasamy et al., 2010; Nordmann et al., 2011b). Clinical evidence suggests antibiotics select for *bla*$_{\text{NDM-1}}$ in gut strains and the gene subsequently enters surface waters, probably via human wastes (Hawkey and Jones, 2009). The *bla*$_{\text{NDM-1}}$ gene has spread globally via international travel in exposed individuals (Hawkey and Jones, 2009; Petersen *et al*., 2015).

Although the above global pathway is probable, the main originating cause of *bla*$_{\text{NDM-1}}$ within the New Delhi urban environment is unclear, especially the relative role of community, hospital, and sewage treatment plant (STP) effluents as sources of *bla*$_{\text{NDM-1}}$ and carbapenem-resistant enteric bacteria (CRE) across the city. Therefore, we quantified levels of *bla*$_{\text{NDM-1}}$, three integron-related genes (*int1*, *int2*, and *int3*), 16S-rRNA genes, total coliforms (TC), faecal coliforms (FC), and CRE isolates in New Delhi surface waters at 49 locations in winter and summer samples.

Integrons are defined here as mobile gene cassettes composed of a gene-encoding integrase (e.g., *int*1), a recognition and recombination of cassette (att I) and a promoter (Pc) for expression of the cassettes (Hall and Collis, 1995). Based on previous data, class 1 markers tend to be most associated with waste releases followed by class 3 and class 2 markers (Rapa and Labbate, 2013). *int1* and *int3* are often found in freshwater and soil proteobacteria, whereas *int2* is a part of marine γ-proteobacteria (Deng et al., 2015; Li et al., 2013). Quantifying the abundance of integron gene cassettes is useful in potentially understanding *bla*$_{\text{NDM-1}}$ dispersal because they often are associated with faecal releases to the environment (Andersson and Levin, 1999; Courvalin, 1994; Gillings et al., 2015; Kruse and Srum, 1994; Leverstein-van Hall et al., 2002). These data were used to quantify season differences in environmental CRE and *bla*$_{\text{NDM-1}}$ exposure, the relative contribution of community, hospital and STP wastewater sources to proximal levels, and dominant CRE species found in sewer drains and the Yamuna River that impacts residents via environmental exposures.

This study is globally relevant because India is the largest consumer of antibiotics for personal use in the world and β-lactams are among the most commonly used antibiotics in India (Boeckel et al., 2014). We suspect antibiotic misuse in places like India partially explains the early evolution of CRE strains, including *bla*$_{\text{NDM-1}}$ positive isolates. Therefore, although New Delhi presents an extreme case, it is a template for understanding AMR spread in any large city without adequate wastewater management; a common scenario in the developing and emerging world.

2. Materials and Methods

2.1 Study Area and Sampling Program

Sampling across the New Delhi wastewater network included hospital effluents, open and sub-surface sewer drains, STPs and final receiving waters. The network comprised 20 drain sites, 12 hospital waste outfalls, effluents from 12 STPs, and five sites along the Yamuna River, which is the ultimate receptacle for wastewaters from the city (see Supporting Information (SI) Fig. S1 and Tables S1-S3 for locations and details). Due to the myriad of wastewater sources to the network, it was not possible to exactly quantify contributing population numbers (i.e., as cohorts) or hospital versus community antibiotic use in an exact manner. Therefore, comparisons among community, hospital, and STP sources were based on detected *bla*$_{\text{NDM-1}}$, other genes and bacterial numbers per wastewater catchment area. This allowed a block experimental design that allowed two-sample comparisons of genes and bacteria per catchments or locations with different contributors.

Water column samples were collected in January/February ("Winter") and May/June ("Summer") 2014 from all locations. The following were quantified in samples (at least in triplicate): TC, FC, and CRE colony forming units (CFUs), and temperature (T), pH, total dissolved solids (TDS), and

specific conductivity. Water samples were sub-sampled in triplicate for qPCR analyses of *bla*$_{\text{NDM-1}}$, integron 1, 2 and 3 gene cassette markers (*int1*, *int2*, and *int3*), and 16S rRNA genes.

2.2 Initial sample collection and processing

All the samples were collected in sterile 500-mL containers and returned to laboratory transported on ice for subsequent microbial and molecular biological analysis. All microbial plating and culturing work was performed within 24 hours of sampling, whereas sub-samples for molecular work were frozen at -20^{o}C for subsequent DNA extraction and qPCR. At the time of sampling, wastewater temperature, pH, TDS, and specific conductivity were measured to describe the water conditions under which samples for the microbiological and molecular biological analyses were collected.

2.3 Microbial culturing and plating

Samples for TC, CRE, and FC were serially diluted in sterile phosphate buffer solution (PBS) and plated in triplicate (per dilution) on Rapid HiColiform Agar (Himedia, India), HiCrome KPC Agar Base (Himedia, India) at 37˚ C for 24 h, and M-FC Agar Base (Himedia, India) at 45˚ C for 24 h, respectively. CFUs were estimated according to manufacturer's instructions. Resistant colonies were selected from the KPC plates and re-streaked to purity to allow 16S sequencing and phenotyping. Isolate identities were verified using strain-specific Biochemical Test Kits (Himedia, India) and included more than just KPC strains. Therefore, identified carbapenem-resistant isolates are referred to as CRE herein.

2.4 DNA extraction and qPCR assays

DNA from frozen water and wastewater samples, and pure culture isolates were extracted using the Fast Soil DNA extraction kit and a Ribolyzer according to manufacturer's instructions. DNA

from pure cultures was amplified using bacterial 338F and 1046R primers (Huber et al., 2007; Yu et al., 2005); preheating at 95˚C for 5 min, 39 cycles of denaturation at 95˚C for 45 s, annealing at 60˚C for 45 s, extension at 72˚C for 45 s and final extension at 72˚C for 7 min. Agarose gels were run to examine the primary PCR products and the 16S-rRNA was sequenced. Extracted DNA from the field samples was stored at -20˚ C for subsequent qPCR analysis. Specific genes reported here include $bla_{\text{NDM-1}}$, *int1*, *int2* and int3, and 16S-rRNA genes were also quantified to estimate the total eubacterial population size. The probes/primers used in qPCR are provided in Table S4 (SI).

All genes were quantified in triplicate using the BioRad CFX-96 system (BioRad, USA). All reactions were performed with serially-diluted DNA standards and DNA-free negative controls. Correlation coefficients for the calibration curves were > 0.99 and all the log gene abundance values were in the linear range of detection.

2.5 Isolate identification and characterization

To identify and characterize the CRE isolates, pure cultures were developed, DNA extracted, and 16S-rRNA (Pace, 1997) were amplified using bacterial 338F and 1046R primers. Purified PCR products were sequenced using Sanger Shotgun sequencing. Sequences were compared with the GenBank database (Benson et al., 2013) using Standard Nucleotide BLAST tool, and strains were identified based on percentage similarities with strains within the database. Biochemical test kits were used to confirm isolates and all isolates were screened for carriage of $bla_{\text{NDM-1}}$ using PCR.

2.6 Statistical analysis and data visualization

All the data analysis was done using Excel 2007 (Microsoft Corporation, USA) and SPSS Version 19.0 (Chicago, IL). Point data (e.g., CRE concentrations) were analyzed using bivariate correlation employing the Spearman's non-parametric methods on log-transformed data because normality

could not be assumed for all datasets. The paired sample t-test was conducted for comparing hospital versus community catchments. Statistical significance was always defined by 95% confidence intervals ($p < 0.05$), although data trends were assumed to be important when $p < 0.1$ because of the complex environments from which the samples were collected.

Spatial analysis of CRE concentrations in surface waters and population densities across the city was performed using ArcGIS 10.1. The aim was to visualize spatial relationships between detected CRE at locations in surface waters across the city and areas of differing population density. Due to differences in data format and the complexity of relationships between areal population density data and point-based CRE data, direct statistical analysis was not possible. However, mapped visualizations were developed to permit qualitative comparisons, which was the best option given the available data.

3. Results and Discussion

Wastewater samples were collected from 12 hospitals, 12 STPs, 20 major drains, and from five locations along the Yamuna River in New Delhi in summer and winter of 2014 (see Fig. S1 and SI for site details). Mean seasonal concentrations of TC, FC, CRE, $bla_{\text{NDM-1}}$, i*nt1*, *int2*, and i*nt3* were quantified across locations, which are summarized in Table S5 (see SI). The concentrations of TC, FC, CRE, $bla_{\text{NDM-1}}$, *int1*, and *int3* were consistently highest in hospital effluents in samples from both seasons, except for FC in summers which were highest in the samples collected from STPs (Table S5). In summer samples, hospitals had at least 10-fold higher levels of CRE ($p = 0.002$) and 100-fold higher levels of $bla_{\text{NDM-1}}$ ($p = 0.015$) compared with the other sampling locations. In contrast, TC, FC, CRE, $bla_{\text{NDM-1}}$, *int1,* and *int3* concentrations were not statistically different among drain, STP, and Yamuna River samples ($p > 0.05$). *int3* concentrations were

significantly higher than *int1* in winter drain samples (p = 0.012), which to our knowledge, has not been observed in previous surface water studies. *int2* concentrations were always low.

Bivariate correlation analysis was performed on all data, and Figure 1 showed significant, positive correlations between FC, and CRE (r = 0.903, p = 0.004, n = 49) and $bla_{\text{NDM-1}}$ (r = 0.787, p = 0.003, n=49) concentrations, respectively, based on all samples collected across the city. Both CRE (r = 0.839, p = 0.007, n=49) and $bla_{\text{NDM-1}}$ (r = 0.794, p = 0.008, n=49) concentrations also significantly correlated with TC levels. Significant correlations of FC with both CRE and $bla_{\text{NDM-1}}$ suggests a close association between surface water CRE and faecal matter, exemplifying the potential impact of sewage releases on CRE and $bla_{\text{NDM-1}}$ exposures to humans from the city's surface waters. Not surprisingly, CRE and $bla_{\text{NDM-1}}$ significantly correlated across all samples (r = 0.888, p = 0.004).

To assess possible relationships between selected MGEs and $bla_{\text{NDM-1}}$, the three integrons were quantified in all hospital, drain, STP and the river samples, and correlations between $bla_{\text{NDM-1}}$ and each was assessed. Overall, *int1* and *int3* concentrations significantly correlated with $bla_{\text{NDM-1}}$ gene abundances in most settings and in both the seasons (Table S6), whereas *int2* and bla_{NDM-1} levels displayed no significant relationship. These data are consistent with observed correlations between integrons and other ARGs in faecal-impacted waters (Leverstein-van Hall et al., 2002), although co-carriage of *int1* and $bla_{\text{NDM-1}}$ was only observed in 52%, 28%, 45% and 57% of the CRE isolates from the drain, STP, hospital and river samples, respectively (see Table S7). This implies that *int1* and $bla_{\text{NDM-1}}$ are present in Delhi surface waters, but they are only sometimes associated with the same specific isolates.

With regards to seasonal variation, higher concentrations of all bacterial and genetic markers were seen in winter samples, probably due to greater seasonal antibiotic use (Boeckel et al., 2014; Kotwani and Holloway, 2011), and lower water use and surface water flows in the winter (Ahammad *et al.*, 2014). As background, antibiotics are often inappropriately used in India for viral infections, which are much more common in the winter. Thus, lower water use which results in lesser dilution coupled with higher antibiotic use in winter, results in higher concentration of bacterial and gene abundances in the winter.

To understand seasonal variations on the bacterial carriage of $bla_{\text{NDM-1}}$, we normalized $bla_{\text{NDM-1}}$, *int1* and *int3* gene levels per bacterial cell (i.e., 16S gene levels) in samples (Fig. 2). Although higher absolute abundances of CRE and $bla_{\text{NDM-1}}$ are apparent in the winter (Table S5), normalised data imply greater carriage of $bla_{\text{NDM-1}}$ per cell in the summer (Fig. 2). The only exception is in hospital wastes in the winter, which appear to have especially high levels $bla_{\text{NDM-1}}$ per cell, presumably due to very high hospital antibiotic use in the winter (Lamba *et al.*, 2017).

Although Fig. 2 and Table S5 data appear contradictory, observed differences can be explained by seasonal differences in receiving water conditions. We suspect higher normalised gene levels in the summer are due to warmer surface water temperatures (see Fig. 3); i.e., faecal CRE that enter surface waters in the summer in wastes probably survive longer due to warmer temperatures. Conversely, lower temperatures in the winter enhance faecal bacteria die-off. This is consistent with Walsh et al. who predicted higher frequencies of plasmid transfer, including of $bla_{\text{NDM-1}}$, in the summer due to high temperatures in New Delhi (Walsh et al. 2014). On a positive note, normalised $bla_{\text{NDM-1}}$ levels were consistently lower in STP effluents, which implies STPs, in relative terms, contribute fewer $bla_{\text{NDM-1}}$ per cell in Delhi surface waters than other sources. Unfortunately, only ~40% of human wastes in New Delhi are treated in STPs (Central Pollution

Control Board, 2013; Central Pollution Control Board, 2005), implying increasing STP coverage should reduce AMR exposures in Delhi surface waters, assuming antibiotic use is also curbed.

To differentiate levels of AMR contributed by community versus hospital sources, we compared CRE, *bla*$_{\text{NDM-1}}$, *int1* and *int3* levels in drains with hospitals in their catchment and drains without apparent hospital contributions (Fig. 4). Higher levels of CRE and *bla*$_{\text{NDM-1}}$ were evident in hospital-impacted drains compared with community waste-only drains in both seasons, which was suggested earlier (Lamba et al., 2017), implying foci of antibiotic use, such as hospitals, result in locally greater environmental AMR exposures (Henninger and Ku, 2003). This is consistent with studies on hospital environs in Bangladesh (Islam et al. 2017).

High use of antibiotics in hospitals is a particular problem in India and, combined with inadequate STP coverage, appear to particularly contribute to AMR spread in New Delhi. As background, most of the hospitals in New Delhi actually have their own wastewater treatment systems, but observed elevated abundances of TC, FC, CRE, and *bla*$_{\text{NDM-1}}$ in hospital effluents (Table S5) suggest treatment systems are either inappropriate or poorly maintained. Interestingly, *int1* and *int3* levels do not consequentially differ between drains with and without hospitals, implying these integron cassettes are not necessarily associated with CRE and *bla*$_{\text{NDM-1}}$ from the hospitals. This is consistent with the occasional co-carriage of *int1* and *bla*$_{\text{NDM-1}}$ in CRE isolates (Table S7).

The presence of high levels of CRE and *bla*$_{\text{NDM-1}}$ in the hospital wastewaters indicates a potential risk of spreading these resistant determinants within the environment and ultimately to human populations (Gómez et al., 2010). The CRE and *bla*$_{\text{NDM-1}}$ contributed from hospitals do get diluted in the sewers; hence lower abundances of coliforms, CRE, *bla*$_{\text{NDM-1}}$ and integron genes are apparent compared to the hospitals. Usually, sewer lines receive water from local rural and urban

residential areas, nearby hospitals, villages and agricultural areas (Lamba et al. 2017). People residing in densely populated urban areas use more and more expensive antibiotics, resulting in higher absolute levels of self-prescription relative to less developed areas (Tamhankar et al. 2018). Though the presence of better sanitation systems in urban areas implies greater waste treatment for "conventional" pollutants, this does not appear to translate into lower local AMR levels, possibly due to higher population densities and antibiotic use, but also because conventional treatment systems were never designed to remove AMR genes (Gómez et al., 2010).

Data in Fig. 2 suggest STPs do reduce normalised levels of AMR and MGE genes, but absolute levels remain relatively high, even in STP effluents (Lamba and Ahammad, 2017). In contrast to the more developed urban areas, economically deprived areas (i.e., slums) often have no central sanitation and-or inadequate health care facilities. Hence it can be inferred that, although less "untreated" waste is released from developed urban areas, per capita AMR discharges may be higher than slum areas. In contrast, slum areas contribute more untreated faecal matter to community surface waters. Interestingly, the sewer drains with the highest CRE levels are primarily central drains in the SE corner of Delhi (see Fig. 6), which is the "downstream" end the city. This implies CRE released citywide may be progressively accumulating in sewer drains as wastewater flows across the city, suggesting greater human exposures to CRE are more based on the direction of flow rather factors like local wealth demographics.

Further, the city was divided based upon the population density and spatial analysis was done using ArcGIS 10.1 to contrast locations of greatest population density on the prevalence of CRE in the drains and river (see Fig. 5). Spatial analysis shows the highest human population densities are in east central Delhi straddling the Yamuna River, which is immediately "upstream" of drain locations with the highest levels CRE across the city. This does not imply a direct and statistical

cause-and-effect, but it hints that greater upstream population densities translate into higher exposure levels to CRE in downstream waters. This downstream effect is particularly acute in the winter (see Fig. 5), although a similar trend is apparent in both seasons.

In order to determine the types of CRE strains in different environmental locations, isolates were purified and identified from hospital, drain, STP and the river samples, which are summarized in Table 1. All noted gram-negative isolates are pathogens associated with nosocomial- and community-associated infections in humans (Anton et al., 2010; Nordmann et al., 2009), and include strains on the World Health Organization list of pathogens in urgent need of new antibiotics (World Health Organization, 2017). Among the CRE strains isolated (n = 4077), 82% of the hospital wastewater isolates, 75% from sewer drains, 43% from STPs and 71% from river samples contained $bla_{\text{NDM-1}}$ (Table S6). Since local human populations are most exposed to drain and river water, the proportional number of different CRE isolates were estimated for each source (Fig. 6). The most common CRE isolates found in sewer drains were *Pseudomonas putida* (39%), *Acinetobacter baumanni* (20%) and *Pseudomonas montelli* (19%). In contrast, Yamuna River samples were dominated by *Klebsiella pneumonia* (26%), *Klebsiella pneumoniae subsp. Pneumoniae* (17%) and *Acinetobacter baumanni* (16%). *Klebsiella* spp. and *Acinetobacter* spp. strains can cause serious health issues, such as urinary tract and bloodstream infections (Peleg and Hooper, 2011).

4. Conclusions

The burden of AR is continuously increasing and is now recognized as a major threat to the public health for treating infectious disease. The present study reveals a grim scenario to the residents of New Delhi due to CRE exposure from wastewater discharges. Clearly, AMR genes and bacteria

are being released to the New Delhi water environment from different sources with hospitals potentially being particularly important. However, hospitals are only one source and a much wider problem exists, which roots from a combination of widespread antibiotic overuse and inadequate urban sanitation. Although it cannot be proved with certainty, this combination is likely changing community health. Therefore, solutions that combine altered human behaviour and improved infrastructure are both urgently needed.

Regardless, high levels of CRE and *bla*$_{\text{NDM-1}}$ in New Delhi surface waters imply a consequential environmental exposure risk to people residing in the city. Improving community sanitation and water quality, encouraging the more prudent use of antibiotics, improving infection control practices, and increasing waste treatment are all needed. We strongly suspect unless all these actions are done, the AMR problem in places like New Delhi will continue to get worse. More prudent use of antibiotics is clearly and urgently needed; however, increased coverage of well-managed and appropriate waste treatment is also critical to reducing AMR exposures via wastewater sources.

REFERENCES

Ahammad Z. S, Sreekrishnan T. R., Hands C. L., Graham. D. W., 2014. Increased Waterborne blaNDM-1 Resistance Gene Abundances Associated with Seasonal Human Pilgrimages to the Upper Ganges River. Environ. Sci. Technol. 48, 3014–3020.

Andersson, D.I., Levin, B.R., 1999. The biological cost of antibiotic resistance. Curr. Opin. Microbiol. 2, 489–493.

Anton Y. Peleg, M.B., B.S., M.P.H. and David C. Hooper, M.., 2010. Hospital-Acquired Infections Due to Gram-Negative Bacteria. N. Engl. J. Med. 362, 1804–1813.

Arcilla, M.S., van Hattem, J.M., Haverkate, M.R., Bootsma, M.C.J., van Genderen, P.J.J., Goorhuis, A., Grobusch, M.P., Lashof, A.M.O., Molhoek, N., Schultsz, C., Stobberingh, E.E., Verbrugh, H.A., de Jong, M.D., Melles, D.C., Penders, J., 2016. Import and spread of

extended-spectrum beta-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet. Infect. Dis. 17, 78–85.

Benson, D. a., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2013. GenBank. Nucleic Acids Res. 41, 36–42.

Boeckel, T.P. Van, Gandra, S., Ashok, A., Caudron, Q., Grenfell, B.T., Levin, S.A., Laxminarayan, R., 2014. Global antibiotic consumption 2000 to 2010 : an analysis of national pharmaceutical sales data. Lancet Infect. Dis. 14, 742–750.

Central Pollution Control Board, 2005. Status of Sewage Treatment in India. 1-97.

Central Pollution Control Board, 2013. Performance evaluation of sewage treatment plants under NRCD. 1-128.

Christgen, B., Yang, Y., Ahammad, S.Z., Li, B., Rodriquez, D.C., Zhang, T., Graham, D.W., 2015. Metagenomics Shows That Low-Energy Anaerobic−Aerobic Treatment Reactors Reduce Antibiotic Resistance Gene Levels from Domestic Wastewater. Environ. Sci. Technol. 49, 2577–2584.

Courvalin, P., 1994. Transfer of Antibiotic Resistance Genes between Gram-Positive and Gram-Negative Bacteria. Antimicrob. Agents Chemother. 38, 1447–1451.

D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W.L., Schwarz, C., Froese, D., Zazula, G., Calmels, F., Debruyne, R., Golding, G.B., Poinar, H.N., Wright, G.D., 2011. Antibiotic resistance is ancient. Nature 477, 457–461.

Deng, Y., Bao, X., Ji, L., Chen, L., Liu, J., Miao, J., Chen, D., Bian, H., Li, Y., 2015. Resistance integrons : class 1, 2 and 3 integrons. Ann. Clin. Microbiol. Antimicrob. 14, 1–11.

Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.-G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. Int. Soc. Microb. Ecol. 9, 1269–79.

Gómez, M., Díaz, M.T., Araujo, M., Sueiro, R., Garrido, J., 2010. Wastewater treatment plants as redistributors of resistance genes in bacteria. WIT Trans. Ecol. Environ. 135, 83–94.

Hall, R.M., Collis, C.M., 1995. Mobile gene cassettes and integrons: Capture and spread of genes by site-specific recombination. Mol. Microbiol. 15, 593–600.

Hawkey, P.M., Jones, A.M., 2009. The changing epidemiology of resistance. J. Antimicrob. Chemother. 64, 3-10.

Henninger, A., Ku, K., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. Clin. Microbiol. Infect. 9, 1203–1214.

Huber, J.A., Mark Welch, D.B., Morrison, H.G., Huse, S.M., Neal, P.R., Butterfield, D.A., Sogin, M.L., 2007. Microbial Population Structures in the Deep Marine Biosphere. Science (80-.). 318, 97–100.

Islam, M.A., Islam, M., Hasan, R., Hossain, M.I., Nabi, A., Rahman, M., Goessens, W.H.F., Endtz, H.P., Boehm, A.B., Faruque, S.M., 2017. Environmental spread of New Delhi metallo-β-lactamase-1-producing multidrug-resistant bacteria in Dhaka, Bangladesh. Appl. Environ. Microbiol. 83, 00793-17.

Jin, L., Wang, R., Wang, X., Wang, Q., Zhang, Y., Yin, Y., Wang, H., 2017. Emergence of mcr-1 and carbapenemase genes in hospital sewage water in Beijing, China. J. Antimicrob. Chemother. 73, 84–87.

Kotwani, A., Holloway, K., 2011. Trends in antibiotic use among outpatients in New Delhi, India. BMC Infect. Dis. 11, 1-9.

Kruse, H., Srum, H., 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Appl. Environ. Microbiol. 60, 4015–4021.

Kumarasamy, K.K., Toleman, M. a, Walsh, T.R., Bagaria, J., Butt, F., Balakrishnan, R., Chaudhary, U., Doumith, M., Giske, C.G., Irfan, S., Krishnan, P., Kumar, a V, Maharjan, S., Mushtaq, S., Noorie, T., Paterson, D.L., Pearson, a, Perry, C., Pike, R., Rao, B., Ray, U., Sarma, J.B., Sharma, M., Sheridan, E., Thirunarayan, M. a, Turton, J., Upadhyay, S., Warner, M., Welfare, W., Livermore, D.M., Woodford, N., 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect. Dis. 10, 597–602.

Lamba, M., Ahammad, S.Z., 2017. Sewage treatment effluents in Delhi: A key contributor of β-lactam resistant bacteria and genes to the environment. Chemosphere 188, 249–256.

Lamba, M., Graham, D.W., Ahammad, S.Z., 2017. Hospital Wastewater Releases of Carbapenem-Resistance Pathogens and Genes in Urban India. Environ. Sci. Technol. 51, 13906–13912.

Lamba M., Sreekrishnan T. R., Ahammad Z. S., 2017. Sewage mediated transfer of antibiotic resistance to River Yamuna in Delhi, India. J. of Environ Chem Eng. 1-8.

Leverstein-van Hall, M.A., Paauw, A., Box, A.T.A., Blok, H.E.M., Verhoef, J., Fluit, A.C., 2002. Presence of Integron-Associated Resistance in the Community Is Widespread and Contributes to Multidrug Resistance in the Hospital. J. Clin. Microbiol. 40, 3038–3040.

Li, B., Hu, Y., Wang, Q., Yi, Y., Woo, P.C.Y., Jing, H., Zhu, B., Liu, C.H., 2013. Structural Diversity of Class 1 Integrons and Their Associated Gene Cassettes in Klebsiella pneumoniae Isolates from a Hospital in China. PLoS One 8, 1–9.

Mazel, D., 2006. Integrons: agents of bacterial evolution. Nat. Rev. Microbiol. 4, 608–620.

Nordmann, P., Cuzon, G., Naas, T., 2009. The real threat of Klebsiella pneumoniae carbapenemase-. Lancet Infect. Dis. 9, 228–36.

Nordmann, P., Naas, T., Poirel, L., Carbapenemases, C.A., 2011a. Global Spread of Carbapenemase- producing Enterobacteriaceae. Emerg. Infect. Dis. 17, 1791–1798.

Nordmann, P., Poirel, L., Carrër, A., Toleman, M. a., Walsh, T.R., 2011b. How to detect NDM-1 producers. J. Clin. Microbiol. 49, 718–721.

Nordmann, P., Poirel, L., Mark A., T., Timothy R., W., 2011c. Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J. Antimicrob. Chemother. 66, 689–692.

Pace, N.R., 1997. A molecular view of microbial diversity and the biosphere. Science 276, 734–740.

Papp-Wallace, K.M., Endimiani, A., Taracila, M.A., Bonomo, R.A., 2011. Carbapenems: Past, Present, and Future. Antimicrob. Agents Chemother. 55, 4943–4960.

Peleg, A.Y., Hooper, D.C., 2011. Hospital-Acquired Infections Due to Gram-Negative Bacteria. N. Engl. J. Med. 362, 1804–1813.

Petersen, T.N., Rasmussen, S., Hasman, H., Carøe, C., Bælum, J., Schultz, A.C., Bergmark, L., Svendsen, C.A., Lund, O., Sicheritz-Pontén, T., Aarestrup, F.M., 2015. Meta-genomic analysis of toilet waste from long distance flights ; a step towards global surveillance of infectious diseases and antimicrobial resistance. Nat. Publ. Gr. 1–9.

Public Health Foundation of India, 2011. Antibiotic Use and Resistance in India, Global Antibiotic Resistance Partnership-India National Working Group. 1-74.

Rapa, R. A., Labbate, M., 2013. The function of integron-associated gene cassettes in Vibrio species: The tip of the iceberg. Front. Microbiol. 4, 1–7.

Tamhankar, A.J., Karnak, S.S., Lundborg, C.S., 2018. Determinants of antibiotic consumption - Development of a model using partial least squares regresion based on data from India. Sci. Reports. 8,6421.

Walsh, Timothy R., J.W. et al, 2014. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect. Dis. 3099, 1–11.

Wilson, M.E., Chen, L.H., 2012. NDM-1 and the role of travel in its dissemination. Curr. Infect. Dis. Rep. 14, 213–226.

World Health Organization, 2017. Global Priority List of Antibiotic-Resistant Bacteria To Guide Research, Discovery, and Development of New Antibiotics. 1-7.

Yong D., Toleman M.A., Giske C.G., Cho H.S., Sundman K., Lee k., Walsh T.R., 2009. Characterization of a New Metallo-β-Lactamase Gene, blaNDM-1, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in Klebsiella pneumoniae Sequence Type 14 from India. Antimicrob. Agents Chemother. 53, 5046–5054.

Yu, Y., Lee, C., Kim, J., Hwang, S., 2005. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 89, 670–679.

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Author Contributions Statement

The study was designed and supervised by ZSA. ML and ZSA collected all the samples. ML performed all the experiments. ML, SG and RS performed the analyses. ML, ZSA, DWG and TRS analyzed the data and prepared the manuscript.

Competing financial interests: The author(s) declare no competing financial interests.

Figure Captions

Fig. 1 Relationships among fecal coliform (FC), carbapenem-resistant enteric bacteria (CRE) and *bla*$_{\text{NDM-1}}$ levels in drain, river, STP, and hospital samples collected across New Delhi.

Fig. 2 Normalized abundances of *bla*$_{\text{NDM-1}}$, *int1* and *int3* in New Delhi surface waters in the summer and winter. Hospital and STP refer to the effluents from each source, whereas Drain and River samples were collected from the drain and river banks. Standard errors are shown as error bars (n = 12 for hospitals, 20 for drains, 12 for STPs and 5 for river).

Fig. 3 Effect of ambient water temperature in the drains and Yamuna River on the normalised levels of *bla*$_{\text{NDM-1}}$ (per 16S-rRNA level).

Fig. 4 Abundance of CRE isolates, *bla*$_{\text{NDM-1}}$, *int1* and *int3* in Delhi sewer drains at the bottom of drainage catchments with hospitals (i.e., community plus hospital inputs) and without hospitals (only receiving community wastes). A hospital within a catchment increases exposure risk to CRE and *bla*$_{\text{NDM-1}}$ via surface waters, especially in the winter. Error bars indicate standard errors.

Fig. 5 Spatial distribution of seasonal CRE exposures across New Delhi. [Map was made using ArcGIS 10.1]

Fig. 6 Relative prevalence of different CRE isolates from (a) drain and (b) river samples

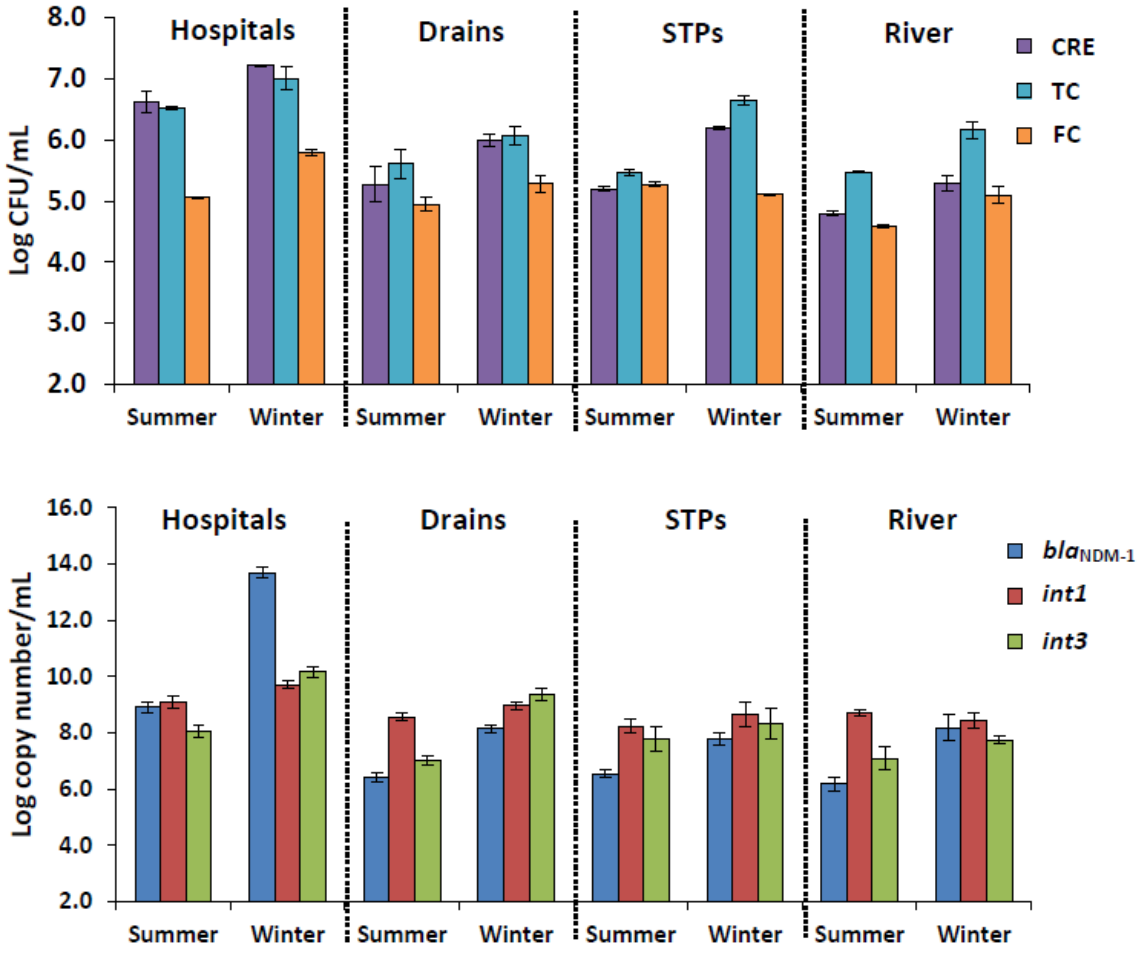
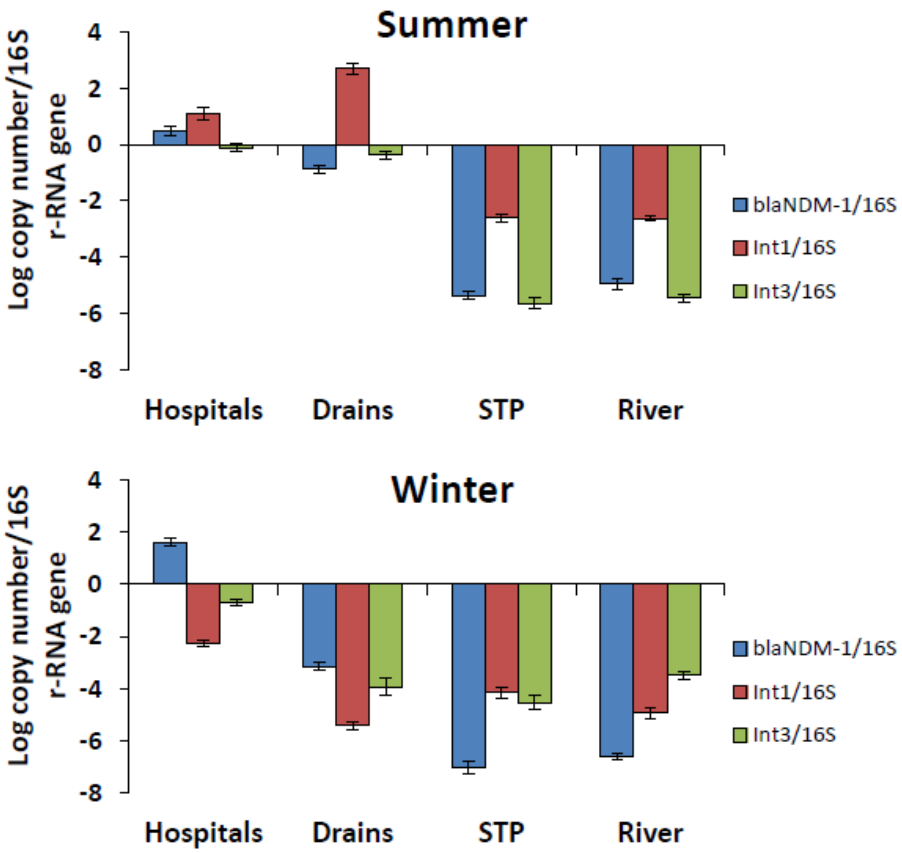


Fig. 1 Abundance of total coliform (TC), faecal coliform (FC), carbapenem resistant enteric bacteria (CRE), bla_{NDM-1}, class 1 and 3 integron gene cassettes (*int1* and *int3*, respectively) in different New Delhi surface waters in summer and winter samples. Error bars refer to standard errors (n = 12 for hospitals, 20 for drains, 12 for STPs and 5 for river).



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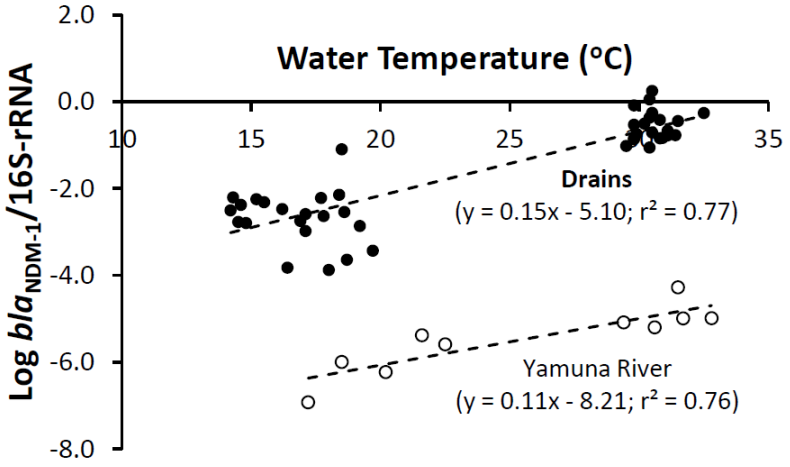

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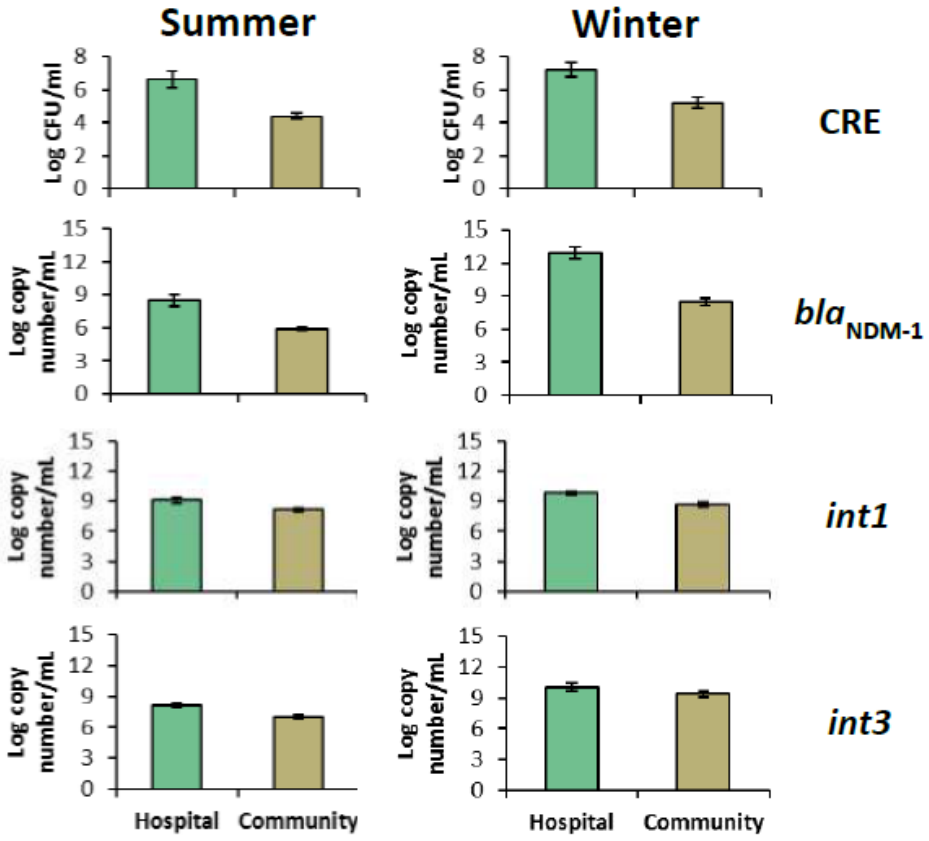

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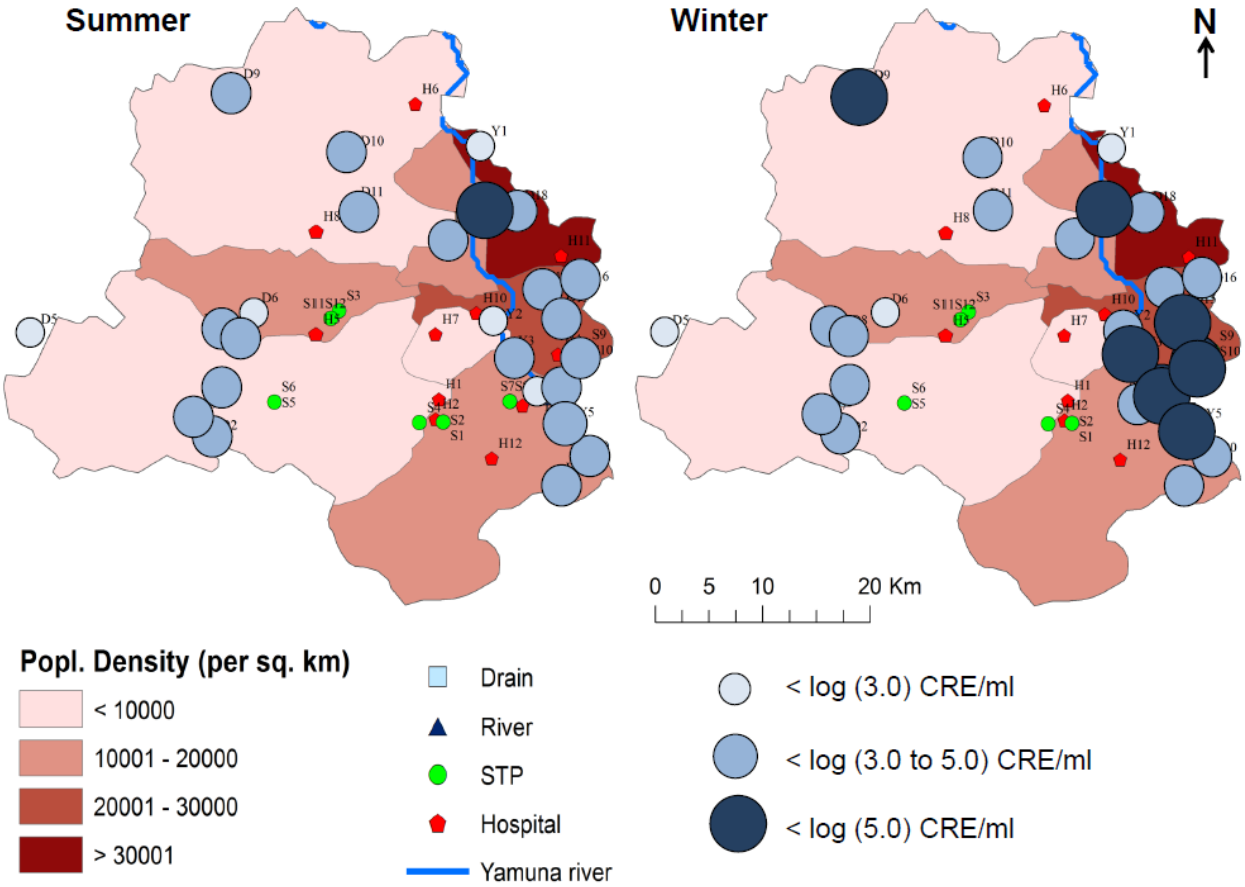

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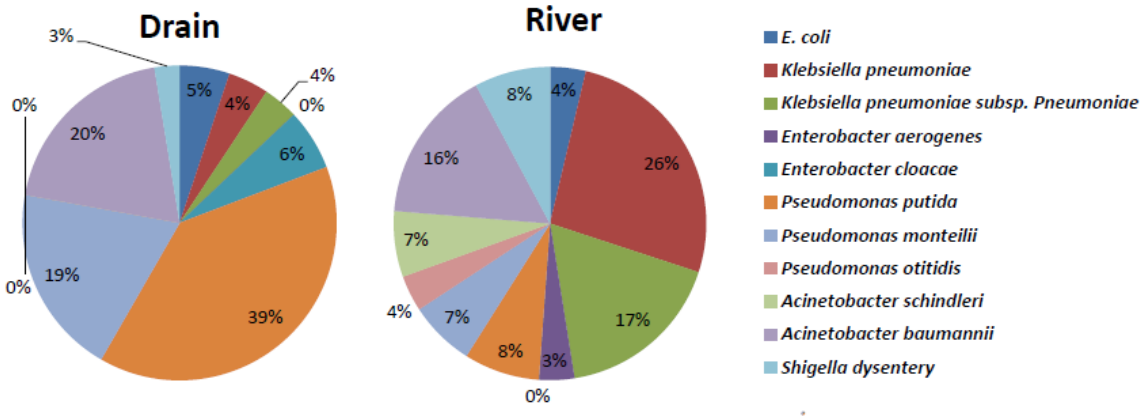

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