Lamba M, Graham DW, Ahammad SZ. [Hospital wastewater releases of carbapenem-resistance pathogens and genes in urban India](#). *Environmental Science & Technology* 2017. DOI: 10.1021/acs.est.7b03380

Copyright:

This document is the Accepted Manuscript version of a Published Work that appeared in final form in *Environmental Science & Technology*, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <https://doi.org/10.1021/acs.est.7b03380>

DOI link to article:

<https://doi.org/10.1021/acs.est.7b03380>

Date deposited:

16/10/2017

Embargo release date:

26 September 2018

This work is licensed under a [Creative Commons Attribution-NonCommercial 3.0 Unported License](#)

**Hospital wastewater releases of carbapenem-resistance pathogens and genes
in urban India**

Manisha Lamba¹, David W. Graham², & S.Z. Ahammad^{1*}

1. Department of Biochemical Engineering and Biotechnology, Indian Institute of
Technology Delhi, Hauz Khas, New Delhi-110016, India
2. School of Civil Engineering & Geosciences, Newcastle University,
Newcastle upon Tyne- NE1 7RU, 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

Increasing antibiotic resistant hospital-acquired infections and limited new antibiotic discovery are jeopardizing human health at global scales, although how hospitals themselves fuel AMR in the wider environment is largely unknown. Antibiotic resistance (AR) from hospitals in countries like India is potentially problematic because of high antibiotic use, overcrowding, and inadequate wastewater containment. Here we quantified fecal coliforms (FC), carbapenem-resistant Enterobacteriaceae (CRE), *bla*_{NDM-1}, and selected extended-spectrum β -lactam (ESBL) resistant bacteria and genes in 12 hospital wastewater outfalls and five background sewer drains across New Delhi over two seasons. Hospital wastewaters had up to nine orders of magnitude greater concentrations of CRE bacteria and *bla*_{NDM-1} than local sewers (depending on the hospital), implying hospitals contribute high concentrations of AR relative to community sources in Delhi, especially during the winter. Significant correlations were found between FC levels (a fecal indicator), and CRE ($r = 0.924$; $p = 0.005$), *bla*_{NDM-1} ($r = 0.934$, $p = 0.009$), and ESBL-resistant bacteria ($r = 0.913$, $p = 0.010$) levels across hospital wastewaters, respectively, implying elevated CRE and *bla*_{NDM-1} are of patient origin. However, of greater importance to global health, microbial culturing found 18 to 41% of wastewater CRE isolates ($n = 1447$) were on the WHO “critical pathogen” list in urgent need of new antibiotics, and 55% of CRE isolates from larger hospitals carried at least one *bla*_{NDM-1} gene. Wastewater releases from New Delhi hospitals may pose a greater AR exposure risk to residents than believed, implying in-hospital antibiotic use must be better controlled and more effective waste treatment is needed for hospital wastewaters.

Introduction

Antibiotic resistance (AR) is a global public health concern. In fact, emergence of resistant and untreatable phenotypes in hospital-acquired infections and limited new antibiotic discovery is threatening return to a pre-antibiotic era^{1,2}. Wastewater releases from hospitals contribute to this problem. Antibiotics are routinely used to treat infections, but treatment results in antibiotic residues, and resistant bacteria (ARB) and genes (ARGs) being excreted in patient wastes, entering hospital wastewater systems³. Waste-born nosocomial and commensal ARB carrying ARGs are then released to the wider environment into sewers, exposing individuals away from hospitals to AR of hospital origination. Although many gut flora die-off upon release to sewers, they can exchange ARGs to environmental strains via horizontal gene transfer (HGT) on plasmids, transposons and integrons^{4,5}. As such, hospital antibiotic use can lead to wider ARB and ARG dissemination via wastewater, becoming a potential health concern, especially in parts of world with inadequate wastewater treatment and community sanitation.

In emerging countries, such as India, this problem is particularly acute. Local domestic wastewater treatment is often not adequate, and clean, safe water is not assured⁶. As an example, only ~40% of wastewater in New Delhi is treated^{7,8}; therefore, ARB and ARGs released in hospital wastes to community sewer drains pose a particular exposure risk due to the inadequate urban infrastructure. This is especially key relative to hospitals in Delhi because antibiotic use is often high; overcrowding is common^{9,10}; and reliable internal wastewater management is often lacking. As such, hospital wastewater releases may particularly contribute to AR spread and exposures in places like India, especially to the very poor who frequently reside near open drains. For the above reasons, AR infections are more common, causing higher per patient treatment costs, prolonged morbidity, and increased mortality rates⁹⁻¹³ due to nosocomial

transmission of multidrug-resistant (MDR) organisms, including *Streptococcus pneumoniae*¹⁴, *Mycobacterium tuberculosis*¹⁵, *Salmonella* sp., *Shigella*¹⁶, and *Vibrio cholera*¹⁷. ARB and ARGs released in hospital wastes pose a huge challenge in the emerging world¹⁸ as was suggested by the World Health Organization¹⁹ and other recent reports²⁰.

India is the largest consumer of antibiotics for human use in the world¹⁰, and β -lactam antibiotics are among the most common drugs used in India, including carbapenems²¹. It is not surprising, therefore, first detection of *bla*_{NDM-1} was in a *Klebsiella pneumonia* isolate from an Indian hospital patient in 2008²². *bla*_{NDM-1} codes for a metallo-beta-lactamase (NDM-1) that can confer MDR²³. After initial clinical detection, *bla*_{NDM-1} was soon found in Delhi surface waters (in 2010), and now is present in >100 countries^{24–26}, including in strains functionally resistant to all antibiotics, including colistin^{26,27}. However, *bla*_{NDM-1} is only one example of hospital-born AR spread via wastes. Extended spectrum β -lactamase (ESBL) *Enterobacteriaceae* were detected in Lebanese hospital wastes²⁸, MDR *E. coli* in Malaysian hospital wastes (including *bla*_{NDM-1})²⁹, and in Spanish hospital wastewater bacteria³⁰ carrying class 1³¹ or class 3³² integrons

Overall, evidence show hospital wastewaters are a potentially important source of AR to the environment, especially in places like New Delhi. However, little is known about how hospital wastes impact the nature and abundance of ARB and ARGs released to the environment, especially how hospital wastewater levels compare with background levels in community sewage. To address this question, ARB/ARGs from 12 hospital waste outfalls and five sewer drains in New Delhi were quantified and characterized over two seasons. Focus here was on carbapenem-resistant *Enterobacteriaceae* (CRE), *bla*_{NDM-1}, and selected ESBL ARGs and ARBs to guide mitigation efforts in India, although results have global implications because extreme

resistant phenotypes in places like India have the potential to spread globally via international travel and other pathways.

Materials and Methods

Study sites and samples collection: Wastewater samples were collected in triplicate from sewage outfalls to community drains from 12 hospitals in New Delhi in February/March (“winter”) and May/June (“summer”) 2014. The hospitals have been anonymised for confidentiality, but size and other data are provided in Table S1 (Supporting Information; SI), which are the best available. The hospitals were chosen based upon various criteria, including: public versus private management, bed capacity, location, and medical approaches, including allopathic and unani medicine. Most of the hospitals surveyed here are general public hospitals (government) that use both conventional and allopathic approaches in treatment. In parallel to hospital wastewater sampling, replicate wastewater samples were collected from five sewer drains in New Delhi to contrast community versus hospital sources of AR to the environment. Only drains without hospitals in their catchments were sampled, including: the Najafgarh Drain (28°31’25’’N 76°54’51’’E), Palam Drain (28°32’25’’N 77°01’10’’E), Bawana Escape Drain (28°75’44’’N 77°18’28’’E), Ali Drain (28°30’32’’N 77°18’50’’E) and Sarita Vihar Drain (28°31’30’’N 77°19’12’’E).

Both hospital and drain wastewater sampling was performed in the summer and winter to assess seasonal differences, such as temperature and drug use trends in each season³³. Specific samples were collected in sterile 500-mL containers, returned to the laboratory on ice in coolers, and stored at 4°C for further analysis. Microbial culturing was performed within 12 hours of sampling. To quantify wastewater conditions, temperature, pH, total dissolved solids (TDS) and conductivity were measured in-situ using hand-held probes (detailed in Table S2).

118
119 **Microbial Culturing of coliforms and ARB:** Microbial culturing was performed to quantify
120 the abundance of fecal coliform (FC), total coliform (TC), ESBL-resistant bacteria (ESBL) and
121 CRE using selective media, and to identify putative pathogens in hospital and drain wastewaters.
122 Prior to plating, samples were serially diluted in sterile phosphate buffer solution (PBS), and then
123 were plated at different dilutions in triplicate on M-FC Agar (FC), Rapid HiColiform Agar (TC),
124 HiCrome ESBL Agar (ESBL ARB), HiCrome KPC Agar (to capture CRE ARB). The
125 HiColiform, HiCrome ESBL and HiCrome KPC plates were incubated at 37°C for 24 h, whereas
126 M-FC plates were incubated at 45°C for 24 h.

127 Phenotypically-resistant isolates from hospital wastewaters (only) were randomly selected
128 from CRE (n = 1447) and ESBL (n = 1200) plates, and DNA extracted using the FastDNA Spin
129 kit for Soil (MP Biomedicals, USA), according to manufacturer's instructions. Isolated DNA
130 was amplified using bacterial 338F and 1046R primers using conditions summarised in Table S3.
131 Conserved 16S-rRNA gene sequences (per isolate) were analyzed using Sanger sequencing and
132 identified by BLAST. Isolate identities were verified using Biochemical Test kits (Himedia,
133 India). DNA from all the isolates was amplified using NDM-1-F and NDM-1-R to verify the
134 presence or absence of *bla*_{NDM-1} in each isolate, using conditions summarized in Table S3.

135
136 **Quantification of selected ARGs and MGE genes using quantitative PCR (qPCR):** Samples
137 for DNA extraction were either directly filtered through sterile 0.22-µm membrane disc filters
138 (Pall Life Science, USA) or pelleted by centrifugation at 13,000 rpm for 30 mins. Subsequent
139 DNA extraction employed the FastDNA Spin kit for Soils (MP Biomedicals, USA). Extracted
140 DNA was stored at -20°C prior to subsequent qPCR analysis. Prior to qPCR, DNA samples were

diluted (1:100) with nuclease free water to minimise background interferences of the qPCR reactions. Four ARGs (*bla*_{TEM}, *bla*_{OXA}, *bla*_{CTX}, *bla*_{NDM-1}), the integrase gene for class one integrons (*int1*), and 16S-rRNA gene were quantified using qPCR. All reactions were performed in triplicate using the BioRad CFX C1000 System (BioRad, Hercules, CA USA). All runs were performed with positive standards and negative controls. Standards probe target DNA cloned into plasmids at different dilutions, whereas negative controls were nuclease-free water. Sample preparations for each gene and programs used for qPCRs are summarised in Table S3.

Data Analysis: Two types of statistical tests were used; bivariate correlation analysis between individual measured parameters in wastewater samples, and two-sample tests comparing differences according to hospital size and season. For analyses here, small and large hospitals were defined by estimated patient admissions. As background, many hospitals in India admit many more patients than their listed bed capacity, including hospitals within this study. Therefore, for hospital size comparisons, the 12 hospitals were sub-divided into two groups of six with “small” hospitals having admissions of between 150 to 691 patients and large hospitals having admission from 1950 to 7270 (see SI for details).

All data analyses were performed using Excel 2007 (Microsoft Office 2007, Microsoft Corp, USA) and SPSS ver. 19.0 (IBM Inc, USA). Bivariate correlation analysis was performed using the Spearman’s non-parametric method to obtain correlation coefficients (*r*) and *p*-values. Paired sample *t*-tests were used to compare hospital sizes or seasons. All data were log-transformed before analysis; statistical significance was defined by 95% confidence intervals (*p* < 0.05).

Result and Discussion

Carbapenem- and ESBL-resistant gram-negative pathogens in hospital wastewaters are a health concern in places like New Delhi due to an insufficient urban wastewater infrastructure. Although nominal wastewater flows from hospitals are small compared to the wider community (Table S4), hospitals are major points of antibiotic use and release patient wastes containing a wide range of AR strains. Therefore, knowing and quantifying bacterial and other constituents in hospital waste streams, including AR pathogens, is critical for reducing wider dissemination and impact of hospital-originated infectious agents.

Within this context, summer and winter samples were collected from wastewater outfalls of 12 hospitals across New Delhi just prior to entry into community sewage drains. TC, FC, ESBL-resistant, and CRE abundances; selected ESBL- (*bla*_{CTX}, *bla*_{OXA}, *bla*_{TEM}) and CRE-related (*bla*_{NDM-1}) ARGs; and *int1* genes (as a reflection of HGT) were quantified and sorted according to season (summarised in Tables S5 and S6) and other factors. Bivariate correlation analysis was performed on all data, and significant positive correlations were found between FC levels, and CRE ($r = 0.924$; $p = 0.005$), ESBL-resistant bacteria ($r = 0.913$, $p = 0.010$), TC ($r = 0.921$, $p = 0.008$), and *bla*_{NDM-1} gene ($r = 0.934$, $p = 0.009$) concentrations in hospital wastewaters across the city (see Figure 1). FC levels also significantly correlated with *int1* levels ($r = 0.77$, $p = 0.04$; see Figure S2), and with *bla*_{CTX}, *bla*_{OXA}, and *bla*_{TEM} abundances among samples, but *int1* did not significantly correlate with *bla*_{NDM-1} ($p = 0.25$). Overall, data show a close association between fecal bacterial levels in hospital wastewaters, and levels of CRE and ESBL-resistance isolates and genes, which are released into the environment.

To better understand AR release patterns, wastewater data were segregated for each hospital per sampling season (see Figure 2, and Tables S5 and S6). Although not always statistically

significant, FC, CRE and ESBL-resistant bacterial abundances, and *bla*_{NDM-1} and *int1* concentrations were always higher in hospital wastewaters in winter samples. Seasonal differences were greatest for *bla*_{NDM-1} abundances, which were three to nine orders of magnitude higher in the winter versus summer. Parallel hospital antibiotic use data was not available (functionally impossible to obtain in India), but seasonal differences almost certainly relate to differences in winter versus summer antibiotic use due to higher levels of infectious disease in the winter³³. Abundances of CRE also were higher in the winter, but differences between seasons were not as profound. This is possibly explained by individual phenotypes carrying multiple *bla*_{NDM-1} genes, which is common in extreme resistant phenotypes³⁴. Overall, results suggest greater exposures to *bla*_{NDM-1} exist in the winter from hospital wastes, which should be considered in public health AR risk assessments.

To further explain differences in *bla*_{NDM-1}, other factors were examined. Inspection of AR data among hospitals hinted size may play a role in disproportionately higher releases in some hospitals. Therefore, ARG and ARB hospital wastewater data were sorted according to hospital size (i.e., small = 150-691 admissions, large = 1950-7270 admissions), and larger hospitals were found to release significantly higher concentrations of FC, CRE and *bla*_{NDM-1} on a mass-per-wastewater volume basis (see Figure 3). Paired t-test analysis shows FC ($p = 0.046$), CRE phenotypes ($p = 0.009$) and *bla*_{NDM-1} ($p = 0.009$) (and also *bla*_{CTX}, $p = 0.020$; see SI) all were significantly higher in larger versus smaller hospitals. ESBL-resistant phenotypes ($p = 0.065$), *bla*_{OXA} ($p = 0.105$) and *bla*_{TEM} ($p = 0.139$) also showed increasing trends with hospital size.

A “hospital size” effect has been observed before in Sub-Saharan African hospitals, where larger hospitals contributed higher bacterial loads in their wastewaters compared with smaller hospitals³⁵, although no explanations were provided. It was initially suspected here overcrowding

in larger hospitals might explain this trend, with hospitals H1, H2, H5, H6 and H7 (all of which are large) reporting admittance rates three to four times their bed capacity (see Table S1). However, many hospitals in India over admit. Therefore, differences between these five and the other hospitals are not definitive because, if a hospital does not report overcrowding, it not does mean overcrowding does not exist. Regardless, mean *bla*_{NDM-1} levels in wastewater from H1, H2, H5, H6 and H7 were significantly higher than the other seven hospitals (i.e., 11.29 ± 2.11 versus 8.87 ± 2.15 log (copy number/mL)), implying overcrowding may be important, although more work is needed to verify this speculation.

To place hospital wastewater AR data into a relative exposure context, five complimentary urban sewer drains in catchments without hospitals (to avoid bias) were sampled to quantify and contrast AR levels in “typical” Delhi community wastes (see Tables S7 and S8 for individual drain data). Significantly higher levels of TC, FC, ESBL-resistant, and CRE strains were present in hospital versus community wastes ($p < 0.01$ based on paired t-tests) and, with one exception, all ARGs assayed were significantly higher in the hospital wastes ($p < 0.02$). Of particular note, however, is the huge difference in *bla*_{NDM-1} levels between hospital and community wastes in the winter; almost nine orders of magnitude greater. Given *bla*_{NDM-1} levels significantly correlate with 16S-rRNA gene levels in the hospital wastewaters (see Figure S3; ~2.3% of 16S-rRNA genes), data indicated particularly high levels of *bla*_{NDM-1} per cell (i.e., 16S-rRNA) are being released from hospitals, especially compared with *bla*_{NDM-1} in the community drains.

To place these ARG data into a medical context, extensive microbial culturing and isolation was performed to identify specific CRE and ESBL-resistant in the hospital wastes. In large hospital wastewaters, 953 CRE (Figure 4) and 748 ESBL-resistant (Figure S4) strains were identified, whereas 494 and 452 CRE and ESBL-resistant isolates, respectively, were identified

in the small hospitals. *Klebsiella pneumoniae* (26%), *Pseudomonas putida* (19%) and *Klebsiella pneumonia subsp. pneumonia* (17%) were the most common CRE isolates in wastes from the large hospitals, whereas *Acinetobacter baumannii* (25%) and *Klebsiella pneumonia* (14%) were the predominant CRE phenotypes in the smaller hospitals (accession numbers are provided for isolates in Table S9). In terms of ESBL-resistant isolates, *E. coli* (26%) was predominant in the larger hospitals, whereas *Pseudomonas putida* (25%) was dominant in smaller hospitals. Further genetic characterisation of the isolates showed 55% of CRE isolates from large hospitals and 27% from small hospitals carried at least one *bla*_{NDM-1} gene; i.e., DNA from each isolate was amplified (using NDM-1-F and NDM-1-R) and amplicons sequenced, and relative *bla*_{NDM-1} prevalence was verified. The most common carriers of *bla*_{NDM-1} were *Klebsiella pneumoniae*, *E. coli*, *Acinetobacter baumannii* and *Pseudomonas putida*. Although these observations are not statistical, they indicate consequential carriage of *bla*_{NDM-1} in medically-relevant strains are being released to the wider environment in hospital wastewaters.

Of particular importance from a global health perspective is the high number of CRE isolates in Delhi hospital wastewaters on the WHO pathogen list in urgent need of new therapeutic options. The WHO recently published a list of “priority pathogens” in critical need of new antibiotics³⁶, which include carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and ESBL-producing *Enterobacteriaceae*. Forty-one percent and 18% of our CRE isolates from small and large hospital wastewaters, respectively, are on the “critical” list, implying large numbers of functionally untreatable pathogens are being released to surface waters from New Delhi hospitals. Given the warm climate, such strains readily spread through the open drains, exposing millions of people to serious infectious disease.

Wider implications of these observations are not yet known. Nosocomial infections are a major cause of morbidity and mortality³⁷, and are a major challenge for public safety. Infections caused by gram-negative bacteria, such as has been detected and isolated here, are of particular concern since such microbes can be efficient in exchanging ARGs, including single genes (e.g., *bla*_{NDM-1}) that confer MDR to antibiotics. A recent report from United States highlighted that in ICUs these gram-negative bacteria are accountable for more than 70% of the nosocomial infections (predominately ventilator-associated pneumonia and urinary tract infection), and similar statistics have been reported from other parts of the world^{38,39}. In 2002, the estimated number of nosocomial infections in U.S. was about 1.7 million and the number of deaths was estimated at ~99,000. Of these, 80% of deaths were due to hospital acquired pneumonia (36%), bloodstream infection (31%) and urinary tract infections (13%)³⁷. The increasing resistance of such gram-negative bacteria towards different antibiotics further worsens the situation, especially where the emergence of new AR is outcompeting development of new antibiotics⁴⁰.

The burden of AR continues to increase and is as a major threat to the public health, especially the treatment of infectious disease⁴¹. Data herein show ARB and ARGs in hospital wastewaters in New Delhi pose a particular health risk because they include extremely MDR phenotypes, including “untreatable” pathogens. We suspect high levels are due to hospital overuse of critical antibiotics, overcrowding, and generally ineffective hospital wastewater treatment plants. As such, to reduce health risk, it is crucial more prudent hospital (and wider) antibiotic use be adopted, although installing and maintaining more effective hospital wastewater treatment systems also is needed. Treatment options that better target ARB and ARGs, including secondary and tertiary wastewater technologies, are needed as well as strategies for reducing HGT to environmental strains⁴². Characterizing hospital wastes herein is an important starting

point for interventions, but use must be reduced and waste management improved in reduce AR spread, especially in places like New Delhi without a fully developed urban wastewater infrastructure.

Supporting Information

Additional information has been provided online, including: a list of hospitals where wastewater samples were collected (Table S1); water quality conditions for samples collected from hospitals and community drains (Table S2); primers for target genes in PCR and qPCR (Table S3); design discharge levels from hospital wastewater treatment plants and community drains (Table S4); detailed abundance data on TC, FC, and ESBL-resistant and CRE bacteria in wastewater samples from the hospitals in the summer and winter (Table S5); abundances of 16S-rDNA genes, *bla*_{NDM-1}, *bla*_{CTX}, *bla*_{OXA}, *bla*_{TEM} and *int1* in hospital wastewater samples in the summer and winter (Table S6); abundances of TC, FC, and ESBL-resistant and CRE bacteria in sewer drain samples in the summer and winter (Table S7); abundances of *bla*_{NDM-1}, *bla*_{CTX}, *bla*_{OXA} and *bla*_{TEM} in sewer drain samples in the summer and winter (Table S8); and accession numbers for CRE isolates from this study (Table S9). Also included are figures displaying the following: relationships between ESBL-resistant bacteria and FC levels, and the relative abundance of *bla*_{CTX}, *bla*_{OXA} and *bla*_{TEM}, in hospital wastewaters (Figure S1); relationships between *int1* gene abundances, and FC and *bla*_{NDM-1} levels in hospital wastewaters (Figure S2); relationships between *bla*_{NDM-1} and 16S-rRNA gene levels in hospital wastewaters (Figure S3); and proportional abundances of ESBL-resistant isolates as a function of hospital size in the hospital wastewaters (Figure S4).

Authors contribution: SZA planned the experiment, SZA and ML collected the samples, ML analysed the samples, ML, SZA and DWG analysed data and prepared the manuscript.

Acknowledgements

Funding for ML and SZA was provided by Department of Science and Technology, Government of India (EASE-TEC, RP03251), whereas funding for DWG was provided by an UK EPSRC Impact Acceleration Award (EP/K503885/1).

References

- (1) Roberts, R. R.; Hota, B.; Ahmad, I.; Ii, R. D. S.; Foster, S. D.; Abbasi, F.; Schabowski, S.; Kampe, L. M.; Ciavarella, G. G.; Supino, M.; et al. Hospital and Societal Costs of Antimicrobial-Resistant Infections in a Chicago Teaching Hospital: Implications for Antibiotic Stewardship. *Clin. Infect. Dis.* **2009**, 8 (49), 1175–1184.
- (2) DL, M.; Lk, A.; Phillips, L.; FC, T.; Jr, M. J. E.; RP, G. Antimicrobial use and resistance in eight US hospitals: complexities of analysis and modeling . Intensive Care Antimicrobial Resistance Epidemiology Project and National Nosocomial Infections Surveillance System Hospitals. PubMed Commons. *Infect. Control Hosp. Epidemiol.* **1998**, 19 (6), 388–394.
- (3) Henninger, A.; Ku, K. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clin. Microbiol. Infect.* **2003**, 9 (12), 1203–1214.
- (4) Stalder, T.; Barraud, O.; Casellas, M.; Dagot, C.; Ploy, M. C. Integron involvement in environmental spread of antibiotic resistance. *Front. Microbiol.* **2012**, 3 (APR), 1–14.
- (5) Mazel, D. Integrons: agents of bacterial evolution. *Nat. Rev. Microbiol.* **2006**, 4 (8), 608–620.
- (6) Ahammad, Z. S.; Sreekrishnan, T. R.; Hands, C. L.; Knapp, C. W.; Graham, D. W. Increased waterborne bla NDM-1 resistance gene abundances associated with seasonal human pilgrimages to the upper Ganges river. *Environ. Sci. Technol.* **2014**, 48 (5), 3014–3020.
- (7) Central Pollution Control Board. Status of Sewage Treatment in India. **2005**, 1-97.

- 330 (8) Central Pollution Control Board. Performance evaluation of sewage treatment plants
331 under NRCD. **2013**, 1-128.
- 332 (9) Flaherty JP1, W. R. Nosocomial infection caused by antibiotic - resistant organisms in the
333 intensive - care unit. *Infect. Control Hosp. Epidemiol.* **1996**, *17* (4), 236–248.
- 334 (10) Public Health Foundation of India. Antibiotic Use and Resistance in India. Global
335 Antibiotic Resistance Partnership-India National Working Group. **2011**, 1-74.
- 336 (11) Mendes, R. E.; Castanheira, M.; Toleman, M. A.; Sader, H. S.; Jones, R. N.; Walsh, T. R.
337 Characterization of an integron carrying blaIMP-1 and a new aminoglycoside resistance
338 gene, aac(6')-31, and its dissemination among genetically unrelated clinical isolates in a
339 Brazilian hospital. *Antimicrob. Agents Chemother.* **2007**, *51* (7), 2611–2614.
- 340 (12) Mascini, E. M.; Bonten, M. J. M. Vancomycin-resistant enterococci : consequences for
341 therapy and infection control. *Clin. Microbiol. Infect.* **2005**, *11* (4), 43–56.
- 342 (13) Sacks, L. V; Pendle, S.; Orlovic, D.; Blumberg, L.; Constantinou, C. A Comparison of
343 Outbreak- and Nonoutbreak-Related Multidrug-Resistant Tuberculosis Among Human
344 Immunodeficiency Virus – Infected Patients in a South African Hospital. *Clin. Infect. Dis.*
345 **1999**, *29* (February), 96–101.
- 346 (14) Crewe-brown, H. H.; Karstaedt, A. S.; Saunders, G. L.; Khoosal, M.; Jones, N.; Wasas,
347 A.; Klugman, K. P. Streptococcus pneumoniae Blood Culture Isolates from Patients with
348 and without Human Immunodeficiency Virus Infection: Alterations in Penicillin
349 Susceptibilities and in Serogroups or Serotypes. *Clin. Infect. Dis.* **1997**, *25*, 1165–1172.

- (15) Wilkinson, D.; Crump, J.; Pillay, M.; Aw, S. Nosocomial transmission of tuberculosis in Africa documented by restriction fragment length polymorphism. *Trans. R. Soc. Trop. Med. Hyg.* **1997**, *91* (3), 318–319.
- (16) D.G. Pillay, J.A. Karas, A. Pillay, A. W. S. Nosocomial transmission of Shigella type 1. *J. Hosp. Infect.* **1997**, *37* (3), 199–205.
- (17) Wells, J. O. Y. G.; Hutwagner, L.; Tauxe, R. V; Mintz, E. D. First do no harm: making oral rehydration solution safer in a cholera epidemic. *Am. J. Trop. Med. Hyg.* **1999**, *60* (6), 1051–1055.
- (18) Verlicchi, P.; Galletti, a.; Petrovic, M.; BarcelÓ, D. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. *J. Hydrol.* **2010**, *389* (3-4), 416–428.
- (19) World Health Organization. WHO Global Strategy for Containment of Antimicrobial Strategy for Containment of Antimicrobial Resistance. **2001**, 1–96.
- (20) O'Neill, J. Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations. **2014**, 1-16.
- (21) Boeckel, T. P. Van; Gandra, S.; Ashok, A.; Caudron, Q.; Grenfell, B. T.; Levin, S. A.; Laxminarayan, R. Global antibiotic consumption 2000 to 2010 : an analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* **2014**, *14* (8), 742–750.
- (22) Yong D.; Toleman M. A.; Giske C. G.; Cho S. H.; Sundman K.; Lee K.; Walsh T. R. Characterization of a New Metallo- β -Lactamase Gene, blaNDM-1, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in Klebsiella

- 371 pneumoniae Sequence Type 14 from India. *Antimicrob. Agents Chemother.* **2009**, *53* (12),
372 5046–5054.
- 373 (23) Papp-Wallace, K. M.; Endimiani, A.; Taracila, M. A.; Bonomo, R. A. Carbapenems: Past,
374 Present, and Future. *Antimicrob. Agents Chemother.* **2011**, *55* (11), 4943–4960.
- 375 (24) Nordmann, P.; Naas, T.; Poirel, L.; Carbapenemases, C. A. Global Spread of
376 Carbapenemase- producing Enterobacteriaceae. *Emerg. Infect. Dis.* **2011**, *17* (10), 1791–
377 1798.
- 378 (25) Nordmann, P.; Poirel, L.; Mark A., T.; Timothy R., W. Does broad-spectrum ??-lactam
379 resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections
380 caused by Gram-negative bacteria? *J. Antimicrob. Chemother.* **2011**, *66* (4), 689–692.
- 381 (26) Kumarasamy, K. K.; Toleman, M. a; Walsh, T. R.; Bagaria, J.; Butt, F.; Balakrishnan, R.;
382 Chaudhary, U.; Doumith, M.; Giske, C. G.; Irfan, S.; et al. Emergence of a new antibiotic
383 resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and
384 epidemiological study. *Lancet Infect. Dis.* **2010**, *10* (9), 597–602.
- 385 (27) Nordmann, P.; Poirel, L.; Carrër, A.; Toleman, M. a.; Walsh, T. R. How to detect NDM-1
386 producers. *J. Clin. Microbiol.* **2011**, *49* (2), 718–721.
- 387 (28) Moubareck, C.; Daoud, Z.; Hakimé, N. I.; Hamzé, M.; Mangeney, N.; Matta, H.;
388 Mokhbat, J. E.; Rohban, R.; Sarkis, D. K.; Doucet-Populaire, F. Countrywide spread of
389 community- and hospital-acquired extended-spectrum β -lactamase (CTX-M-15)-
390 producing Enterobacteriaceae in Lebanon. *J. Clin. Microbiol.* **2005**, *43* (7), 3309–3313.

- 391 (29) Thong, K. L.; Lim, K. T.; Yasin, R.; Yeo, C. C.; Puthucheary, S. Characterization of
392 multidrug resistant ESBL-Producing *Escherichia coli* isolates from hospitals in Malaysia.
393 *J. Biomed. Biotechnol.* **2009**, Article ID 165637, 1-10.
- 394 (30) Gómez, M.; Díaz, M. T.; Araujo, M.; Sueiro, R.; Garrido, J. Waste water treatment plants
395 as redistributors of resistance genes in bacteria. *WIT Trans. Ecol. Environ.* **2010**, *135*, 83–
396 94.
- 397 (31) Oberlé, K.; Capdeville, M. J.; Berthe, T.; Budzinski, H.; Petit, F. Evidence for a complex
398 relationship between antibiotics and antibiotic-resistant *Escherichia coli*: From medical
399 center patients to a receiving environment. *Environ. Sci. Technol.* **2012**, *46*, 1859–1868.
- 400 (32) Barraud, O.; Casellas, M.; Dagot, C.; Ploy, M. C. An antibiotic-resistant class 3 integron
401 in an *Enterobacter cloacae* isolate from hospital effluent. *Clin. Microbiol. Infect.* **2013**, *19*
402 (7), 3–5.
- 403 (33) Kotwani, A.; Holloway, K. Trends in antibiotic use among outpatients in New Delhi,
404 India. *BMC Infect. Dis.* **2011**, *11*, 99.
- 405 (34) Paul, D.; Bhattacharjee, A.; Bhattacharjee, D.; Dhar, D.; Maurya, A. P.; Chakravarty, A.
406 Transcriptional analysis of *bla* NDM-1 and copy number alteration under carbapenem
407 stress. *Antimicrob. Resist. Infect. Control* **2017**, *6* (1), 26.
- 408 (35) Laffite, A.; Kilunga, P. I.; Kayembe, J. M.; Devarajan, N.; Mulaji, C. K.; Giuliani, G.;
409 Slaveykova, V. I.; Pot??, J. Hospital effluents are one of several sources of metal,
410 antibiotic resistance genes, and bacterial markers disseminated in sub-Saharan urban
411 rivers. *Front. Microbiol.* **2016**, *7* (JUL), 1–14.

- (36) World Health Organization. Global Priority List of Antibiotic-Resistant Bacteria To Guide Research, Discovery, and Development of New Antibiotics. **2017**, 1-7.
- (37) Klevens, R. M.; Edwards, J. R.; Richards Jr., C. L.; Horan, T. C.; Gaynes, R. P.; Pollock, D. A.; Cardo, D. M. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Heal. Rep* **2007**, *122* (2), 160–166.
- (38) Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. NHSN annual update : antimicrobial - resistant pathogens associated with healthcare - associated infections : annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and PubMed Commons. *Infect. Control Hosp. Epidemiol.* **2008**, *29* (11), 996–1011.
- (39) Weinstein, R. A.; Gaynes, R.; Edwards, J. R. Overview of Nosocomial Infections Caused by Gram-Negative Bacilli. *Clin. Infect. Dis.* **2005**, *41* (6), 848–854.
- (40) Davies, J.; Davies, D. Origins and Evolution of Antibiotic Resistance. *Microbiol. Mol. Biol. Rev.* **2010**, *74* (3), 417–433.
- (41) Mulvey, M. R.; Simor, A. E. Antimicrobial resistance in hospitals : How concerned should we be ? *Can. Med. Assoc. J.* **2009**, *180* (4), 408–415.
- (42) Lamba, M.; Ahammad, S. Z. Sewage treatment effluents in Delhi: A key contributor of β -lactam resistant bacteria and genes to the environment. *Chemosphere* **2017**, *188*, 249–256.

432 **Figure Legends**

433 **Figure 1** Relationships between carbapenem-resistant Enterobacteriaceae (CRE), *bla*_{NDM-1}
434 abundance, ESBL-resistant bacteria (ESBL), and total coliform bacteria (TC), and fecal coliform
435 bacterial levels in hospital wastewater outfalls from across New Delhi.

436 **Figure 2** Concentration of FC, CRE, *bla*_{NDM-1}, *int1* in wastewaters from each New Delhi
437 hospital. Data normalized to patient number. Error bars refer to standard errors.

438 **Figure 3** Abundance of faecal coliforms, CRE, *bla*_{NDM-1} and *int1* in wastewaters from large
439 (n=6) and small (n=6) hospitals in New Delhi in winter samples. *Statistically significant
440 difference (Paired t-test; CRE and *bla*_{NDM-1}, p = 0.009; Faecal Coliforms, p = 0.046). Boxes
441 represent the 25 to 75% percentile range and bars represent the standard error.

442 **Figure 4** Proportional abundance of CRE bacteria as a function of genus and hospital sizes in
443 hospital wastewaters across New Delhi. Data reported per wastewater volume basis. Small
444 hospital CRE isolates, n = 494; Large hospital CRE isolates, n = 953.

445

446

447

448

449

**Hospital wastes seed carbapenem-resistance
pathogens to Indian urban water systems**

Figure 1

Figure 2

Figure 3

Figure 4