Screening and Absolute Quantification of a β -lactamase Resistance Gene NDM-1 in Lake Sediment

Rajeev Ranjan (✉ ce16resch01004@iith.ac.in)

IITH: Indian Institute of Technology Hyderabad <https://orcid.org/0000-0001-5378-9621>

Shashidhar Thatikonda

IITH: Indian Institute of Technology Hyderabad

Research Article

Keywords: Antimicrobial resistance (AMR), NDM-1, sediment, qPCR, hierarchical clustering

Posted Date: June 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-608035/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The extensive usage of antibiotics in humans and veterinary medicine and their discharge into the aquatic environment hasten the growth, selection, and horizontal transmission of ARGs in a given bacterial community. New Delhi Metallo- β -lactamase-1 (NDM-1) is an enzyme that hydrolyzes a wide range of β -lactams antibiotics, including carbapenems. The rapid distribution of NDM-1 harboring bacteria accounts for a significant public health menace worldwide. The presence of the NDM-1 inhibited the potential of β -lactam antibiotics for treating infections caused by bacterial strains carrying such resistances, leaving minimal treatment options available. NDM-1 harboring bacteria have been detected in clinical specimens and environmental compartments where bacterial infections are ubiquitous. In this study, identification and absolute quantification of NDM-1 in sixteen lake sediment samples collected in and around Hyderabad, India, was carried out using a real-time quantitative polymerase chain reaction (qPCR) and results were expressed in gene copy number/ng (nanogram) of template DNA. 13 samples (out of 16) shown a positive signal for NDM-1 during qPCR analysis. Durgamcheru lake, Kandi lake, and Singur dam showed a negative signal for the NDM-1 during qPCR analysis among the tested samples. The remaining sampling locations tested positive with the highest gene copy number/ng of template DNA observed in the Amberpet STP (71.8). Hierarchical clustering analysis was performed to categorize the sampling location into different clusters based on pollution sources and observed results expressed in the form of a dendrogram.

1. Introduction

In present times, the medical application of antibiotics is quintessential for treating against a vast array of microbial infections (Rather et al. 2017). Abusive antibiotic usage has substantially evolved in the elicitation of antibiotic-resistant bacteria (ARB), making it a worldwide concern. World Health Organization (WHO 2004) proclaimed antimicrobial resistance (AMR) to be a public health crisis and needs managerial implication to handle with utmost caution. Antibiotic resistance represents bacteria's ability to confer resistance against antibiotic effects, for which they were sensitive previously. The bacterial species become resistant to antibiotics via genetic mutations or acquire antibiotic resistance genes (ARGs). However, the proliferation of ARG is due to the exchange of genes among different bacterial species (Von Wintersdorff et al. 2016). Mobile genetic elements (MGEs), including plasmids, transposons, integrons, and genomic islands, play a vital role in the horizontal transmission of ARGs (Gwenzi et al. 2018; Bennett, 2008). The emergence of environmental antibiotic resistance increased gravely in India (Laxminarayan and Chaudhari 2016). Lakes and rivers are regarded as presumptive reservoirs of evolving contaminants (metals, drugs, ARGs). They receive wastewaters comprising several contaminants originated from different sources (Kummerer 2004; Pote et al. 2008; Allen et al. 2010).

Effluents from the domestic wastewater and sewage, industries, hospitals, and urban/agricultural runoff signify a vital source of evolving contaminants (ARB, ARGs, metals) for the receiving environment in developing countries. Effluents released into the sewer systems, lakes, and rivers without the proper degree of treatment leading to their accumulation in the sediments (Mwanamoki et al. 2014; Spindler et

al. 2012; Devarajan et al. 2015a). Several pathogenic microorganisms, including bacteria, viruses, and protozoa, were observed in contaminated surface waters and sediments (Haller et al. 2009; Mwanamoki et al., 2014). Sediments may comprise 100–1000 times higher bacterial density than the surface water (Pote et al. 2010). A high abundance of microorganisms observed in the lake sediment due to the accumulation of heavy metals, phosphorus, nitrogen, and organic matter. It can offer a unique record of anthropogenic and natural contaminants inflows into the aquatic environments (Ip et al. 2004; Roske et al. 2012). Lake sediment and water encompass two diverse habitats, and both playing specific functions in the aquatic ecosystems. The distinct roles ascribed to lake sediment and water may be accountable for the microbial diversity in lakes (Yang et al. 2016). Analysis of microbial determinants in sediments serves as a stable index for assessing long-term water quality risks (Haller et al. 2009; Mwanamoki et al. 2014; Pote et al. 2010; Devarajan et al. 2015b; Thevenon et al. 2012). The aquatic environment is regarded as a hotspot for the attainment and distribution of ARB. It exhibits greater susceptibility for human exposure to ARB and ARGs in lakes and rivers, thereby posing an additional health hazard (Chang et al. 2011).

Antibiotics contamination in the river and lake sediments results from improper waste effluent disposal from drug manufacturing units, domestic households, wastewater treatment plants, animal husbandry units, and runoffs from agriculture (Jurado et al. 2012; Gothwal and Shashidhar, 2015). The residues of antibiotics used in aquaculture and effluent discharge from the pharmaceutical industry are often deposited in sediments. These antibiotics exert selection pressure on the sediments' flora fauna, promoting ARB growth (Kümmerer, 2009; Czekalski et al. 2014). Thus, sediment offers an opportunity to address the prevalence of ARB/ARGs, the potential impact of the resistant bacterial strains emerging from wastewaters, and its transmission to the freshwater microbial community. Hyderabad accounted for approximately one-fifth of India's pharmaceutical exports and is considered India's bulk drug capital. Pharmaceutical effluents are considered a source of severe water pollution in the surrounding areas in recent times. Besides, there is a daunting issue over the prohibition on further industrial expansion by the Indian Ministry of Environment and Forests (MoEF) due to the status of the 'critically polluted' region.

Rapid contamination of waterways and agricultural lands is considered a crucial factor for propagating ARB and ARG in the environment based on the ground inspection of southern Indian states of Andhra Pradesh and Telangana in early 2016. It connects a link between pollution contributing manufacturers and large global drug companies' trades, emphasizing, establishing, and implementing a robust environmental standard at an early stage of the logistics network chain. Hyderabad is under imminent threat from toxic industrial effluents, and active pharmaceutical ingredient (API) loaded waste disposed of in their lakes, rivers, fields, and groundwater.

NDM-1 is an enzyme that hydrolyzes a broad range of β -lactams antibiotics, including carbapenems (Khan et al. 2017). The rapid dissemination of NDM-1 harboring bacteria accounts for a significant public health menace worldwide (Berrazeg et al. 2014). The existence of the NDM-1 inhibited the potential of β -lactam antibiotics to treat infections caused by bacterial strains carrying such resistances. The NDM-1 harboring bacteria have been detected in clinical specimens (Perry et al. 2011; Islam et al. 2012 and 2014) and the aquatic environment where bacterial infections are ubiquitous. The study revealed the

presence of the NDM-1 producing bacteria in contaminated surface water or drinking water (Walsh et al. 2011; Toleman et al. 2012). The NDM-1 gene is found on self-transmittable plasmids that carry many other ARGs (Walsh and Toleman 2011). The extensive usage of antibiotics in humans and veterinary medicine and their discharge into the aquatic environment hasten the growth, selection, and horizontal transmission of ARGs in a particular bacterial community (Andersson and Hughes 2014). In recent times, India has observed a significant rise in antibiotic resistance in clinics, especially with the development of NDM-1 carrying superbug (Kumarsamy et al. 2010). The newly discovered NDM-1 enzyme exhibited resistance via hindering a more extensive array of antibiotics in the beta-lactam group (Martínez-Martínez & González-López, 2014). The study was conducted to evaluate the environmental prevalence and dissemination of the NDM-1 gene in lakes and river sediment and sludge from a sewage treatment plant (STP) surrounding Hyderabad using a real-time qPCR to understand the distribution of the NDM-1 gene among sampling locations.

2. Material And Methods

2.1 Sample location and collection

The study area extends from 17° 23' 13.7040" N and 78° 29' 30.0624" E surrounding Hyderabad. A minimum and maximum temperature of 11.60°C and 40.50°C respectively, with an average annual rainfall of 73.55 cm, were observed in the region. Hyderabad inhabits 625 square kilometres along the Musi river banks, situated on the Deccan Plateau of South India at a typical altitude of 542 meters. Sixteen sediment samples were collected in the first week of January 2019 from geographically distinct lakes and rivers around Hyderabad (Fig. 1). At each sampling location (50–200 m on both banks of lakes and river), approximately 100 gram of surface sediment (0–5 cm depth) was collected by using a sterile falcon tube as sub-samples from various points with low current speeds to draw the fine-grained sediment (Zhang et al. 2019). Samples were transferred to the laboratory in an ice-packed container and kept at 4°C for DNA isolation after collection.

2.2 Isolation of DNA from sediment samples

Subsequent isolation of DNA from lake sediment achieved within 48 hrs of sample collection using the soil DNA isolation kit–NucleoSpin® by Macherey-Nagel GmbH & Co. K.G. and stored at -20°C for qPCR assay. The study employed Eon™ Microplate Spectrophotometer (BioTek Instruments, Inc., USA) to estimate the purity and concentration of extracted DNA. Typically, the ratio of A_{260}/A_{280} values varying between 1.8–2.0 reflects the purity of extracted DNA.

2.3 Primer Design:

The primer used in the analysis was designed using reference nucleotide sequences for NDM-1 in GenBank under accession number FN396876.1 (*Klebsiella pneumoniae* plasmid pKpANDM-1 sequence carrying new Metallo-beta-lactamase gene blaNDM-1, isolate KP-05-506). Primer-BLAST was performed to design specific primer pairs on the target sequence and then commercially synthesized by Eurofins

Genomics India Pvt Ltd. The designed primer sequence comprises of forward primer sequence 5'-GTACTGGCGTAACCCTTCACA – 3' and the reverse primer sequence 5'- CATTTCATGGCGGGCAGGATAA – 3' for amplifying a 121 base pair sequence during qPCR. Primer-BLAST was employed to verify the designed primer's selectivity on the National Center for Biotechnology Information (NCBI) database (<http://www.ncbi.nlm.nih.gov>). BLASTN analysis of designed primers for a real-time qPCR assay exhibited a 100% homogeneity in-silico with the NDM-1 encoding gene.

2.4 Real-time qPCR assay

Assays involving a real-time qPCR optimization conducted using a real-time qPCR system CFX-96, Bio-rad Laboratories (India) Private Limited using SYBR green chemistry. Protocols followed as per T.B. Green™ Premix Ex Taq™ (Tli RNaseH Plus) purchased from DSS Takara Bio India Private Ltd. Undiluted 1 µL (microliter) final DNA extracts used in triplicate during the experiment. All reactions carried out for analysis comprising a reaction mixture of 10 µL per well prepared via different components in definite proportion. The final volume of reaction mixture comprises 4.2 µL of template DNA, 21 µL of Sybrgreen Master mix, 0.8 µL of 10 µM (micromolar) Forward primer, 0.8 µL of 10 µM Reverse primer, and 15.1 µL of PCR Grade Water. The plate setup for each reaction comprises of an unknown sample in triplicate, a negative control (N.C.) and a no template control (NTC) in duplicate and five known standards of NDM-1 [100 pg (pictogram)/µL, 10 pg/µL, 1 pg/µL, 100 fg (femtogram)/µL and 10 fg/µL). The optimal cycling protocol consists of one initial cycle of denaturation and polymerase activation step at 95°C for 30 secs. It was further, followed by 40 cycles of denaturation at 95°C for 5 secs, 40 cycles of annealing at 60°C for 30 secs, strand extension at 72°C for 30 secs and 1 cycle of melt curve from 65–95°C, 0.2°C increments at 10 secs/step. The standards of NDM-1 were synthesized commercially by Bioartis Life Sciences Private Limited, Hyderabad, using known quantities of cloned target genes.

2.5 Hierarchical clustering and Statistical analyses

Hierarchical clustering was conducted using the ward's linkage method followed by Euclidean distance measurements to group sampling locations in clusters based on their similarity. Shapiro-Wilk test was performed using R software to correlate the NDM-1 gene copies distribution among sampling locations (Chen et al. 2019).

3. Results

3.1 Amplification and Absolute quantification of the NDM-I gene

The system evaluated several lakes and river sediment with possible NDM-1 bearing strains from 16 different geographic origins and further quantified using a real-time qPCR system CFX-96, Bio-Rad. Amplification and quantification were performed by running the unknown sample in triplicate with the known standards of NDM-1 and results expressed in gene copy number/ng of template DNA. Data analysis and gene copy number calculation was accomplished using CFX maestro software provided along with the real-time qPCR system CFX-96 thermal cycler, and results summarized in Table 1. In this study, thirteen samples were detected positive for the NDM-1 gene among the collected samples, whereas

three samples tested negative during qPCR analysis. The Cq values of analyzed samples were found to be varying between 30 to 35 for positive amplification of the NDM-1 gene. The cut-off cycle threshold value was determined as 35 for positive amplification, and the optimal number of amplification cycles fixed as 40 for the qPCR assay. The signal obtained above 35 was considered non-specific for any test sample. Repeated runs in triplicates with the standards of the NDM-1 gene shown a significant coefficient of correlation (r^2) value varying between 0.92 to 1 and reaction efficiency between 90.4 to 109.9 %. The standard curve technique was used to evaluate the number of gene copies of the NDM-1 gene/ nanogram of template DNA in test samples during qPCR analysis.

Table 1
Absolute quantification of NDM-1 in sediment DNA collected from different lakes, dam, and STP surrounding Hyderabad

S.No.	Sampling locations	Quantification Cycle (Cq)	Coefficient of Determination (r^2)	Efficiency (%)	Gene copy number/ng of template DNA
S1	Alwal lake	32.43 ± 0.33	0.979	90.4	62.41
S2	Amberpet STP	34.17 ± 0.088	0.981	98	71.08
S3	Ameenpur lake	30.80 ± 0.449	0.969	92.6	44.25
S4	Durgamcheru lake	-	-	-	-
S5	Fox Sagar lake	34.21 ± 0.240	0.953	109.9	16.80
S6	Himayat Sagar lake	31.30 ± 0.986	0.940	108.8	4.00
S7	Hussain Sagar lake	32.26 ± 0.399	0.984	109.9	4.30
S8	Kandi lake	-	-	-	-
S9	Downstream of Manjeera dam	30.36 ± 0.193	0.994	90.6	46.02
S10	Mir alam tank lake	31.17 ± 0.516	0.994	93.3	11.90
S11	Mominpet lake	31.69 ± 0.500	0.945	105.3	4.15
S12	Nagole lake	32.20 ± 0.016	1.000	97.6	22.19
S13	Osman Sagar lake	30.96 ± 0.199	0.999	100.4	66.57
S14	Safilguda lake	30.36 ± 0.176	0.998	90.4	67.58
S15	Saroor Sagar lake	33.65 ± 0.171	0.921	107.5	3.34
S16	Downstream of Singur dam	-	-	-	-

3.2 Hierarchical Clustering Analysis

The analysis was performed to categorize samples based on their pollution sources. It divided data into four clusters, involving Ward's linkage method followed by Euclidean distance measurements. The clusters obtained in the dendrogram by cluster analysis (Fig. 2) employ sixteen characteristic peaks considered clustering variables, referring to lake sediment samples (S1-S16) from different geographically distinct water bodies. The dendrogram implemented a single-linkage rescaled distance cluster. There were four clusters referred to as A, B, C, and D involving sixteen common constituents. The rescaled distance cluster performed based on pollution sources impacting NDM-1 in the dendrogram (Fig. 2) identified Cluster A's sediment samples comprised of water bodies used as reservoirs for drinking water supply. It may be noted that both the Singur dam (S16) and Manjeera dam (S9) were built on the river Manjeera and used for irrigation, hydropower generation, and drinking water supply to Hyderabad city. Cluster B comprises sub-clusters involving Himayat Sagar (S6) and Osman Sagar (S13) as both of these lakes lie closer and parallel to one another.

Both Osman Sagar and Himayat Sagar were constructed on the Musi River to provide a supplementary drinking water supply for Hyderabad and protect against a flood. The rapid population growth, use of fertilizers in agriculture, and various anthropogenic activities severely deteriorated the lake's water quality. Thus, S6 and S13 sediment samples showed eroded soil from soil erosion and runoffs from agricultural activities. Cluster C comprised of water bodies polluted due to domestic sewage and industrial effluents discharge, including Amberpet STP (S2), Fox Sagar lake (S5), Hussain Sagar lake (S7), Mir Alam tank (S10), Nagole lake (S12), Safilguda lake (S14), and Saroor Sagar lake (S15). Amberpet STP (S2) is the largest STP in the country based on Up-flow Anaerobic Sludge Blanket (UASB) process. It receives the combined discharge from domestic sewage and industrial effluents. Fox Sagar lake (S5) is supposed to be the second most polluted lake in Hyderabad. Due to the discharge of pollutants from nearby industrial and residential areas, a drastic deterioration in the lake's water quality is observed.

Hussain Sagar lake (S7) is regarded as the largest lake in the vicinity. It got severely contaminated due to the constant discharge of domestic sewage and untreated industrial effluents for several years. A considerable increase in contamination level was observed for past decades, leading to the accumulation of pollutants in lake sediments that cause a potential threat of contributing pollution to the surrounding groundwater. Mir Alam Tank lake (S10) is located to the south of the Musi river, constructed mainly to fulfill the water demand of Hyderabad and its industrial areas. Nagole lake (S12) is located east of Hyderabad city on the inner ring road and the Northern bank of the Musi river. It receives much sewage inflows from the nearby city, industrial areas, and Hussian Sagar lake. Safilguda lake (S14) is located in a residential suburb in Hyderabad. The lake's water quality declined due to agricultural pesticides' inflow and untreated industrial and domestic sewage discharge. Saroor Sagar lake (S15) is one of the significant water bodies in Hyderabad and is constructed for agricultural and drinking purposes. The inflow of solid waste, domestic sewage, and untreated industrial effluents into the lake's catchment area leads to a severe accumulation of pollutants.

Cluster D comprised of water bodies polluted due to domestic sewage and wastewater discharge, including Alwal lake (S1), Ameenpur lake (S3), Durgamcheru lake (S3), Kandi lake (S8), and Mominpet

lake (S11). All Lake (S1) is an artificial lake that lies in the close-by region of Hyderabad that is polluted due to garbage dumping and domestic sewage discharge from surrounding areas. Ameenpur Lake (S3) is an artificial lake located on the north-western fringes of Hyderabad. The lake lies amid an urban sprawl, surrounded by villages and modern apartments, and receives domestic sewage discharge from the surrounding areas. Durgamcheru lake (S3) is referred to as a secret lake, has already been recorded with a low biological oxygen demand due to effluent discharge from commercial complexes and residential buildings. Cluster A and B lie near each other, as observed from the dendrogram. Cluster C and D lie near each other but away from clusters A and B. The clustering patterns appeared consistent from clustering analysis between water bodies based on observed data and the pollution sources noted from the dendrogram.

3.3 Statistical analyses

Shapiro-Wilk test was performed using R software to correlate NDM-1 gene copies distribution among sampling locations. The variation in the distribution of gene copies number of NDM-1 gene among sampling locations is big enough to be statistically significant. ($\alpha = 0.05$, $p\text{-value} = 0.022$). Since $p\text{-value} < \alpha$, it is assumed that the gene copies number is not normally distributed (Fig. 3).

4. Discussion

Among tested sediment samples, NDM-1 genes were observed in thirteen samples whereas, three samples, Durgamcheru lake (S4), Kandi lake (S8), and downstream of Singur dam (S16), were tested negative to NDM-1. Among the positive tested sediment samples, the highest gene copy number was detected in Amberpet STP sludge (71.08). The combined discharge of domestic sewage and industrial effluents contributes to a significant pollution level in this sampling location. STPs are considered an abundant source of ARGs and a hotspot for their release into the environment (LaPara et al. 2011; Chen et al. 2016). The increasing effluent disposal is, in fact, due to Hyderabad's position as leading bulk drug producers (40% of the national production) and constituting about 50% of exporting of drugs as well, thus making it the hub for pharmaceutical companies (Lübbert et al. 2017). The observed gene copy number from Amberpet STP demarcates that ever since the Patancheru–Amberpet pipeline's induction, there is a gradual deterioration of the local river's quality. Besides, they also serve as carriers of ARGs flowing through almost 100 villages from its drainage basin, making it more pernicious to public health (Lübbert et al. 2017). A significant variation in the distribution of the gene copies number of NDM-1 gene was observed among sampling locations.

A low to high variation in the distribution of the NDM-1 gene in lake sediment among sampling locations might be depending on several factors, including microbial diversity, season and conductivity, pH, metals, moisture (Eramo et al. 2020). Other factors that could be vital for hastening the NDM-1 sediment loading include the concentrations of absorbed antibiotics, nutrients exchange rate, sediment age, and selection factors in the sediment's mobile bed (Eramo et al. 2020). Sampling locations (S4, S8, S16) were tested negative for NDM-1 due to the absence of the beta-lactamase gene. In contrast, sampling locations (S6,

S7, S11) exhibited a low gene copy number due to a small concentration of the beta-lactamase gene (Fig. 4).

It may be because ARG annotations are primarily governed by amphenicol, macrolide, tetracycline, sulfonamide, and fluoroquinolone resistance genes in an anthropogenically impacted lake and river sediment (Eramo et al. 2020). In Hyderabad, several rivers and lake water were recorded with a broader range of pollutants. These environmental pollutants elicit antimicrobial resistance in microorganisms. It is attributed primarily to the rapid increase in antibiotic usage identified in aquaculture and intensive farming activities besides pharmaceutical treatment and wastewater treatment plants (Bengtsson-Palme et al. 2014). The increasing ARG prevalence observed among Indian sediment samples derived from reference sites; local drug manufacturers probably reflect that emissions of a higher degree of antibiotics in these regions are more likely attributed to ARG dissemination in nearby environments (Flach et al. 2015). In the present study, qPCR analysis of sediment samples from different sampling locations revealed the presence of the NDM-1 gene from lakes and rivers, indicating augmentation of resistant bacterial strains in the effluent-laden environment. The finding obtained was supported by the investigation conducted by Lübbert et al. 2017, which revealed there is a rapid distribution of β -lactamase and carbapenems producing pathogens among 24 different sampling locations in Hyderabad. The abundance of ARGs in drinking water supplies and reservoirs has posed significant public health concerns in recent times. However, reports on resistant genes involving TEM and SHV were investigated in drinking water samples (Xi et al. 2009).

The conducted work provides the first insight into the NDM-I gene's occurrence in the reservoir used as a drinking water supply (Manjeera dam). The prevalence of ARG in drinking water sediments is due to conventional water treatment such as sedimentation and sand filtration (Pei et al. 2007). Pollutants from industrial effluent discharge traced in nearby lake sediments exhibited a more significant impact as the observed copy number of NDM-1 in the Safilguda lake and Nagole lake was significantly higher. Similar results exhibited in the study conducted by Bielen et al. 2017 showed effluent discharge from two manufacturing sides comprised of higher concentration ranges of antibiotics resulting in a more significant proportion of culturable ARB in the aquatic environment. Due to technological advancement, industrialization, followed by urbanization, is essential for sustainability in today's world. Despite its significance, excessive usage has presently been at stake due to environmental complications. For instance, the aquatic environment, which remains the essential life support for floral and faunal sustenance, devastated due to human-made pollution caused by domestic, agricultural, and industrial activities (Rashmi et al. 2017)

5. Conclusion

The study demonstrated a high precision of a real-time qPCR analysis for detecting the NDM-1 gene in river/ lake sediments. Durgamcheru lake, Kandi lake, and Singur dam showed a negative signal for the NDM-1 during qPCR analysis among the tested samples. The highest gene copy number/ng of template DNA observed in the Amberpet STP (71.8). The proliferation of the NDM-1 gene among disease-causing

bacteria led to the emergence of multi-drug resistant genes resistant in all current-generation antibiotics. NDM-1 producers in samples collected from water bodies have significant inferences for people residing in the city and surrounding area dependent on public water supply and sanitation amenities. The occurrence and rapid dissemination of carbapenems resistance caused by the NDM-1 gene worldwide monitored gravely to circumvent any risk of an epidemic. Therefore, early identification of the NDM-1 gene harboring pathogens and preventing their spread must be performed in any region.

Strength and limitations

The present analysis's quality is a broad array of sampling locations, specific representation of locations, and the usage of sophisticated techniques of real-time qPCR. There is the presence of billions of bacteria in each collected sediment sample. The qPCR used to evaluate the existence of the NDM-1 in the sediment samples, a positive outcome for almost every gene in any sample comprising sewage is anticipated.

Declarations

Conflicts of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Funding

The authors thank the University Grant Commission (UGC), New Delhi, for providing financial assistance and IIT Hyderabad for providing lab facilities for the smooth conduct of this work.

Data Availability Statement

All data generated or used during the study appear in the submitted article.

References

Rather, I. A., Kim, B. C., Bajpai, V. K., & Park, Y. H. (2017). Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. *Saudi journal of biological sciences*, 24(4), 808-812. World Health Organization. (2004). *The World health report: 2004: changing history*. World Health Organization. Von Wintersdorff, C. J., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., & Wolffs, P. F. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in microbiology*, 7, 173. Gwenzi, W., Musiyiwa, K., & Mangori, L. (2018). Sources, behavior, and health risks of antimicrobial resistance genes in wastewaters: A hotspot reservoir. *Journal*

of Environmental Chemical Engineering, 102220. Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British journal of pharmacology*, 153(S1), S347-S357. Laxminarayan, R., & Chaudhury, R. R. (2016). Antibiotic resistance in India: drivers and opportunities for action. *PLOS Medicine*, 13(3). Kummerer K. (2004). Resistance in the environment. *J Antimicrob Chemother.* 54(2):311–20. Pote J, Haller L, Loizeau JL, Bravo AG, Sastre V, Wildi W. (2008). Effects of a sewage treatment plant outlet pipe extension on the distribution of contaminants in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. *Bioresource Technol.* 2008; 99(15):7122–31. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology.* 8(4):251–9. Mwanamoki PM, Devarajan N, Thevenon F, Atibu EK, Tshibanda JB, Ngelinkoto P, et al. (2014). Assessment of pathogenic bacteria in water and sediment from a water reservoir under tropical conditions (Lake MaVallee), Kinshasa Democratic Republic of Congo. *Environmental monitoring and assessment* 186(10):6821–30. Spindler A, Otton LM, Fuentesfria DB, Corcao G. (2012). Beta-lactams resistance and presence of class 1 integron in *Pseudomonas* spp. isolated from untreated hospital effluents in Brazil. *Antonie van Leeuwenhoek.* 102(1):73–81. Devarajan N, Laffite A, Ngelikoto P, Elongo V, Prabakar K, Mubedi JI, et al. (2015a). Hospital and urban effluent waters as a source of accumulation of toxic metals in the sediment receiving system of the Cauvery River, Tiruchirappalli, Tamil Nadu, India. *Environmental science and pollution research* 22: 12941-12950. Haller L, Pote J, Loizeau JL, Wildi W. (2009). Distribution and survival of fecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. *Ecol Indic.* 9(3):540–7. Pote J, Bravo AG, Mavingui P, Ariztegui D, Wildi W. (2010). Evaluation of quantitative recovery of bacterial cells and DNA from different lake sediments by Nycodenz density gradient centrifugation. *Ecol Indic.* 10(2):234–40. Ip CCM, Li XD, Zhang GS, Farmer JG, Wai OWH, Li Y.S. (2004). Over one hundred years of trace metal fluxes in the sediments of the Pearl River Estuary, South China. *Environ Pollut.* 132(1):157–172. Roske K, Sachse R, Scheerer C, Roske I. (2012). Microbial diversity and composition of the sediment in the drinking water reservoir Saidenbach (Saxonia, Germany). *Syst Appl Microbiol.* 35:35–44. Yang J, Jiang H C, W, G, Liu W, Zhang GJ. (2016). Distinct factors shape aquatic and sedimentary microbial community structures in the lakes of Western China. *Front Microbiol.* 7:1782. Devarajan N, Laffite A, Graham ND, Meijer M, Prabakar K, Mubedi JI, et al. (2015b). Accumulation of clinically relevant antibiotic-resistance genes, bacterial load, and metals in freshwater lake sediments in central Europe. *Environ Sci Technol.* 49(11):6528–37. Thevenon F, Adatte T, Wildi W, Pote J. (2012). Antibiotic-resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). *Chemosphere.* 86(5):468–76. Chang, H., Wan, Y., Wu, S., Fan, Z., & Hu, J. (2011). Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. *Water Research*, 45(2), 732-740. Jurado, A.; Vázquez-Suñé, E.; Carrera, J.; López de Alda, M.; Pujades, E.; Barceló, D. (2012). Emerging Organic Contaminants in Groundwater in Spain: A Review of Sources, Recent Occurrence, and Fate in a European Context. *Sci. Total Environ.*, 440, 82–94. Gothwal, R., & Shashidhar, T. (2015). Antibiotic pollution in the environment: a review. *Clean–Soil, Air, Water*, 43(4), 479-489. Kümmerer, K. (2009). The presence of pharmaceuticals in the environment due to human use—present knowledge and future challenges. *Journal of environmental management*, 90(8), 2354-2366. Czekalski, N., Díez, E. G., &

Bürgmann, H. (2014). Wastewater as a point source of antibiotic-resistance genes in the sediment of a freshwater lake. *The ISME Journal*, 8(7), 1381-1390.

Khan, A.U., Maryam, L. & Zarrilli, R. (2017). Structure, Genetics, and Worldwide Spread of New Delhi Metallo- β -lactamase (NDM): a threat to public health. *BMC Microbiol* 17, 101.

Berrazeg M, Diene S, Medjahed L, Parola P, Drissi M, Raoult D, Rolain J. (2014). New Delhi Metallo-beta-lactamase around the world: an eReview using Google Maps. *Euro Surveill* 19: pii=20809.

Perry JD, Naqvi SH, Mirza IA, Alizai SA, Hussain A, Ghirardi S, Orenga S, Wilkinson K, Woodford N, Zhang J, Livermore DM, Abbasi SA, Raza MW. (2011). Prevalence of fecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. *J Antimicrob Chemother* 66:2288 –2294.

Islam MA, Talukdar PK, Hoque A, Huq M, Nabi A, Ahmed D, Talukder KA, Pietroni MA, Hays JP, Cravioto A, Endtz HP. (2012). Emergence of multidrug-resistant NDM-1-producing Gram-negative bacteria in Bangladesh. *Eur J Clin Microbiol Infect Dis* 31:2593–2600.

Islam MA, Nabi A, Rahman M, Islam M, Ahmed D, Faruque AS, Hossain A, van Belkum A, Endtz HP. (2014). Prevalence of fecal carriage of NDM-1- producing bacteria among patients with diarrhoea in Bangladesh. *J Med Microbiol* 63:620–622.

Walsh TR, Weeks J, Livermore DM, Toleman MA. (2011). 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* 11:355–362.

Toleman MA, Bugert JJ, Nizam SA. (2012). Extensively drug-resistant New Delhi Metallo- β -lactamase-encoding bacteria in the environment, Dhaka, Bangladesh. *Emerg Infect Dis* 21:1027–1030.

Walsh TR, Toleman MA. (2011). The new medical challenge: why NDM-1? Why Indian? *Expert Rev Anti Infect Ther* 9:137–141.

Andersson DI, Hughes D. (2014). Microbiological effects of sub-lethal levels of antibiotics. *Nat Rev Microbiol* 12:465– 478.

Kumarasamy, KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the U.K.: a molecular, biological, and epidemiological study. *Lancet Infect Dis*. 10:597–602.

Martínez-Martínez, L., & González-López, J. J. (2014). Carbapenemases in Enterobacteriaceae: types and molecular epidemiology. *Enfermedades infecciosas y microbiología clinica*, 32, 4-9.

Zhang L, Zhao T, Wang Q, Li L, Shen T, Gao G. (2019). Bacterial community composition in aquatic and sediment samples with spatiotemporal dynamics in large, shallow, eutrophic Lake Chaohu, China. *Journal of Freshwater Ecology*, 34:1, 575-589.

Chen Y, Su Jian-Qiang, Zhang J et al. (2019). High-throughput profiling of antibiotic resistance gene dynamic in a drinking water river-reservoir system. *Water Research* 149, 179-189.

Lara TM, Burch TR, 615 McNamar PJ, Tan DT, Yan M, Eichmiller JJ. (2011). Tertiary-Treated Municipal Wastewater is a Significant Point Source of Antibiotic Resistance Genes into Duluth-Superior Harbor. *Environ Sci Technol* 45: 9543-9549.

Chen QL, An XL, Li H, Su JQ, Ma YB, Zhu YG. (2016). Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. *Environ Int* 92: 1-10.

Lübbert, C., Baars, C., Dayakar, A., Lippmann, N., Rodloff, A. C., Kinzig, M., & Sörgel, F. (2017). Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with the dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens. *Infection*, 45(4), 479–491.

Eramo, A.; Medina, W. R. M.; Fahrenfeld, N. L. (2020). Factors associated with elevated levels of antibiotic resistance genes in sewer sediments and wastewater. *Environ. Sci.: Water Res. Technol.*, 6, 1697-1710.

Bengtsson-Palme J., Fredrik B., Jerker F., Erik K., and D. G. Joakim L. (2014). Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a

polluted lake in India. *Front Microbiol.*, 2014; 5: 648. Flach, C. F., Johnning, A., Nilsson, I., Smalla, K., Kristiansson, E., and Larsson, D. G. (2015). Isolation of novel Inc A/C and Inc N fluoroquinolone resistance plasmids from an antibiotic-polluted lake. *J. Antimicrob. Chemother.* 70, 2709–2717. Xu, C., Zhang, Y., Marrs, C. F., Ye, W., Simon, C., Foxman, B., & Nriagu, J. (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. *Appl. Environ. Microbiol.*, 75(17), 5714-5718. Pei, R., J. Cha, K. H. Carlson, and A. Pruden (2007). Response of antibiotic resistance genes (ARG) to biological treatment in dairy lagoon water. *Environ. Sci. Technol.* 41:5108-5113. Bielen A., Šimatović A., Kosić-Vukšić J., Senta I., Ahel M., Babić S., Jurina T., González Plaza J.J., Milaković M., Udiković-Kolić N. (2017). Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries. *Water Res.*, 126:79–87. Rashmi H. B., S.K. Bharti, Moloya G., Sucharitha D., Anita & Subha G. (2017). Antibiotic Resistance: Role of Fruits and Vegetables in the Food Basket. *Int. J. Pure App. Biosci.*, 5 (4): 169-173.

Figures

Figure 1

Sampling sites surrounding Hyderabad city, India

Figure 2

Dendrogram obtained by cluster analysis

Figure 3

Gene copy number distribution among sediment samples

Figure 4

Quantile-Quantile (Q-Q) plot showing distribution of the NDM-1 gene copies among sampling locations