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RESEARCH PAPER

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

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Abstract
New Delhi Metallo-β-lactamase-1(NDM-1) is an enzyme that hydrolyzes a wide range of β-lactams antibiotics, including carbapenems. The presence of the NDM-1 inhibits the potential of β–lactam antibiotics in treating infections caused by bacterial strains carrying such resistances, thus leaving minimal treatment options available. Due to this, the rapid distribution of NDM-1 harboring bacteria accounts for a significant public health menace worldwide. These 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. Thirteen samples (out of sixteen) displayed a positive signal for NDM-1 during the qPCR analysis with the highest gene copy number/ng of template DNA (71.8) being observed in the Amberpet STP. Three samples, samples those from Durgamcheru lake, Kandi lake, and Singur dam, were negative for the NDM-1 during the qPCR analysis. Hierarchical clustering analysis was performed to categorize the sampling location into different clusters based on pollution sources and the observed results were expressed in the form of a dendrogram.

Keywords: Antibiotics resistance gene (ARG), NDM-1, Sediment, qPCR, Hierarchical clustering

INTRODUCTION

In present times, the medical application of antibiotics is quintessential for treating against a vast array of microbial infections (Rather et al., 2017). Substantial abusive antibiotic usage has resulted in the evolution of antibiotic-resistant bacteria (ARB), thus making it a worldwide concern. Antibiotic resistance represents a 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 by acquiring 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 gravity of the situation can be seen from the fact that, in 2004 itself, the World Health Organization (WHO) proclaimed antimicrobial resistance (AMR) to be a public health crisis that needed to be handled with utmost caution.

It has been found that the environmental antibiotic resistance has increased gravely in

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India (Laxminarayan & Chaudhari, 2016). Lakes and rivers are being regarded as presumptive reservoirs of evolving contaminants (metals, drugs, ARGs) as they receive wastewaters comprising several contaminants that originate from different sources (Kummerer, 2004; Pote et al., 2008; Allen et al., 2010). In developing countries especially effluents from domestic wastewater and sewage, industries, hospitals, and urban/agricultural runoff signify a vital source of evolving contaminants (ARB, ARGs, metals) for the receiving environment. Effluents are released into the sewer systems, lakes, and rivers without the requisite degree of treatment, thus resulting in 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, have been observed in contaminated surface waters and sediments (Haller et al., 2009; Mwanamoki et al., 2014). It has been observed that sediments may comprise 100–1000 times higher bacterial density than surface water (Pote et al., 2010). A high abundance of microorganisms has also been observed in lake sediments due to the accumulation of heavy metals, phosphorus, nitrogen, and organic matter. Lake 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 are two diverse habitats, with 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 by the drug manufacturing units, domestic households, wastewater treatment plants, animal husbandry units, and runoffs from agriculture (Jurado et al., 2012; Gothwal & Shashidhar, 2015). The residues of antibiotics used in aquaculture and the discharge of effluents from the pharmaceutical industry are often deposited as sediments in the rivers and lakes. These antibiotics and effluents exert selection pressure on the sediments' flora and fauna, resulting in ARB growth (Kümmerer, 2009; Czekalski et al., 2014). Thus, the study of the sediments offers an opportunity to address the existence of ARB/ARGs, the potential impact of the resistant bacterial strains emerging from wastewaters, and its transmission to the freshwater microbial community. Hyderabad accounts 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, especially in recent times. Rapid contamination of waterways and agricultural lands was found to be 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 establishes a link between pollution contributing manufacturers and large global drug companies' trades, and indicates the urgency for 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 its 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). NDM-1 harboring bacteria have been detected in clinical specimens (Perry et al., 2011; Islam et al. 2012 & 2014) and aquatic environments where bacterial infections are ubiquitous. The study conducted by Walsh et al., 2011 and Toleman et al., 2012 revealed the presence of the NDM-1 producing bacteria in contaminated surface water and drinking water. The NDM-1 gene is

found on self-transmittable plasmids that carry many other ARGs (Walsh & 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 & 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) in and around Hyderabad using a real-time qPCR to understand the distribution of the NDM-1 gene among sampling locations.

MATERIAL AND METHODS

The DMS coordinates of the study area (Hyderabad, Telangana, India) are 17° 23' 13.7040" N and 78° 29' 30.0624" E surrounding Hyderabad. The temperature of the region usually ranges from 11.60°C to 40.50°C, and it witnesses an average annual rainfall of 73.55 cm. Hyderabad is situated on the Deccan Plateau of South India at an altitude of 542 meters and inhabits 625 square kilometres along the banks of the Musi River. Sixteen sediment samples were collected in the first week of January 2019 from geographically distinct lakes and rivers in and around Hyderabad (Fig.1). At each sampling location (50-200 m on both banks of the identified lakes and river), approximately 100 gram of surface sediment (0-5 cm depth) was collected using sterile falcon tube (Zhang et al., 2019). Samples thus collected were transferred to the laboratory in ice-packed containers and kept at 4 °C for DNA isolation.

Isolation of DNA from lake sediment was 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.

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-

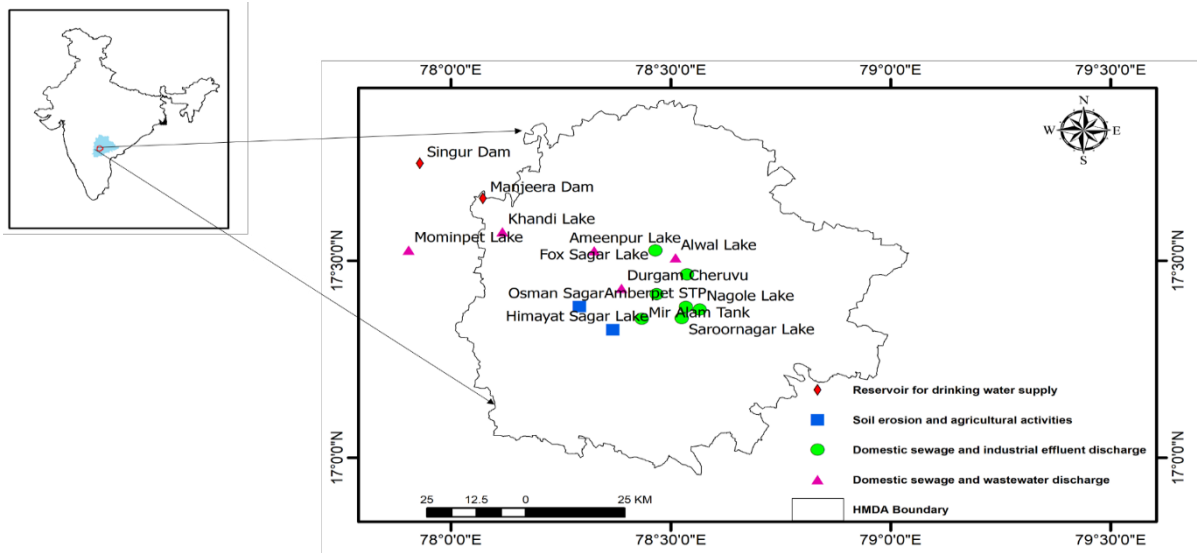

Fig 1. Sampling sites surrounding Hyderabad city, India

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 comprised of forward primer sequence 5′- GTACTGGCGTAACCCTTCACA -3′ and reverse primer sequence 5′- CATTCATGGCGGGCAGGATAA -3′ which were used 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.

Assays involving a real-time qPCR optimization were conducted using a real-time qPCR system CFX-96 of Bio-rad Laboratories (India) Private Limited using SYBR green chemistry. Protocols followed were as per T.B. Green™ Premix Ex Taq ™ (Tli RNaseH Plus) purchased from DSS Takara Bio India Private Ltd. 1 µL (microliter) undiluted final DNA extracts were used in triplicate during the experiment. All reactions carried out for analysis comprised of a reaction mixture of 10 µL per well, prepared via different components in definite proportions. The final volume of reaction mixture comprised of 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 comprised of an unknown sample in triplicate, a negative control (NC) 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 consisted 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, with 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.

Hierarchical clustering was conducted using Ward's linkage method followed by Euclidean distance measurements in order 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).

RESULTS AND DISCUSSION

The system evaluated several lakes and river sediment with possible NDM-1 bearing strains from 16 different geographic origins, using a real-time qPCR system CFX-96, Bio-Rad. Amplification and quantification were performed by running the unknown samples in triplicate with the known standards of NDM-1with the results being 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, the results have been summarized in Table 1.

In this study, among the sixteen collected samples, thirteen samples were detected positive for the NDM-1gene, while three samples tested negative during the qPCR analysis. The Cq values of analyzed samples were found to be varying from 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 at 40 for the qPCR assay. The signal obtained above 35 was considered non-specific for any test sample. Repeated run in triplicates with the standards of the NDM-1 gene showed a significant coefficient of correlation (r^2) value varying between 0.92 and 1 while the reaction efficiency varied between 90.4 to 109.9 %. During the qPCR analysis, 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.

Hierarchical Clustering Analysis was carried out in order to categorize samples based on

Table 1. Absolute quantification of NDM-1 in sediment DNA collected from different lakes, dams, and STP in and around 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	-	-	-	-

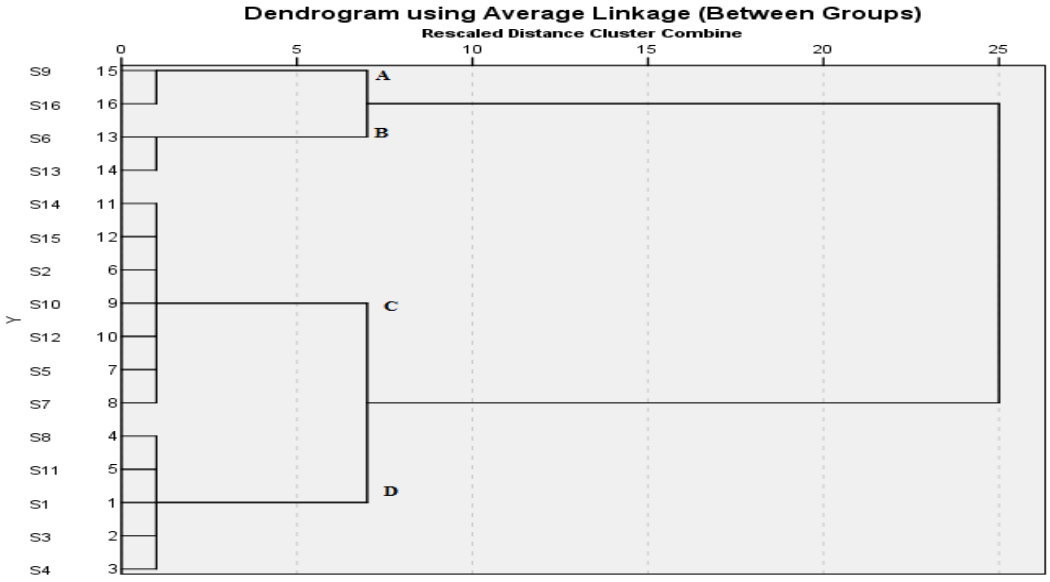

Fig 2. Dendrogram obtained by cluster analysis

their pollution sources. Ward's linkage method followed by Euclidean distance measurements was used to categorize data into four clusters. The clusters obtained in the dendrogram by cluster analysis (Fig. 2) employ different 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

herein after 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 (Fig2) identified Cluster A's sediment samples which comprises of water bodies (S9 and S16) that were being used as reservoirs for drinking water supply. It may be noted that both the Manjeera dam (S9) and Singur dam (S16) were built on the river Manjeera and water from them is being 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) since both these lakes are close to and parallel to each other.

Both Himayat Sagar (S6) and Osman Sagar (S13) lakes were constructed on the Musi River to provide supplementary drinking water supply to Hyderabad and to protect the city against floods. Rapid population growth, use of fertilizers in agriculture, and various anthropogenic activities however 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, which includes 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), was found to be polluted due to domestic sewage and industrial effluents discharge. Amberpet STP (S2) is the largest STP in the country based on Up-flow Anaerobic Sludge Blanket (UASB) process. It receives combined discharge from both 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 which has resulted in, a drastic and visible deterioration in the lake's water quality.

Hussain Sagar lake (S7) is regarded as the largest lake in Hyderabad. Over the past few decades, it has become increasingly contaminated due to the constant discharge of domestic sewage and untreated industrial effluents. The accumulation of pollutants in lake sediments are today a potential pollution threat to the surrounding groundwater. Mir Alam Tank lake (S10), located to the south of the Musi river, was 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 of the sewage from the nearby industrial areas, and Hussian Sagar lake. Safilguda lake (S14) is located in a residential suburb in Hyderabad. Its water quality has declined due to inflow of agricultural pesticides and untreated industrial and domestic sewage discharge. Saroor Sagar lake (S15) is one of the significant water bodies in Hyderabad and was constructed for agricultural and drinking purposes. The inflow of solid waste, domestic sewage, and untreated industrial effluents into the lake's catchment area has led to a severe accumulation of pollutants. Cluster D consisting of Alwal lake (S1), Ameenpur lake (S3), Durgamcheru lake (S3), Kandi lake (S8), and Mominpet lake (S11) was also found to be polluted due to domestic sewage and wastewater discharge. Alwal Lake (S1) is an

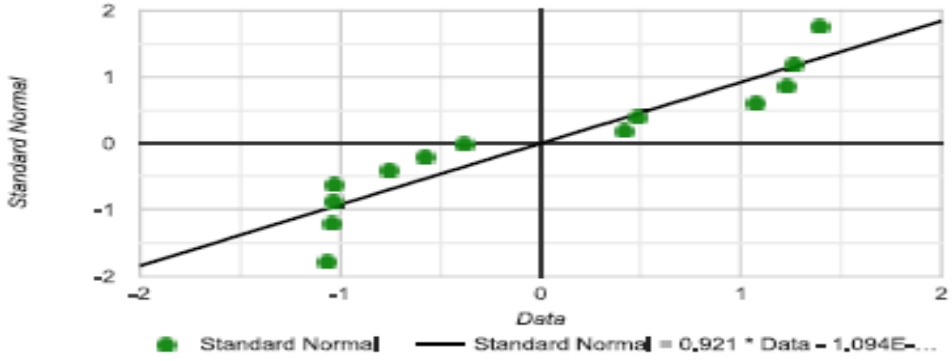

Fig 3. Quantile-Quantile (Q-Q) plot showing distribution of the NDM-1 gene copies among sampling locations

artificial lake in the vicinity of Hyderabad, it was found to be 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. It 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; it however been recorded with a low biological oxygen demand due to effluent discharge from commercial complexes and residential buildings. As can be observed from the dendrogram, Clusters A and B lie close to each other. Clusters C and D lie close each other but are away from clusters A and B.

Shapiro-Wilk test was performed using R software to correlate distribution of NDM-1 gene copies among the sampling locations. The variation in the distribution of gene copies number of the NDM-1 gene among sampling locations is large enough to be statistically significant [significance level (α) = 0.05, probability value (p-value) = 0.022]. Since p-value < α, it is assumed that the gene copies number is not normally distributed (Fig. 3).

Among the sixteen sediment samples tested, NDM-1 genes were observed in thirteen samples while the remaining three samples namely, Durgamcheru lake (S4), Kandi lake (S8), and downstream of Singur dam (S16), tested negative for NDM-1. Among the sediment samples that tested positive, the highest gene copy number was detected in Amberpet STP sludge (71.08). It was observed that the combined discharge of domestic sewage and industrial effluents contributed 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 a leading bulk drug producers (40% of the national production) and exporter (about 50% of drugs produced is exported), thus making it the hub for pharmaceutical companies (Lübbert et al., 2017). The observed gene copy number from Amberpet STP indicates that ever since the induction of Patancheru–Amberpet pipeline, there has been a gradual deterioration of the quality of the local river. Besides, the river also serves as a carrier 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 due to several factors, including microbial diversity, season and conductivity, pH, metals, and 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

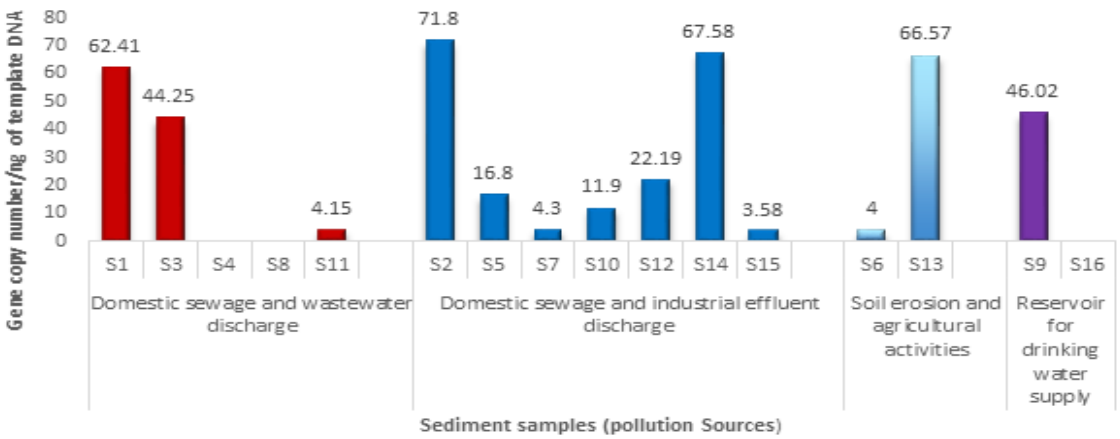

Fig 4. Gene copy number distribution among sediment samples

factors in the sediment's mobile bed (Eramo et al. 2020). Sampling locations (S4, S8, and S16) were tested negative for NDM-1 due to the absence of the beta-lactamase gene. In contrast, sampling locations (S6, S7, and S11) exhibited a low gene copy number due to a small concentration of the beta-lactamase gene (Fig. 4). The small concentration 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, waters of several rivers and lakes were found to contain a broader range of pollutants which elicit AMR in microorganisms. AMR 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 was observed among Indian sediment samples derived from sampling sites. The 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 that there was 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. 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 since the observed copy number of NDM-1 in the Safilguda lake and Nagole lake were significantly higher. Similar results exhibited in the study conducted by Bielen et al., 2017 showed effluent discharge from two manufacturing sites comprised of higher concentration ranges of antibiotics resulting in a more significant proportion of culturable ARB in the aquatic environment. For instance, the aquatic environment, which remains the essential life support for floral and faunal sustenance, has been devastated due to human-made pollution caused by domestic, agricultural, and industrial activities (Rashmi et al., 2017).

CONCLUSION

The study applied the real-time qPCR for the detection of the NDM-1 gene in river and lake sediments. Of the tested samples, Durgamcheru lake, Kandi lake, and Singur dam showed a negative signal for the NDM-1 during qPCR analysis. The highest gene copy number/ng of template DNA was 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 to all current-generation antibiotics. NDM-1 producers in samples collected from water bodies have significant inferences for people residing in the city and surrounding areas dependent on public water supply and sanitation amenities. The occurrence and rapid dissemination of carbapenems resistance caused by the NDM-1 gene worldwide is being monitored to circumvent any risk of an epidemic. Therefore, early identification of the NDM-1 gene harboring pathogens and preventing their spread must be carried out in any region.

GRANT SUPPORT DETAILS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, ethical issues, concerning plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K. A., Davies, J. and Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. Nature Reviews Microbiology, 8(4):251–259.

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

Bengtsson-Palme, J., Boulund, F., Fick, J., Kristiansson, E. and Larsson, D. G. J. (2014). Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front Microbiol., 5, 648.

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.

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

Bielen, A., Šimatović, A., Kosić-Vukšić, J., Senta, I., Ahel, M., Babić, S., Jurina, T., González Plaza, J. J., Milaković, M. and Udiković-Kolić, N. (2017). Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries. Water Res., 126, 79–87.

Chang, H., Wan, Y., Wu, S., Fan, Z. and 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.

Chen, Q. L., An, X. L., Li, H., Su, J. Q., Ma, Y. B. and Zhu, Y. G. (2016). Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environ Int., 92, 1-10.

Chen, Y., Su, J-Q and 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.

Czekalski, N., Díez, E. G., and 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.

Devarajan, N., Laffite, A., Ngelikoto, P., Elongo, V., Prabakar, K. and Mubedi, J. I., 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.

Devarajan, N., Laffite, A., Graham, N. D., Meijer, M., Prabakar, K. and Mubedi, J. I., 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), 6528–6537.

Eramo, A.; Medina, W. R. M. and 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.

Flach, C. F., Johnning, A., Nilsson, I., Smalla, K., Kristiansson, E. and Larsson, D. G. J. (2015). Isolation of novel Inc A/C and Inc N fluoroquinolone resistance plasmids from an antibiotic-polluted lake. J. Antimicrob. Chemother., 70, 2709–2717.

Gothwal, R. and Shashidhar, T. (2015). Antibiotic pollution in the environment: a review. Clean–Soil, Air, Water, 43(4), 479-489.

Gwenzi, W., Musiyiwa, K. and Mangori, L. (2018). Sources, behaviour, and health risks of antimicrobial resistance genes in wastewaters: A hotspot reservoir. Journal of Environmental Chemical Engineering,

102220.
Haller, L., Pote, J., Loizeau, J.L., and 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), 540–547.
Ip, C. C. M., Li, X. D., Zhang, G. S., Farmer, J. G., Wai, O. W. H. and 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.
Islam, M. A., Talukdar, P. K., Hoque, A., Huq, M., Nabi, A., Ahmed, D., Talukder, K. A., Pietroni, M. A., Hays, J. P., Cravioto, A. and Endtz, H. P. (2012). Emergence of multidrug-resistant NDM-1-producing Gram-negative bacteria in Bangladesh. Eur J Clin Microbiol Infect Dis., 31, 2593–2600.
Islam, M. A., Nabi, A., Rahman, M., Islam, M., Ahmed, D., Faruque, A. S., Hossain, A., van Belkum, A. and Endtz, H. P. (2014). Prevalence of fecal carriage of NDM-1- producing bacteria among patients with diarrhoea in Bangladesh. J Med Microbiol., 63, 620–622.
Jurado, A., Vàzquez-Suñé, E., Carrera, J., López de Alda, M., Pujades, E. and 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.
Khan, A.U., Maryam, L. and Zarrilli, R. (2017). Structure, Genetics, and Worldwide Spread of New Delhi Metallo-β-lactamase (NDM): a threat to public health. BMC Microbiol., 17**,** 101.
Kummerer, K. (2004). Resistance in the environment. J Antimicrob Chemother. 54(2), 311–320.
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.
Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., Bagaria, J., Butt, F. and 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.
Lara, T. M., Burch, T.R., McNamar, P. J., Tan, D.T., Yan, M. and Eichmiller, J. J. (2011). Tertiary-Treated Municipal Wastewater is a Significant Point Source of Antibiotic Resistance Genes into Duluth-Superior Harbor. Environ Sci Technol., 45, 9543-9549.
Laxminarayan, R. and Chaudhury, R. R. (2016). Antibiotic resistance in India: drivers and opportunities for action. PLOS Medicine, 13(3), e1001974.
Lübbert, C., Baars, C., Dayakar, A., Lippmann, N., Rodloff, A. C., Kinzig, M. and 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.
Martínez-Martínez, L. and González-López, J. J. (2014). Carbapenemases in Enterobacteriaceae: types and molecular epidemiology. Enfermedades infecciosas y microbiologia clinica, 32, 4-9.
Mwanamoki, P.M., Devarajan, N., Thevenon, F., Atibu, E. K., Tshibanda, J.B. and 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), 6821–6830.
Pei, R., Cha, J., Carlson, K. H. and Pruden, A. **(**2007). Response of antibiotic resistance genes (ARG) to biological treatment in dairy lagoon water. Environ. Sci. Technol., 41(14)**,** 5108-5113.
Perry, J. D., Naqvi, S. H., Mirza, I. A., Alizai, S. A., Hussain, A., Ghirardi, S., Orenga, S., Wilkinson, K., Woodford, N., Zhang, J., Livermore, D. M., Abbasi, S. A. and Raza, M. W. (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.
Pote, J., Haller, L., Loizeau, J. L., Bravo, A.G., Sastre, V. and 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., 99(15), 7122–7131.
Pote, J., Bravo, A. G., Mavingui, P., Ariztegui, D. and 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), 234–240.
Rashmi, H. B., Bharti, S. K., Gogoi, M., Devi, S., Ganguly, A. and Ganguly, S. (2017). Antibiotic Resistance: Role of Fruits and Vegetables in the Food Basket. Int. J. Pure App. Biosci., 5 (4), 169-173.
Rather, I. A., Kim, B. C., Bajpai, V. K. and Park, Y. H. (2017). Self-medication and antibiotic resistance: Crisis, current challenges, and prevention. Saudi journal of biological sciences, 24(4), 808-812.
Roske, K., Sachse, R., Scheerer, C. and Roske, I. (2012). Microbial diversity and composition of the sediment in the drinking water reservoir Saidenbach (Saxonia, Germany). Syst Appl Microbiol., 35,

35–44.
Spindler, A., Otton, L. M., Fuentefria, D.B. and 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.
Thevenon, F., Adatte, T., Wildi, W. and Pote, J. (2012). Antibiotic-resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). Chemosphere, 86(5), 468–476.
Toleman, M. A., Bugert, J. J. and Nizam, S. A. (2012). Extensively drug-resistant New Delhi Metallo-β-lactamase-encoding bacteria in the environment, Dhaka, Bangladesh. Emerg Infect Dis., 21, 1027–1030.
Von Wintersdorff, C. J., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B. and Wolffs, P. F. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Frontiers in microbiology, 7, 173.
Walsh, T. R. and Toleman, M. A. (2011). The new medical challenge: why NDM-1? Why Indian? Expert Rev Anti Infect Ther., 9, 137–141.
Walsh, T. R., Weeks, J., Livermore, D. M. and Toleman, M. A. (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.
World Health Organization. (2004). The World health report: 2004: changing history. World Health Organization.
Xu, C., Zhang, Y., Marrs, C. F., Ye, W., Simon, C., Foxman, B. and Nriagu, J. (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Appl. Environ. Microbiol., 75(17), 5714-5718.
Yang, J., Jiang, H. C., Liu, W. and Zhang, G. J. (2016). Distinct factors shape aquatic and sedimentary microbial community structures in the lakes of Western China. Front Microbiol., 7, 1782.
Zhang, L., Zhao, T., Wang, Q., Li, L., Shen, T. and 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.