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# Assessment of heavy metal contamination and Hg-resistant bacteria in surface water from different regions of Delhi, India



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## KEYWORDS

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River Yamuna

**Abstract** The present study aims to monitor the surface water quality of different regions in Delhi (India). With many physical and chemical properties, all samples had a high load of pollution in which Najafgarh drain (Nd) exhibited maximum and laboratory tap water (Ltw) minimum contamination. Water samples contained notable amounts of heavy metals including Cr, Cd, As, Cu, Pb and Hg. A total of 88 Hg-resistant bacteria were isolated from all the regions except Ltw. Among all the samples, the density of Hg-resistant bacteria was highest in sample of Nd and their morphotype heterogeneity was highest in sample collected from river Yamuna nearby Kashmiri gate (Kg). Different strains showed different patterns of resistance to different heavy metals and antibiotics. Multiple antibiotic resistance (MAR) indices were high in two samples, the highest reported in a sample taken from river Yamuna nearby Majnu ka tila (Mkt) (0.34). The 12.5% and 24.45% isolates showed  $\beta$ - and  $\alpha$ -hemolytic natures, respectively that might be of pathogenic concern. In this account, high concentrations of heavy metals and their resistant bacteria in surface water have severely damaged the quality of water and their resources and produced high risk to the associated life forms.

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## 1. Introduction

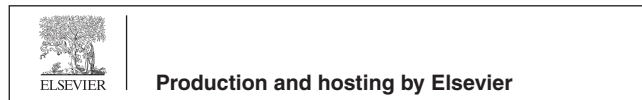
Over the past few decades, high industrial density as a result of increasing socioeconomic development has generated a

tremendous amount of pollution. Industrial effluents being continuously discharged into rivers are gradually deteriorating our global environment. Unlike most of the organic substances that can be metabolized by natural microbiota, heavy metals being indestructible, persist in the environment for a long time. Heavy metal is the collective term used for those elements (metals, metalloids, lanthanides and actinides) which have atomic density greater than  $5 \text{ g cm}^{-3}$  (Nies, 1999). Heavy metals are extremely hazardous and present in the environment beyond their permissible limits. They accumulate in the biological systems and concentrate in the food chain at each trophic level (Tao et al., 2012). These events bring serious challenges to

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the survival of microorganisms and plants, and cause cancers and neurological disorders in humans and animals. Heavy metals like copper (Cu), chromium (Cr)(III), zinc (Zn), manganese (Mn), cobalt (Co) and molybdenum (Mo) have some biological importance at low concentrations, but their high concentrations and long-term exposures produce detrimental effects on several biomolecules. On the other hand, mercury (Hg), cadmium (Cd), chromium (Cr)(VI), arsenic (As) and lead (Pb) are very toxic even at very low concentrations (Nies, 1999; Oyetibo et al., 2010).

Among various heavy metals, Hg is poisonous to all living beings and its high concentration is of major public health concern. Hg is released into the environment through atmospheric deposition, coal-fired power station, gold mining, cement production, non-ferrous metal production and from various other industrial sources. Moreover, some natural activities such as volcanic eruption, forest fire and erosion are also responsible for notable emission of Hg in the environment (Wang et al., 2004). In the biogeochemical cycle, Hg undergoes many physical and chemical transformations, thereby existing in three different forms i.e. Hg(0) in metallic form, Hg(II) and Hg(I) in inorganic forms and R-Hg<sup>+</sup> (where R is phenol or methyl group) in organic form.

The presence of Hg in the environment typically results in low microbial abundance. This situation occurs because relatively low genetic diversity of microbial communities acclimatizes to Hg, and induces a compositional change (Rasmussen et al., 2008). Besides, Hg tolerance in acclimatized microbial communities also varies in response to different concentrations of Hg in different environments. Hg resistance is determined by the *mer* operon that is located on plasmid, transposons, integrons and genomic DNA, frequently linked with the antibiotic resistance genes (Nascimento and Chartone-Souza, 2003). These genes are mobile elements and are often transferable to other bacterial species via horizontal gene transfer (Jan et al., 2012). As a consequence, antibiotic resistance genes also disseminate together with heavy metal resistance genes even in the absence of frequent antibiotics used due to co-selection of the linked markers (Wireman et al., 1997). Since multiple antibiotic resistance in microorganisms poses a potential health risk to humans and animals for the treatment of infectious diseases, this event is a matter of high concern for the therapeutic discipline. Bioremediation of Hg by isolating such type of resistant bacteria has long been a subject of interest in recent studies; however the contributions of Hg resistant bacteria in multiple heavy metal and antibiotic resistance and monitoring of contaminated regions are rarely focused.

In this perspective, the present investigation was undertaken to estimate the heavy metal pollution along with the assessment of heavy metal and antibiotic resistance in Hg-resistant bacteria isolated from surface water of different regions in Delhi (India).

## 2. Materials and methods

### 2.1. Study sites

Delhi, the capital of India along with its extended suburbs is the second highest populated city in the world. Around 22.7 million people reside in this region as per world popula-

tion data sheet released by the US's Population Reference Bureau (2013). Inside the territory of Delhi, river Yamuna flows for about 22 km length that enters from Wazirabad barrage and leaves at the Okhla barrage. This river is the primary source of drinking water in the city. Although the city contributes only to 0.4% catchment area of this river (CPCB, 2006–07), 70% of the total pollution in this river releases from this region. Eighteen drains along with industrial effluents continuously running off into river awfully deteriorate the water quality of river Yamuna.

### 2.2. Sampling

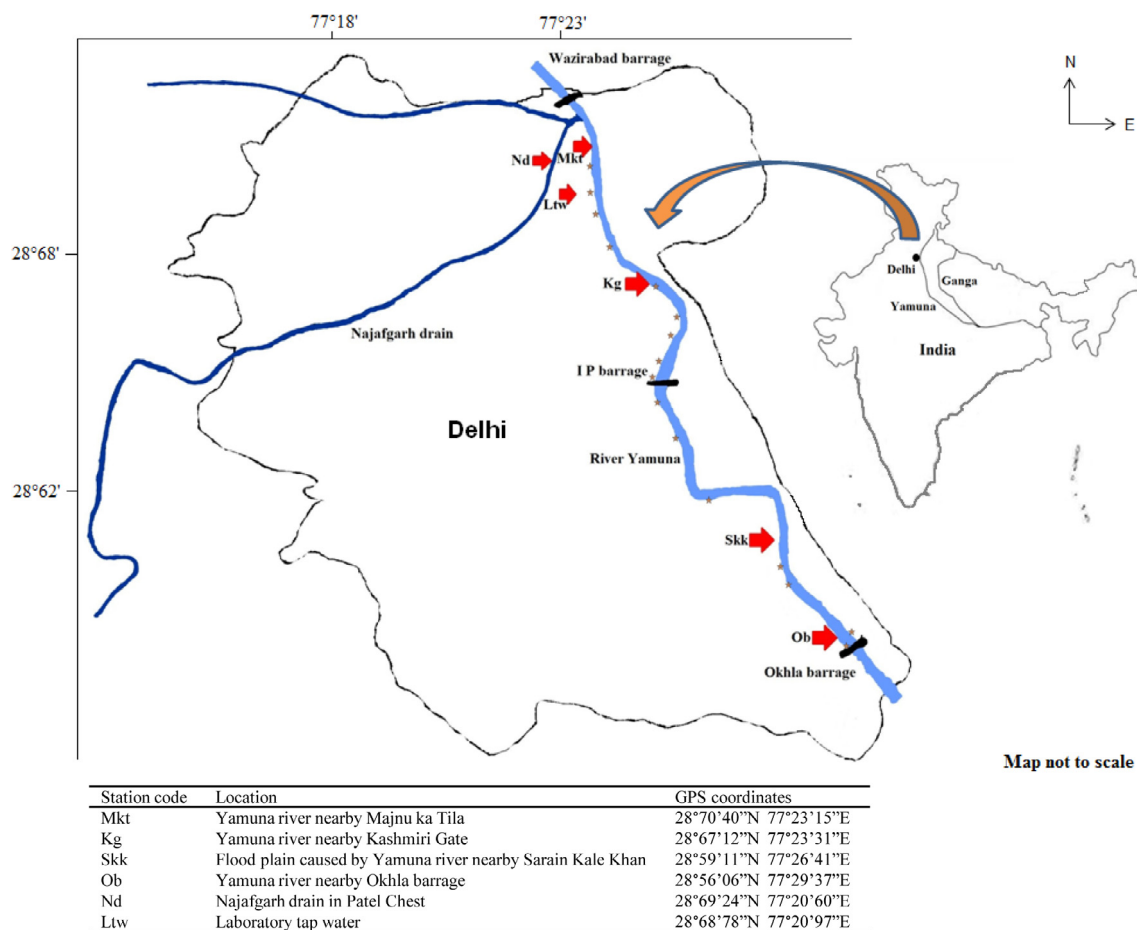
The present study was conducted in six different aquatic regions of Delhi during July–August, 2013. Samples Mkt, Kg and Ob were taken from surface water of the river Yamuna nearby Majnu ka Tila, Kashmiri Gate and Okhla barrage, respectively. Samples Skk, Nd and Ltw were taken from the flood plain of river Yamuna nearby Sarai Kale Khan, surface water of Najafgarh drain nearby Vallabhbhai Patel Chest Institute and tap water of our laboratory, respectively. All samples were collected in sterile bottles and stored at 4 °C. The global positioning system (GPS) of each sampling site is mentioned in Fig. 1.

### 2.3. Physicochemical qualities and heavy metal composition

Physicochemical qualities of water samples were determined for parameters such as pH, temperature, electrical conductivity (EC), total dissolved solids (TDS), chloride, nitrate and alkalinity. For heavy metal composition, water samples were acid digested (HNO<sub>3</sub>:HCl in 1:3), filtered through filter paper no. 1 (Whatman Inc., NJ, USA) and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700x ICP-MS, USA). Hg was detected using Hg analyzer MA 5840 (Electronics Corporation of India Ltd., India) (O'Dell et al., 1994). The chemical qualities of water samples (for physicochemical analysis and heavy metal concentrations) were compared with the standard limits set by various regulatory bodies including World Health Organization (WHO) (2011), U.S. Environmental Protection Agency (US EPA) (2009), and Bureau of Indian Standards (BIS) (2012) (Table 1).

### 2.4. Isolation of Hg-resistant bacteria

Hg-resistant bacteria were isolated by serial dilution method. For the same, 1 ml water sample was serially diluted in sterile saline water (0.85%, w/v) and shaken vigorously for 5 min. Then, 0.1 ml aliquot of each dilution was spread onto 25 mg/l Hg (in the form of HgCl<sub>2</sub>) (filter sterilized) containing Luria-Bertani (LB) agar (HiMedia, India) plates. After incubation at 37 °C for 24–72 h, the CFU/ml of bacteria was calculated for those plates which had the number of colonies ranging from 30 to 300. Morphologically different colonies were picked up and purified by repeated sub-culturing on the same medium. Further, all bacterial isolates were categorized based on Gram's staining reaction. They were stored in LB agar plates and 30% (v/v) glycerol stocks at 4 °C and –20 °C, respectively for further analysis.



**Figure 1** Geographical positions of sampling in Delhi (India). Sign  $\blacktriangleright$  represents different sampling sites and sign  $\star$  represents dumping sites of drains in river Yamuna.

### 2.5. Minimum inhibitory concentrations (MICs) of heavy metals

Minimum inhibitory concentrations (MICs) of six heavy metals were determined for all the isolates. Bacterial colonies were streaked onto LB agar amended with increasing heavy metal concentrations until the growth completely ceased (Kathiravan et al., 2011). The plates were incubated at 37 °C for 48 h. Heavy metals used in different concentrations included Cr(VI) (50–1600 mg/l), Cd (50–1600 mg/l), Cu (50–3200 mg/l), As (50–1200 mg/l), Pb (50–4500 mg/l) and Hg (50–100 mg/l). Positive controls were set by growing the test isolates in the absence of heavy metals under similar conditions. The lowest concentration of heavy metal which did not favor the growth of an organism was considered MIC.

### 2.6. Antibiotic resistance test

Antibiotic resistance in bacteria was determined using Hi antibiotic disk Combi 506 (HiMedia, India). Different antibiotics included Ciprofloxacin (CIP) (5 µg), Ofloxacin (OF) (5 µg), Sparfloxacin (SPX) (5 µg), Gatifloxacin (GAT) (5 µg), Teicoplanin (TEI) (30 µg), Azithromycin (AZM) (15 µg),

Vancomycin (VA) (30 µg), Doxycycline HCl (DO) (30 µg). An aliquot of 0.1 ml freshly grown culture was spread on Mueller-Hinton agar plate and antibiotic disk was mounted on it under aseptic conditions. After 18–24 h of incubation at 37 °C, growth inhibition zone was measured using zone scale. Based on Clinical and Laboratory Standards Institute (CLSI) (2012) standard limits for the zone of inhibition, bacteria were categorized as resistant, intermediate or susceptible. Multiple antibiotic resistance (MAR) index of each water sample was determined using the equation  $a/(b \times c)$ , where 'a' is the number of antibiotics to which 'c' number of bacteria scored resistance from a sample, and 'b' represents the total number of antibiotics tested (Tao et al., 2010).

### 2.7. Hemolytic test

Freshly cultured bacteria were streaked onto sheep blood agar plates (HiMedia, India). After 18–24 h of incubation at 37 °C, bacteria were categorized for the pathogenicity by changed color of blood in the medium. Color of blood changed from red to yellow gray/dark green and yellow/transparent was considered  $\alpha$ -hemolysis and  $\beta$ -hemolysis, respectively; however, no change in color of the blood came under  $\gamma$ -hemolysis (Carey et al., 2007).

**Table 1** Physicochemical and metal analyses in different water samples and their standard limit of various regulatory bodies.

Parameters	Water samples						Standard limit		
	Mkt	Kg	Skk	Ob	Nd	Ltw	WHO (2011)	US EPA (2009)	BIS (2012)
pH	7.83	7.59	8.66	8.97	7.15	7.61	NG <sup>a</sup>	6.5–8.5	6.5–8.5
EC ( $\mu\text{S cm}^{-1}$ )	821	397	335	147	1070	245	NS <sup>b</sup>	NS	NS
Salinity (mg/l)	409	198	160	185	526	125	NS	NS	NG
TDS (mg/l)	621	306	242	131	802	190	NG	500	500
Chloride (mg/l)	130	30	20	20	240	20	NG	250	250
Nitrate (mg/l)	4	2	5	12	10	2	50	10	45
Alkalinity (mg/l)	70	40	70	90	400	60	NG	NS	200
<i>Heavy metals (mg/l)</i>									
Cr	0.08	0.38	0.06	0.09	0.94	0.06	0.05	0.10	0.05
Cd	0.002	0.008	0.001	0.003	0.004	0.003	0.003	0.005	0.003
Cu	0.15	0.40	0.05	0.22	0.52	0.07	2.00	1.30	0.05
As	0.02	0.13	0.14	0.05	0.02	0.00	0.01	0.01	0.01
Pb	0.34	0.68	0.45	0.46	0.49	0.49	0.01	0.015	0.01
Hg	0.004	0.003	0.003	0.004	0.008	0.001	0.006 <sup>c</sup>	0.002 <sup>c</sup>	0.001 <sup>c</sup>

<sup>a</sup> NGL: No guideline, not of health concern at levels found in drinking water.

<sup>b</sup> NS: Not specified.

<sup>c</sup> Hg as inorganic form only.

## 2.8. Statistical analysis

Pearson product-moment correlation analysis was carried out for determining the heavy metal association in water samples. Statistical significance for the analysis was set at  $P < 0.05$ .

## 3. Results and discussion

### 3.1. Physicochemical and heavy metal analyses

Water collected from the Najafgarh drain (sample Nd) had highest load of pollution among all the studied sites. Water quality of river Yamuna (sample Mkt) just after receiving effluent from the drain was also considerably affected. The pH of water in samples Skk and Ob notably exceeded its permissible ranges of limit. EC and salinity were higher in samples Nd and Mkt than other samples; however no specific guideline is made for their limits. Also, TDS values were very high in samples Nd and Mkt. On the other hand, chloride, nitrate and alkalinity in almost every sample were detected within standard limits (Table 1).

Among all the sites, samples Nd and Kg had very high concentrations of heavy metals, and samples Mkt, Skk and Ob had moderate concentrations. It is noteworthy that even in tap water, some metals like Cr, Cu and Pb were reported beyond the standard limits. All the sites had Pb in highest concentrations, while Hg and Cd in lowest concentrations among all the considered heavy metals. The concentrations of Pb were almost similar in all the samples and estimated far above from the standard allowed values. Also, Cr in all samples crossed the standard limits set by BIS and WHO. Cd was present in insignificant amounts in all samples except in sample Kg. As was not detected in tap water; however, its high concentrations were found in other samples. Cu concentrations in all studied samples were below the standard limits of WHO and US EPA. The concentrations of Hg in all samples except sample Ltw

crossed the standard limits of WHO and US EPA; while in the perspective of BIS, it was estimated within the prescribed limit (Table 1).

The correlation matrix of the various heavy metals among water samples collected from different sites in Delhi is interpreted in Table 2. Cr was positively correlated with Cu and Hg. Cd also exhibited positive correlation with Pb. These correlations between different heavy metals deduced that their concentrations in different aquatic sites could be linked due to their common sources of origin.

Evidence blames that industrial effluents were the primary source of heavy metal contamination in the river Yamuna. Singh and Kumar (2006) found substantial levels of contamination of heavy metals in soil, water and vegetation grown peri-urban area of Delhi. High concentration of Cr(VI) in water might be due to the electroplating industries in Wazirabad, Badli and Mangolpuri areas, while coal-fired power station in proximity of the river might be the primary cause of Hg emission in the water. Sehgal et al. (2012) stated that lead battery-based units and vehicular pollution in the city were significantly responsible for an increase in the level of Pb in Delhi. Moreover, the absence of minimum flow of water ( $10 \text{ m}^3/\text{sec}$ ) in river Yamuna around Delhi stretch is also the one of the main reasons for the high contamination in water (Upadhyaya et al., 2011). This situation brings an into focus the persistence of heavy metals in the river's basin for an extended period, which results in their deposition into the surrounding sediments and soil (Sehgal et al., 2012). Ground water in the vicinity of Najafgarh drain and other related locations have also been reported to receive alarmingly high contamination of heavy metals due to this reason (Shekhar and Sarkar, 2013).

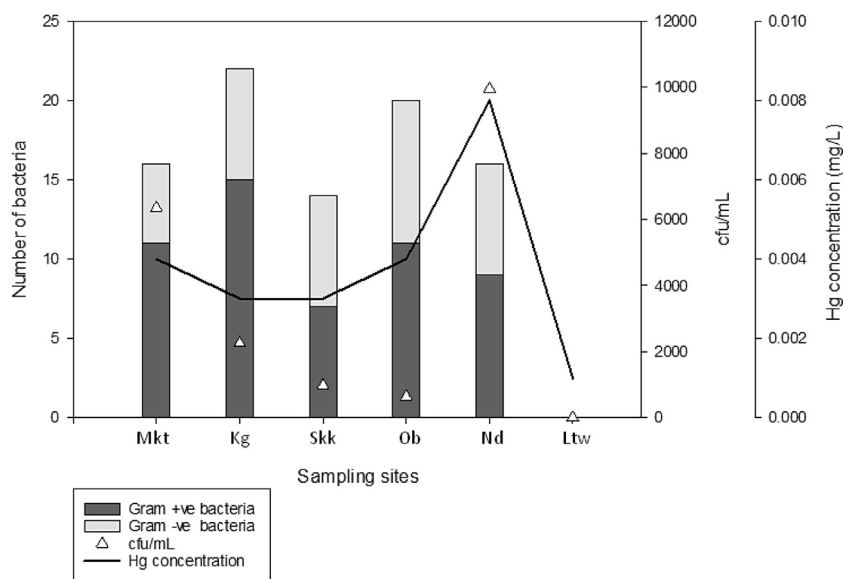
### 3.2. Hg-resistant bacteria

The details of Hg-resistant bacteria isolated from different samples are provided in Fig. 2. Hg-resistant bacteria were

**Table 2** Correlation of metal levels in different water samples.

	Cr	Cd	Cu	As	Pb	Hg
Cr	1.000	0.178 (0.736)	<b>0.911(0.012)*</b>	-0.131 (0.804)	0.325 (0.529)	<b>0.837(0.038)*</b>
Cd		1.000	0.452 (0.368)	0.548 (0.260)	<b>0.873(0.023)*</b>	-0.160 (0.763)
Cu			1.000	-0.021 (0.968)	0.500 (0.313)	0.759 (0.080)
As				1.000	0.492 (0.321)	-0.186 (0.724)
Pb					1.000	-0.121 (0.819)
Hg						1.000

\* Correlation is significant at the 0.05 level (2-tailed).

**Figure 2** Abundance and morphotype heterogeneity of Hg-resistant bacteria along with Hg concentration in different water samples.

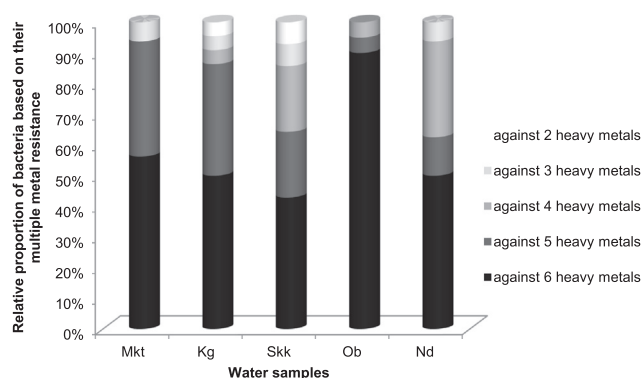
widespread in the samples that had a high amount of Hg, while no bacterium was encountered from the sample Ltw that contained very low concentration of Hg. However, bacterial abundance was inconsequential with the Hg concentration at the sampling sites. Bacterial density was highest in sample Nd followed by sample Mkt and Kg, though their morphotype heterogeneity was highest in sample Kg. However, sample Skk depicted the lowest density and heterogeneity of Hg-resistant bacteria among all the studied sites. More precisely, among a total of 88 isolates, samples Mkt and Nd harbored 16 morphologically different bacteria and samples Kg, Skk and Ob 22, 14 and 20, respectively. Both Gram-positive and Gram-negative types of bacteria were reported for Hg resistance. Overall, Gram-positive bacteria were in high proportion in most of the samples, representing 60.23% of the total isolates. The spore-forming capability of Gram-positive bacteria can provide an additional resistance in stress situations, thereby dominating in Hg-contaminated sites.

In contrast to culture-independent techniques, which provide information regarding total species diversity and specific genes diversity of the community, culture-dependent methods tend to represent Hg-resistant microbial subpopulation without any discrimination (Rasmussen et al., 2008). Previously, many studies have focused on Hg-resistant isolates from different environmental conditions for different aspect of investiga-

tions. Jan et al. (2012) characterized bacterial isolates resistant to organic Hg from diverse wet locations of India. Acclimatization of Hg-resistant microflora in the fecal-oral route of the human and primates has also got much speculation on account of Hg amalgam dental fillings (Wireman et al., 1997; Summers et al., 1993; Ready et al., 2003). All these studies also demonstrated that Hg acclimatization in inhabiting microflora prevails mainly by the selective pressure of the Hg. Hg in water is very soluble and bioavailable, which significantly influences the emergence of Hg-resistant microorganisms in the aquatic system. On the other hand, Hg in soil persists more as non-bioavailable forms in clay, which limits the Hg availability for existing population (Ruggiero et al., 2011).

### 3.3. Heavy metal resistance

Multiple heavy metal resistance among Hg-resistant bacteria was very common. Each bacterium had very different resistance profile. Bacteria resistant to all tested six heavy metals were prevalent in all samples, with maximum isolates (90%) belonging to the sample Ob. Bacteria resistant to five heavy metals were also accountable, comprising 37.5% and 36.36% isolates from the samples Mkt and Kg, respectively. Besides, two bacteria one from each samples Kg and Skk were resistant to only single heavy metal (Cu) apart from Hg (Fig. 3).



**Figure 3** Multiple heavy metal resistance profiles in Hg-resistant bacteria isolated from different water samples.

For the effect of Cr(VI), maximum bacteria (52.77%) were tolerant to the range of its 400–800 mg/l concentrations. For Cd, the majority of the bacteria (34.01%) showed MIC to 800 mg/l concentration of metal. Besides, 29.55% bacteria were not resistant to Cd, showing the highest percentage of bacterial susceptibility to any heavy metal. Also, a very high percentage of bacteria (28.41%) could not resist As(III). On the other hand, 100% isolates were resistant to Cu and also much (96.59%) isolates showed resistance to Pb. Such prevalence of resistance to Pb and Cu among the Hg-resistant isolates in the environment might be due to widespread existence of their concomitant regulating factors for co-selection. Moreover, to their resistant isolates, 48% and 77% bacteria were tolerant to the above level of 1200 mg/l Cu concentrations and 1600 mg/l Pb concentrations, respectively, showing a very high level of threshold of Cu and Pb for Hg-resistant isolates. On the other hand, despite the selection pressure of Hg on the bacterial isolation, the threshold of Hg for the bacterial tolerance was lowest among all the tested heavy metals. Only 3.42% of the total bacteria, representing one and two isolates from the samples Mkt and Kg, respectively could cover at MIC value to 100 mg/l of Hg. However, most of the isolates (68.18%) showed tolerance to the Hg in concentration of < 50 mg/l (Table 3). Other investigators also reported a very few bacteria to be Hg resistant at its high concentrations (Alam et al., 2011; Verma et al., 2001).

Heavy metal resistant bacteria are considered the biological indicators of heavy metal contamination. Such bacteria potentially contribute to biogeochemical cycling of heavy metals. But in response to increased resistance to different heavy metals, they also represent negative impact on ecosystem functioning by decreasing the expression of several enzymes (Sabry et al., 1997; De Souza et al., 2006). Bacteria resistant to particular heavy metal might also acquire resistance to other heavy metals. Previously, many have assessed abundance of Cr(VI) resistant bacteria from the high Cr-contaminated sites, showing resistance to Cr(III), Ni, Zn, Cu, Cd and Hg (Alam et al., 2011; Verma et al., 2001).

Certainly, two different mechanisms of co-selection regulate for the multiple heavy metal resistance. These include co-resistance where genetically linked different resistance factors transfer concomitantly and cross-resistance in which the same factor is responsible for resistance to more than one structurally dissimilar compound (Baker-Austin et al., 2006).

The former process is much more common and several conjugative potential resistant markers such as *Hg-Cu-Zn-Cr*, *Hg-Cu-Cr*, *Co-Ni-Cr* and *Cd-Co-Zn* have been identified in various isolates (Malik and Aleem, 2011; Verma et al., 2001). However, studies underlying for the cross-resistance to multiple heavy metals did not receive much attention. A paradigm of this compliance have been noticed in the study of Nakajima et al. (1995), where overexpression of single *robA* gene in *Escherichia coli* showed increased resistance to multiple metals like Ag, Cd and Hg and multiple antibiotics like tetracycline, chloramphenicol and novobiocin.

### 3.4. Antibiotic resistance pattern

Antibiotic resistance patterns of Hg-resistant bacteria are presented in Table 4. Of all the bacteria, maximum isolates (56.82%) were resistant to antibiotic TEI in which the greatest number of bacteria (68.75%) belonged to sample Mkt followed by 65.0% from sample Nd. Also, a significant number of bacteria (44.32%) were resistant to antibiotic VA. Low percentages of bacteria, 12.5% and 14.77% were resistant to antibiotics SPX and GAT, respectively. Hg-resistant isolates were highly sensitive to antibiotics CIP and OF, and only two different bacteria could resist to these antibiotics.

Previous studies also made corroborative evidence for Hg-resistant bacteria, carrying resistance to multiple antibiotics (Summers et al., 1993). In the study of Meredith et al. (2012), concomitant antibiotic and Hg resistance in various genera of gastrointestinal microflora of fecal brook trout was shown as supportive feature for multiple selective genes transfer. Skurnik et al. (2010) have suggested that exposure to Hg in the environment might be a specific driving force for the carriage of antibiotic resistance genes.

MAR index is the resistance indicators of disseminating contamination of antimicrobial agents in the particular area. The extended MAR index (>0.2) value upsets the environmental ethic of protecting the natural resources (Krumperman, 1985). High MAR index value was reported in sample Mkt (0.34), followed by in sample Ob (0.24). Other samples Kg, Skk and Nd had lower MAR index (Fig. 4). The average MAR index value for all the samples was also high i.e. 0.22.

### 3.5. Pathogenicity concern

Among all bacteria, 12.5% and 24.45% isolates showed  $\beta$ - and  $\alpha$ -hemolysis, respectively. Considering individual samples, sample Skk encompassed maximum  $\beta$ -hemolytic bacteria (35.72%), insinuating the high concern of infectious diseases in stagnant water. Sample Nd showed high percentages of (18.75%)  $\beta$ -hemolytic bacteria and (37.5%)  $\alpha$ -hemolytic bacteria, indicating drain as a prominent source for waterborne diseases. There was high percentage of bacteria (37.5%) from sample Mkt, which exhibited  $\alpha$ -hemolytic nature. However, samples Ob and Mkt did not load  $\beta$ -hemolytic bacteria (Fig. 5).

Many previous reports admit the risk of infectious diseases from contaminated aquatic regions. Researchers such as Skariyachan et al. (2013) and Pavlov et al. (2004) have focused on the existence of hemolytic bacteria in water bodies. Being environmental conditions same for the presence of higher

**Table 3** Heavy metal resistance in Hg-resistant bacteria isolated from different water samples.

Metals	Concentrations (mg/l)	Number of resistant bacteria (%) in different water samples					Total resistant bacteria (%) (n = 88)
		Mkt (n = 16)	Kg (n = 22)	Skk (n = 14)	Ob (n = 20)	Nd (n = 16)	
Cr(VI)	0	1 (6.25)	2 (9.09)	1 (7.14)	–	1 (6.25)	5 (5.68)
	100	4 (25.0)	3 (13.64)	–	5 (25.0)	2 (12.5)	14 (15.91)
	400	2 (12.5)	4 (18.18)	2 (14.28)	1 (5.0)	1 (6.25)	10 (11.36)
	800	7 (43.75)	10 (45.45)	10 (71.43)	13 (65.0)	6 (37.5)	46 (52.27)
	1200	1 (6.25)	3 (13.64)	1 (7.14)	1 (5.0)	3 (18.75)	9 (10.23)
	1600	1 (6.25)	–	–	–	3 (18.75)	4 (4.55)
Cd	0	4 (25.0)	8 (36.36)	6 (42.86)	2 (10.0)	7 (43.75)	26 (29.55)
	100	–	–	–	–	–	–
	400	8 (50.0)	9 (40.91)	3 (21.43)	3 (15.0)	6 (37.5)	30 (34.01)
	800	1 (6.25)	–	1 (7.14)	4 (20.0)	–	6 (6.81)
	1200	–	1 (4.55)	–	5 (25.0)	2 (12.5)	8 (9.09)
	1600	3 (18.75)	4 (18.18)	4 (28.57)	6 (30.0)	1 (6.25)	18 (20.45)
As(III)	0	4 (25.0)	6 (27.28)	7 (50.0)	1 (5.0)	7 (43.75)	25 (28.41)
	100	–	1 (4.55)	–	1 (5.0)	1 (6.25)	3 (3.41)
	400	8 (50.0)	4 (18.18)	1 (7.14)	2 (10.0)	1 (6.25)	16 (18.18)
	800	1 (6.25)	4 (18.18)	2 (14.28)	5 (25.0)	4 (25.0)	16 (18.18)
	1200	3 (18.75)	7 (31.82)	4 (28.57)	11 (55.0)	3 (18.75)	28 (31.82)
Cu	0	–	–	–	–	–	–
	100	–	–	–	–	–	–
	400	–	1 (4.55)	1 (7.14)	–	1 (6.25)	3 (3.41)
	800	4 (25.0)	11 (50.0)	6 (42.86)	–	5 (31.25)	26 (29.55)
	1200	5 (31.25)	3 (13.64)	1 (7.14)	7 (35.0)	1 (6.25)	17 (19.32)
	1600	6 (37.5)	5 (22.73)	4 (28.57)	7 (35.0)	8 (50.0)	30 (34.01)
	2400	1 (6.25)	2 (9.09)	2 (14.28)	6 (30.0)	1 (6.25)	12 (13.64)
Pb	0	–	1 (4.55)	2 (14.28)	–	–	3 (3.41)
	100	–	–	–	–	–	–
	800	1 (6.25)	5 (22.73)	1 (7.14)	–	1 (6.25)	8 (9.09)
	1600	4 (25.0)	1 (4.55)	1 (7.14)	2 (10.0)	1 (6.25)	9 (10.23)
	2400	2 (12.25)	1 (4.55)	3 (21.43)	1 (5.0)	4 (25.0)	11 (12.5)
	3200	6 (37.5)	10 (45.50)	4 (28.57)	12 (60.0)	5 (31.25)	37 (42.05)
	> 3200	3 (18.75)	4 (18.18)	3 (21.43)	5 (25.0)	5 (31.25)	20 (22.73)
Hg	50	9 (56.25)	12 (54.55)	11 (78.57)	14 (70.0)	14 (87.5)	60 (68.18)
	75	6 (37.5)	8 (36.36)	3 (21.43)	6 (30.0)	2 (12.5)	25 (28.41)
	100	1 (6.25)	2 (9.09)	–	–	–	3 (3.41)

**Table 4** Antibiotic resistance in Hg-resistant bacteria isolated from different water samples.

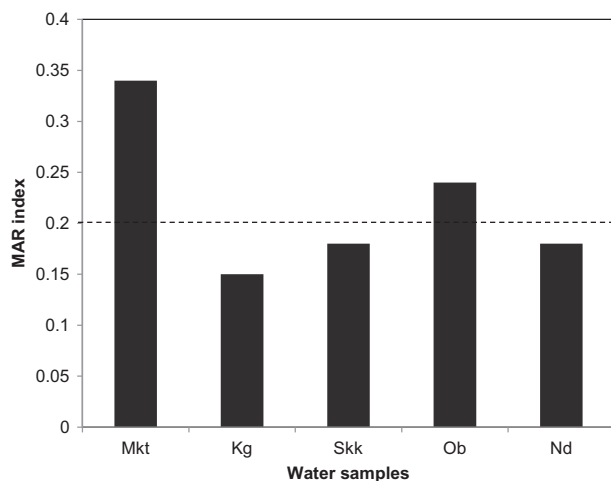
Antibiotics ( $\mu\text{g}/\text{disk}$ )	No. of resistant variants (%) from different water samples					Total resistant variants (%)
	Mkt	Kg	Skk	Ob	Nd	
Azithromycin (15)	6 (37.5)	4 (18.18)	4 (28.57)	4 (20.0)	2 (12.5)	20 (22.73)
Vancomycin (30)	9 (56.25)	9 (40.91)	4 (28.57)	11 (55.0)	6 (37.5)	39 (44.32)
Doxycycline HCl (30)	8 (50.0)	3 (13.64)	nr	6 (30.0)	3 (18.75)	20 (22.73)
Ciprofloxacin (5)	1 (6.25)	nr	nr	nr	1 (6.25)	2 (2.27)
Ofloxacin (5)	1 (6.25)	1 (4.55)	nr	nr	nr	2 (2.27)
Sparfloxacin (5)	4 (25.0)	1 (4.55)	2 (14.29)	2 (10.0)	2 (12.5)	11 (12.5)
Gatifloxacin (5)	4 (25.0)	1(4.55)	4 (28.57)	2 (10.0)	2 (12.5)	13 (14.77)
Teicoplanin (30)	11 (68.75)	11 (50.0)	8 (57.14)	13 (65.0)	7 (43.75)	50 (56.82)

nr = no resistance.

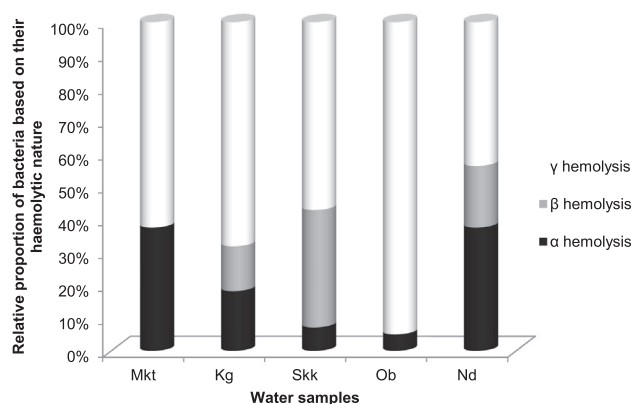
organisms and several microorganisms, there is a high chance that pathogenic bacteria may adhere and colonize into humans, when contaminated water is used for personal hygiene (Berg et al., 2005). In result, people with weakened or/and compromised immune system get infections, and their severe cases fall into death.

#### 4. Conclusion

With the advent of high anthropogenic inputs, documented outcomes revealed predominant geochemical pollution and heavy metal resistant bacteria in surface water from different



**Figure 4** MAR index of Hg-resistant bacteria in different water samples. Dash line represents MAR threshold value (0.2) to differentiate the low and high risk.



**Figure 5** Pathogenicity concern in Hg-resistant bacteria isolated from different water samples.

sites of Delhi (India). Continuously increasing load of harmful chemicals and the resistant microbes against them are causing severe damage to the water bodies. The use of those water bodies by humans and other organisms is being increasingly dangerous for their survival. These anthropogenically disturbed wet regions, as a long-term selective pressure of Hg, are the good pools of Hg-resistant bacteria. These bacteria, being highly tolerant to multiple heavy metals, play the important role in biogeochemistry. Moreover, dissemination of their antibiotic resistance genes among the pathogenic bacteria induce a significant risk for medical treatment of infectious diseases.

These situations enforce an urgent call for regeneration of water in Delhi and its adjoining area. For the same, water needs to be continuously monitored, and suitable remediation techniques should be applied.

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