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Sewage treatment effluents in Delhi: A key contributor of β-lactam resistant bacteria and genes to the environment

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HIGHLIGHTS

- Presence of high levels of antibiotic resistance (ARB and ARGs) in STP effluents in New Delhi.
- Significant correlations between ARB and FC as well as ARGs and integron genes were observed.
- High abundance of carbapenem resistant *Escherichia coli* and *Pseudomonas aeruginosa* in the STP effluents.
- STP having chlorination unit and anaerobic digester performed better in treating ARB and ARGs.

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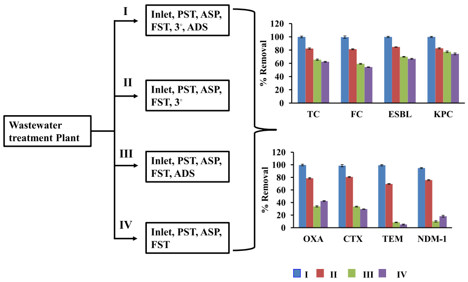
GRAPHICAL ABSTRACT



ABSTRACT

Rapid emergence of antibiotic resistance (AR) in developing countries is posing a greater health risk and increasing the global disease burden. Lack of access to safe drinking water, poor sanitation and inadequate sewage treatment facilities in these countries are fueling the problem associated with emergence of AR. Rapid proliferation of AR mediated by treated and untreated discharges from sewage treatment plants (STPs) is a prime public health concern. This study aims to understand the occurrence, fate, and routes of proliferation of carbapenem (KPC) and extended spectrum β-lactam (ESBL) resistant bacteria, and selected resistant genes in the samples collected from different unit operations in 12 STPs in New Delhi over two seasons. Strong correlation observed between faecal coliform levels and KPC ($R = 0.95$, $p = 0.005$, $n = 60$) and ESBL ($R = 0.94$, $p = 0.004$, $n = 60$) resistant bacteria levels indicates possible association of resistant bacteria with faecal matter. Different unit operations in STPs proved inefficient in treating resistant bacteria and genes present in the wastewater. However, inclusion of tertiary treatment (chlorination) unit and anaerobic digester in the present STPs resulted in better removal of AR. Significant correlations between antibiotic resistant genes (ARGs) and integron levels indicates a potential for higher rate of AR proliferation in the environment. Microbial culturing indicated the presence of clinically significant drug-resistant pathogens such as *Escherichia coli*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Acinetobacter baumannii*, *Shigella dysentery* and *Aeromonas caviae* in the STP effluents. The emergence and spread of resistant bacteria through STP effluents poses exposure risk for the residents of the city.

Abbreviations: AR, Antibiotic resistance; ESBL, Extended spectrum β-lactam; KPC, Carbapenem; ARB, Antibiotic resistant bacteria; ARG, Antibiotic resistant gene; TC, Total coliform; FC, Faecal coliform; STP, Sewage treatment plant; PST, Primary settling tank; FST, Final settling tank; AD, Anaerobic digester; PS, Sludge collected from PST; FS, Sludge collected from FST; ADS, Sludge collected from AD; ASP, Activated sludge process; PBS, Phosphate buffer solution; DO, Dissolved oxygen; TDS, Total dissolved solids; COD, Chemical oxygen demand; BOD, Biological oxygen demand; WHO, World health organization; MLD, Million litres per day; MGD, Million gallons per day.

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1. Introduction

Antibiotic resistance (AR) is a global public health concern (Petrie et al., 2015; Storteboom et al., 2010). Inappropriate and indiscriminate use of antibiotics is a known cause for the spread of AR in the environment (Kümmerer, 2003a). Indiscriminate use of antibiotics for treating different infections in humans, animals, and agricultural activities results in the development of resistance among the gut microbiota which is subsequently released into the natural environments through the fecal matter (Kümmerer, 2004). In addition, the antibiotics consumed by humans and animals are not always metabolized completely and are thus discharged with their feces (Hirsch et al., 1999). These residual antibiotics present in the sewage create selective pressure for the proliferation of resistant bacteria (Zhang and Li, 2011). Sewage carries residual antibiotics, antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARG) along with household organic pollutants into the sewage treatment plants (STPs) for treatment before getting discharged into the surface water bodies (Xu et al., 2015). The STPs play a crucial role for increasing AR proliferation in the environment. The presence of excessive nutrients and diverse bacterial communities in the STPs creates an environment appropriate for redistribution and dissemination of resistant determinants in the microbes present in the wastewater (Lapara et al., 2011; Schlüter et al., 2007). Previous studies have reported that the present STPs cannot eliminate the residual antibiotics and other stress causing persistent chemicals thus they can promote AR by creating pressure to the bacterial population (Göbel et al., 2005; Kümmerer, 2003b; Tuckfield and Lindell, 2005; Zhang and Li, 2011). A previous study has shown higher abundance of *E.coli* resistant to ciprofloxacin and cephalothin in the treated effluent compared to raw sewage (Ferreira et al., 2007), highlighting the potential risk of spread of ARB and their resistant determinants in the environment and ultimately to the human population through the discharge of treated/untreated sewage (Gómez et al., 2010). Similarly, higher abundance of resistance determinants have been reported in the aeration basin and tertiary (UV) treatment unit (Auerbach et al., 2007 Lapara et al., 2011) Higher abundance of ARB and higher frequency of development of multi-resistant microbes is observed in the treated effluent compared to the raw influent (Lapara et al., 2011; Silva et al., 2006). Additionally, high incidence of plasmid transfer has been reported in the primary and secondary clarifiers of STPs (Merlin et al., 2011; Szczepanowski et al., 2004). The co-existence of antibiotics and the bacteria in the STPs increases the possibility of development of novel resistance mechanisms (Canto and Baquero, 2008). Increase in the resistance among the microbes in surface water and ground water have also been reported and the authors suggested that it was due to the discharge of the STP effluents (McKeont et al., 1995; Harwood et al., 2000; Xu et al., 2015). Further, the association of ARGs with transposons and integrons present in the plasmid facilitates horizontal gene transfer which ultimately results in faster dissemination of resistant genes and even co-selection for other resistance genes (Mazel, 2006; Zhang et al., 2009a, b). Various studies have reported the presence of integron genes in plasmids of microbes in the STPs (Stalder et al., 2014; Tennstedt et al., 2003; Zhang et al., 2009a, b). Integrons have been detected from all the stages of STPs including the treated effluent, indicating a higher chance of faster proliferation of AR in the environment (Stalder et al., 2012). Previous studies have shown that activated sludge processes are ineffective in eliminating the bacteria harboring ARGs and the integrons (Ferreira et al., 2007;Zhang et al., 2009a, b). STPs are considered as the hotspot for horizontal gene transfer (Stalder et al., 2012) and they play a significant role in the development of multi-drug resistant microbes (Szczepanowski et al., 2004).

Efficient treatment of wastewater is among our most important defenses against the rapid dissemination of ARB and ARGs in the environment (Christgen et al., 2015). The presence of residual antibiotics, ARB and their resistant determinants in the treated wastewater is of primary concern since this is the principal route for the entry of these contaminants in the water bodies. The situation is even more alarming in the developing countries like India due to poor sanitation, lack of proper sewage treatment facilities, inadequate management and maintenance of existing treatment systems. The World Health Organization (WHO) also highlighted the lack of data on AR in India and reported AR as the most serious health risk in India (World Health Organization, 2014). Despite the need of extensive national data to drive policies for reducing AR in India, very few reports exist on AR in the country (Laxminarayan and Chaudhury, 2016). The present study aims to reduce the knowledge gaps on AR in India by evaluating the occurrence, fate, and routes of the proliferation of ARB and ARGs, in different unit operations of 12 major STPs in New Delhi, India. Different unit operations in STPs respond differently towards the fate of residual antibiotics, ARB and ARGs. Hence, it is very crucial to understand the response of ARB and ARGs to different unit operations at STPs to achieve their complete removal. Resistant microorganisms and respective ARGs which are responsible for exhibiting resistance to prominent representatives of the β-lactam group of antibiotics [extended spectrum β-lactam(ESBL) and carbapenem (KPC)] were selected for the present study since β-lactam are the highest consumed antibiotics in India (Boeckel et al., 2014). Therefore, occurrence of β-lactam resistant pathogens in the environment is more likely among ARB. Additionally, the abundance and fate of integron genes responsible for AR proliferation are analyzed to understand their role in the dissemination of ARGs.

2. Methods

2.1. Study sites and samples collection

Almost all the STPs in Delhi use aerobic activated sludge process technology with either surface aeration or submerged aeration for the treatment of sewage generated in the city. The estimated sewage generation in Delhi is about 3800 million litres per day (MLD) and the installed treatment capacity is only 2330 MLD. But only 1398 MLD of treatment capacity of the STPs are being used for treating the sewage as on an average less than 60% of the installed capacity is utilized (Central Pollution Control Board, 2013; Central Pollution Control Board, 2005). Samples were collected from different unit operations of 12 major STPs in Delhi. In most of the STPs, after the primary screening (bar screen followed by grit chamber), the influent goes through a grit chamber to the primary settling tank (PST). In PST, most of the suspended solids get separated, and liquid goes to the secondary treatment systems which are mostly aerobic biological systems. The treated effluent from secondary treatment system goes to the final settling tank (FST) for clarification. Clarified effluent either goes to the tertiary treatment system or the final discharge lines. Only 2 out 12 STPs in the present study have tertiary treatment unit (chlorination). The sludge from PST and FST goes to the anaerobic digester (AD) wherever anaerobic digester is available; otherwise the sludge from PST and FST are discharged untreated. The sludge is then used as manure in agricultural lands. The scheme of the STPs is shown in Fig. S1 and details of the STPs are given in Table S1.

Water samples were collected from the inlet and outlet of the respective stages of operations in the STPs to assess the performance of the individual units as well as the complete treatment scheme. Sludge samples were collected from PST, FST, and discharge line of AD. Triplicate water and sludge samples were collected from each unit operation. Samples were collected in both