

**STUDY ON ANTIBIOTIC RESISTANCE
TRANSMISSION AND PROLIFERATION IN
DELHI AND DEVELOPMENT OF POSSIBLE
MITIGATION STRATEGY**

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**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
BIOTECHNOLOGY**

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DELHI AND DEVELOPMENT OF POSSIBLE
MITIGATION STRATEGY**

by

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DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
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Submitted

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to the



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Certificate

This is to certify that the thesis entitled “**Study on Antibiotic Resistance Transmission and Proliferation in Delhi and Development of Possible Mitigation Strategy**” being submitted by **Ms. Manisha Lamba**, Indian Institute of Technology Delhi for the award of the degree of **Doctor of Philosophy**, is a record of the bonafide work carried out by her, which has been prepared under my supervision in conformity with the rules and regulations of the Indian Institute of Technology Delhi. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

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Manisha Lamba

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 hospitals and sewage treatment plants (STPs) is a prime public health concern. The present study aims to estimate the abundances of antibiotic resistance bacteria (ARB), antibiotic resistant genes (ARGs) and genes assisting AR proliferation (integrons) in various environmental settings (hospitals, drains, STPs and Yamuna River) in New Delhi. Samples collected from the outfalls of 12 major hospitals, 20 drains, 12 STPs and 5 sites of the river Yamuna were analyzed to estimate the abundances of coliforms, ARB [ESBL (Extended spectrum β -lactam) and carbapenem resistant bacteria], ARGs (*bla*_{CTX}, *bla*_{OXA}, *bla*_{TEM}, *bla*_{NDM-1}) and integron genes (*int1*, *int2* and *int3*) in summer and winter seasons. Higher abundances of ARB and ARGs were observed in the hospital effluents compared to the samples collected from STPs, drains and river. Inefficiency of the present STPs to remove AR, poor connectivity between drains and STPs has worsened the situation of the river Yamuna. Higher abundance and significant correlation between faecal coliform (FC) and ARB at all the sites indicates possible association of ARB with faecal matter. Strong correlation between ARGs and integrons (*int1* and *int3*) indicates a faster proliferation of AR in the environment. The abundance of ARB and ARGs were higher in winter than summer. ARB isolates obtained from the samples were dominated by gram-negative pathogenic ARB. Complete treatment of the entire sewage generated using tertiary treatment techniques could prevent the introduction of these emerging pollutants in the environment. Treatment studies in various bioreactors showed that the use of

hybrid sponge reactor along with the tertiary treatment using UV/Ozone was superior to ASP and septic tank in reducing both the conventional and emerging pollutants. Though complete removal of coliforms and ARB was observed by treating the wastewater using the hybrid sponge reactor followed by UV or ozone but substantial levels of ARGs were observed. Therefore, different advanced and effective treatment technologies need to be developed and evaluated for complete removal of ARGs along with the ARB. Combinations of different tertiary treatment techniques should also be tested to reduce the burden of AR in the environment.

सार

विकासशील देशों में एंटीबायोटिक प्रतिरोध (एआर) के तेजी से उभरने से स्वास्थ्य खतरा बढ़ रहा है और वैश्विक बीमारी का बोझ बढ़ रहा है। इन देशों में सुरक्षित पेयजल, गरीब स्वच्छता और अपर्याप्त सीवेज उपचार की सुविधा का अभाव एआर के उभरने से जुड़ी समस्या को भरना है। अस्पताल और सीवेज उपचार संयंत्रों (एसटीपी) से इलाज और अनुपचारित निर्वहन द्वारा मध्यस्थता में एआर की तेजी से प्रसार एक प्रमुख सार्वजनिक स्वास्थ्य चिंता का विषय है। वर्तमान अध्ययन में एंटीबायोटिक प्रतिरोधी बैक्टीरिया (एआरबी), एंटीबायोटिक प्रतिरोधी जीन (एआरजी) और जीन की बहुतायत का अनुमान लगाने का लक्ष्य नई दिल्ली में विभिन्न पर्यावरण सेटिंग्स (अस्पतालों, नालियों, एसटीपी और यमुना नदी) में एआर प्रसार को बढ़ावा देना है। यमुना नदी के 12 प्रमुख अस्पतालों, 20 नालियों, 12 एसटीपी और 5 स्थलों की चपेट में एकत्र किए गए नमूनों का विश्लेषण किया गया है कि कोलिफॉर्म, एआरबी [ईएसबीएल (विस्तारित स्पेक्ट्रम β -लैक्टम) और कार्बापेमेनम प्रतिरोधी बैक्टीरिया], एआरजी (ब्लैक्टिक्स, ब्लॉएक्सए, ब्लैटेम, ब्लैन्डएम -1) और ग्रीष्म और सर्दियों के मौसम में इंटीग्रॉन जीन (इंट 1, इंट 2 और इंट 3)। एसआरपी, नालियों और नदी से एकत्र किए गए नमूनों की तुलना में एआरबी और एआरजी के उच्च बहुतायत अस्पताल में प्रदूषित किए गए थे। एआर हटाने के लिए मौजूदा एसटीपी की अक्षमता, नालियों और एसटीपी के बीच खराब संपर्क के कारण यमुना नदी की स्थिति खराब हो गई है। सभी जगहों पर मल-कॉलीफार्म (एफसी) और एआरबी के बीच उच्च बहुतायत और महत्वपूर्ण सहसंबंध, मलबा पदार्थ के साथ एआरबी का संभव संघ इंगित करता है। एआरजी और इंटीग्रॉस (इंट 1 और इंट 3) के बीच मजबूत संबंध वातावरण में एआर के तेजी से प्रसार को दर्शाता है। सर्दियों में गर्मियों की तुलना में एआरबी और एआरजी की बहुतायत अधिक थी नमूने से प्राप्त एआरबी आइसोलेट्स ग्राम-नकारात्मक रोगजन्य एआरबी का वर्चस्व थे। तृतीयक उपचार तकनीकों का उपयोग करते हुए पूरे सीवेज का पूरा इलाज पर्यावरण में इन उभरते प्रदूषकों की शुरुआत को रोक सकता है। विभिन्न बायोरेक्टरों में

उपचार के अध्ययन से पता चला है कि यूवी / ओजोन का इस्तेमाल करने वाले तृतीयक उपचार के साथ संकर स्पंज रिएक्टर का उपयोग एएसपी और सेंट्रिक टैंक से बेहतर था, जो परंपरागत और उभरते प्रदूषक दोनों को कम करने में था। हालांकि कोलिफोर्म्स और एआरबी को पूरी तरह से हटाने से हाइब्रिड स्पंज रिएक्टर का इस्तेमाल करते हुए अपशिष्ट जल के इलाज के बाद यूवी या ओजोन के बाद से देखा गया था लेकिन एआरजी के पर्याप्त स्तर पर मनाया गया। इसलिए, एआरबी के साथ एआरजी को पूरी तरह से हटाने के लिए विभिन्न उन्नत और प्रभावी उपचार तकनीकों को विकसित और मूल्यांकन की जरूरत है। पर्यावरण में एआर के भार को कम करने के लिए विभिन्न तृतीयक उपचार तकनीकों के संयोजन का भी परीक्षण किया जाना चाहिए।

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Nomenclature

AD	Anaerobic Digester
ADS	Sludge collected from anaerobic digester
AR	Antibiotic Resistance
ARB	Antibiotic Resistance Bacteria
ARGs	Antibiotic Resistance Genes
ASP	Activated Sludge Process
BOD	Biological Oxygen Demand
C	Chlorination
COD	Chemical Oxygen Demand
D	Diffusers (Submerged Aeration)
DO	Dissolved Oxygen
EML	Essential medicine List
ESBL	Extended Spectrum β lactam
FC	Faecal Coliforms
FS	Sludge collected from final settling tank
FST	Final Settling Tank
HAI	Hospital Acquired Infections
HRT	Hydraulic Retention Time
<i>int1</i>	Class 1 integron
<i>int2</i>	Class 2 integron
<i>int3</i>	Class 3 integron
KPC	Carbapenem Resistant Bacteria
MIC	Minimum Inhibitory Concentration
MGD	Million Gallons per Day
MLD	Million Litres per Day
PS	Sludge collected from primary settling tank
PST	Primary Settling Tank

qPCR	Quantitative Polymerase Chain Reaction
RAS	Return Activated Sludge
SA	Surface Aeration
SRT	Sludge Retention Time
STPs	Sewage Treatment Plants
TC	Total Coliforms
TDS	Total Dissolved Solids
TPD	Tonnes per day
WHO	World Health Organization
WWTPs	Wastewater Treatment Plants