

FINDING THE LINK BETWEEN METAL POLLUTION AND ANTIBIOTIC RESISTANCE

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**DEPARTMENT OF BIOCHEMICAL ENGINEERING AND
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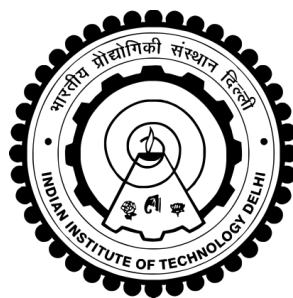
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CERTIFICATE

This is to certify that the thesis entitled, “**Finding the link between metal pollution and antibiotic resistance**” being submitted by **Ms. Sonia Gupta** to 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 our 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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Abstract

Emergence of antibiotics resistance (AR) represents a significant global health problem in today's society. Metal exposure makes not only bacteria resistant to heavy metals but also antibiotics. In this study, efforts were made to find the link between heavy metals pollution and AR in the different environment by estimating the abundance of different bacteria resistant to heavy metals and antibiotics as well as different resistant genes mediating resistance to metals and antibiotics and also the mobile genes, present in low and high metal polluted environments in U.K (Tyne River and Ouseburn River) and India (Ganga River, Yamuna River, and 2 STPs). Co-resistance studies were carried out by applying different combinations of heavy metals and antibiotics on the different pathogenic strains to estimate the role of heavy metals in the emergence and proliferation of AR. Genetic analysis was performed to find out the possible mechanism for the co-selection of heavy metals and AR in the different bacterial strains. High concentrations of heavy metals Pb, Cd, and Zn were observed in river Tyne at Featherstone and West Allen. In contrast, heavy metals such as Co, Ni, Cu, and Cr were detected in a very high concentration in the river Ganga at Kanpur and in river Yamuna. Heavy metals were detected in a low concentration in river Ouseburn and river Ganga at Rishikesh and Haridwar. The river Tyne at site Warks Burn has the lowest concentration of heavy metals as this site is relatively pristine with little or no human influences. The abundance of resistant bacteria was found higher in high polluted sites, Kanpur, at river Ganga and in river Yamuna compared to less polluted sites at river Ouseburn and river Ganga at Rishikesh and Haridwar and in high and low metal polluted sites of river Tyne. This was possibly due to a higher abundance of resistance genes in river Yamuna and river Ganga at Kanpur. The lower abundance of resistant bacteria and resistance genes in river Tyne might be due to a highly toxic environment in the river resulting from the high concentration of toxic heavy metals. The

abundance of heavy metals, resistant bacteria, as well as resistant genes, was observed higher in STPs. The higher abundance of resistant bacteria and resistance genes, along with a high concentration of heavy metals at Kanpur and in river Yamuna as well as in STPs, showed the association of heavy metals with the abundance of resistant bacteria and resistance genes. The network analysis indicates a significantly positive correlation between heavy metals concentration and abundance of resistant bacteria and resistance genes as well as between MRB and ARB, MRGs and ARGs, MRB and ARGs, and ARB and MRGs suggesting the co-occurrence of metal and AR in the environment. The abundance of pathogenic MRB, belonging to genera *Pseudomonas*, *Acinetobacter*, *Aeromonas*, *Bacillus*, and members of family Enterobacteriaceae, was observed higher in high polluted sites compared to low polluted sites. Co-resistance studies revealed that heavy metals tend to induce AR in different bacterial strains. Heavy metal Cu showed maximum tendency in inducing resistance against all the antibiotics with Zn inducing resistance to meropenem, erythromycin, ciprofloxacin, and ESBL; Pb to meropenem, ciprofloxacin, and ESBL; Ni to ciprofloxacin, erythromycin, and tetracycline; Co to ciprofloxacin and erythromycin; Cd to meropenem and ciprofloxacin and Cr to tetracycline. The genetic analysis indicates that the co-selection of heavy metals and AR in the different bacterial strains might be due to the close association of different MRGs and ARG on the plasmid DNA of bacterial strains (co-resistance) as well as due to the dominating efflux mechanism for resistance to both heavy metals and antibiotics.

सार

एंटीबायोटिक्स प्रतिरोध (एआर) का उभरना आज में एक महत्वपूर्ण वैश्विक स्वास्थ्य समस्या का प्रतिनिधित्व करता है समाज। धातु जोखिम न केवल बैक्टीरिया को भारी धातुओं के लिए प्रतिरोधी बनाता है, बल्कि एंटीबायोटिक भी बनाता है। में इस अध्ययन में, भारी धातुओं के प्रदूषण और एआर के बीच की कड़ी को खोजने का प्रयास किया गया भारी धातुओं के प्रतिरोधी विभिन्न जीवाणुओं की प्रचुरता का अनुमान लगाकर विभिन्न वातावरण और एंटीबायोटिक्स के साथ-साथ धातुओं और एंटीबायोटिक्स के प्रतिरोध में मध्यस्थता करने वाले विभिन्न प्रतिरोधी जीन और भी मोबाइल जीन, ब्रिटेन में कम और उच्च धातु प्रदूषित वातावरण में मौजूद (टाइन नदी और नदी) और भारत (गंगा नदी, यमुना नदी, और 2 एसटीपी)। सह-प्रतिरोध पर भारी धातुओं और एंटीबायोटिक दवाओं के विभिन्न संयोजनों को लागू करके अध्ययन किया गया विभिन्न रोगजनक उपभेदों के उद्भव और प्रसार में भारी धातुओं की भूमिका का अनुमान लगाने के लिए या चलो। के सह-चयन के लिए संभावित तंत्र का पता लगाने के लिए आनुवंशिक विश्लेषण किया गया था भारी बैक्टीरिया और विभिन्न जीवाणु उपभेदों में ए.आर. भारी धातुओं की उच्च सांद्रता पीबी, सी.डी, और जन को फेदरस्टोन और वेस्ट एलन में टाइन नदी में देखा गया था। इसके विपरीत, भारी धातु जैसे कि सह, नी, घन, और सीआर कानपुर में गंगा नदी में बहुत अधिक मात्रा में पाए जाते थे और यमुना नदी में। ओउसेबुरं और नदी में कम सांद्रता में भारी धातुओं का पता लगाया गया ऋषिकेश और हरिद्वार में गंगा नदी। साइट वाक्स बर्न में टाइन नदी सबसे कम है इस साइट के रूप में भारी धातुओं की सांद्रता अपेक्षाकृत कम या कोई मानव प्रभाव नहीं है। गंगा नदी में उच्च प्रदूषित स्थलों, कानपुर में प्रतिरोधी बैक्टीरिया की प्रचुरता पाई गई और नदी में कम प्रदूषित स्थलों की तुलना में यमुना नदी में और ऋषिकेश में गंगा नदी और हरिद्वार और टाइन नदी के उच्च और निम्न धातु प्रदूषित स्थलों में। यह संभवतः एक के कारण था कानपुर में यमुना नदी और गंगा नदी में प्रतिरोध जीनों की अधिकता है। कम टाइन नदी में प्रतिरोधी बैक्टीरिया और प्रतिरोध जीन की बहुतायत अत्यधिक विषाक्त होने के कारण हो सकती है नदी में वातावरण विषाक्त भारी धातुओं की उच्च सांद्रता के परिणामस्वरूप होता है। भारी धातुओं, प्रतिरोधी बैक्टीरिया, साथ ही साथ प्रतिरोधी जीन की बहुतायत अधिक देखी गई एसटीपी। एक उच्च के साथ प्रतिरोधी बैक्टीरिया और प्रतिरोध जीन की अधिकता कानपुर और यमुना नदी में और साथ ही एसटीपी में भारी धातुओं की सांद्रता ने दिखाया प्रतिरोधी बैक्टीरिया और प्रतिरोध जीन की प्रचुरता के साथ भारी धातुओं का जुड़ावा नेटवर्क विश्लेषण भारी धातुओं एकाग्रता के बीच एक महत्वपूर्ण सकारात्मक सहसंबंध को इंगित करता है और प्रतिरोधी बैक्टीरिया और प्रतिरोध जीन की बहुतायत के साथ-साथ एमआरबी और एआरबी, एमआरजी के बीच और एआरजी, एमआरबी और एआरजी, और एआरबी और एमआरजी धातु और एआर की सह घटना का सुझाव देते हैं। पर्यावरण में। रोगजनक एमआरबी की बहुतायत, जेने *स्यूडोमोनास* से संबंधित है, *एसिनेटोबैक्टर*, *एरोमोनस*, *बेसिलस* और परिवार *एंटेरोबैक्टीरिया* के सदस्य देखे गए कम प्रदूषित साइटों की तुलना में उच्च प्रदूषित साइटों में उच्च। सह-प्रतिरोध अध्ययनों से पता चला है कि

भारी धातुएँ विभिन्न जीवाणु उपभेदों में एआर को प्रेरित करती हैं। भारी धातु घन अधिकतम दिखाया जन उत्प्रेरण प्रतिरोध के साथ सभी एंटीबायोटिक दवाओं के खिलाफ प्रतिरोध उत्प्रेरण की प्रवृत्ति मेरोपेनेम, एरिथ्रोमाइसिन, सिप्रोफ्लोक्सासिन, और ईएसबीएल; पीबी से मेरोपेनेम, सिप्रोफ्लोक्सासिन, और ईएसबीएल; नी से सिप्रोफ्लोक्सासिन, एरिथ्रोमाइसिन और टेट्रासाइक्लिन; सिप्रोफ्लोक्सासिन और एरिथ्रोमाइसिन के लिए सह; को सी.डी. मेरोपेनेम और सिप्रोफ्लोक्सासिन और सीआर से टेट्रासाइक्लिन। आनुवंशिक विश्लेषण इंगित करता है कि सह-अलग-अलग बैक्टीरियल उपभेदों में भारी धातुओं और एआर का चयन बंद होने के कारण हो सकता है बैक्टीरिया के उपभेदों के प्लास्मिड डीएनए (सह-प्रतिरोध) पर विभिन्न एमआरजी और एआरजी का सहयोग साथ ही भारी धातुओं और दोनों के प्रतिरोध के लिए वर्चस्व वाले फ्लक्स तंत्र के कारण एंटीबायोटिक दवाओं।

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Nomenclature

AR	Antibiotic Resistance
ARB	Antibiotic Resistance Bacteria
ARGs	Antibiotic Resistance Genes
MRB	Metal Resistance Bacteria
MRGs	Metal Resistance Genes
MIC	Minimum Inhibitory Concentration
MGEs	Mobile Genetic Elements
TC	Total Coliforms
FC	Fecal Coliforms
ESBL	Extended Spectrum- β -Lactam
Cipro	Ciprofloxacin
Ery	Erythromycin
Tetra	Tetracycline
Mero	Meropenem
Chl	Chloramphenicol
DO	Dissolved Oxygen
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
TS	Total Solids
TSS	Total Suspended Solids
TDS	Total Dissolved Solids
FST	Final Settling Tank
PST	Primary Settling Tank
qPCR	Quantitative polymerase chain reaction
PCR	Polymerase chain reaction
WHO	World Health Organization
WWTPs	Waste Water Treatment Plants
AD	Anaerobic Digester
FS	Sludge collected from final settling tank
<i>int1</i>	Class 1 integron

<i>int2</i>	Class 2 integron
<i>int3</i>	Class 3 integron
CFU	Colony Forming Units
<i>Tn</i>	Tansposons
STP	Sewage Treatment Plants
UK	United Kingdom
DNA	Deoxy Ribonucleic Acid
MADI-TOF	Matrix Assisted Desorption Ionisation-Time of Flight