INVESTIGATION OF CLASSICAL AND NON-CLASSICAL MECHANISMS UNDERLYING ANTIMICROBIAL RESISTANCE AND VIRULENCE IN BACTERIA

DIPANNITA GHOSH

KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

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by

DIPANNITA GHOSH

Kusuma School of Biological Sciences

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DEDICATED TO

My mother,

whose love is my foundation and resilience my compass.

CERTIFICATE

This is to certify that the thesis titled "**Investigation of classical and non-classical mechanisms underlying antimicrobial resistance and virulence in bacteria**", being submitted by **Ms. Dipannita Ghosh** to the Indian Institute of Technology Delhi for the award of the degree of "Doctor of Philosophy" is a record of the bonafide research carried out by her under my supervision and guidance, and conforms to the rules and regulations of Indian Institute of Technology Delhi, India. The results prescribed in this thesis have not been submitted in part or full to any other university or institute for the award of any degree/diploma.

Prof. Vivekanandan Perumal
Professor
Kusuma School of Biological Sciences
Indian Institute of Technology Delhi
New Delhi-110016, India

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Dipannita Ghosh

ABSTRACT

Emergence of bacterial pathogens with increased drug resistance and virulence is increasingly becoming a major challenge in terms of treatment and clinical outcomes worldwide, including in South Asian countries. It has become imperative to not only monitor the prevalence and spread of these pathogenic bacteria carrying classical or well-known resistance and virulence markers, but it is also essential to explore non-classical mechanisms, such as changes in the epigenome and chromosome conformation, which can confer higher survival advantage to bacteria leading to increased pathogenic potential.

Here, we first investigated the prevalence of antimicrobial resistance genes (ARGs) in uropathogenic *E. coli* (UPEC) isolates from paediatric patients with community-acquired urinary tract infections (UTIs) presenting to community health centers at four different geographic locations across the Indian subcontinent. We found that CTX-M-type extended-spectrum beta-lactamases (ESBLs) are the most common ARGs in our communities, which mirrors the global shift to CTX-M-type ESBL from TEM- and SHV-type ESBLs. We found the high-risk clonal group, ST131, to be the most common causative pathogen of paediatric UTIs reported in this study, with majority of these clones harboring CTX-M-15 beta-lactamase. Our results also indicate the existence of high-risk clones that carry multiple beta-lactamases including NDM-5, CTX-M-15, OXA-1 and TEM-4 in Indian communities, which is quite alarming and highlights the need for increased surveillance to devise better prevention and treatment strategies.

Next, we explored the epigenetic signatures associated with the recently emergent hypervirulent pathotype of *Klebsiella pneumoniae* (hvKp). We observed that hvKp has overall higher levels of adenine (G<u>A</u>TC) and cytosine (C<u>C</u>WGG) methylation compared to its classical counterpart (cKp). Virulence genes classically recognized as genetic markers of hvKp are

hypermethylated compared to other genes not directly linked to the hypervirulent phenotype. Furthermore, we found several differentially methylated genes (DMGs) in hvKp compared to cKp, including metal ion transporters, multidrug efflux pumps, transcriptional regulators of stress response and genes associated with biofilm formation, which are known to be associated with bacterial pathogenicity. Our results indicate that hypermethylation of GATC and CCWGG motifs are unique epigenetic signatures of hvKp isolates.

Lastly, we investigated how triclosan, a widely used non-antibiotic antimicrobial, affects the chromosome structure of *Escherichia coli*. We mapped the structural landscape of wild-type and Δ*dcm E. coli* chromosomes under triclosan stress using Hi-C to identify triclosan-induced chromosomal interaction domains (CIDs). Two CIDs were common to the wild-type and Δ*dcm E. coli*, including a CID with a common boundary at *fabI* gene, which encodes the triclosan target. All mutations and structural variants under triclosan stress were observed within or in close proximity to triclosan-induced CIDs. Absence of Dcm methylation impacts short-range interactions in triclosan stress. Single-base resolution methylome maps reveal hypermethylation of adenines (G<u>A</u>TC; in wild-type and Δ*dcm*) and cytosines (C<u>C</u>WGG; in wild-type) in the two common triclosan-induced CIDs. Furthermore, global gene expression profiling identified enrichment of highly expressed genes within the two common CIDs. These findings indicate a pivotal role for spatial reorganization of the bacterial chromosome during antimicrobial stress response and provides a new molecular framework to understand antimicrobial adaptation.

Overall, our findings indicate that it is crucial to examine both classical and non-classical mechanisms regulating bacterial physiology and pathogenesis, as this can lead to the discovery of novel drug targets, diagnostic markers or improved strategies for treating bacterial infections.

थीसिस का सार

दवा प्रतिरोध और विषाणु में वृद्धि के साथ जीवाणु रोगज़नक़ों का उद्भव दक्षिण एशियाई देशों सहित दुनिया भर में उपचार और नैदानिक परिणामों के मामले में एक बड़ी चुनौती बनता जा रहा है। न केवल शास्त्रीय या सुविख्यात प्रतिरोध और विषाणु मार्कर वाले इन रोगजनक जीवाणुओं की व्यापकता और प्रसार की निगरानी करना अनिवार्य हो गया है, बल्कि गैर-शास्त्रीय तंत्रों का पता लगाना भी आवश्यक है, जैसे कि एपिजेनोम और क्रोमोसोम संरचना में परिवर्तन, जो बैक्टीरिया को अधिक जीवित रहने का लाभ दे सकता है जिससे रोगजनक क्षमता में वृद्धि हो सकती है।

यहां, हमने सबसे पहले भारतीय उपमहाद्वीप में चार अलग-अलग भौगोलिक स्थानों पर सामुदायिक स्वास्थ्य केंद्रों में आने वाले सामुदायिक-अधिग्रहित मूत्र पथ संक्रमण (UTIs) वाले बाल रोगियों से यूरोपैथोजेनिक *एस्चेरिचिया कोलाई* (*Escherichia coli*) आइसोलेट्स में रोगाणुरोधी प्रतिरोध जीन (ARGs) की व्यापकता की जांच की। हमने पाया कि सीटीएक्स-एम-प्रकार (CTX-M-type) विस्तारित-स्पेक्ट्रम बीटा-लैक्टामेस (ESBLs) हमारे समुदायों में सबसे आम एआरजी (ARGs) हैं, जो टीईएम- (TEM-) और एसएचवी- (SHV-) प्रकार ईएसबीएल (ESBLs) से सीटीएक्स-एम-प्रकार (CTX-M-type) ESBLs में वैश्विक बदलाव को दर्शाते हैं। हमने उच्च-जोखिम वाले क्लोनल समूह ST131 को इस अध्ययन में रिपोर्ट किए गए बच्चों से जुड़े UTIs का सबसे आम प्रेरक रोगज़नक़ पाया, इन क्लोनों में से अधिकांश में CTX-M-15 बीटा-लैक्टामेज़ मौजूद है। हमारे परिणाम उच्च-जोखिम वाले क्लोनों के अस्तित्व का भी संकेत देते हैं जिनमें कई बीटा-लैक्टामेस जैसे कि NDM-5, CTX-M-15, OXA-1 और TEM-4 ESBLs एक साथ मौजूद हैं जो काफी चिंताजनक है और भारत में बेहतर रोकथाम और उपचार रणनीति तैयार करने के लिए बढ़ी हुई निगरानी की आवश्यकता पर प्रकाश डालता है।

इसके बाद, हमने *क्लेबसिएला निमोनिया* (*Klebsiella pneumoniae*) के हाल ही में उभरे हाइपरविरुलेंट पैथोटाइप (hvKp) से जुड़े एपिजेनेटिक मार्करों की खोज की। हमने देखा कि hvKp में इसके शास्त्रीय

समकक्ष (cKp) की तुलना में एडेनिन (G<u>A</u>TC) और साइटोसिन (C<u>C</u>WGG) मिथाइलेशन का समग्र स्तर उच्च है। hvKp के आनुवंशिक मार्करों के रूप में शास्त्रीय रूप से पहचाने जाने वाले विषाणु जीन अन्य जीन (जो सीधे हाइपरविरुलेंट फेनोटाइप से जुड़े नहीं होते हैं) की तुलना में हाइपरमेथिलेटेड होते हैं। इसके अलावा, हमें सीकेपी की तुलना में एचवीकेपी में कई विभेदित मिथाइलेटेड जीन (DMGs) मिले, जिनमें धातु आयन ट्रांसपोर्टर, मल्टीड्रग एफ्लक्स पंप, तनाव प्रतिक्रिया के ट्रांसक्रिप्शनल नियामक और बायोफिल्म गठन से जुड़े जीन शामिल हैं, जिन्हें जीवाणु रोगजनन से जुड़ा माना जाता है। हमारे परिणामों से संकेत मिलता है कि GATC और CCWGG रूपांकनों का हाइपरमेथिलेशन hvKp आइसोलेट्स के अद्वितीय एपिजेनेटिक हस्ताक्षर हैं।

अंत में, हमने जांच की कि ट्राइक्लोसन, एक व्यापक रूप से इस्तेमाल किया जाने वाला गैर-एंटीबायोटिक रोगाणुरोधी, *एस्चेरिचिया कोलाई* (*E. coli*) की गुणसूत्र संरचना को कैसे प्रभावित करता है। हमने ट्राइक्लोसन से जुड़े क्रोमोसोमल इंटरेक्शन डोमेन (CIDs) की पहचान करने के लिए Hi-C का उपयोग करके ट्राइक्लोसन के कारण तनाव के तहत मूल और Δ*dcm E. coli* क्रोमोसोम के संरचनात्मक परिदृश्य को मैप किया। दो CIDs मूल और Δ*dcm E. coli* के लिए समान थे, जिसमें से एक CID की सीमा पर *fabI* जीन मिला हैं। *fabI* जीन ट्राइक्लोसन के ड्रग-लक्ष्य को एनकोड करता है। ट्राइक्लोसन के कारण होने वाले सभी डीऐनए उत्परिवर्तन (म्यूटेशन) और संरचनात्मक वेरिएंट ट्राइक्लोसन से जुड़े CIDs के भीतर या उनके करीब देखे गए। डीसीएम मिथाइलेशन की अनुपस्थिति ट्राइक्लोसन द्वारा तनाव में कम दूरी के क्रोमोसोमल इंटरैक्शन को प्रभावित करती है। सिंगल-बेस रिज़ॉल्यूशन मिथाइलोम मानचित्र पहले चर्चा की गयी दो समान ट्राइक्लोसन-कारण CIDs में एडेनिन (G<u>A</u>TC: मूल और Δ*dcm E. coli* में) और साइटोसिन (C<u>C</u>WGG: केवल मूल *E. coli* में) के हाइपरमेथिलेशन को दिखाते हैं। जीन अभिव्यक्ति प्रोफाइलिंग ने ट्राईक्लोसन से जुड़े दो समान CIDs के भीतर अत्यधिक व्यक्त जीनों के संवर्धन की पहचान की। ये निष्कर्ष रोगाणुरोधी दवाओं के कारण होने वाले जीवाणु तनाव के दौरान जीवाणु गुणसूत्र के स्थानिक

पुनर्गठन के लिए एक महत्वपूर्ण भूमिका दर्शाते हैं और रोगाणुरोधी अनुकूलन की एक नई आणविक समझ प्रदान करते हैं।

कुल मिलाकर, हमारे निष्कर्ष बताते हैं कि बैक्टीरियल फिजियोलॉजी और रोगजनन को विनियमित करने वाले शास्त्रीय और गैर-शास्त्रीय दोनों तंत्रों की जांच करना महत्वपूर्ण है, क्योंकि इससे बैक्टीरिया संक्रमण के इलाज के लिए उपन्यास दवा लक्ष्य, नैदानिक मार्कर या बेहतर रणनीतियों की खोज हो सकती है।

Table of Contents

List of Figures

List of Tables

Abbreviations

AAC	Aminoglycoside Acetyltransferases
APHs	Aminoglycoside Phosphotransferases
ACT	AmpC Type β-Lactamases
ARG	Antibiotic Resistance Gene
AST	Antibiotic Susceptibility Testing
ARMA	Antimicrobial Resistance Mapping Application
CPS	Capsule Synthesis
CTX-M	Cefotaxime-Hydrolysing B-Lactamase
CIDs	Chromosomal Interaction Domains
COGs	Clusters Of Orthologous Groups of Proteins
CHCs	Community Health Centers
Dam	Deoxyadenosine Methylase
DNA	Deoxyribonucleic Acid
DEGs	Differentially Expressed Genes
DMGs	Differentially Methylated Genes
Dcm	DNA Cytosine Methyltransferase
DND	DNA Degradation
Dps	DNA-Binding Protein from Starved Cells
ACP	Enoyl-Acyl Carrier Protein
ENA	European Nucleotide Archive
ESBL	Extended-Spectrum Beta-Lactamase
Fis	Factor For Inversion Stimulation
GTA	Glutaraldehyde
HU	Heat-Unstable Protein

HAC	High Accuracy
H-NS	Histone-Like Nucleoid Structuring Protein
HGT	Horizontal Gene Transfer
IS	Insertion Sequence
Indels	Insertions/Deletions
IHF	Integration Host Factor
iTOL	Interactive Tree of Life
KDE	Kernel Density Estimation
cKp	Classical Klebsiella Pneumoniae
hvKp	Hypervirulent Klebsiella Pneumoniae
KR	Knight And Ruiz
Lrp	Leucine-Responsive Regulatory Protein
LPS	Lipopolysaccharide
LB	Luria–Bertani
MFS	Major Facilitator Superfamily
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
MIC	Minimum Inhibitory Concentration
MGEs	Mobile Genetic Elements
ModFrac	Modified Fraction
MLST	Multi Locus Sequence Types
MDR	Multidrug-Resistant
NCBI	National Center for Biotechnology Information
NDM	New Delhi Metallo-B-Lactamase
NAAMs	Non-Antibiotic Antimicrobials
NAPs	Nucleoid-Associated Proteins

Ori	Origin
OPA	Orthophthalaldehyde
OMVs	Outer Membrane Vesicles
OXA	Oxacillinases
ONT	Oxford Nanopore Technologies
FFPE	Formalin-Fixed Paraffin-Embedded
AMR	Antimicrobial Resistance
CARD	Comprehensive Antibiotic Resistance Database
CDS	Coding Sequence
PBS	Phosphate Buffer Saline
TE	Tris [tris(hydroxymethyl)aminomethane] EDTA (ethylenediaminetetraacetic acid)
NAD	Nicotinamide Adenine Dinucleotide
PRPP	Phosphoribosylpyrophosphate
PMQR	Plasmid-Mediated Quinolone Resistance
PCR	Polymerase Chain Reaction
PEGEs	Putative Extra-Chromosomal Genetic Elements
Pap	Pyelonephritis-Associated Pilus
QAC	Quaternary Ammonium Compounds
R-M	Restriction-Modification
RT-qPCR	Reverse-Transcription Quantitative PCR
RNA	Ribonucleic Acid
RNAPs	RNA Polymerase Clusters
RBPs	RNA-Binding Proteins
SRA	Sequence Read Archive
ST	Sequence Type

SNPs	Single Nucleotide Polymorphisms
SMRT	Single-Molecule Real-Time
SMR	Small Multidrug Resistance
SMC	Structural Maintenance of Chromosomes
SVs	Structural Variants
SHV	Sulfhydryl Reagent Variable
TEM	Temoneira
Ter	Terminus
WHO	World Health Organization
UTIs	Urinary Tract Infections
UPEC	Uropathogenic *Escherichia coli*
VSP	Very Short Patch
WGS	Whole Genome Sequencing

Thesis outline

Investigation of classical and non-classical mechanisms underlying antimicrobial resistance and virulence in bacteria

- Chapter 1: Review of literature
- Chapter 2: To perform genomic characterization of uropathogenic *E. coli* isolates from paediatric patients presenting with community-acquired urinary tract infections
- Chapter 3: To perform comparative methylome analysis of classical and hypervirulent *Klebsiella pneumoniae* clinical isolates
- Chapter 4: To investigate the effect of triclosan on the higher-order chromosome structure of *E. coli* and its association with changes in the *E. coli* genome, methylome and transcriptome profiles
- Chapter 5: Summary
- Future directions