Presentations

&

Publications

List of Conferences and Presentations:

1. **Suraj Shukla**, Devarshi Gajjar (2023) Whole genome sequencing based surveillance study of virulence factors in Indian *Klebsiella pneumoniae* genomes. **Poster** presented in Two Day International Conference on 'Microbial Odyssey: Converging Biotechnology and Industry' and '6th Prof. V. V. Modi Memorial Lecture' during 28th-29th December 2023 at The M. S. University of Baroda, Vadodara, Gujarat.

2. **Suraj Shukla**, Ashutosh Bagchi, Devarshi Gajjar (2022) Diversity and distribution of *β*-lactamase genes circulating in Indian isolates of resistant *Klebsiella pneumoniae*. **Poster** presented during 'An International Conference on Antimicrobial Resistance and Microbiome under Changing Climate', held from October 10–12, 2022, at Pondicherry University, Puducherry, India.

3. **Suraj Shukla**, Devarshi Gajjar (2020) Antimicrobial resistance (AMR) profile of *Klebsiella pneumoniae* using whole genome sequencing (WGS). **Oral** presentation in the 4th Prof. V. V. Modi memorial lecture series and a one-day National Seminar on "Genomics and Metagenomics of Microbial Ecosystems" on February 10, 2020, at The M. S. University of Baroda, Vadodara, Gujarat.

List of Awards:

- Received **BEST AWARD (Gold Medal)** for the Poster Presentation entitled "Diversity and distribution of *β*-lactamase genes circulating in Indian isolates of resistant *Klebsiella pneumoniae"* during 'An International 4 Conference on Antimicrobial Resistance and Microbiome under Changing Climate' held from October 10–12, 2022, at Pondicherry University, Puducherry, India.

List of Publications:

Shukla, S.; Desai, S.; Bagchi, A.; Singh, P.; Joshi, M.; Joshi, C.; Patankar, J.; Maheshwari, G.; Rajni, E.; Shah, M.; Gajjar, D. Diversity and Distribution of β-Lactamase Genes Circulating in Indian Isolates of Multidrug-Resistant *Klebsiella pneumoniae*. *Antibiotics* 2023, *12*, 449.

Shukla, S., Upadhyaya, H., Sisodiya, P., Kosara, S., & Gajjar, D. (2023). Genotypic, phenotypic, and in silico analysis of carbapenem-resistant Klebsiella pneumoniae. *Indian Journal of Biochemistry and Biophysics (IJBB)*, *60*(9), 673-680.

Rajni, Ekadashi, **Suraj Shukla**, Swati Duggal, P. K. Khatri, and Devarshi Gajjar. "Report on carbapenemase-producing rare sequence types of *Escherichia coli* and *Enterobacter hormaechei*." *Biomedicine* 42, no. 1 (2022): 84-90.

Shukla, S., Joshi, P., Trivedi, P., Akinwotu, O., Gajjar, D. (2023). Genomic Islands in *Klebsiella pneumoniae*. In: Mani, I., Singh, V., Alzahrani, K.J., Chu, DT. (eds) Microbial Genomic Islands in Adaptation and Pathogenicity. Springer, Singapore.

Rathod, M., **Shukla, S.**, Sanapala, P. *et al.* Genetic Diversity in Antimicrobial Resistance Determinants Among Pathogenic *Pseudomonas aeruginosa* in India. *Curr Microbiol* **82**, 189 (2025). https://doi.org/10.1007/s00284-025-04174-5

Certificate of Presentation

This is to certify that

Mr. Suraj Shukla has presented a poster entitled

Whole Genome Sequencing. based surveillance..... genomes.

during the 6th **Prof. V. V. Modi Memorial lecture and Two Day International Conference on "Microbial Odyssey: Converging Biotechnology and Industry"** held on 28th and 29th December 2023 organized by **Department of Microbiology and Biotechnology Centre, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India.**

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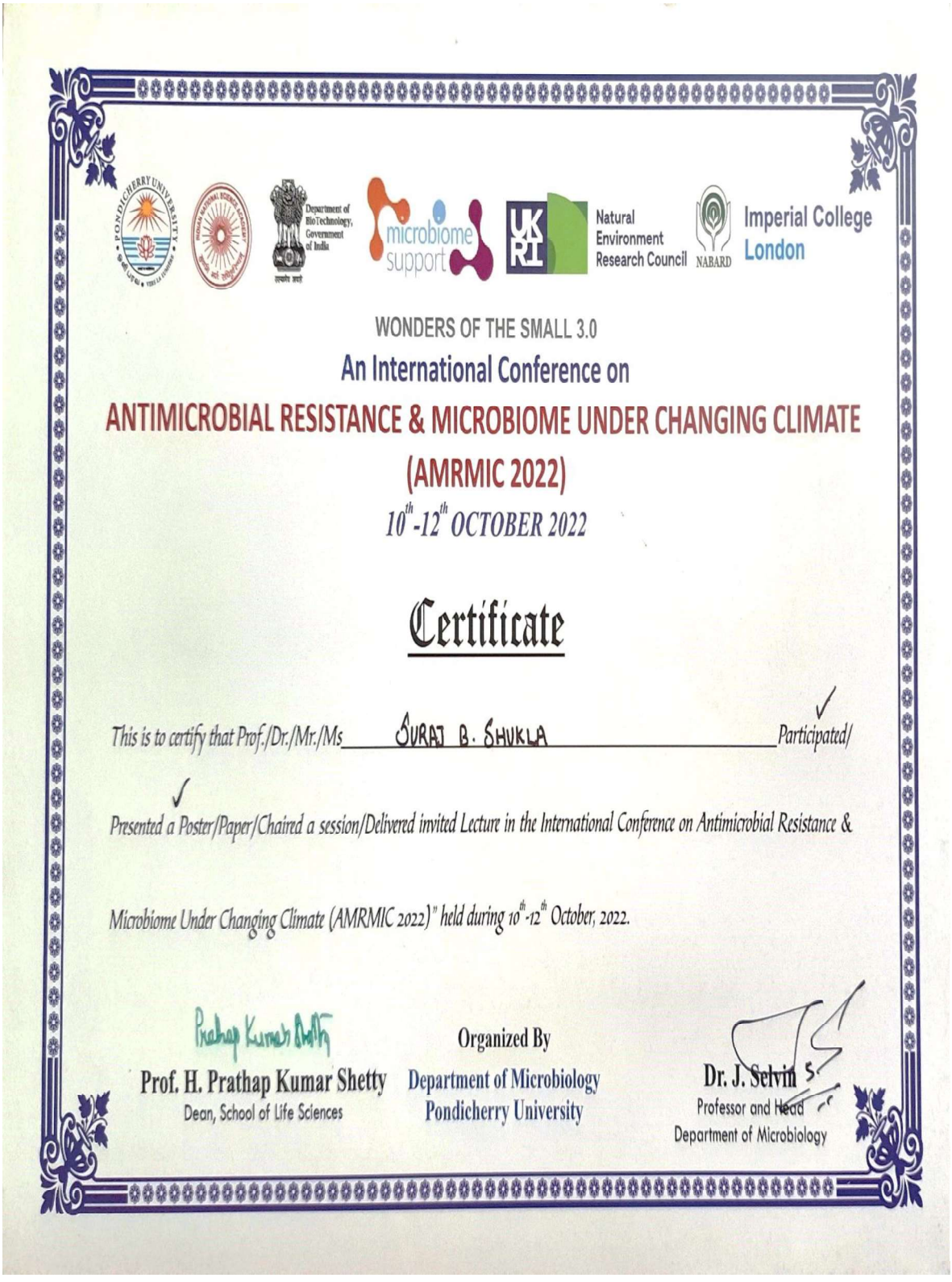

WONDERS OF THE SMALL 3.0

An International Conference on

ANTIMICROBIAL RESISTANCE & MICROBIOME UNDER CHANGING CLIMATE

(AMRMIC 2022)

10th-12th OCTOBER 2022

Certificate

This is to certify that Prof./Dr./Mr./Ms SURAJ B. SHUKLA ✓ Participated/

✓ Presented a Poster/Paper/Chaired a session/Delivered invited Lecture in the International Conference on Antimicrobial Resistance & Microbiome Under Changing Climate (AMRMIC 2022)" held during 10th-12th October, 2022.

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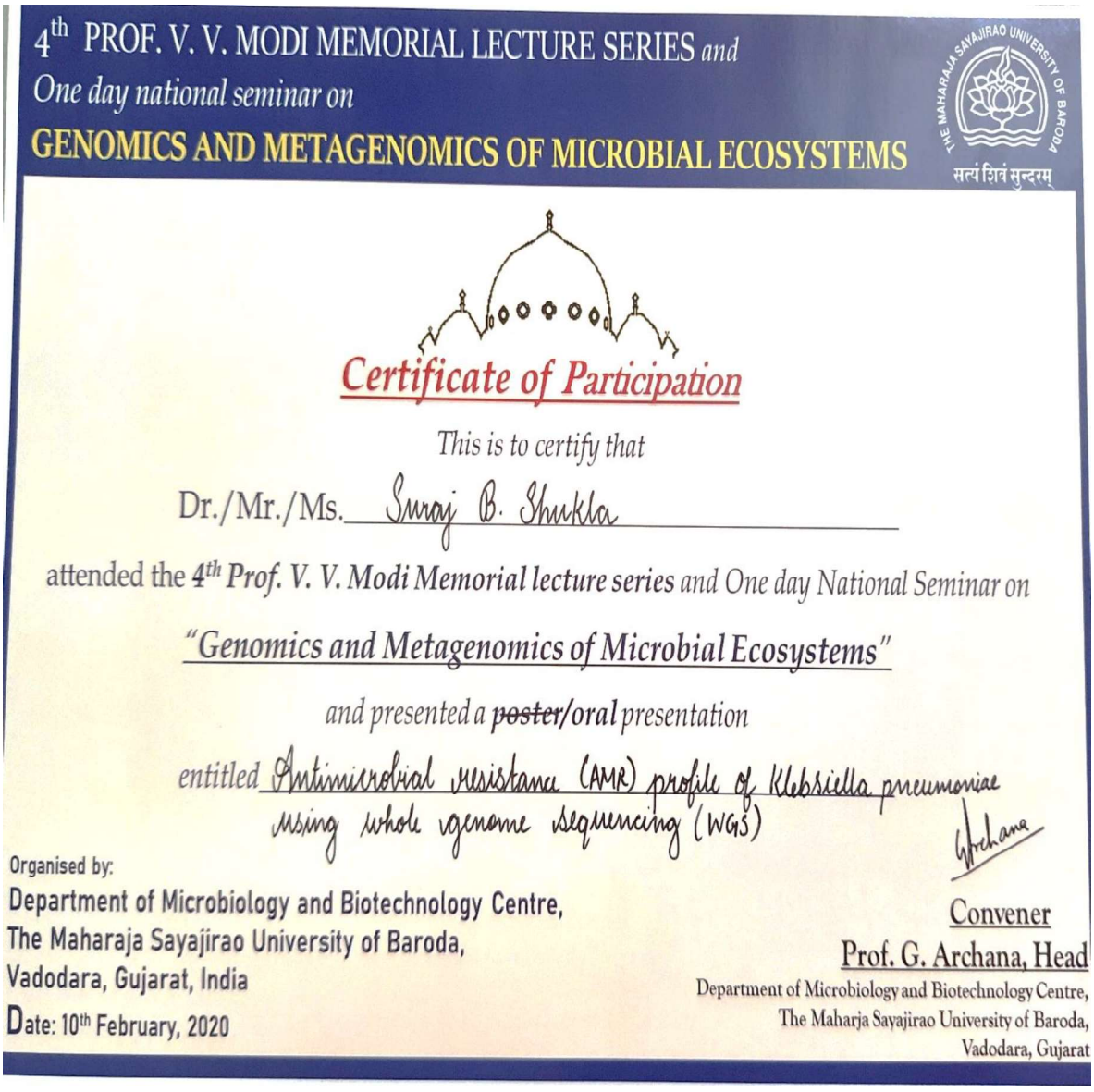

4th PROF. V. V. MODI MEMORIAL LECTURE SERIES *and*
One day national seminar on
GENOMICS AND METAGENOMICS OF MICROBIAL ECOSYSTEMS

THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA
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This is to certify that

Dr./Mr./Ms. Suraj B. Shukla

attended the *4th Prof. V. V. Modi Memorial lecture series and One day National Seminar on*

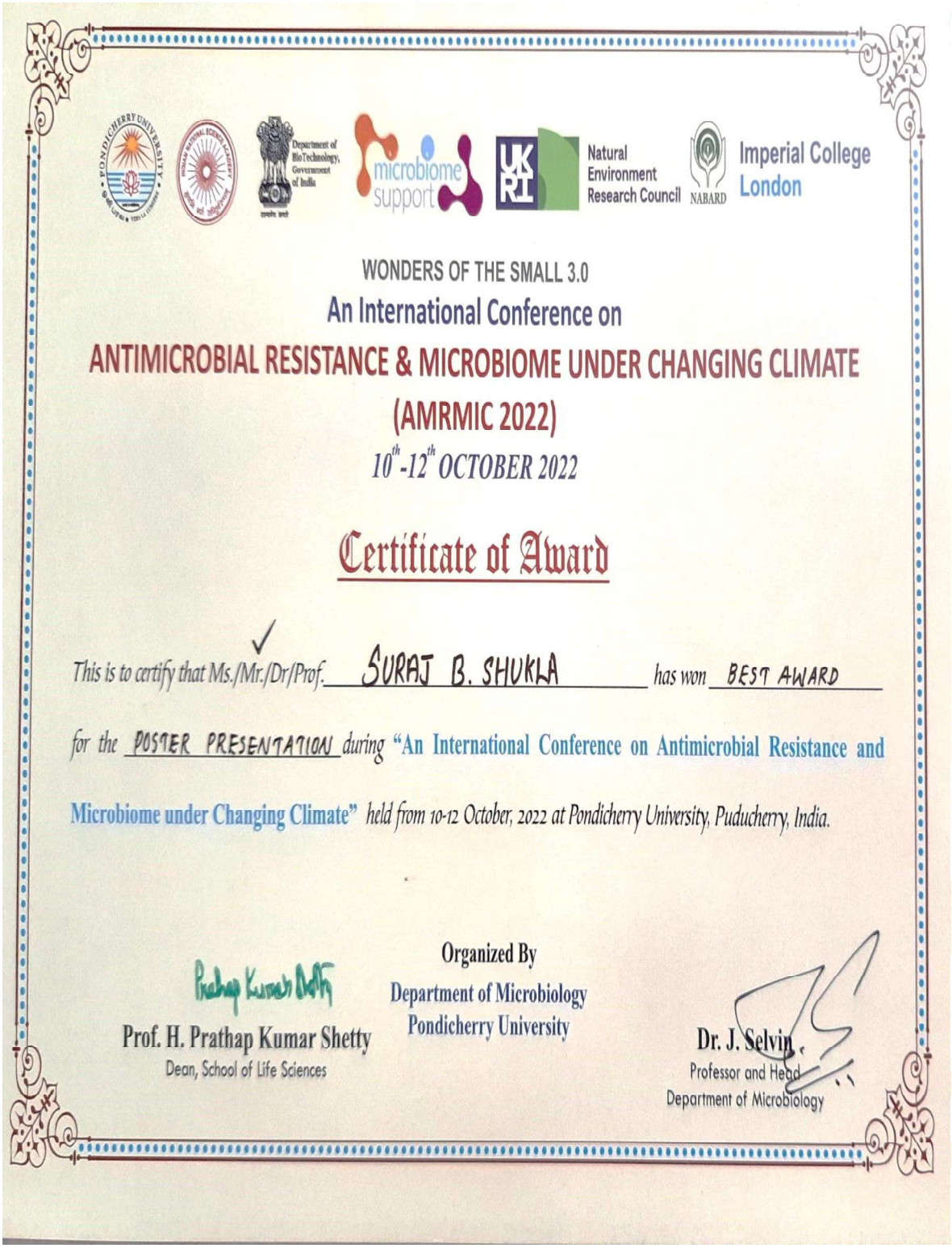
"Genomics and Metagenomics of Microbial Ecosystems"

and presented a ~~poster~~/oral presentation

entitled Antimicrobial resistance (AMR) profile of Klebsiella pneumoniae using whole genome sequencing (WGS)

Organised by:
Department of Microbiology and Biotechnology Centre,
The Maharaja Sayajirao University of Baroda,
Vadodara, Gujarat, India
Date: 10th February, 2020

Convener
Prof. G. Archana, Head
Department of Microbiology and Biotechnology Centre,
The Maharja Sayajirao University of Baroda,
Vadodara, Gujarat



WONDERS OF THE SMALL 3.0

An International Conference on

ANTIMICROBIAL RESISTANCE & MICROBIOME UNDER CHANGING CLIMATE

(AMRMIC 2022)

10th-12th OCTOBER 2022

Certificate of Award

This is to certify that Ms./Mr./Dr/Prof. SURAJ B. SHUKLA has won BEST AWARD

for the POSTER PRESENTATION during "An International Conference on Antimicrobial Resistance and Microbiome under Changing Climate" held from 10-12 October, 2022 at Pondicherry University, Puducherry, India.

Prof. H. Prathap Kumar Shetty
Dean, School of Life Sciences

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Article

Diversity and Distribution of β-Lactamase Genes Circulating in Indian Isolates of Multidrug-Resistant *Klebsiella pneumoniae*

Suraj Shukla [1,†], Siddhi Desai [1,†], Ashutosh Bagchi [2], Pushpendra Singh [3], Madhvi Joshi [4,*], Chaitanya Joshi [4], Jyoti Patankar [5], Geeti Maheshwari [6], Ekadashi Rajni [7], Manali Shah [8] and Devarshi Gajjar [1,*]

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Abstract: *Klebsiella pneumoniae* (Kp) has gained prominence in the last two decades due to its global spread as a multidrug-resistant (MDR) pathogen. Further, carbapenem-resistant Kp are emerging at an alarming rate. The objective of this study was (1) to evaluate the prevalence of β-lactamases, especially carbapenemases, in Kp isolates from India, and (2) determine the most prevalent sequence type (ST) and plasmids, and their association with β-lactamases. Clinical samples of *K. pneumoniae* (n = 65) were collected from various pathology labs, and drug susceptibility and minimum inhibitory concentrations (MIC) were detected. Whole genome sequencing (WGS) was performed for n = 22 resistant isolates, including multidrug-resistant (MDR) (n = 4), extensively drug-resistant (XDR) (n = 15), and pandrug-resistant (PDR) (n = 3) categories, and genomic analysis was performed using various bioinformatics tools. Additional Indian MDRKp genomes (n = 187) were retrieved using the Pathosystems Resource Integration Center (PATRIC) database. Detection of β-lactamase genes, location (on chromosome or plasmid), plasmid replicons, and ST of genomes was carried out using CARD, mlplasmids, PlasmidFinder, and PubMLST, respectively. All data were analyzed and summarized using the iTOL tool. ST231 was highest, followed by ST147, ST2096, and ST14, among Indian isolates. bla_{ampH} was detected as the most prevalent gene, followed by $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$. Among carbapenemase genes, $bla_{OXA\text{-}232}$ was prevalent and associated with ST231, ST2096, and ST14, which was followed by $bla_{NDM\text{-}5}$, which was observed to be prevalent in ST147, ST395, and ST1437. ST231 genomes were most commonly found to carry Col440I and ColKP3 plasmids. ST16 carried mainly ColKP3, and Col(BS512) was abundantly present in ST147 genomes. One Kp isolate with a novel MLST profile was identified, which carried $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, and $bla_{TEM\text{-}1}$. ST16 and ST14 are mostly dual-producers of carbapenem and ESBL genes and could be emerging high-risk clones in India.

Keywords: whole genome sequencing; β-lactamases; MLST; plasmid replicons; *Klebsiella pneumoniae*

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Citation: Shukla, S.; Desai, S.; Bagchi, A.; Singh, P.; Joshi, M.; Joshi, C.; Patankar, J.; Maheshwari, G.; Rajni, E.; Shah, M.; et al. Diversity and Distribution of β-Lactamase Genes Circulating in Indian Isolates of Multidrug-Resistant *Klebsiella pneumoniae*. *Antibiotics* **2023**, *12*, 449. https://doi.org/10.3390/antibiotics12030449

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1. Introduction

Klebsiella pneumoniae (Kp), a member of the *Enterobacteriaceae* family, is one of the commensal organisms in the gastrointestinal tract of healthy humans and animals [1]. Since the last two decades, Kp has gained importance because of its worldwide spread

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Genotypic, phenotypic, and *in silico* analysis of carbapenem-resistant *Klebsiella pneumoniae*

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Due to an increase in serious infections and a lack of efficient therapies, *Klebsiella pneumoniae* has recently gained more recognition. The production of carbapenemases is one of the most common strategies by which *K. pneumoniae* acquire resistance to carbapenems which is considered the last resort of antibiotics. Previously collected isolates from different clinical settings and on the basis of their genetic profile, mainly the absence and presence of single or dual carbapenemases (OXA-181, OXA-232, NDM-1, NDM-5, NDM-5+OXA-181, and NDM-1+OXA-232), mutations in porins, and efflux pumps, seven isolates (M40, M52, M39, J20, M53, M49, and M17B) were selected. Its phenotypic resistance against two carbapenem drugs (ertapenem and meropenem) was checked and we found NDM-5 followed by OXA-181 and NDM-5+OXA-181 carrying isolates showed high MIC values. Further, no significant differences were observed either in the presence of efflux pumps or mutations in porins among isolates. By molecular docking, among single amino acid differences between OXA-181 and OXA-232 and with two amino acids differences between NDM-1 and NDM-5, OXA-232 and NDM-5 showed a higher binding affinity than OXA-181 and NDM-1 with both antibiotics. It is concluded that the presence of specific carbapenemases or combinations of the same can drastically increase MIC values. The presence of NDM-5, and OXA-181, or their combinations is more fatal than NDM-1+OXA-232.

Keywords: Carbapenemases, *Klebsiella pneumoniae*, Minimum inhibitory concentration (MIC), Molecular docking, Whole genome sequencing

Resistance to carbapenem is one of the growing concerns in healthcare-associated infections caused by *Klebsiella pneumoniae* (*Kp*). The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) identified carbapenem-resistant *Enterobacteriaceae* (CRE) as one of the most significant dangers[1]. Extended-spectrum-lactamase-producing (ESBLs) *Enterobacteriaceae*, particularly *K. pneumoniae*, are the most common cause of healthcare-associated infections, and carbapenem therapy is their primary treatment[2]. Due to a limited number of drugs available for the carbapenem-resistant strains, carbapenem-resistant *Kp* and other CRE are grouped under the critical priority pathogens.

In particular, some distinct molecular mechanisms that may cause carbapenem resistance in *K. pneumoniae* could arise due to carbapenemase production, efflux pumps, and porin mutations[3]. The carbapenemases are divided into Ambler classes A (serine penicillinases), B (metallo-β-lactamases), and D (oxacillinases), which are the primary cause of carbapenem resistance in *Enterobacteriaceae*[4]. The majority of carbapenem resistance is linked to the presence of carbapenemases, the most prevalent of which are found in *Enterobacteriaceae* as Class A (*bla*KPC), Class B (*bla*IMP, *bla*NDM, *bla*VIM), and Class D (*bla*OXA-48-like) types[5]. A recent study from India reported the dominance of OXA-48-like (*bla*OXA-232 & *bla*OXA-181) and NDM type (*bla*NDM-5 & *bla*NDM-1), while other carbapenemases such as *bla*KPC, *bla*VIM, and *bla*IMP were rarely detected[6].

With the exception of monobactams, New Delhi metallo-lactamase (NDM), a carbapenemase, that has the ability to gravely endanger global health, was first discovered in a Swedish patient who had previously been hospitalised in India[7]. NDM-5, which was first discovered in *E. coli*, had Valine at position 88 and Methionine at position 154 replaced by Leucine[8]. Compared to NDM-1, NDM-5 exhibits higher

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Research Article

Report on carbapenemase-producing rare sequence types of *Escherichia coli* and *Enterobacter hormaechei*

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ABSTRACT

Introduction and Aim: Carbapenem Resistant *Enterobacteriaceae* (CRE) have emerged at an alarming rate. Multi locus sequence typing (MLST) is an important parameter for identifying drug resistant organisms. The present study was carried out for elucidating the mechanisms of CRE and MLSTs associated with CRE.

Materials and Methods: CRE (n=14) were obtained from various clinical samples and subjected to Rapidec Carba NP (CNP) test and multiplex polymerase chain reaction (M-PCR) and five isolates proceeded for whole genome sequencing (WGS). β-lactamase (*bla*) genes were analysed using Resfinder and CARD tool. Bioinformatics tools: mlplasmids, plasmid finder, mobile element finder, and Center for Genomic Epidemiology (CGE) toolbox were used.

Results: All isolates (n=14) were positive for CNP and *bla* genes using M-PCR. Isolates (J21, J22, J23, J27) were identified as *Escherichia coli* while (J34) was *Enterobacter hormaechei.* MLST showed *E. coli* isolates (J21& J22) as ST648; *E. coli* (J23) was ST940; *E. coli* (J27) was ST 2851, and *E. hormaechei* (J34) was closest to ST1325. Genes bla_{TEM}, bla_{NDM} & bla_{ampC} were found to be present in all isolates; bla_{CTX-M} was present in all *E. coli* isolates but not in *E. hormaechei*. bla_{OXA} was present in *E. coli* (J23) and in *E. hormaechei* (J34); while ESBL bla_{SFO-1} in *E. hormaechei* (J34).

Conclusion: ESBLs (bla_{TEM} & bla_{CTXM}) and metallo beta-lactamase -MBL (bla_{NDM}) cause carbapenem resistance in rare sequence types of *E. coli* while; ESBL (bla_{SFO-1}) and MBL (bla_{NDM}) cause carbapenem resistance in *E. hormaechei*.

Keywords: β-lactamases; multi drug resistance; *Enterobacteriaceae;* MLST; whole genome sequencing.

INTRODUCTION

Enterobacteriaceae are a large family of Gram-negative bacteria commonly present as normal commensal flora in the gastrointestinal (GI) tract of humans. There is a growing trend of resistance seen to commonly used antibiotics amongst these isolates (1). Carbapenems are last resort agents used to treat infections caused by organisms that are resistant to other classes of antibiotics. However, Carbapenem Resistant *Enterobacteriaceae* (CRE) are emerging at an alarming rate and pose a significant global threat (2). Infections commonly associated with CRE are due to *Klebsiella* spp., *Escherichia* spp., and *Enterobacter* spp(3). *Enterobacter cloacae* complex is considered an emerging pathogen responsible for causing the second most common nosocomial infections; while *Escherichia* spp. is reported to spread in the environment (drinking water, soil, poultry farms, etc)(4). Resistance to carbapenems occurs because of various mechanisms. These include hyperproduction of AmpC type or extended-spectrum β-lactamases (ESBLs) and carbapenemases coupled with outer membrane porin loss, hyperproduction of efflux pumps, and decreased affinity of penicillin-binding proteins. Carbapenem resistance due to the acquisition of carbapenemases is widely reported. Three types of carbapenemases are commonly identified in the *Enterobacteriaceae* family. These are mainly; Ambler class A – *Klebsiella pneumoniae* carbapenemase (KPC), class β-Metallo-beta-lactamase (MBL), and class D-Oxacillinase (OXA) types (1). Originally, carbapenem resistance was found in *K. pneumoniae* isolates harboring the KPC, however, clinical isolates of *Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae*, etc. have been identified to be carbapenem-resistant due to the presence of KPC, MBLs, and OXA(3). The emergence of carbapenem resistance among *Enterobacteriaceae* isolates due to carbapenemases is particularly worrisome because this is plasmid-mediated. These bacteria are a leading cause of

Genomic Islands in *Klebsiella pneumoniae* 13

Suraj Shukla, Purvi Joshi, Pinal Trivedi, Oluwatosin Akinwotu, and Devarshi Gajjar

Abstract

Genomic Islands (GI) of *Klebsiella pneumoniae* include integrative and conjugative elements (ICEs), prophages, integrons, and transposons belonging to a group of genetic elements transferred horizontally and have integrated into the genome of *K. pneumoniae*. Integrative and conjugative elements of *K. pneumoniae* (ICE*Kp*) are flanked by direct repeats, encode the yersiniabactin (*ybt*) locus, a mobilization locus-type 4 secretion system (T4SS), and other variable regions based on which they are classified into 14 types (ICE*Kp*1–14). Their sizes range from 75–200 kb and their chromosomal insertion site is mostly one of the four tRNA-Asn sites. Each *K. pneumoniae* genome can harbor one to six prophages; accounting for 0.1–8% of the genome. The site of phage integration could be either the tRNA or ABC transporter permease SapC. Class I integrons are the most commonly found integrons in *K. pneumoniae*. They contain three essential components for the capture of external genes: an integrase, attI site, and an outwardly oriented promoter (Pc) that controls transcription of the captured genes. Conjugative transposons (CTn) in *K. pneumoniae* are associated with resistance (Tn916 and Tn6009) and hypervirulence (Tn6497).

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