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Article

Diversity and distribution of β-lactamase genes circulating in Indian isolates of multi-drug 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,*]

1 Department of Microbiology and Biotechnology Centre, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara- 390 002, Gujarat, India
2 Amity University of Noida, Noida – 201 313, Uttar Pradesh, India
3 ICMR- National Institute of Research in Tribal Health, Jabalpur- 482 003, Madhya Pradesh, India
4 Gujarat Biotechnology Research Centre, Department of Science and Technology, Government of Gujarat, Gandhinagar- 382 011, Gujarat, India
5 Microdiagnostika Labs, Vadodara – 390 007, Gujarat, India
6 Toprani Advanced Lab Systems, Vadodara- 390 020, Gujarat, India
7 Department of Microbiology, Mahatma Gandhi University of Medical Sciences & Technology, Jaipur- 302 015, Rajasthan, India
8 Desai Metropolis Health Service Pvt. Ltd., Surat-395 001, Gujarat, India.
* Correspondence: madhvimicrobio@gmail.com (M.J.); devarshi.gajjar-microbio@msubaroda.ac.in (D.G.)
† These authors contributed equally to this work.

Abstract: *Klebsiella pneumoniae* (*Kp*) has gained prominence in the last two decades due to its global spread as a multi-drug 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, (2) determine the most prevalent sequence type (ST) & plasmids, and their association with β-lactamases. Clinical samples of *K. pneumoniae* (n=65) were collected from various pathology lab, drug susceptibility and minimum inhibitory concentrations (MIC) were detected. Whole genome sequencing (WGS) was done for (n=22) resistant isolates and WGS analysis was performed using various bioinformatics tools. Additional Indian MDR *Kp* genomes (n=187) were retrieved using Pathosystems Resource Integration Center (PATRIC) database. Detection of β-lactamase genes, location, plasmid replicons, and ST type of genomes were carried out using CARD, mlplasmids, PlasmidFinder, and PubMLST respectively. All data were analyzed and summarized using iTOL tool. ST231 was highest, followed by ST147, ST2096 & ST14 among Indian isolates. *bla*$_{\text{AmpH}}$ was detected as the most prevalent gene followed by *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1}}$. Among carbapenemase genes, *bla*$_{\text{OXA-232}}$ was prevalent and associated with ST231, ST2096 and ST14, which was followed by *bla*$_{\text{NDM-5}}$ which was observed to prevalent in ST147, ST395 &ST437. 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 novel MLST profile was identified, which carried *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{TEM-1}}$. ST16 &ST14 from this study, which is mostly dual producer of carbapenem and ESBL genes, could be emerging high-risk clones in India.

Keywords: Whole genome sequencing; β-lactamases; MLST; Plasmid replicons; *Klebsiella pneumoniae*

1. Introduction

Klebsiella pneumoniae (*Kp*), a member of the *Enterobacteriaceae* family, is one of the commensal organisms in the GI tract of healthy humans and animals [1]. Since the last two decades, *Kp* has gained importance because of its worldwide spread as a multi-drug resistant (MDR) pathogen. Further, *Kp* poses a great concern since the acquisition of plasmids and transposons carrying antibiotic resistance

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genes are not restricted to horizontal transfer to other *Kp* strains but also other enteric bacteria [1]. The broad host range plasmids (INCX3, IncA/C, IncN, and IncL) acquired by *Kp* have made it the harbinger of resistance determinants among other enterobacteria [2]. Consequently, *Kp* has been declared as an "Priority 1: CRITICAL" pathogen by world health organization (WHO) in 2017 (https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed) while it was listed in Indian priority pathogen list (IPPL) in 2021 (https://cdn.who.int/media/docs/default-source/searo/india/antimicrobial-resistance/ippl_final_web.pdf?sfvrsn=9105c3d1_6), due to the increasing antibiotic resistance (ABR), including last-resort antibiotics such as carbapenems, colistin and tigecycline. Further, Carbapenem-Resistant *Enterobacteriaceae* (CRE) are emerging at an alarming rate; hence, surveillance studies of MDR *Kp* become highly important.

The epidemiological features of carbapenem-resistant *Kp* have been reviewed previously [3]. The global presence of carbapenem resistance in *Kp* is mainly due to the presence of isolates containing class A type *β*-lactamase (*bla*$_{KPC}$); class B type metallo-*β*-lactamase (*bla*$_{NDM}$) and Class D type oxacillinase (*bla*$_{OXA-48}$) with little geographical variation. For e.g. Greece, Taiwan, Columbia, USA, Canada, and China have much more strains that produce *bla*$_{KPC}$ and *bla*$_{NDM}$ than do strains that produce *bla*$_{OXA-48}$, which are far less common in those nations. While in the Arabian Peninsula and India *bla*$_{OXA-48}$ and *bla*$_{NDM}$ producers are common while *bla*$_{KPC}$ producers are rare.

The resistance rate against carbapenems and extended-spectrum *β*-lactamases (ESBLs) has been worryingly increasing in India in the past few years. A report from India has manifested an increase in carbapenem resistance rates from 9% in 2008 to 44% in 2010 [4]. Another report from a tertiary hospital in India reported 24.6% resistance to ESBLs in 2007 [5]. In 2017, carbapenem-resistance was as high as 44% reported from India [6]. In 2018 a significant rise in resistance level of ESBLs (45.1-93.1%) were reported from India; the highest resistance of 84.9% was reported against cephalosporins [7]. A report from North India suggested 29.4% resistance rate against carbapenems [8]. Recently, the overall prevalence of multidrug-resistance (MDR: isolate that has developed resistance to at least one antimicrobial agent from three or more antimicrobial categories [9]) among Indian *Kp* isolates was reported 58.0%. Further, they also reported that tigecycline and colistin are the most effective drugs so far [10]. However, extensively drug resistant (XDR: resistant to at least one agent in all antimicrobial groups except two or fewer, i.e. bacterial isolates remain susceptible to only one or two categories [9]) isolates with co-resistance against carbapenem and colistin [6, 11] and pandrug-resistant (PDR: resistant to any antimicrobial agent [9]) isolates have also been reported recently from India [12].

Genome analysis of multidrug-resistant organisms provides us with all important information about phylogroup and the number of genes and plasmids in MDR bacteria at the same time. The multi locus sequence typing (MLST) aids in identifying region-specific sequence types (STs) and their association with AMR genes. In recent times, extensive sequencing of pure genomes of *Kp* has given huge momentum in providing a consolidated snapshot of resistance. Here we report the comparison of all whole-genome sequences of *Kp* genomes from India. We leveraged a collection of 209 genomes including genomes of our 22 isolates available at PATRIC database (https://www.patricbrc.org) and reported the most prevalent MLST, plasmid replicons, and AMR genes and its location in genomes for Indian isolates.

2. Results

We investigated the genomic diversity of *Klebsiella pneumoniae* genomes circulating in Indian subcontinent using genomes (n=209) reported from 2012 to early 2021 **(Figure 1)**. The analysis revealed that 50 different sequence types were circulating in India of which ST231 (33.49%; n=70) is the most prevalent followed by ST147 (10.04%; n=21), ST2096 (7.17%; n=15) and ST14 (5.74%; n=12) while other STs; (ST43, ST395, ST16, ST11, ST15, ST23, ST35, ST48, ST101, ST307, ST437, ST515, ST420, ST42 were found in at least 2 genomes, while rest 32 different STs were found individually) were detected in less than 10 genomes. A genome (**SAMN17492641**) had unique combinations of 7 housekeeping genes (*gapA:198, infB:1, mdh:2, pgi:1, phoE:10, rpoB:4, tonB:764*), which was submitted to

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Institut Pasteur MLST database and nidentified as ovel ST5438. Majority of the genomes have human origin (92.82%; n=194) of which (49.28%; n=103) isolates belonged to blood stream infections (BSIs) followed by respiratory tract infections (RTIs) (27.75%; n=58), urinary tract infections (UTIs) (8.61%; n=18) and central nervous system (CNS) (0.95%: n=2), while a single isolate was found for each infection such as nosocomial, wound, sepsis, implant infection and the data for 9 isolates were not found and mentioned in **Figure 1** as not available. Few of them were from environmental origin (6.69%; n=14) and the sample origin of one genome (**SAMN07713993**) was missing and mentioned in **Figure 1**.

Regarding the State wise distribution of *Klebsiella* genomes, highest number of genomes were reported from the southern states of India (63.15%; n=132) that include (Tamil Nadu n=128 and Kerala n=4) followed by Eastern states (15.13%; n=32) which include (West Bengal n=21, Assam n=10 and Odisha n=1), Western states (12.44%; n=26) which include (Gujarat n=22 and Maharashtra n=4), Northern part (7.17%; n=15) which include (New Delhi n=11, Uttar Pradesh n=2 and Punjab n= 2) and in central part of India (1.43%; n=3) were collected from Madhya Pradesh shown in **Figure 2**. The geographic location for one isolate (Biosample: **SAMEA7026324**) was not found. State wise distribution for STs mentioned in **Figure 2**, the presence of ST231 was most common among Indian states as it detected in all regions; ST147 was detected in Tamil Nadu, Gujarat, West Bengal, and Uttar Pradesh; Both ST2096, ST16, and ST42 was detected in Tamil Nadu and Gujarat; Presence of ST14 was detected in Tamil Nadu, West Bengal, Gujarat and New Delhi; ST43 was found in Tamil Nadu, New Delhi, Assam, and Uttar Pradesh; presence of ST11 was seen in Tamil Nadu, Gujarat, and Kerala; ST395, ST35, ST437, ST420 these are few STs that only belonged to Tamil Nadu; ST23 and ST101 both were detected in Tamil Nadu and West Bengal; ST15 was detected in Tamil Nadu, Gujarat, and West Bengal; ST48 was detected in Assam and West Bengal; ST515 was found in Kerala and Tamil Nadu, and ST307 was only found in Assam.

The percentage of isolates harboring the β-lactamase genes is shown in **Figure 3**. The carbapenemase genes; *bla*$_{\text{OXA-48-like}}$ (*bla*$_{\text{OXA-48}}$, *bla*$_{\text{OXA-232}}$ *&* *bla*$_{\text{OXA-181}}$) were found to be most circulating among Indian isolates, where *bla*$_{\text{OXA-232}}$ is the most prevalent carbapenemase found in (46.41%; n=97) of the total genomes and is most frequently carried by ST231 genomes (61.85%, n=60). Besides ST231, ST2096 (12.37%, n=12), ST14 (8.24%, n=8), and the remaining 18% of *bla*$_{\text{OXA-232}}$ were majorly found in ST147, ST395, ST23, and ST437 genomes. All *bla*$_{\text{OXA-232}}$ genes were located on plasmids except 2 genomes (**SAMN13440776** and **SAMN22238555**), Also 2 copies of *bla*$_{\text{OXA-232}}$ gene were detected in 2 genomes (**SAMN07690982** and **SAMN17492640**). *bla*$_{\text{OXA-181}}$ (11.48%; n=24) was found mainly in ST43, ST147& ST16. 2 genomes (**SAMN08612379** and **SAMN17492649**) had *bla*$_{\text{OXA-181}}$ on chromosome while the rest was detected on plasmids. *bla*$_{\text{OXA-48}}$ was found in only 2 genomes that belong to ST101 and both were present on plasmid. *bla*$_{\text{NDM}}$ and *bla*$_{\text{KpC}}$ were not observed to be as prevalent as *bla*$_{\text{OXA-232}}$ in India. Four variants of *bla*$_{\text{NDM}}$ were detected, namely, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-4}}$, *bla*$_{\text{NDM-5}}$, and *bla*$_{\text{NDM-7}}$. Among these, *bla*$_{\text{NDM-5}}$ (18.18%; n=38) was observed to be most prevalent followed by *bla*$_{\text{NDM-1}}$ (5.74%; n=12) while *bla*$_{\text{NDM-4}}$ & *bla*$_{\text{NDM-7}}$ each were found only in a single isolate belonging to ST11 and ST711 respectively. Total of *bla*$_{\text{NDM-5,}}$ (28.94%; n=11) was carried by ST147, one of the clinically prevalent STs found in India. Besides this, *bla*$_{\text{NDM-5}}$ was also detected in other clinically less prevalent STs; ST395, ST437, ST16, ST14 and ST101. All *bla*$_{\text{NDM-5}}$ were detected on plasmids among genomes except 2 genomes (**SAMN08612379** and **SAMN17492649**). *bla*$_{\text{NDM-1}}$ was detected mainly in ST14 and ST11. Single isolates of ST231, ST147, ST273, ST624 and ST2816 also carried *bla*$_{\text{NDM-1}}$, whereas ST231 genomes were not found to carry any of the *bla*$_{\text{NDM}}$ variants except one genome carrying *bla*$_{\text{NDM-1}}$. All *bla*$_{\text{NDM-1}}$ was detected on plasmids, while one genome (**SAMN09604002**) had 3 copies of *bla*$_{\text{NDM-1}}$ and all were present on plasmid. *bla*$_{\text{KpC-2}}$ was also found to be present only in 4 genomes of ST147 and 1 genome of ST101 and all were detected on plasmids, circulation of carbapenemase genes among Indian genomes also shown in **Figure 4**. Production of dual carbapenemase with five different combinations, *bla*$_{\text{NDM-5}}$*+bla*$_{\text{OXA-232}}$ was detected in ST147(n=4), ST437(n=3), ST2096(n=2), ST395(n=2), and ST14(n=1); *blaNDM-5+blaOXA-181* was detected in ST16(n=5) and ST147(n=4); *bla*$_{\text{NDM-5}}$*+bla*$_{\text{OXA-48}}$ was detected in ST101(n=2); *bla*$_{\text{NDM-1}}$*+bla*$_{\text{OXA-232}}$ was detected in ST14(n=3), and *bla*$_{\text{NDM-1}}$*+bla*$_{\text{OXA-181}}$ was seen in ST14(n=1), ST11(n=1), and ST42(n=1).

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Among various ESBLs, *bla*$_{CTX-M-15}$ (80.38%, n=168) was abundantly present across genomes of various STs. Majority of ST231, ST147, ST2096, ST14, ST16, ST43, ST395, ST11, ST15, ST23, ST45, ST48, ST307 and ST437 genomes carried *bla*$_{CTX-M-15}$. Only the less prevalent STs, that is ST515, ST420 & ST101 did not carry *bla*$_{CTX-M-15}$. *bla*$_{CTX-M-15}$ among genomes of different STs, detected on both plasmids and chromosomes, only genomes of ST16 had all *bla*$_{CTX-M-15}$ on plasmids. Double copy of *bla*$_{CTX-M-15}$ gene was detected in ST231 (n=9) and all were found on chromosome and one genome (**SAMN17915109**) in ST231 had triple copy of this gene, located on chromosome. While four other genomes had double copy of *bla*$_{CTX-M-15}$ gene belonged to ST15, ST23, and ST152, all were present on plasmids. Among genomes 39.7%, n=83 isolates showed co-existence of *bla*$_{CTX-M-15}$ and *bla*$_{OXA-232}$, the co-existence was mainly found in genomes of ST231 (77.14%; n=54/70) followed by ST2096 (66.66%; n=10/15), ST14 (50%; n=6/12), ST437 (100%; n=3/3) and ST23 (66.66%; n=2/3) while few genomes of ST147, ST395 & ST16 showed co-existence. Apart from *bla*$_{CTX-M-15}$ two other ESBLs *bla*$_{SHV-1}$ (37.79%; n=79) & *bla*$_{OXA-1}$ (28.70%; n=60) were also found predominant in genomes. *bla*$_{SHV-1}$ was majorly carried by genomes of ST231 (72.15%, n=57) followed by ST16 (5.06%, n=4), ST101 (3.79%, n=3), ST515 (3.79%, n=3) & ST48 (2.53%, n=2). Interestingly, we did not find any *bla*$_{SHV-1}$ in the genomes of other prevalent STs such as ST147, ST2096, ST14, ST43, ST395, ST11, ST15, ST23, ST35, ST307 and ST437. All *bla*$_{SHV-1}$ genes were found to be located on chromosomes, except one genome (**SAMN14402361**) of ST16, and one genome (**SAMN14402361**) of ST101 detected with two copies of this gene, one was on chromosome while another was on plasmid. *bla*$_{OXA-1}$ was not detected in genome of prevalent ST231, it was detected highest in ST2096 (21.66%; n=13) followed by ST14 (18.33%; n=11), ST147 (10%; n=6) & ST395 (10%; n=6). *bla*$_{OXA-1}$ was also detected in a few genomes of other prevalent STs such as ST16, ST11, ST15, ST35, ST48 and ST307. One genome of each ST2096, ST48, and ST11, and three genomes of ST14 had *bla*$_{OXA-1}$ gene on chromosome, while rest was present on plasmids among STs.

Genes of BSBLs, *bla*$_{TEM-1}$ (74.16%; n=155) was most frequently found in ST231 (42.58%, n=66) followed by ST147 (9.03%, n=14), ST2096 (7.74%, n=12), ST14 (5.80%, n=9), ST16 (4.51%, n=7), ST43 (3.87%, n=6) and ST395 (3.22%, n=5). The co-existence of *bla*$_{OXA-232}$ with *bla*$_{TEM-1}$ was found in 41.14% (n=86) genomes among different STs, while 65.55% (n=137) genomes were found to carry both, *bla*$_{CTX-M-15}$ and *bla*$_{TEM-1}$, co-occurrence of these three genes (one carbapenemase- *bla*$_{OXA-232}$, and two ESBLs- *bla*$_{CTX-M-15}$ and *bla*$_{TEM-1}$) was detected in 37.79% (n=79) of the genomes. All *bla*$_{TEM-1}$ genes among genomes were detected on plasmids, except 2 genomes of each ST231 and ST43. *bla*$_{SHV-11}$ (25.35%; n=53) was found to be circulating mainly in ST147 (39.62%; n=21) followed by ST43 (13.20%; n=7) and ST395 (7.54%; n=4). Apart from this, some other prevalent STs such as ST11, ST23, and ST437 also carried *bla*$_{SHV-11}$ in their genome, notably the most prevalent ST231 along with ST2096, ST14, and ST16 did not carry *bla*$_{SHV-11}$ gene except one genome of ST14 and ST16. *bla*$_{SHV-11}$ was found to be located on chromosomes, except **SAMN22238552** (ST16), while **SAMN10712994** had two copies of *bla*$_{SHV-11}$, of which one was present on chromosome while another was detected on plasmid.

Apart from most frequent carbapenemases, ESBLs, BSBLs, some other β-lactamases like *bla*$_{AmpH}$, which is a penicillin-binding protein that is related to *AmpC* were found highest (85.16%, n=176) across genomes, and all *bla*$_{AmpH}$ genes were found on chromosomes among genomes. *bla*$_{SHV-28}$ (13.39%, n=28) was detected in ST2096, ST14, ST15, and ST307, while no other STs had *bla*$_{SHV-28}$, and all *bla*$_{SHV-28}$ genes were detected on chromosomes, except one genome (**SAMN08612378**) of ST14. Variants of *bla*$_{SHV}$ (*bla*$_{SHV-5}$, *bla*$_{SHV-12}$, *bla*$_{SHV-25}$, *bla*$_{SHV-27}$, *bla*$_{SHV-31}$, *bla*$_{SHV-33}$, *bla*$_{SHV-36}$, *bla*$_{SHV-60}$, *bla*$_{SHV-71}$, *bla*$_{SHV-75}$, *bla*$_{SHV-187}$); *bla*$_{CTX-M}$ (*bla*$_{CTX-M-163}$, *bla*$_{CTX-M-238}$); *bla*$_{TEM}$ (*bla*$_{TEM-214}$, *bla*$_{TEM-243}$); *bla*$_{CMY}$ (*bla*$_{CMY-4}$, *bla*$_{CMY-6}$, *bla*$_{CMY-59}$) along with *bla*$_{OXA-9}$, *bla*$_{DHA-1}$, and *bla*$_{LAP-2}$ were also found in few genomes.

Regarding plasmids, a diverse set of plasmid combinations were found to be circulating in Indian genomes and different plasmid combinations were observed to be associated with specific ST (**Figure 5)**. For instance, most ST231 genomes carried Col440I and ColKP3 while few genomes of ST231 carried ColRNAI. ColRNAI was mainly associated with ST2096 and ST23. ST14 and ST2096 mainly carried ColKP3. Col(BS512) was abundantly present in ST147 genomes, while frequently not carried by ST231, ST14 & ST2096. ColpVC was seen to be associated mainly with ST43 and little with ST147 and ST437. Among prevalent STs, Col440II was carried by ST16, ST23, and ST101. Regarding IncF plasmids, different sets of IncF plasmids were observed to be associated with different ST.

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IncFIA, IncFIB(pQil), IncFII(K) and IncF(pAMA1167-NDM-5) are abundantly present in ST231 genomes, whereas ST14 harbored only IncFII(K) and IncFIB(K), which were not carried by ST231. Few STs; ST395, ST147, ST43 & ST11 were found to be associated with IncFIB(pQil). IncHI1B(pNDM-MAR) was carried mainly by ST2096, ST14 & ST43. IncFII was carried by the genomes of ST147, ST395, ST14, & ST16. IncFIB(pNDM-Mar) was detected mainly in ST2096 & ST14. IncR was observed to be strongly associated with ST147 while IncFIB(pKPHS1), IncFII(pKPX1) and ColpVC were also detected in genomes of ST147.

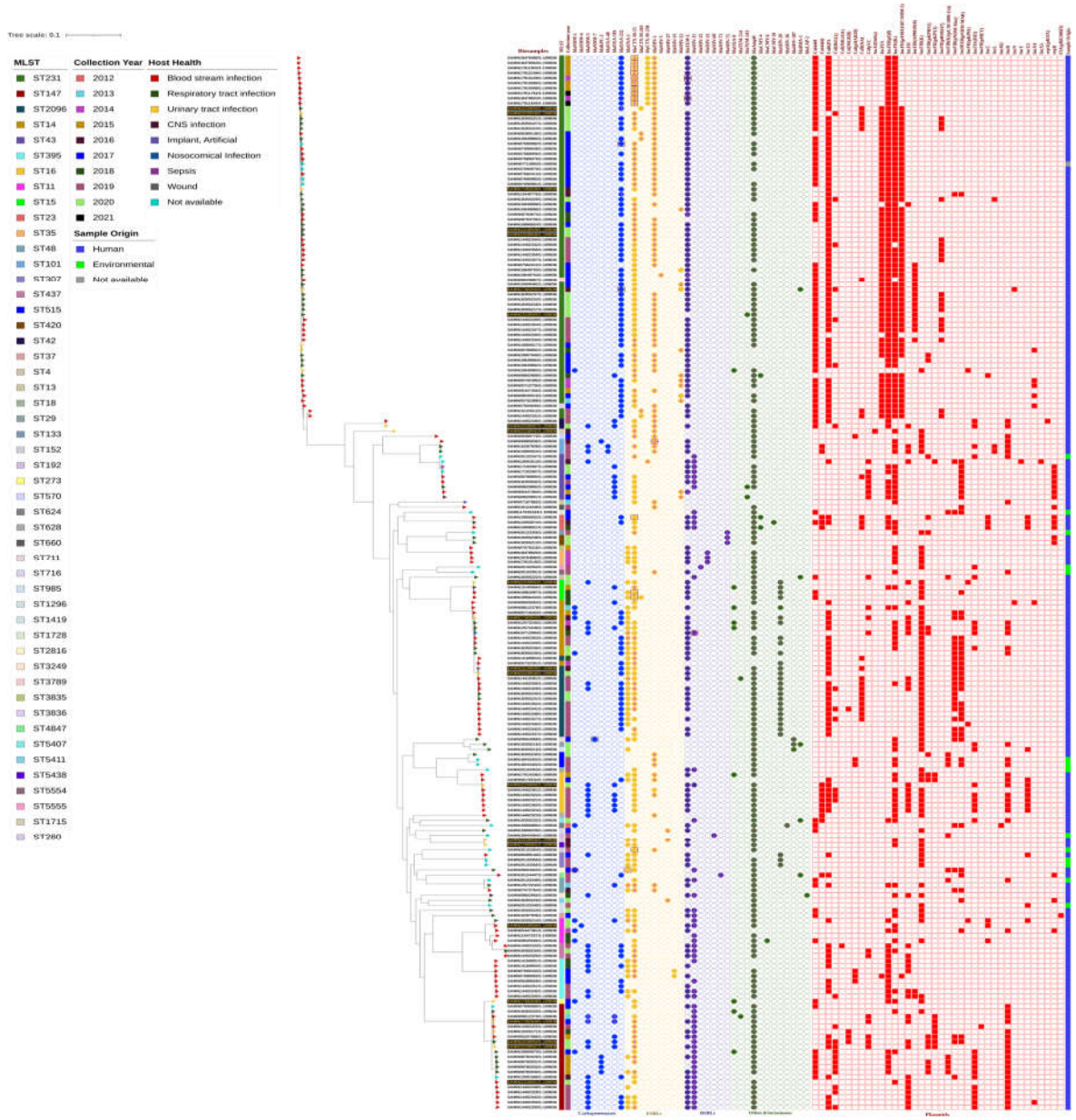

Figure 1. Genomic analysis of *β*-lactamase genes & plasmid replicons circulating in *Kp* genomes from India. Biosamples in yellow denote the lab isolates, black denotes retrieved genomes; Circles with filled colour (blue- carbapenemase, yellow- ESBLs, purple- BSBLs, and green- other *β*-lactamases) for beta-lactamase genes denote presence of genes, unfilled denotes absence of genes; C inside circle denotes location of genes on chromosome, without C denotes gene on plasmid; Red ring outside circle denotes 3 copies of gene, Dark red square outside the circle denotes 2 copies of gene, C&P denotes one is on chromosome and another is on plasmid. For plasmids, red colour filled square denotes presence of plasmid, unfilled denotes absence. iTOL was used to create this image.

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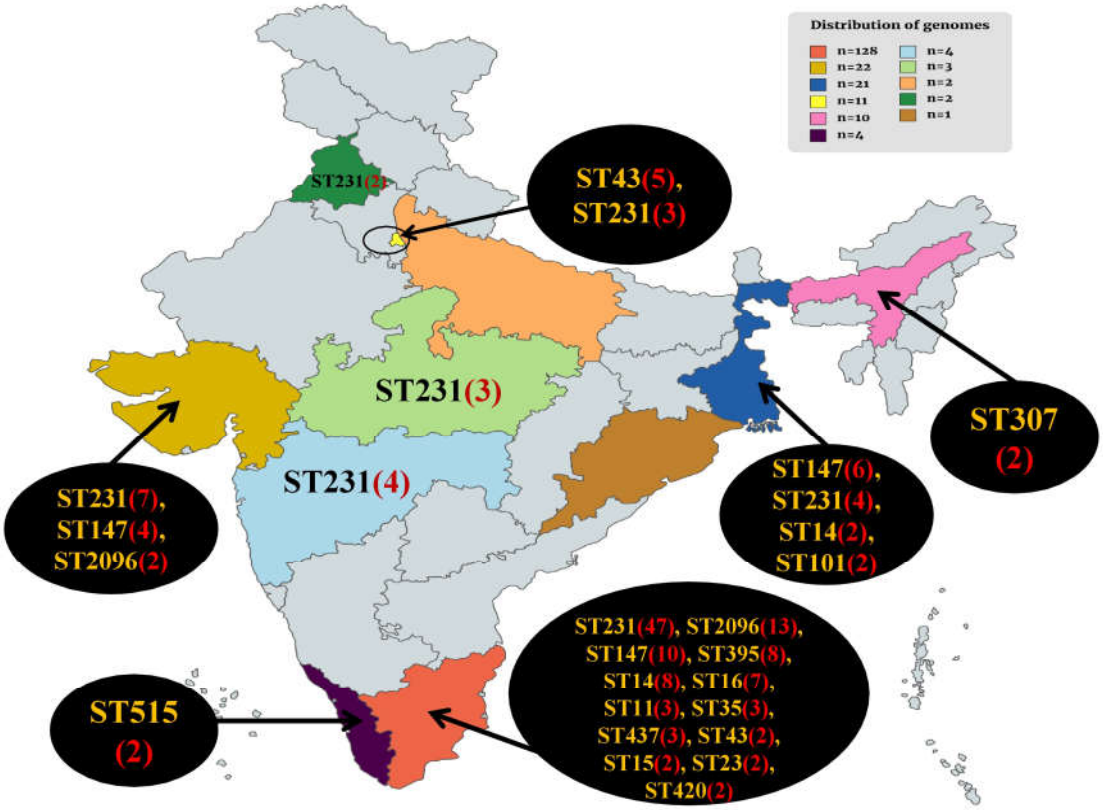

Figure 2. Geographic location of genome collection and state-wise ST distribution: ST found atleast in 2 genomes were shown in this figure. In legend (n) represents the number of genomes while colours represent to particular states. carrot red (n=128)- Tamil Nadu; mustard yellow (n=22)- Gujarat; cyan (n=21)- West Bengal; yellow (n=11)- New Delhi; pink (n=10)- Assam; purple (n=4)- Kerala, sky blue (n=4)- Maharashtra; pastel green (n=3)- Madhya Pradesh; orange (n=2)- Uttar Pradesh; green (n=2)- Punjab; brown (n=1)- Orrisa. Digits in red font & brackets denote number of STs. MapChart online tool was used to generate this image.

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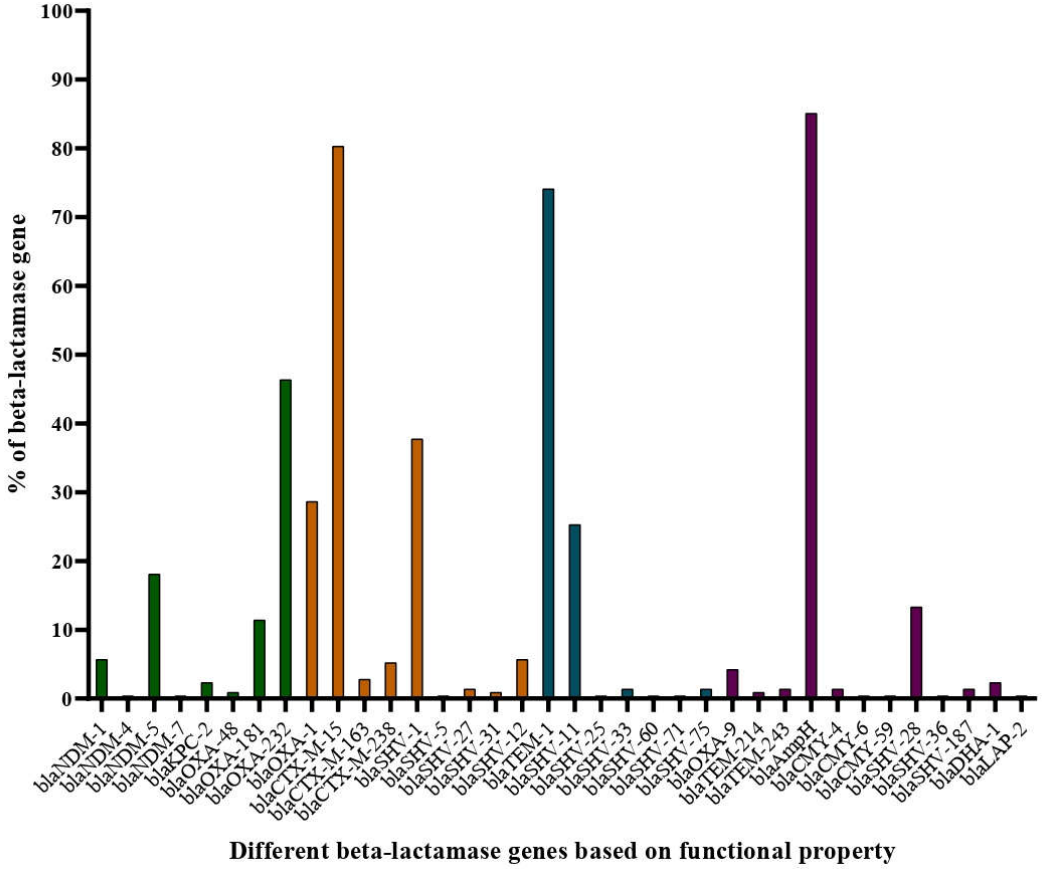

Figure 3. Percentage distribution of *β*-lactamase genes among isolates, bars in graph indicating individual *β*-lactamase gene in which bars in green indicates carbapenemase gene; orange indicates ESBLs; blue indicates BSBLs; and purple indicates other *β*-lactamase genes. GraphPad Prism 8.4.2 tool was used to create this graph.

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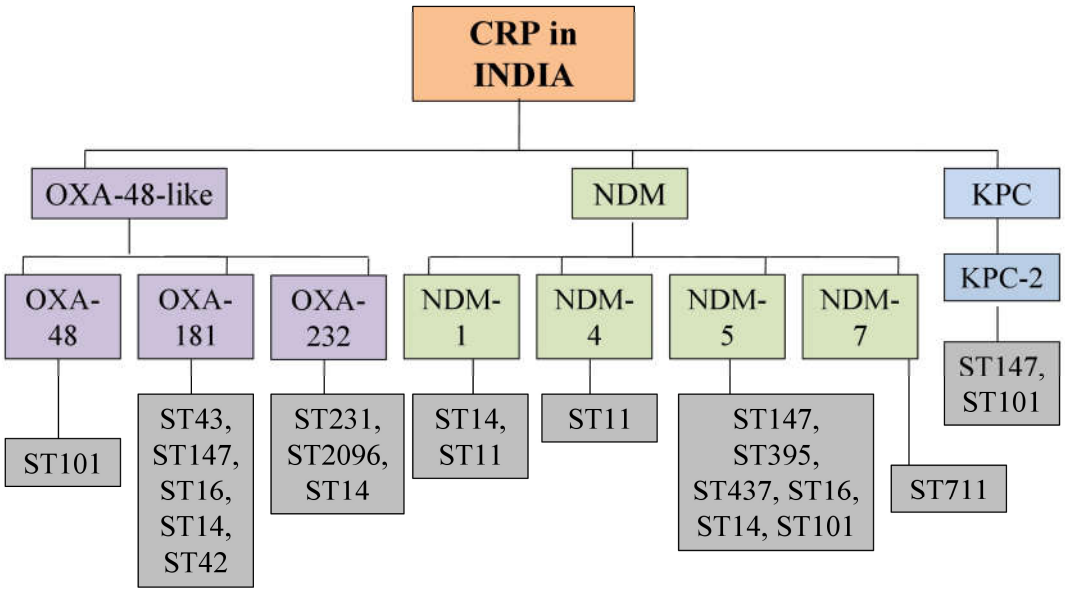

Figure 4. Distribution of carbapenemase genes circulating in India. CRP- carbapenem resistant pattern.

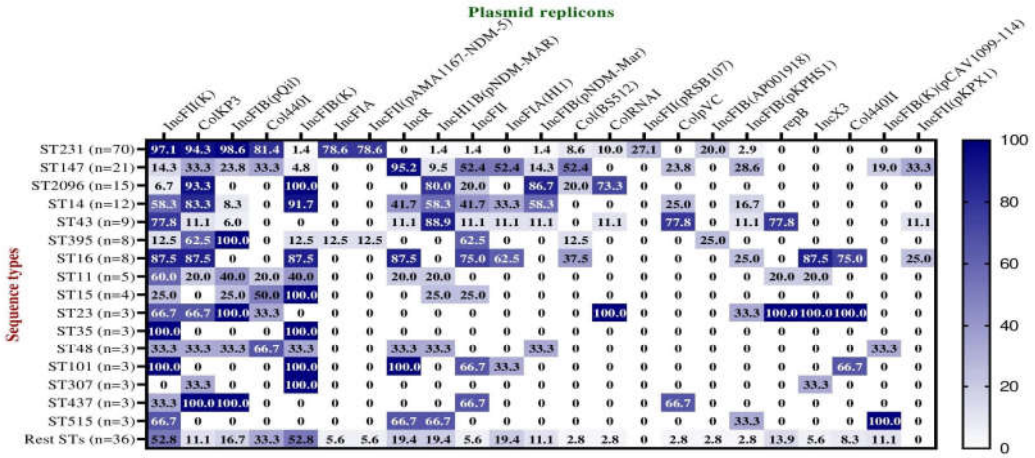

Figure 5. Heatmap of percentage distribution of plasmid replicons among STs. (n) indicates total number of particular ST. On scale; dark blue indicates 100%, white indicates 0%. GraphPad Prism 8.4.2 tool was used to generate this heatmap.

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Table 1. List of Isolates with resistance category & biosample accession number submitted in NCBI (Bioproject no: **PRJNA694019**).

Isolates	Resistance Category	Biosample accession no.	Isolates	Resistance Category	Biosample accession no.
M2	XDR	**SAMN17492638**	M50	PDR	**SAMN22238548**
M6	XDR	**SAMN17492640**	M51	XDR	**SAMN22238549**
M10	MDR	**SAMN17492641**	M52	XDR	**SAMN22238550**
M17B	XDR	**SAMN17492642**	M53	XDR	**SAMN22238551**
M39	MDR	**SAMN17492648**	M54	XDR	**SAMN22238552**
DJ	PDR	**SAMN17492649**	M55	XDR	**SAMN22238553**
M34A	MDR	**SAMN22238541**	M56	XDR	**SAMN22238554**
M40	MDR	**SAMN22238542**	M57	XDR	**SAMN22238555**
M47	PDR	**SAMN22238545**	M58	XDR	**SAMN22238556**
M48	XDR	**SAMN22238546**	M59	XDR	**SAMN22238557**
M49	XDR	**SAMN22238547**	ST1	XDR	**SAMN22238558**

3. Discussion

β-lactams are widely prescribed antibiotics for treating *Klebsiella* infections, and carbapenems are one of the last resort drugs used to treat highly-resistant ones. Public health is currently under immediate threat from the advent of *Kp* that is resistant to carbapenem. To track infections and resistance quickly and affordably, WGS is being employed more and more in research and public health labs. In the present study, 72.24% (n=151) isolates were detected with at least one carbapenemase gene (either *bla*$_{OXA}$ or *bla*$_{NDM}$) and 19.20% (n=29) isolates were detected with dual carbapenemase genes in their genome. The two major types of carbapenemase genes namely *bla*$_{OXA\text{-}48\text{-}like}$ & *bla*$_{NDM1/5}$ were detected, and these were mainly associated with ST231 and ST147 respectively. To date, 47 variants of *bla*$_{OXA\text{-}48\text{-}like}$ and 43 variants of *bla*$_{NDM}$ have been reported (https://www.ncbi.nlm.nih.gov/pathogens/refgene) so far, but fortunately only 4 variants of *bla*$_{NDM}$ and 3 variants of *bla*$_{OXA\text{-}48\text{-}like}$ were detected in *Kp* genomes circulating in India.

Recently, *Kp* genomic surveillance studies from India were reported mostly from South India [13, 14]. To the best of our knowledge, in this report *Klebsiella* genomes from Western India were included for the first time in surveillance study. Our finding regarding the most common STs; ST231, followed by ST147 corroborates with these studies however, the third most common ST we found was ST2096 while it was ST14 in the earlier studies. Next, we found *bla*$_{OXA\text{-}232}$ as the most prevalent carbapenemase and it was mostly associated with ST231. Similar observations have been reported from South and North India [14, 15] The rapid dissemination of *bla*$_{OXA\text{-}232}$ in ST231 can be correlated with the diverse set of mobile genetic elements found neighboring *bla*$_{OXA\text{-}232/181}$ which include numerous insertion sequences and transposons of Tn3 family [16].

bla$_{OXA\text{-}232}$ was 1st identified in *Kp* and *E. coli* isolated from three patients who had been transported from India to France in 2011 [17]. Since then, outbreaks of *bla*$_{OXA\text{-}232}$ *Kp* have been reported worldwide and diverse STs have been identified including ST14 & ST15 in China [18], ST16 in Thailand [19], ST147 in Germany [20], ST231 & 2096 in France [21], ST307 & ST101 in Netherlands [22], ST437 & ST395 in India from this study. *bla*$_{OXA\text{-}232}$ and *bla*$_{OXA\text{-}181}$, both belong to *bla*$_{OXA\text{-}48\text{-}like}$ group, we found 24 genomes that contained *bla*$_{OXA\text{-}181}$ and 2 genomes of ST101 with *bla*$_{OXA\text{-}48}$. *bla*$_{OXA\text{-}48}$ producing *Kp* from Europe and Africa have been found to belong to ST395 [17]. *bla*$_{OXA\text{-}181}$ is currently considered the 2nd most common global *bla*$_{OXA\text{-}48\text{-}like}$ derivative after *bla*$_{OXA\text{-}48}$ [23]. Until 2007, *bla*$_{OXA\text{-}181}$ was considered endemic in India as it was reported as the most common *bla*$_{OXA\text{-}48\text{-}like}$ carbapenemase from India. However, it is possible that *bla*$_{OXA\text{-}181}$ was misreported because of a biased pool of samples from a few centers that had molecular diagnosis facilities. From the current scenario, it is evident that *Kp* with

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bla$_{OXA-232}$ and *bla*$_{OXA-181}$ are endemic in India with a higher prevalence of *bla*$_{OXA-232}$ (as observed in the present study). *bla*$_{OXA-48}$ is currently the most common *bla*$_{OXA-48-like}$ enzyme followed by *bla*$_{OXA-181}$ globally [23]. It is interesting to find that though *bla*$_{OXA-181}$ was first reported from India its prevalence is currently less compared to *bla*$_{OXA-232}$. *bla*$_{OXA-232}$ differs from *bla*$_{OXA-181}$ by a single amino acid substitution, and the genetic environment surrounding the *bla*$_{OXA-232}$ was initially very similar to the environment surrounding *bla*$_{OXA-181}$ [24]. The similarities of the genes, transposons, and plasmids between *bla*$_{OXA-181}$ and *bla*$_{OXA-232}$ suggested a common origin and transposition followed by the subsequent evolution of *bla*$_{OXA-232}$ from *bla*$_{OXA-181}$. However, in the last decade, the genetic environment of *bla*$_{OXA-232}$ (especially from India) has attained vast diversity as suggested by the MGEs found associated with it [16]. Future studies using long-read sequencing are warranted for a greater number of isolates from India to give a detailed understanding of the exchanges occurring that lead to the successful dissemination of *bla*$_{OXA-232}$ (particularly in ST231) and not *bla*$_{OXA-181/48}$ in India.

Another important observation in the present study was the selected number (n=29) of isolates that co-harbored *bla*$_{OXA-48-like}$ and *bla*$_{NDM-1/5}$. Combination of *bla*$_{NDM-5}$+*bla*$_{OXA-232}$ was highest (n=11) among 29 dual producers. Only 2 genomes (**SAMN08612379** and **SAMN17492649**) of ST147 with *bla*$_{OXA-181}$+*bla*$_{NDM-5}$ combination found to carry both carbapenemase genes on chromosome, rest dual producers had both genes on plasmids. Genomes of ST16 in this study had *bla*$_{OXA-181}$ were seen to co-exist with *bla*$_{NDM-5}$. Dual carbapenemase producers have been also reported from South Korea [25], Italy [26], Saudi Arabia [27], Iran [28], and Algeria [29]. *bla*$_{NDM-5}$ was mostly carried by ST147 genomes that belonged to bloodstream infections from Tamil Nadu, while other genomes of ST147 were found to carry *bla*$_{KPC-2}$ that belonged to respiratory tract infection from West Bengal. Plasmid replicon IncFIB(K)(pCAV1099-114) was present only in those *bla*$_{KPC-2}$ producing isolates of ST147. Total 65.78% (n=25/38) of *bla*$_{NDM-5}$ co-existed with *bla*$_{OXA-48-like}$. Patients from South Korea, The United States, and Nepal who had traveled to India or the Indian subcontinent were reported to have *bla*$_{OXA-48-like}$ and *bla*$_{NDM-5}$ [25, 30, 31]. The presence of carbapenemase duplex (*bla*$_{OXA-48-like}$ and *bla*$_{NDM-1/5}$) among the genomes could lead to pan-carbapenem-resistant isolates and hence conditions leading to the origination of such duplexes need to be addressed for tackling them. A group evaluated the worldwide spread and genotype distribution of human clinical isolates of *bla*$_{NDM}$ producing *Kp* and found that *bla*$_{NDM}$ was present in all 5 continents and dispersed among numerous STs [32]. The lack of any dominating lineages suggests that there aren't any *bla*$_{NDM}$ positive *Kp* clones that are obviously high-risk. *bla*$_{NDM}$ positive *Kp* strains are frequently reported to be associated with ST14 [33-35]. Another prevalent sequence type in many investigations is ST11 [33, 34]. It should be noted that ST11, which is the most common ST of carbapenem-resistant *Kp* in China, mostly carries *bla*$_{KPC-2}$ rather than *bla*$_{NDM}$ [36]. Even though there isn't enough evidence to prove that ST11, ST14, ST15, and ST147 are epidemic clones that are mediating the global spread of *bla*$_{NDM}$, their prevalence across several nations calls for more research.

Although *bla*$_{NDM}$ has been found on bacterial chromosomes [12, 37], the majority of carriage occurs on plasmids, which are essential for dissemination. Several different plasmid replicon types have been identified to carry *bla*$_{NDM}$, the *Enterobacteriaceae* include 20 different replicon types of *blaNDM* carrying plasmids, including the IncC, IncB/O/K/Z, IncFIA, IncFIB, IncFIC, IncFIII, IncHI1, IncHI2, IncHI3, IncN, IncN2, IncL/M, IncP, IncR, IncT, IncX1, IncX3, IncX4, IncY, and ColE10 types [35, 37, 38]. This indicates that different plasmids have acquired *bla*$_{NDM}$ on several occasions, and it also emphasizes the worrisome the fact many different plasmids are involved in the horizontal transfer of *bla*$_{NDM}$. We also found ambiguity regarding location of *bla*$_{CTX-M-15}$ among genomes that is still unclear, while other β-lactamase genes were dominantly found on either plasmid or chromosome.

There are a few shortcomings of this study; first – the antibiotic susceptibility data of publicly available genomes was not included for the analysis as the data was not available; second – this is a biased population of isolates that were randomly selected by the respective research/clinical lab, and third – majority of the isolates belonged to South India especially Tamil Nadu. Hence there is a need for genome-based surveillance from other parts of the country. Further, the exact correlation as well

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as copy number of *bla* genes with respective plasmids/chromosomes can only be achieved by long-read sequencing and this is warranted for future work.

4. Materials and Methods

4.1. Sample Collection, Identification and Antibiotic Susceptibility Testing

Clinical Samples (n=65) were collected from various pathology labs of Gujarat, India. This included samples from urine, blood, sputum, stool, broncho-alveolar fluid (BAL), wound swab and endotracheal aspirate (ET). Antimicrobial susceptibility tests were performed according to CLSI guidelines **(https://clsi.org/meetings/susceptibility-testing-subcommittees/clsi-and-ast/).** Among all (n=65) isolates, we found 22 isolates were resistant to different classes of antibiotics and further confirmed as *Klebsiella pneumoniae* by 16S rRNA sequencing. The minimum inhibitory concentrations (MIC) of selected isolates were also determined using CLSI guidelines **(https://clsi.org/)** and (data provided in Supplementary 1) classified [9] as MDR, XDR, and PDR **(Table 1)**. To explore the resistome and plasmidome of these resistant isolates, we performed whole genome sequencing (WGS).

4.2. Genomic DNA Extraction, NGS Library Preparation and Whole Genome Sequencing

Genomic DNA for 22 isolates of *Kp* was extracted using XpressDNA Bacterial kit (MagGenome, India). Whole genome sequencing of 6 isolates (M2, M6, M10, M17B, M39 & DJ) was performed using Ion Torrent (S5-0083-GGI) NGS platform (ThermoFisher Scientific, MA, USA) and another 16 isolates (M34A, M40, M47, M48, M49, M50, M51, M52, M53, M54, M55, M56, M57, M58, M59 & ST1) were sequenced using Illumina MiSeq platform (Illumina, CA, USA). Ion Xpress™ Plus gDNA fragment library preparation kit (ThermoFisher Scientific, MA, USA) and Nextera XT DNA Library Prep Kit (Illumina, CA, USA) were used to prepare NGS libraries, respectively as per instructions given in the manual. The sequences were submitted to NCBI, accession number for each sequence is included in Table 1.

4.3. Analysis of Whole Genome Sequencing Data

Raw data was analyzed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and filtered using FastX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Filtered reads were assembled *De novo* using SPAdes (http://cab.spbu.ru/software/spades/) and quality assessment of assembled sequences was done using QUAST v5.0.2 (http://quast.sourceforge.net/quast). Assembled genomes were annotated using Prokka v1.13.3 (https://github.com/tseemann/prokka). The assembly data was deposited on NCBI (Bioproject no:**PRJNA694019**).

4.4. Retrieval and Analysis of Publicly Available Genomes of Klebsiella pneumoniae

Publicly available genome sequences of MDR *Kp* (n=187) deposited from India were downloaded from PATRIC database (https://www.patricbrc.org/) till Sept-2021. To access genomes deposited from India with antimicrobial resistance properties filters such as 'India', 'Antimicrobial resistance' & 'Genome quality:good' were used. The sequences in FASTA format, which were previously assembled and had good quality were retrieved and used for combine analysis with our samples. Biosample's information such as collection year, geographic location, sample origin, and host health for each genome was also collected from PATRIC.

MLST profile for all isolates in PATRIC database were mentioned in metadata except for few isolates, sequence types for our (n=22) isolates and remaining from PATRIC database were determined using MLST v2.0.4 of Centre for Genomic Epidemiology (CGE) tool box (https://cge.cbs.dtu.dk/services/MLST/). For detection of STs, 7 housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB, tonB*) and their allelic combinations were used to generate STs. All isolates (n=209) were analyzed to detect the *β*-lactamase genes using Resistance Gene Identifier (RGI) from The

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Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/analyze/rgi). Location of β-lactamase genes in the genome was detected using mlplasmids v2.1.0 (https://sarredondo.shinyapps.io/mlplasmids/) (Arredondo-Alonso et al., 2018). Plasmids were detected using PlasmidFinder v2.0.1 of CGE tool box (https://cge.cbs.dtu.dk/services/PlasmidFinder/). The single nucleotide polymorphism (SNP) based phylogenetic tree was generated using CSI Phylogeny 1.4 and MGH78578 (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=272620) was used as a reference strain. iTOL v6 (https://itol.embl.de/) was used to visualize the phylogeny and genomic profile of isolates.

5. Conclusions

Collectively, this is the first surveillance analysis of carbapenem resistant *Kp* Pan-India genomes, which includes WGS of 194 clinical, 14 environmental, and 1 unknown strain that were collected from various geographic locations of India between 2012 and early 2021. In this surveillance analysis of MDR *Kp* circulating in India, ST231 was found to be most predominant ST. We also identified one novel ST5438 in our isolates. In regards with carbapenemase genes *bla*$_{\text{OXA-232}}$ was the most circulating followed by *bla*$_{\text{NDM-5}}$ while *bla*$_{\text{CTX-M-15}}$ was highest among ESBLs followed by *bla*$_{\text{SHV-1}}$ and these genes can be targeted for diagnostic purposes. IncFII(K) was the most frequent plasmid replicon belonged to Inc type while ColKP3 was detected as 2nd most prevalent among Col type plasmids. ST147 is already known as high-risk clones globally, while ST16 and ST14 from this study, which is mostly dual producer of carabapenem and ESBL genes, could be emerging high-risk clones in India. Our study suggests an unmet need of future large-scale multi-regional genomic surveillance of multi-drug resistant resistant *Kp* isolates with collaboration across different states of India, especially from Nothern and western India.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Table S1: Antibiotic susceptibility and categorization (MDR, XDR, and PDR) of isolates.

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References

1. Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS microbiology reviews 2017;41(3):252-75. doi: 10.1093/femsre/fux013
2. Ho PL, Cheung YY, Lo WU, Li Z, Chow KH, Lin CH, et al. Molecular characterization of an atypical IncX3 plasmid pKPC-NY79 carrying bla KPC-2 in a *Klebsiella pneumoniae*. Current microbiology 2013;67(4):493-8. doi: 10.1007/s00284-013-0398-2
3. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. Frontiers in microbiology 2016;7:895. doi: 10.3389/fmicb.2016.00895
4. Parveen RM, Harish BN, Parija SC. Emerging carbapenem resistance among nosocomial isolates of *Klebsiella pneumoniae* in South India. Int J Pharma Bio Sci 2010;1(2):1-1.
5. Shahid M, Malik A, Akram M, Agrawal LM, Khan AU, Agrawal M. Prevalent phenotypes and antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* at an Indian tertiary care hospital: plasmid-mediated cefoxitin resistance. International journal of infectious diseases 2008;12(3):256-64. doi: 10.1016/j.ijid.2007.08.008
6. Pragasam AK, Shankar C, Veeraraghavan B, Biswas I, Nabarro LE, Inbanathan FY, et al. Molecular mechanisms of colistin resistance in *Klebsiella pneumoniae* causing bacteremia from India—a first report. Frontiers in microbiology 2017;7:2135. doi: 10.3389/fmicb.2016.02135
7. Odsbu I, Khedkar S, Lind F, Khedkar U, Nerkar SS, Orsini N, et al. Trends in resistance to extended-spectrum cephalosporins and carbapenems among *Escherichia coli* and *Klebsiella spp.* isolates in a district in Western India during 2004–2014. International Journal of Environmental Research and Public Health 2018;15(1):155. Doi: 10.3390/ijerph15010155
8. Jaggi N, Chatterjee N, Singh V, Giri SK, Dwivedi P, Panwar R, et al. Carbapenem resistance in *Escherichia coli* and *Klebsiella pneumoniae* among Indian and international patients in North India. Acta Microbiologica et ImmunologicaHungarica 2019;66(3):367-76. doi: 10.1556/030.66.2019.020
9. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection 2012;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x
10. Karki D, Dhungel B, Bhandari S, Kunwar A, Joshi PR, Shrestha B, et al. Antibiotic resistance and detection of plasmid mediated colistin resistance mcr-1 gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. Gut pathogens 2021;13(1):1-6. doi: 10.1186/s13099-021-00441-5
11. Dey S, Gaur M, Sahoo RK, Das A, Jain B, Pati S, et al. Genomic characterization of XDR *Klebsiella pneumoniae* ST147 co-resistant to carbapenem and colistin–The first report in India. Journal of Global Antimicrobial Resistance 2020;22:54-6. doi: 10.1016/j.jgar.2020.05.005
12. Rodrigues C, Desai S, Passet V, Gajjar D, Brisse S. Genomic evolution of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal group 147. Microbial genomics 2022;8(1). doi: 10.1099/mgen.0.000737
13. Sundaresan AK, Vincent K, Mohan GB, Ramakrishnan J. Association of Sequence types, Antimicrobial Resistance and Virulence Genes in Indian isolates of *Klebsiella pneumoniae*: A Comparative Genomics Study. Journal of Global Antimicrobial Resistance 2022. doi: https://doi.org/10.1016/j.jgar.2022.05.006
14. Shankar C, Jacob JJ, Sugumar SG, Natarajan L, Rodrigues C, Mathur P, et al. Distinctive mobile genetic elements observed in the clonal expansion of carbapenem-resistant *Klebsiella pneumoniae* in India. Microbial Drug Resistance 2021;27(8):1096-104. doi: https://doi.org/10.1089/mdr.2020.0316
15. Naha S, Sands K, Mukherjee S, Saha B, Dutta S, Basu S. OXA-181-like carbapenemases in *Klebsiella pneumoniae* ST14, ST15, ST23, ST48, and ST231 from septicemic neonates: coexistence with NDM-5, resistome, transmissibility, and genome diversity. Msphere 2021;6(1):e01156-20. doi: 10.1128/mSphere.01156-20
16. Shankar C, Mathur P, Venkatesan M, Pragasam AK, Anandan S, Khurana S, et al. Rapidly disseminating blaOXA-232 carrying *Klebsiella pneumoniae* belonging to ST231 in India: multiple and varied mobile genetic elements. BMC microbiology 2019;19(1):1-8. doi: 10.1186/s12866-019-1513-8
17. Potron A, Rondinaud E, Poirel L, Belmonte O, Boyer S, Camiade S, et al. Genetic and biochemical characterisation of OXA-232, a carbapenem-hydrolysing class D β-lactamase from *Enterobacteriaceae*. International journal of antimicrobial agents 2013;41(4):325-9. doi: 10.1016/j.ijantimicag.2012.11.007
18. Jia H, Zhang Y, Ye J, Xu W, Xu Y, Zeng W, et al. Outbreak of Multidrug-Resistant OXA-232-Producing ST15 *Klebsiella pneumoniae* in a Teaching Hospital in Wenzhou, China. Infection and Drug Resistance 2021;14:4395. doi: 10.2147/IDR.S329563
19. Abe R, Akeda Y, Takeuchi D, Sakamoto N, Sugawara Y, Yamamoto N, et al. Clonal dissemination of carbapenem-resistant *Klebsiella pneumoniae* ST16 co-producing NDM-1 and OXA-232 in Thailand. JAC-Antimicrobial Resistance 2022;4(4):dlac084. doi: https://doi.org/10.1093/jacamr/dlac084

20. Xanthopoulou K, Carattoli A, Wille J, Biehl LM, Rohde H, Farowski F, et al. Antibiotic resistance and mobile genetic elements in extensively drug-resistant*Klebsiella pneumoniae* sequence type 147 recovered from Germany. Antibiotics 2020;9(10):675. doi: https://doi.org/10.3390/antibiotics9100675
21. Emeraud C, Birer A, Girlich D, Jousset AB, Creton E, Naas T, et al. Polyclonal Dissemination of OXA-232 Carbapenemase–Producing *Klebsiella pneumoniae*, France, 2013–2021. Emerging Infectious Diseases 2022;28(11):2304-7.
22. Hendrickx AP, Landman F, de Haan A, Witteveen S, van Santen-Verheuvel MG, Schouls LM, et al. bla OXA-48-like genome architecture among carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in the Netherlands. Microbial Genomics 2021;7(5). doi: https://doi.org/10.1099/mgen.0.000512
23. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clinical microbiology reviews 2019;32(2):e00115-18. doi: 10.1128/CMR.00115-18
24. Pitout JD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. Clinical microbiology reviews 2019;33(1):e00102-19. DOI: 10.1128/CMR.00102-19
25. Cho SY, Huh HJ, Baek JY, Chung NY, Ryu JG, Ki CS, et al. *Klebsiella pneumoniae* co-producing NDM-5 and OXA-181 carbapenemases, South Korea. Emerging Infectious Diseases 2015;21(6):1088. doi: 10.3201/eid2106.150048
26. Avolio M, Vignaroli C, Crapis M, Camporese A. Co-production of NDM-1 and OXA-232 by ST16 *Klebsiella pneumoniae*, Italy, 2016. Future Microbiology 2017;12(13):1119-22. doi: 10.2217/fmb-2017-0041
27. Shibl A, Al-Agamy M, Memish Z, Senok A, Khader SA, Assiri A. The emergence of OXA-48-and NDM-1-positive *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. International Journal of Infectious Diseases 2013;17(12):e1130-3. doi: 10.1016/j.ijid.2013.06.016
28. Solgi H, Nematzadeh S, Giske CG, Badmasti F, Westerlund F, Lin YL, et al. Molecular epidemiology of OXA-48 and NDM-1 producing enterobacterales species at a University Hospital in Tehran, Iran, between 2015 and 2016. Frontiers in Microbiology 2020;11:936. doi: 10.3389/fmicb.2020.00936
29. Chaalal N, Touati A, Bakour S, Aissa MA, Sotto A, Lavigne JP, et al. Spread of OXA-48 and NDM-1-producing *Klebsiella pneumoniae* ST48 and ST101 in chicken meat in western Algeria. Microbial Drug Resistance 2021;27(4):492-500. doi; 10.1089/mdr.2019.0419
30. Rojas LJ, Hujer AM, Rudin SD, Wright MS, Domitrovic TN, Marshall SH, et al. NDM-5 and OXA-181 beta-lactamases, a significant threat continues to spread in the Americas. Antimicrobial agents and chemotherapy 2017;61(7):e00454-17. doi: 10.1128/AAC.00454-17
31. Sherchan JB, Tada T, Shrestha S, Uchida H, Hishinuma T, Morioka S, et al. Emergence of clinical isolates of highly carbapenem-resistant *Klebsiella pneumoniae* co-harboring blaNDM-5 and blaOXA-181 or-232 in Nepal. International Journal of Infectious Diseases 2020;92:247-52. doi: 10.1016/j.ijid.2020.01.040
32. Safavi M, Bostanshirin N, Hajikhani B, Yaslianifard S, van Belkum A, Goudarzi M, et al. Global genotype distribution of human clinical isolates of New Delhi metallo-β-lactamase-producing *Klebsiella pneumoniae*; A systematic review. Journal of Global Antimicrobial Resistance 2020;23:420-9. doi: https://doi.org/10.1016/j.jgar.2020.10.016
33. Giske CG, Fröding I, Hasan CM, Turlej-Rogacka A, Toleman M, Livermore D, et al. Diverse sequence types of *Klebsiella pneumoniae* contribute to the dissemination of bla NDM-1 in India, Sweden, and the United Kingdom. Antimicrobial agents and chemotherapy 2012;56(5):2735-8. doi: 10.1128/AAC.06142-11
34. Jain A, Hopkins KL, Turton J, Doumith M, Hill R, Loy R, et al. NDM carbapenemases in the United Kingdom: an analysis of the first 250 cases. Journal of Antimicrobial Chemotherapy 2014;69(7):1777-84. doi: https://doi.org/10.1093/jac/dku084
35. *Yoon EJ, Kang DY, Yang JW, Kim D, Lee H, Lee KJ, et al. New Delhi metallo-beta-lactamase-producing Enterobacteriaceae in South Korea between 2010 and 2015. Frontiers in microbiology 2018;9:571.* doi*: https://doi.org/10.3389/fmicb.2018.00571*
36. Qi Y, Wei Z, Ji S, Du X, Shen P, Yu Y. ST11, the dominant clone of KPC-producing *Klebsiella pneumoniae* in China. Journal of Antimicrobial Chemotherapy 2011;66(2):307-12. doi: 10.1093/jac/dkq431
37. Baraniak A, Izdebski R, Fiett J, Gawryszewska I, Bojarska K, Herda M, et al. NDM-producing *Enterobacteriaceae* in Poland, 2012–14: inter-regional outbreak of *Klebsiella pneumoniae* ST11 and sporadic cases. Journal of Antimicrobial Chemotherapy 2016;71(1):85-91. doi: 10.1093/jac/dkv282
38. Wu W, Feng Y, Tang G, Qiao F, McNally A, Zong Z. NDM metallo-β-lactamases and their bacterial producers in health care settings. Clinical microbiology reviews 2019;32(2):e00115-18. doi: 10.1128/CMR.00115-18