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Importance of Clonal Complex 258 and $IncF_{K2\text{-}like}$ Plasmids among a Global Collection of *Klebsiella pneumoniae* with bla_{KPC}

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ABSTRACT This study was designed to determine the global distribution of clonal complex (CC) 258 and $IncFII_{K2\text{-}like}$ plasmids with bla_{KPC} among 522 global *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* isolates. CC258 (i.e., ST258 [clades I and II], ST11, ST340, and ST512) and ST147 were statistically associated with $IncFII_{K2\text{-}like}$ KPC-containing plasmids and may possess an epidemiological advantage over isolates that harbored non-IncF KPC-harboring plasmids.

KEYWORDS *Klebsiella pneumoniae*, ST258, $IncF_K$ plasmids

The class A *Klebsiella pneumoniae* carbapenemase (KPC) β-lactamases have been extensively reported in *K. pneumoniae* (1). KPCs are present in >100 different *K. pneumoniae* sequence types (STs), but the KPC pandemic is primarily driven by the spread of members of clonal complex (CC) 258, namely, ST258 (clades I, II), ST11, ST340, and ST512 (1).

Several different KPC-containing plasmids (i.e., IncF, IncI2, IncX, IncA/C, IncR, and ColE1) have been identified in CC258 (2); however, the most predominant plasmid type is IncF with FIIk replicons, i.e., $IncFII_{K1}$ ($FIB_{pKPN\text{-}like}$) and $IncFII_{K2}$ ($FIB_{pKPQIL\text{-}like}$) (3). pKpQIL was the prototype of the $IncFII_{K2}$ group and one of the most common bla_{KPC}-harboring plasmids, reported in Israel, the United States, the United Kingdom, Colombia, and Italy (2). pKPN-3 was the prototype of the $IncFII_{K1}$ and was not initially associated with bla_{KPC} but was a virulence plasmid and coresident with pKpQIL within ST258 (2). In the current study, we set out to determine the presence and global distribution of CC258 among a defined population consisting of 522 KPC-producing *K. pneumoniae* isolates from AstraZeneca's (AZ) international surveillance study on antimicrobial resistance (2012 to 2014). We also investigated the association of $IncFII_{K2\text{-}like}$ plasmids containing bla_{KPC} with CC258 strains versus with non-CC258 strains.

The AZ global surveillance program was initiated in 2012 and includes a wide representation of microbiology laboratories among the various continents (4). Up to 100 consecutive nonselected Gram-negative aerobic and facultative bacilli from each of the participating countries/hospitals are included. All organisms are deemed clinically significant based on the criteria of the local investigators and were obtained from urinary tract, skin structure, intra-abdominal, and lower respiratory tract specimens. The countries participating in the surveillance include, in Africa, Egypt, Kenya, Nigeria, and South Africa; in Asia, China, South Korea, Taiwan, and Thailand; in Europe, Austria, Belgium, Bulgaria, Greece, Czech Republic, Denmark, France, Germany, Hungary, Italy,

Received 9 December 2016 **Returned for modification** 30 January 2017 **Accepted** 3 February 2017

Accepted manuscript posted online 6 February 2017

Citation Peirano G, Bradford PA, Kazmierczak KM, Chen L, Kreiswirth BN, Pitout JDD. 2017. Importance of clonal complex 258 and $IncF_{K2\text{-}like}$ plasmids among a global collection of *Klebsiella pneumoniae* with bla_{KPC}. Antimicrob Agents Chemother 61: e02610-16. https://doi.org/10.1128/AAC.02610-16.

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TABLE 1 Primers and target sizes for the characterization of $IncFII_{K2\text{-like}}$ plasmids that contain bla_{KPC}

PCR primer set	Primer	Sequence	Size (bp)	Target	Reference
I	FIIK-repA-F1 FIIK-repA-R1	CTTCACGTCCCGTTTTGATT CGCTTCAGCGCTTCTTTATC	657	IncFII *repA* gene	3
II	QIL-F1 QIL-R1	ACAGGGAGTGCCAGGAAAG TGTATTTGCATGGCGATGAG	2,001	Junction between Tn*4401 tnpR* and upstream $IncFII_{K2}$backbone gene	8
III	Tn4401v-F(3098U) Tn4401v-R1 Tn4401v-R(3781L)	TGACCCTGAGCGGCGAAAGC GCAAGCCGCTCCCTCTCCAG CACAGCGGCAGCAAGAAAGC	604	pKpQIL-associated Tn*4401a* isoform	9
IV	QIL-F2 QIL-R2	GCCTCAGATAGATGCGGTAGC AAGCTGGAGACATGGAATGG	1,831	Junction between Tn*4401* IS*Kpn6* and downstream gene	9
V	QIL-hsdR-F1 QIL-hsdR-R1	GGGTCGTTCACAAAGTCGAT CGTTGAGCACTTCACCAAAA	498	$IncFII_{K2}$-associated type I restriction modification system *hsdR* gene	9
VI	K2-repB-F1 K2-repB-R1	CCATTCCGATCCTTTTCTGA AACGCTACTGTCCAGCCTGT	395	$IncFII_{K2}$ *repFIB* gene	9

Macedonia, Portugal, Poland, Russia, Romania, Slovakia, Spain, Turkey, and the United Kingdom; in Latin America, Argentina, Brazil, Chile, Colombia, Mexico, Uruguay, and Venezuela; in the Middle East, Lebanon, Israel, Syria, and Kuwait; in North America, the United States; and in the South Pacific, Australia, Philippines, and Japan.

The AZ surveillance program does have some drawbacks. It only includes 100 consecutive nonselected Gram-negative aerobic and facultative bacilli from each participating country/hospital/year. It is therefore possible that the program will miss outbreaks of a particular resistance mechanism. The program is also biased toward sites that have AZ representation within that region/city/country (i.e., hospitals that can afford AZ products). Moreover, it only includes isolates from urinary tract, skin structure, intra-abdominal, and lower respiratory tract specimens. The program has some significant advantages as well. It includes a wide representation of microbiology laboratories among the various continents, although Africa and Asia are underrepresented. It only includes clinically significant bacteria and is not biased toward a certain resistance phenotype. Therefore, it provides a snapshot of the types of resistance determinants that are endemic in a specific hospital/region.

Molecular screening for bla_{KPC} was performed on carbapenem-resistant *K. pneumoniae* as described previously (5). Genetic relatedness among the isolates was initially determined using pulsed-field gel electrophoresis (PFGE) (6), and the major pulsotypes (those with >10 isolates per pulsotype) also underwent multilocus sequencing typing (MLST) (7). PCR typing was used to determine the presence of bla_{KPC}s on $IncFII_{K2\text{-like}}$ types of plasmids and to identify the different Tn*4401* isotypes (8, 9). Table 1 illustrates the PCR primers and the respective targets used in this study. $IncFII_{K2\text{-like}}$ plasmids were identified with amplifications with all the primer sets, namely, I, II, III, IV, V, and VI.

PFGE identified four major pulsotypes among 412 isolates (79%) that were designated clusters A ($n = 290$), B ($n = 80$), C ($n = 27$), and D ($n = 15$). We also recognized three minor pulsotypes (those with <10 isolates per pulsotype) among 23 isolates (4%), designated clusters E ($n = 9$), F ($n = 7$), and G ($n = 7$). The remaining isolates ($n = 87$ [17%]) were not clonally related; i.e., they exhibited <60% similar PFGE profiles and did not show patterns similar to those from clusters A to G. MLST identified the different pulsotypes as follows: cluster A, ST258; cluster B, ST11; cluster C, ST147; cluster D, ST512; cluster E, ST189; cluster F, ST15; and cluster G, ST437. ST258 was further differentiated into clades I and II (10). The geographic distribution of the different bla_{KPC}s, STs, and $IncFII_{K2\text{-like}}$ plasmids associated with bla_{KPC} are shown in Table 2.

K. pneumoniae ST258 is a prototype of a high-risk clone and has been largely responsible for the global spread of carbapenem resistance among the *Enterobacteriaceae* (11). Deleo and colleagues (10) performed whole-genome sequencing on a

TABLE 2 Sequence types, global distribution, and presence of $IncFII_{K2\text{-like}}$ plasmids among *K. pneumoniae* with bla_{KPC}

Sequence type	Country of isolation (no. of isolates)	KPC (no. of isolates)	$IncFII_{K2\text{-like}}$ plasmids (no. of isolates)
ST258-I ($n = 165$)	Argentina (33), Belgium (2), China (1), Greece (104), Italy (11), Romania (4), United (10)	KPC-2 (154), KPC-3 (9), KPC-9 (2)	103
ST258-II ($n = 125$)	Austria (1), Belgium (1), Brazil (7), Chile (1), Colombia (2), Germany (2), Greece (1), Israel (7), Italy (72), Mexico (1), United States (28), Venezuela (1)	KPC-2 (22), KPC-3 (103)	85
ST11 ($n = 80$)	Argentina (7), Austria (1), China (19), Brazil (45), Colombia (1), Israel (1), Taiwan (1), United States 3), Venezuela (1)	KPC-2 (73), KPC-3 (6), KPC-12 (1)	33
ST147 ($n = 27$)	Argentina (2), Greece (20), Italy (1), Philippines (1), Romania (1), Venezuela (2)	KPC-2 (26), KPC-3 (1)	19
ST512 ($n = 15$)	Colombia (9), Israel (2), Italy (2), United States (2)	KPC-2 (4), KPC-3 (11)	5
ST189 ($n = 9$)	Colombia (8), Venezuela (1)	KPC-2 (9)	0
ST15 ($n = 7$)	Portugal (6), Colombia (1)	KPC-2 (1), KPC-3 (6)	0
ST437 ($n = 7$)	Brazil (7)	KPC-2 (7)	0
Other ($n = 87$)	Argentina (12), Austria (1), Belgium (2), Brazil (4), China (1), Colombia (17), Czech Republic (1), Greece (5), Italy (10), Israel (5), Philippines (1), Portugal (13), Romania (1), United Kingdom (1), United States (9), Venezuela (4)	KPC-2 (56), KPC-3 (31)	19

global collection of *K. pneumoniae* ST258 isolates and showed that this ST belonged to two well-defined lineages, clade I and clade II. Clade I was associated with KPC-2, and clade II was associated with KPC-3. The majority of *K. pneumoniae* ($n = 290$ [56%]) from our global collection belonged to ST258; clade I ($n = 165$ [32%]) was associated with $bla_{KPC\text{-}2}$ on $IncFII_{K2\text{-like}}$ plasmids. This clade was mostly present in Argentina and Greece and to a lesser extent in Belgium, China, Italy, Romania, and the United States (Table 2). ST258 clade II ($n = 125$ [24%]) was associated with $bla_{KPC\text{-}3}$ on $IncFII_{K2\text{-like}}$ plasmids. This clade was mainly identified in Italy and the United States and to a lesser extent in Austria, Belgium, Brazil, Chile, Colombia, Germany, Greece, Israel, Mexico, and Venezuela (Table 2). No specific association of $bla_{KPC\text{-}2}$ or $bla_{KPC\text{-}3}$ with $IncFII_{K2\text{-like}}$ plasmids was seen (40% of KPC-2 and 54% of KPC-3 were harbored on $IncFII_{K2\text{-like}}$ plasmids) (Table 2).

ST11, which is closely related to ST258, is the major ST among *K. pneumoniae* harboring bla_{KPC} from Asia (especially China) (12) and Latin America (13) and sometimes contains other carbapenemases (14, 15). ST11 was the second-most-common ST in our study ($n = 80$ [15%]) and was associated with $bla_{KPC\text{-}2}$ on $IncFII_{K2\text{-like}}$ plasmids. ST11 was present in Brazil and China and to a lesser extent in Argentina, Colombia, Israel, Taiwan, the United States, and Venezuela (Table 2).

Other STs that belong to CC258 with bla_{KPC} were reported from Colombia (ST512), Italy (ST512), Israel (ST512), Brazil (ST340, ST437), and Greece (ST340) (13). The remaining members of CC258 from our study included ST512 and ST437. ST512, mainly from Colombia, was the fourth-most-common ST ($n = 15$ [3%]) in our collection and was also identified in Israel, Italy, and the United States (Table 2). The ST was associated with $bla_{KPC\text{-}3}$ on $IncFII_{K2\text{-like}}$ plasmids. ST437 ($n = 7$) was identified in Brazil and did not contain IncF plasmids.

K. pneumoniae ST147 is an emerging high-risk clone that was first identified in Greece, where it has been associated with bla_{VIM} and bla_{KPC} (16, 17). NDM (18) and OXA-181 (6) carbapenemases have also been described in ST147 from various countries, such as Switzerland, Iraq, Canada, the United Kingdom, India, and Italy (1). ST147, mainly from Greece, was the third-most-common ST ($n = 27$ [5%]) in our study and was associated with $bla_{KPC\text{-}2}$ on $IncFII_{K2\text{-like}}$ plasmids. ST147 was also present in Argentina, Italy, Philippines, Romania, and Venezuela (Table 2).

The geographic distribution of the other minor STs was as follows: ST189 ($n = 9$) in Colombia and ST15 ($n = 7$) in Portugal (Table 2). The isolates that did not belong to major or minor STs ($n = 87$) showed a global distribution. The IncFII (none k2-like) plasmids ($n = 141$) contained Tn*4401* isotypes a (66 [47%]), b (34 [24%]), and d (5 [4%]); $IncFII_{K2\text{-like}}$ plasmids ($n = 264$) only contained isotype a.

It was recently postulated that the presence of IncF plasmids with FIIk replicons harboring bla_{KPC} is central to the global success of CC258 and that these plasmids have contributed significantly to the evolutionary dominance of ST258 (11). Our molecular

epidemiological data support this hypothesis. The majority of CC258 isolates from our study harbored $IncFII_{K2\text{-like}}$ plasmids containing bla_{KPC}, in contrast to non-CC258 STs (226/392 [58%] versus 38/130 [23%]; $P < 0.0001$). This is especially true for ST258 in that 188/290 plasmids [65%] with bla_{KPC} from this ST belonged to $IncFII_{K2\text{-like}}$ (Table 2). *K. pneumoniae* ST147 was also associated with IncF plasmids, especially $IncFII_{K2\text{-like}}$ (Table 2). Our data suggest that certain successful high-risk *K. pneumoniae* clones (i.e., CC258 and ST147) are linked to specific narrow-host-range IncF plasmids with bla_{KPC}, and this association may possess epidemiological advantages over other clones that carry non-IncF KPC plasmids. It is possible that the maintenance and coevolution of GC258 with $IncF_{K2\text{-like}}$ plasmids provide rapid and continual adaptation opportunities for this CC, providing them with the additional ability to outcompete other *K. pneumoniae* clones. This scenario is consistent with both the macro- and microevolutionary versions of the Red Queen hypothesis of coevolution (11). However, this might be a very simplistic view regarding the role of $IncF_{K2\text{-like}}$ plasmids in the success of CC258, and this CC frequently harbors non-F antimicrobial resistance plasmids (1).

This study was not designed to specifically address the epidemiological advantage attributed to $IncFII_{K2\text{-like}}$ plasmids compared to other features of CC258. The $IncFII_{K2\text{-like}}$ plasmids are clearly the most common KPC-containing plasmids disseminating in *K. pneumoniae* but are not necessarily restricted to CC258 (23% of non-CC258 STs also contained $IncFII_{K2\text{-like}}$ plasmids). To the best of our knowledge, this is the first study to provide a comprehensive overview on the global distribution of different STs with bla_{KPC} and the association of these STs with $IncFII_{K2\text{-like}}$ plasmids in a defined population.

ACKNOWLEDGMENTS

This work was supported in part by a research grant from the Calgary Laboratory Services (10015169) and National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services award number R01AI090155 (to B.K.).

J.D.D.P. previously received research funds from Merck and Astra Zeneca.

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