Plasmid Evolution in Carbapenemase-Producing *Enterobacteriaceae*: A Review

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Running head: Plasmid-mediated evolution of carbapenemases

Abstract

Carbapenem-resistant *Enterobacteriaceae* (CRE) have been listed by the WHO as high-priority pathogens owing to their high association with mortalities and morbidities. Resistance to multiple β-lactams complicates effective clinical management of CRE infections. Using plasmid typing methods, a wide distribution of plasmid replicon groups has been reported in CREs around the world, including IncF, N, X, A/C, L/M, R, P, H, I, and W. We performed a literature search for English research papers, published between 2013 and 2018, reporting on plasmid-mediated carbapenem resistance. A rise in both carbapenemase types and associated plasmid replicon groups was seen, with China, Canada, and the United States recording a higher increase than other countries. bla_{KPC} was the most prevalent, except in Angola and the Czech Republic, where OXA-181 ($n = 50$, 88%) and OXA-48–like ($n = 24$, 44%) carbapenemases were most prevalent, respectively; $bla_{KPC-2/3}$ accounted for 70% ($n = 956$) of all reported carbapenemases. IncF plasmids were found to be responsible for disseminating different antibiotic resistance genes worldwide, accounting for almost 40% ($n = 254$) of plasmid-borne carbapenemases. bla_{CTX-M}, bla_{TEM}, bla_{SHV}, $bla_{OXA-1/9}$, *qnr*, and *aac-(6')-lb* were mostly detected concurrently with carbapenemases. Most reported plasmids were conjugative but not present in multiple countries or species, suggesting limited interspecies and interboundary transmission of a common plasmid. A major limitation to effective characterization of plasmid evolution was the use of PCR-based instead of whole-plasmid sequencing–based plasmid typing.

Keywords: CRE; carbapenem resistance; plasmid typing; replicon types; incompatibility groups

1. Introduction

Prescription of carbapenems are increasing extensively worldwide due to their relative safety and efficacy in resolving most fatal multidrug-resistant (MDR) bacterial infections. Subsequently, this is triggering and selecting resistance to carbapenems among an increasing number of Gram-Negative bacterial pathogens, including *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [1]. The increasing worldwide incidence and prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE), *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, with their very high attributable mortalities ranging from 6.6% to 20%, are considered global threats to human and animal health [2–5]. Subsequently, they have been enlisted as priority 1 critical pathogens by the World Health Organization[6]. Due to the importance of carbapenems in the clinical management of MDR infections, the emergence and rapid dissemination of CREs that are also resistant to fluoroquinolones, aminoglycosides, colistin etc. constrict therapeutic options [7,8]. Although CREs have been mostly isolated from healthcare-associated infections, *Enterobacteriaceae* also cause community-acquired infections, explaining the spread of CREs in the community [9].

Carbapenem resistance is mainly driven by carbapenemases found on mobile genetic elements (MGEs) such as integrons, insertion sequences, transposons, and mobile plasmids that can shuttle carbapenemase genes within and across bacterial cells of the same or different species [10–12]. The ability of plasmids to carry multiple antibiotic resistance genes (ARGs) and be mobilized across same and different species via conjugation make them very important in the molecular epidemiology of CREs [12,13]. This is further complicated by the ability of multiple plasmids, depending on their incompatibility (Inc), to be harbored in a single CRE cell. Coupled with their

extrachromosomal and self-replicative characteristics, plasmids are crucial in bacterial adaptations and survival in unsuitable environments[13,14].

The centrality of plasmids in the epidemiology of antibiotic resistance, necessitates an in-depth study into their structural and genetic characteristics. Plasmid replicon typing is the main technique used in identifying and classifying plasmids carrying virulence and/or ARGs [15]. These typing schemes, which includes the PCR-based replicon typing (PBRT) and plasmid mobility (MOB) typing, can determine whether antibiotic resistance is driven by a dominant or diverse plasmid type(s) [16].

1.1. Literature search strategy

Pubmed was searched for all English research papers using the following search words: carbapenems, carbapenemase, *Enterobacteriaceae* and plasmids. This search yielded a total of 2098 articles after duplications were removed. A search period of six years, from 1/1/2013 to 30/08/2019 was applied, which decreased the number of papers to a total of 862. The title and abstracts of these papers were screened for eligibility according to our hypothesis and research questions, which finally resulted in 102 research papers being included in the analysis. The inclusion criteria included all papers retrieved using the keywords "carbapenem", "carbapenemase", *"Enterobacteriaceae"*, and "plasmid", and reporting plasmid replicon groups associated with carbapenemases in *Enterobacteriaceae* species. We excluded all papers that reported plasmid replicon groups in non-carbapenemase producing *Enterobacteriaceae*. Plasmid sequences of unpublished articles but deposited in Genbank were also included in this review (Supplementary dataset), accession numbers are shown in Table S1.
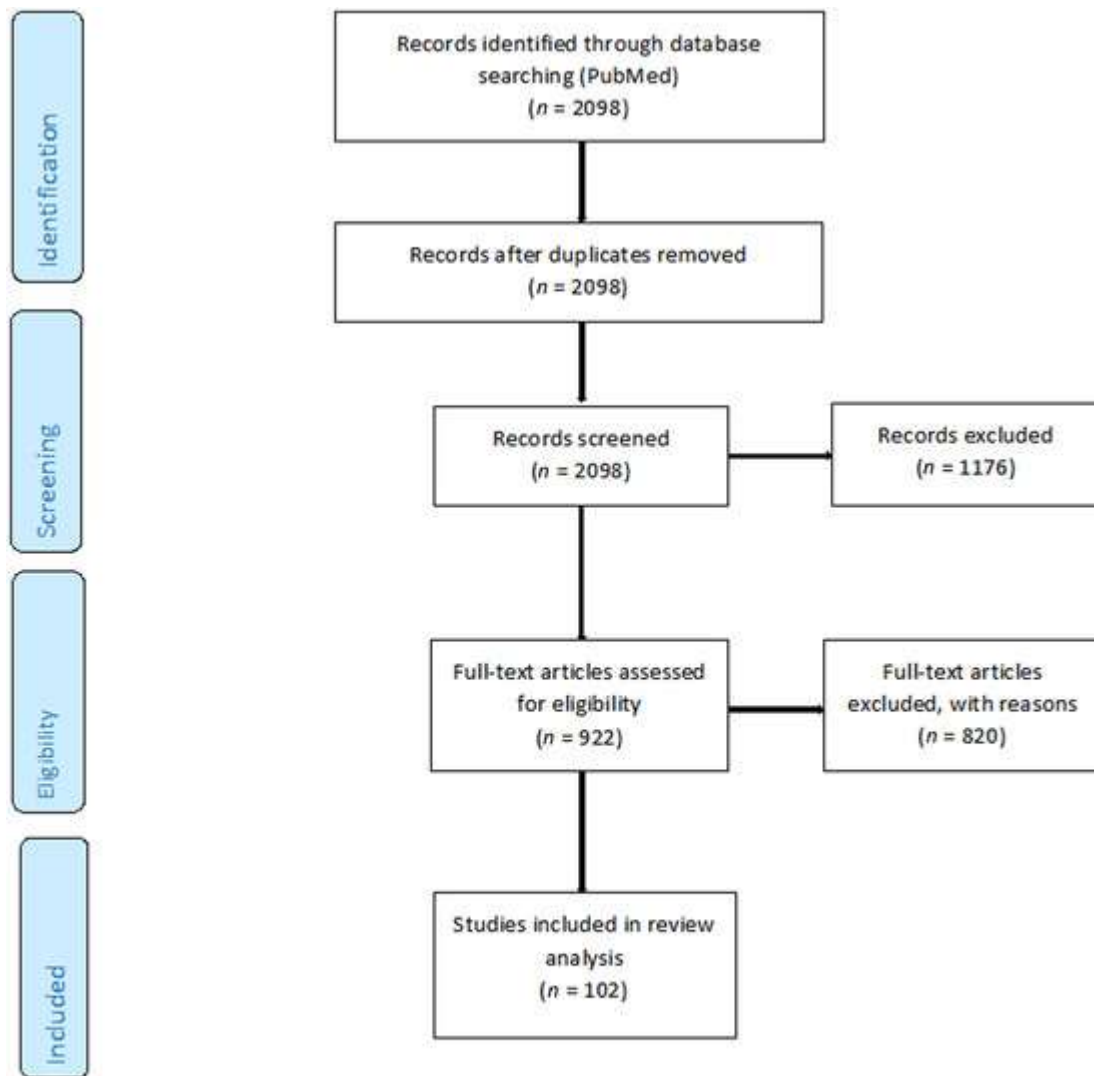
Eligibility: only articles from 2013 to 2019 were assessed. All articles not reporting plasmid replicon groups in carbapenemase-producing *Enterobacteriaceae* were excluded.

Figure 1. PRISMA-adapted flow diagram of included and excluded studies. Adapted from the PRISMA website (http://prisma-statement.org/PRISMAStatement/CitingAndUsingPRISMA.aspx).

1.2. Statistical analysis

All pie and bar charts in this review were constructed after analysis and calculation of the results using Microsoft excel 365®. All charts were also designed using Microsoft Excel 365.

1.3. Evidence before this review

To our knowledge, at least two articles have been published on this topic. A mini review published by Carattoli (2009)[17] focused on plasmid families in *Enterobacteriaceae*, while this review provides an update on plasmid families associated with carbapenemases. The second review published by Mathers (2015)[18], focused on high risk clones in the spread of MDR *Enterobacteriaceae* and associated resistance plasmids. Moreover, they did not report the frequency of carbapenemase genes and their associated plasmid groups, which this review aims to provide, besides looking at all *Enterobacteriaceae* species instead of specific clones.

1.4. Purpose of this review

This quinquennial systematic review aims to provide insights into plasmids mediating the dissemination of carbapenem resistance in *Enterobacteriaceae*. It focusses on the following aspects: classification of carbapenemases, methods used in plasmid classification, plasmid biology and incompatibility plasmid groups, plasmid epidemiology and MGEs associated with Inc groups. Thus, it aims to highlight the frequency and evolution of plasmids carrying carbapenemase genes over the last five years. Information in this review also shows the evolution of the genetic structures in different incompatibility groups, explaining the spread of carbapenemases and plasmids worldwide.

2. Carbapenems used as "last resort" antibiotics

Carbapenems are β-lactam antibiotics that differ from other β-lactams by the presence of a carbon instead of a sulphone at the fourth position of the lactam ring [19]. Carbapenems have broad-spectrum activity against both Gram-Negative and Gram-Positive bacteria, and are usually reserved for serious infections caused by Gram-Negative bacteria (GNB) [20]. However, each carbapenem differs in stability, ability to inhibit or induce β-lactamases, and resistance to β-lactamases [21]. These

characteristics have been used to classify carbapenems into three groups. Group 1 carbapenems such as ertapenem and panipenem, have limited activity against non-fermentative GNB and are suitable for community-acquired infections. Group 2 carbapenems include biapenem, doripenem, imipenem, and meropenem, which are active against non-fermentative GNB and suitable for hospital-acquired infections. Group 3 carbapenems such as PZ-601 (not licensed) comprise of the cationic and dithiocarbamate carbapenems and have extended spectrum of activity; they are also active against methicillin-resistant *Staphylococcus aureus* [22,23]. Carbapenems are usually saved for β-lactamase-producers that are resistant to almost all classes of β-lactams except carbapenems. However, some *Enterobacteriaceae* and other non-fermenters may produce carbapenem-hydrolyzing enzymes that enable them to resist carbapenem activity [23]. Carbapenemase production is thus the major mechanism of carbapenem resistance in *Enterobacteriaceae*.

3. Classification of Carbapenemases

Carbapenemases hydrolyze carbapenems and all other β-lactams [24] by breaking the β-lactam ring structure of β-lactam antibiotics, thus disrupting their function. β-lactamases are classified into different classes, according to their amino acid sequence or their functionality i.e. substrate specificity.

In the 1980s, Ambler grouped β-lactamases into four classes i.e., class A − D, based on their amino acid sequence homology [25]. These classes function on different mechanisms based on the molecules at their active sites. Classes A, C, and D have serine at their active sites and use serine ester hydrolysis mechanism while class B members have a zinc ion(s) at their active sites, which facilitate substrate catalysis [26,27]. Among these four classes, carbapenemases are placed in only three: classes A, B, and D.

The functionality classification scheme consists of three major groups: groups 1 – 3. Group 1 consists of cephalosporinases, group 2 are the penicillinases, cephalosporinases, and broad-spectrum β-lactamases inhibitors, and group 3 is composed of the metallo-β-lactamases (MBLs) [28,29]. In this scheme, carbapenemases are placed in Group 2 (class A and D) and Group 3 (class B), with the former being serine carbapenemases (SBLs) [10,28,29].

3.1. Class A carbapenemases

The first class A carbapenemase to be described was chromosomally located and reported in both clinical and environmental GNB [30]. It was only in the 1990s that plasmid-mediated class A carbapenemases were commonly described in clinical GNB including *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter* species [31]. Both chromosomally and plasmid-mediated carbapenemases are capable of hydrolyzing almost all β-lactams including carbapenems, but SBLs are inhibited by commercially available β-lactamase inhibitors such as clavulanic acid and tazobactam[30,32]. The most commonly described plasmid-mediated class A carbapenemases are the *Klebsiella pneumoniae* carbapenemase (KPC) and Guiana extended-spectrum β-lactamase (GES).

The GES family has more than 20 variants, with GES-1 showing activity towards other β-lactams but not carbapenems[33,34]. Most GES variants have activity towards broad-spectrum cephalosporins but amino acid substitution in other variants extend their activity towards carbapenems [34]. These variants with carbapenemase activity include GES-2, GES-4, GES-5, GES-6, GES-14, GES-16 and GES-18 [33,35–39]. GES-2 is commonly detected in *Pseudomonas spp.*, and it was first identified in a clonal outbreak of *Pseudomomas aeruginosa* in South Africa [40]. Additionally, GES-5 is also described in *Pseudomonas* spp. and *Enterobacteriaceae* and have been

widely reported in South America with few reports in Canada, Czech Republic, Turkey, Portugal, South Africa, and South Korea [12,34,41–44]. Other GES variants are also reported, although rarely [45,46]

KPCs have broad-spectrum activity against almost all β-lactams, including carbapenems, and they are mostly reported in *K. pneumoniae* clinical isolates [47,48]. However, in the last decade, KPC has also been reported in other species of *Enterobacteriaceae*, including *Escherichia coli*, *Enterobacter spp.*, *K. oxytoca*, *P. mirabilis*, *S. marcescens*, *M. morganii*, and *Citrobacter freundii*, among others[48–51]. These carbapenemases are widely distributed worldwide, but they are mostly reported in the United States, where they cause majority of the reported cases[52,53]. In the United States, KPC-producers are usually associated with hospital outbreaks caused by patient-to-patient transmission of clonally related resistant organisms [54]. More than 20 KPC variants have been described, but KPC-2 and KPC-3 are mostly reported and widely distributed [48,55]. KPCs have been reported in several *K. pneumoniae* sequence types (ST), although ST258, ST11 and more recently ST101, are the major players in the pandemic spread [12,54,56–58].

3.2. Class B Metallo-β-lactamases

Class B carbapenemases or MBLs are broad-spectrum β-lactamases capable of hydrolyzing all clinically available β-lactams except monobactams, and are not inhibited by commercially available β-lactamase inhibitors: clavulanic acid, tazobactam, or sulbactam [34,59]. However, MBLs are inhibited by metal ion chelators such as ethylene diamine tetra-acetic acid (EDTA) and dipicolinic acid (DA) [59–62] as their hydrolytic activity is dependent on the interaction between the active site zinc ion (Zn^{2+}) and the β-lactam [61]. The most common MBLs reported in *Enterobacteriaceae* include Verona-integron metallo-β-lactamase (VIM), Imipenemase (IMP) and New Delhi metallo-β-lactamase (NDM) [56,60,61,63,64].

IMP-types were among the acquired MBLs first identified in *Enterobacteriaceae*, the most common variant being IMP-1 [61]. In 1991, IMP-1 was isolated for the first time in *Serratia marcescens* in Japan, and was located on a class 1 integron [34]. Since then, more than 40 variants have been reported in Japan, Taiwan and around the whole world [30].

The first occurrence of VIM-type (VIM-1) β-lactamase was in 1997 in Verona, Italy in a *Pseudomonas aeruginosa* isolate; and VIM-2 was reported in France [60,65–67]. So far, more than 40 variants of VIM have been described, albeit VIM-2 is the most common worldwide [34,68] VIM-2 is usually common in *Pseudomonas* spp. while VIM-1 is common in *Enterobacteriaceae* [17,69]. VIM-types carbapenemases have been reported in more than 17 countries but they are mostly prevalent in Africa and Europe[70]. *K. pneumoniae* species are mostly associated with VIM-variants, followed by *E. cloacae*, *Citrobacter* spp., and *E. coli* in Greece, Spain and rarely in Germany and Czech Republic[70–72]. Since 2014, Sporadic reports of VIM-4-producing *K. pneumoniae* and *E. cloacae* were identified in Mediterranean countries[72].

First emergence of NDM was described in *K. pneumoniae* and *E. coli* clinical isolates in 2009 from a Swedish patient in New Delhi, India [73]. Since August 2010, NDM has spread worldwide to Canada, China, Europe, Japan, South Asia, Africa, Australia and the United States [74–76]. Epidemiologically analysis of the NDM-1 gene shows that it originated from the Indian subcontinent. NDM-1 is the most common variant described worldwide, but NDM-1 to NDM-9 has been published and 12 variants have been assigned [77,78]. NDM-4, NDM-5, NDM-7 were reported to have increased carbapenemase activity than NDM-1 [61,79–81].

3.3. Class D carbapenemases

Class D β-lactamases are called the oxacillin-hydrolyzing enzymes and comprise of more than 200 enzymes, although a few have carbapenemase activity [61]. The most prevalent variants are OXA-48 and OXA-181, which weakly hydrolyze carbapenems [82]. Most OXA variants are commonly reported in *Acinetobacter baumannii* and rarely in *Enterobacteriaceae* [66,83]. This class of β-lactamases is not inhibited by commercially available β-lactamase inhibitors and/or EDTA [82].

Since the emergence of OXA-48, it has been increasingly reported in *Enterobacteriaceae* species including *E. coli*, *Enterobacter* spp., *Citrobacter freundii*, *Klebsiella oxytoca*, *Providencia rettgeri*, and *Salmonella marcescens* [40,84]. Although OXA-48s hydrolyze carbapenems to a lesser extent, their co-occurrence with other resistance mechanisms such as membrane impermeability may result in high-level resistance [40]. OXA-48 is widespread in *Enterobacteriaceae* worldwide and has been reported in countries in the Middle East (Saudi Arabia, Israel), Africa (Libya, Egypt, Algeria, Morocco, South Africa), Asia (Russia, India, China, Taiwan), and South America (Argentina, Brazil, Colombia) [76,85–90]. The geographical distribution frequency of carbapenemase genes reported per country in the articles included in this review are shown in Figure 2.
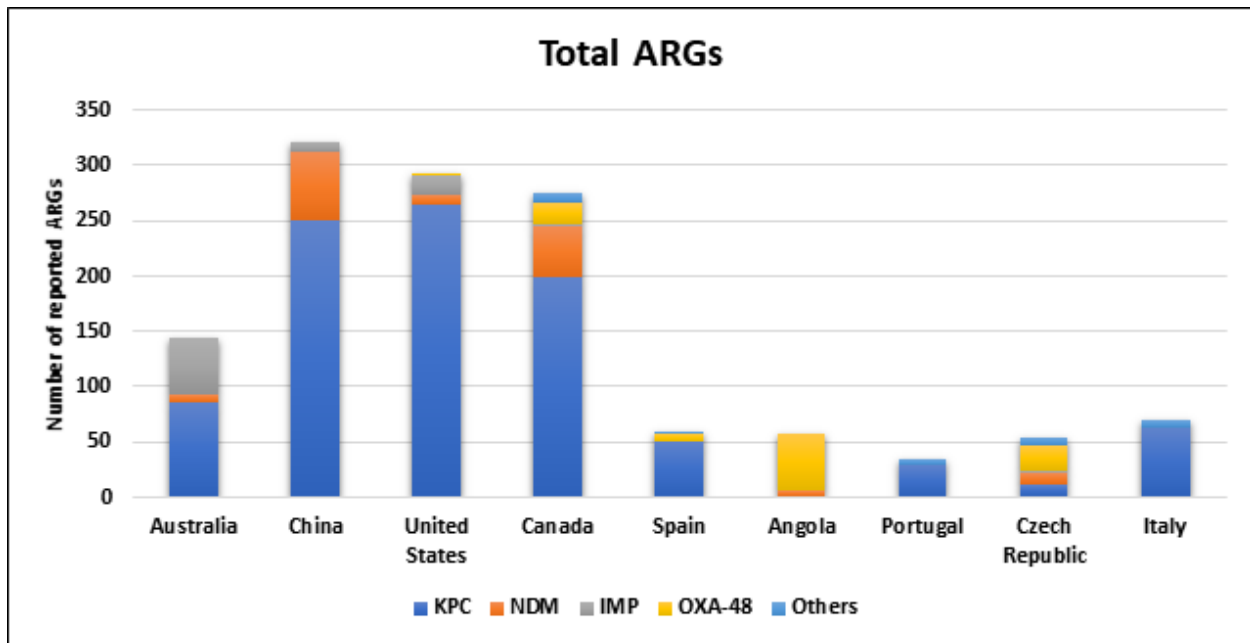
Figure 2. Frequency distribution of carbapenemase genes reported in countries represented by the included articles. KPC (blue bars) were the commonest per country except in Angola and the Czech Republic. China, the United States, and Canada had more included studies and hence, higher carbapenemase incidence. ARGs, antibiotic resistant genes.

4. Methods used in plasmid classification

The identification and classification of plasmids form the foundation of research looking at different plasmid groups in bacteria. Scientists gave m   uch attention to this topic after discovering the role of plasmids in the acquisition and dissemination of virulence and resistance genes by horizontal gene transfer [14]. Classification of plasmids is very important in studying the biology, adaptation, and evolution of microbial populations. Size and number of plasmids in a bacterial cell are usually determined using gel electrophoresis and/or pulsed field gel electrophoresis [91]. Plasmids are classified according to their incompatibility (Inc) or replicon group, which is based on the replication factors expressed by the plasmid in the bacteria.

Incompatibility was determined by introducing a plasmid of unknown replicon group in a recipient with a plasmid of known replicon group. The two plasmids are assigned to the same replicon group if the resident plasmid is eliminated. If this plasmid is not eliminated, the two plasmids are assigned to different incompatibility groups [92]. This method was used for several years to trace the dissemination of antibiotic resistance plasmids and the evolution of new plasmids. Couturier *et al.* (1988) proposed a new method based on hybridization of the major plasmid replicon groups in *Enterobacteriaceae* [93]. This method was labor intensive and almost impossible on large sample sizes. To overcome these limitations, new typing schemes were introduced to facilitate the characterization and epidemiological analysis of resistance plasmids [94].

Numerous plasmid classification schemes, including replicon and degenerate primer MOB typing (DPMT), which respectively targets loci encoding replicons and mobility functions, are widely used in research [15,95,96]. Carattoli *et al.* (2005) developed a PCR-based replicon typing (PBRT) method, which uses five multiplex-PCRs and three simplex PCRs with 18 sets of primers that target the major plasmid replicon groups in *Enterobacteriaceae* [97]. Subsequently, this method was updated to incorporate emerging plasmid replicon groups such as IncR and IncU. Until recently, this method has been useful in the identification and classification of major antibiotic resistance plasmids circulating among *Enterobacteriaceae*. The PBRT scheme increased our knowledge of plasmid diversity and revealed that conjugative plasmids belonging to a few widespread replicon groups carry clinically relevant ARGs [98]. Real-time PCR has also been used with the same principle as the PBRT method, which speeds up the detection and classification of plasmids and reduces human error and contamination [99].

In 2011, a commercially available PCR-based typing kit was introduced, which includes all the modifications that have been incorporated since 2005 to 2010 [100]. This kit contains all reagents and primers needed to perform the PCR, but still uses the same principle as the original PBRT method. The PBRT kit detects 28 replicons and is composed of eight multiplex-PCRs and positive control plasmids for all the PCRs [100]. Even though this method is still labor intensive and time consuming, it may detect more plasmid replicons than the 2005 PBRT scheme [100].

A technique based on plasmid mobility, called DPMT, was introduced by Francia and colleagues in 2006 [101,102]. This technique uses degenerate primers to target relaxase sequences for separating plasmids into MOB types identified by *in silico* MOB typing [95,103]. The MOB typing overcomes replicon typing limitations in that it targets relaxases, of which only one can be encoded in a plasmid. Unlike the PBRT, which detects plasmids at higher resolution, the MOB typing uses lower resolution to classify plasmids [94,98]. However, PBRT and DPMT have been combined to successfully classify plasmids in clinically relevant pathogens[104].

However, these typing schemes have a relatively lower discriminatory power than recent techniques such as plasmid multi-locus sequence typing (pMLST), whole-plasmid sequencing (WPS) and whole-genome sequencing (WGS) [94]. The PBRT methods have a few setbacks such as: i) the presence of multiple replicons in a single plasmid, which complicates plasmid classification; ii) rapid evolution of plasmid replicons; iii) and the presence of hybrid replication regions that make plasmid classification complicated [94,98]. Nevertheless, PCR-based typing methods may be used preliminarily for screening plasmids prior to using higher resolution techniques. All the typing techniques play a major role in plasmid evolution and epidemiology research in different countries worldwide.

pMLST is a tool used to further subtype already known plasmid Inc groups that occur very frequently in bacterial cells [105]. This technique has been used to successfully subtype IncF, IncHI1, IncHI2, IncI1, and IncN plasmids (www.pubmlst.org/plasmid/). Moreover, A/C subtyping was also developed to increase the discriminatory power for plasmid epidemiology studies. Hancock et al. (2017), recommended the use of pMLST and other PCR methods to further subtype A/C plasmids[106]. Garcia-Fernandez and colleagues (2011) suggested that pMLST can be used as a second-line plasmid typing technique after using PCR-based methods to identify plasmids [107]. pMLST has been used for epidemiological description of virulence and resistance plasmids in both human and animal reservoirs; moreover, more plasmid groups can be classified by pMLST[107]. In cases where pMLST is not available and plasmid subtyping is needed, a conventional technique, called Restriction Fragment Length Polymorphism (RFLP), can be used. However, the results produced by this method can be difficult to interpret and may be very subjective [94]

WGS overcomes the defined limitations of typing methods and many plasmids can be typed in a reasonable timescale [108]. According to Carloni and colleagues (2017), plasmid sequencing was able to detect novel plasmids previously not identified over the years by the PBRT scheme[100]. One major advantage of WGS is its ability to provide researchers with sequences of new/unknown plasmids[100]. Short read sequencers such as Illumina and Ion Torrent as well as long read sequencers such as PacBio and Oxford Nanopore are used for WGS or WPS, albeit PacBio is preferred for complete plasmid sequencing and gapless assembly[100]. Long-read sequencers are able to sequence repetitive sequences and/or multiple copies of the same mobile elements, which are usually longer than the read length covered by short-read sequencers; assembly programs will collapse such reads, identifying them as a single contig [11]. Long-read sequencing therefore provides a comprehensive insight into the epidemiology and evolution of plasmids, although it is

more expensive and error prone due to lower throughput or coverage [109]. Subsequently, hybrid (short and long-read) sequencing and assembly, has been proposed and proven to override the deficiencies of both long- and short-read sequencers [43,110]. For instance, Li and colleagues (2018), used Illumina and PacBio to yield high quality sequence reads; *proovread* was used to correct the long reads errors [51].

Plasmid prediction database servers such as Plasmidfinder, pMLST, PLACNET, and plasmidSPAdes enable easy identification and annotation of relevant plasmid sequences from large WGS datasets [43,96,111], as well as assemble plasmids from WGS data [43,96,112,113]. PlasmidFinder is a web-based tool that allows submission of raw or assembled reads, which are searched for through a plasmid replicon database to identify replicons and assign the plasmid to an Inc group[96]. The plasmid constellation network (PLACNET) is a graph-based tool that reconstructs plasmids from short read WGS raw data and is applied in plasmid diversity and adaptation [114]. The PLACNET tool uses three types of data for reconstruction of plasmids: i) scaffold links and coverage; ii) comparison to a reference plasmid; and iii) sequences such as replication initiator proteins [112]. Although, this tool assemble plasmid contigs automatically, it relies on manual trimming of the graph[113].

Furthermore, in 2017, PLACNETw (https://castillo.dicom.unican.es/) was developed based on the PLACNET database, automating all BLAST searches. PLACNETw only extracts the needed plasmid information and the graph-based presentation is automated[115]. In 2016, Antipov and colleagues developed a novel plasmid prediction database (PlasmidSPAdes) which also allows *de-novo* plasmid contigs assembly by manipulating differences in coverage in raw sequence reads [113]. PLACNET and PlasmidSPAdes are Linux-based applications that do not run on Windows and use

raw sequence reads instead of assembled fasta files. A more recent Linux-based application for identifying known plasmid sequences from WGS data is PlasmidSeeker [116], which also use raw reads and *k-mers* abundance to identify plasmid sequences. PlasmidSeeker is unable to assemble plasmid sequences from raw reads *de novo.*

5. Plasmid biology and incompatibility groups

Plasmids are usually double-stranded (ds) extra-chromosomal material or DNA that can replicate independently from the chromosome. These dsDNA materials occur naturally in bacterial cells and are essential for bacterial adaptability and persistence [117]. Thus, bacterial fitness may also be gained under some ecological conditions via the accessory genes carried on these plasmids [118]. For example, increased survival and competitive fitness is seen in bacteria carrying plasmids with heavy-metal resistance genes and ARGs [119]. Plasmids that occur naturally vary in size (1-100s kilobases) and in copy number (1- 100s in a cell).

Plasmids mediate the acquisition and dissemination of ARGs, including carbapenemases, through conjugation [120], which is only achievable by mobile/conjugative plasmids. The conjugative machinery share the same relaxase, a key protein that recognizes the origin of transfer (*ori*T) in conjugation [121]. Conjugative plasmids carry all the genes that are responsible for self-transfer, including the type IV coupling protein (T4CP) and all the components needed for mating channels that assemble a type IV protein secretion systems (T4SS) [121]. These systems are responsible for transporting proteins such as virulence factors and toxins extracellularly. The conjugative T4SS also exports DNA substrates [122].

Hedges and Datta (1971) defined plasmids based on their stability (Inc) and defined four Inc types including: i) the type F pili-producing plasmids, which are susceptible to phage Ff (IncF); ii)

the type I pili-producing plasmids, susceptible to phage Ifl (IncI); iii) plasmids related to N3, susceptible to phage Ike (IncN); iv) and plasmids related to RP4, susceptible to phage PRR1 (IncP) [17]. Numerous plasmid incompatibility (Inc) replicon groups have been associated with carriage of ARGs, thereby facilitating intra- and inter-species transfer.

5.1. Plasmid types and incompatibility groups associated with carbapenemases

To date, 27 major plasmid incompatibility groups are associated with ARGs in *Enterobacteriaceae* [17,93,123]. A wide distribution of plasmid replicon groups has been reported in CREs, including IncF, N, X, A/C, L/M, R, P, H, I, and W. These replicon groups are associated with different carbapenemases and the IncF, A/C, and X being the most prevalent in carbapenemase-producing than the other Inc groups. The most prevalent incompatibility types in *Enterobacteriaceae* are the IncF plasmids, which have been reported in different sources around the world [17,18].

Plasmid host range is usually a term used to describe the range of hosts in which a plasmid can replicate. This host range varies amongst plasmids, and the terms narrow-host range and broad-host range are used for the plasmid host range differentiation [124,125]. Narrow-host range self-transmissible plasmids are mainly of IncF, IncH and IncI types, while L/M, IncN, IncP, and IncW can replicate in broad-host ranges [124,126]. Table 1 and Figures 2-4 show the different ARGs reported in each country and their associated plasmid replicon groups mediating the spread of these genes.

Table 1: Major plasmids mediating carbapenem resistance in *Enterobacteriaceae*

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
Australia	2018	*K. pneumoniae*	ST258	FIB, FII	_	_	*bla*KPC-2	_	Tn*4401*	[169]
		K. oxytoca	_	FII	_	_	*bla*KPC-2	_	_	
		C. farmeri	_	FII, R	_	_	*bla*KPC-2	_	Tn*4401*	
		C. freundii	_	R	_	_	*bla*KPC-2	_	Tn*4401*	
	2016	*S. enterica*	ST19	HI2	339 kb	conjugative	*bla*IMP-4	TEM-1, *sul1*, OXA-1, *aacA4*, *qnrB2*	class 1 integron	[186]
	2015	*E. cloacae*	ST127	FII	_	conjugative	*bla*NDM-1	_	_	[131]
			ST265	X3	_	conjugative	*bla*NDM-1	_	_	
			ST45	L/M	_	conjugative	*bla*IMP-4	TEM-1, SHV, CTX-M, *qnr*, *aac (6')-Ib*	class 1 integron	
			ST1	HI2	_	non-conjugative	*bla*IMP-4	TEM-4, *qnrB2*, *aaCA4*	class 1 integron	
		E. hermannii	ST1	HI2	_	non-conjugative	*bla*IMP-4	*qnrB*, TEM-1, SHV, *aac(6')-Ib*	class 1 integron	
		E. aerogenes	ST45	L/M	_	conjugative	*bla*IMP-4	*qnrB*, TEM-1, *aac(6')-Ib*	*class 1 integron*	
		E. asburiae	ST1	HI2	_	non-conjugative	*bla*IMP-4	TEM-1, *aac(6')-Ib*	class 1 integron	
		E. coli	_	HI2	_	non-conjugative	*bla*IMP-4	*qnrB*, TEM-1, *aac(6')-Ib*	class 1 integron	
		K. pneumoniae	_	HI2, L/M	_	conjugative	*bla*IMP-4	*qnr*, TEM-1, SHV, *aac(6')-Ib*	class 1 integron	
		C. freundii	_	HI2	_	non-conjugative	*bla*IMP-4	TEM-1, SHV, CTX-M, *qnr*, *aac (6')-Ib*	class 1 integron	
		C. koseri	_	HI2	_	non-conjugative	*bla*IMP-4	*qnrB*, TEM-1, *aac(6')-Ib*	class 1 integron	
		P. mirabilis	_	HI2	_	non-conjugative	*bla*IMP-4	*qnrB*, TEM-1, SHV, *aac(6')-Ib*	class 1 integron	
China	2018	*K. pneumoniae*	ST11	FII	_	_	*bla*KPC-2	CTX-M-65, SHV-12, TEM-1	Tn*1721*-Tn*3*-IS*26*	[146]
			ST11	FII, I1	_	_	*bla*KPC-2	CTX-M-55, SHV-12, DHA-1	Tn*1721*-Tn*3*-IS*26*	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
China	2018	*K. pneumoniae*	ST11	FII, N	_	_	*bla*KPC-2	CTX-M-65, SHV-12, TEM-1	Tn*1721*-Tn*3*-IS*26*	146
			ST571	A/C	_	_	*bla*NDM-1	CMY-2, TEM-1	_	
			ST1723	P, FII	_	_	*bla*IMP-4	CTX-M, SHV-12, TEM-1	_	
		K. aerogenes	_	X6		conjugative	*bla*KPC-2	TEM-1	Tn6296 & ISkpn19	104
		P. mirabilis	_	X6		conjugative	*bla*KPC-2	TEM-1	Tn6296 & ISkpn19	
		S. marcescens	_	X6		conjugative	*bla*KPC-2	TEM-1, *qnr*S1	Tn6296 & ISkpn19 (Tn6292)	
		M. morganii	_	X6		conjugative	*bla*KPC-2	_	Tn6296 & ISkpn19	
		E. hormaechei	ST177	FII	109-kb	conjugative	*bla*NDM-1	-	-	193
		E. coli	ST167	FII/FIA	144-kb	conjugative	*bla*NDM-5	*aadA2, aadA5*, TEM-1, *Sul*1, *drfA12, drfA15*	IS*26*	194
			ST167	X3	80-kb	-	*bla*NDM-1	-	IS*Aba125*	195
			ST1114	X3	46-kb	conjugative	*bla*NDM-20	-	IS*Aba125*	196
			ST405	FII	-	-	*bla*NDM-1	-	-	197
			-	X3	46-kb	-	*bla*NDM-1	-	IS*Aba125*	
		C. freundii	-	X3	80-kb	-	*bla*NDM-1	CTX-M-15	IS*Aba125*	
	2017	*K. pneumoniae*	_	_	_	_	*bla*KPC-2	*rmtB*, CTX-M-65, TEM-1, SHV-11, *catA2, fosA, oqxA*	_	185
			ST14	X3	46 161-bp	conjugative	*bla*NDM-5	CTX-M-15	_	176
		E. coli	ST48	X3	47-kb	conjugative	*bla*NDM-1	CTX-M-64, TEM-1b, *sul2, aadA5, rmtB*	IS	144
			ST10	X3	102 512-bp	conjugative	*bla*NDM-5	mcr-1, *aadA2, sul1, dfrA12, aac(3)-IId*	IS*3000*	137
			ST4981	FII	92-kb	conjugative	*bla*NDM-5	mcr-1, TEM-1B, erm	IS*30*	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
China	2016	*K. pneumoniae*	ST105	FI	50-kb	conjugative	bla_{NDM-1}, bla_{IMP-4}	*qnrS1*, *qnrB4*, *aacA4*, CTX-M-15, SHV-1	IS*3000*	[198]
			ST2250	FII	30-kb	conjugative	bla_{NDM-5}	-	IS*3000*	[175]
			ST3835	X3	54-kb	conjugative	bla_{NDM-1}	CTX-M-15, SHV-12, CMY-42, OXA-1		[52]
		C. sakazakii	_	B/O	80-kb	conjugative	bla_{NDM-9}	mcr-1, *fosA3*, CTX-M-55, *qnrS*	IS26	[199]
		P. mirabilis	_	X3	40-kb	conjugative	bla_{NDM-5}	_	IS*3000*	[175]
		E. cloacae	ST231	A/C	130 573-bp	non-conjugative	bla_{NDM-1}	MBL, strA,strB, aadA2, armAmph2, mel, sul1 and sul2, dfrA12, qacE1	class 1 integron	[148]
				X6	10 756-bp	conjugative	bla_{KPC-3}	TEM-1	Tn3-Tn1722	
	2015	*E. cloacae*	ST120	HI2	340-kb	conjugative	bla_{NDM-1}	*armA*, *fosA3*	IS*Aba125*	[184]
			ST93	A/C	55-kb	conjugative	bla_{NDM-1}	*armA*	IS*Aba125*	
			ST88	N	65-kb	conjugative	bla_{NDM-1}	TEM-1, CTX-M-3	IS*Aba125*	
			_	X3	54 035-bp	conjugative	bla_{NDM-1}		IS*5*	[180]
		K. pneumoniae	ST11	FII-FIB	110 786-bp	conjugative	bla_{NDM-1}	*sul1*, *rmtC*	IS*CR3*, IS*Ehe3*	[200]
			Clone B, A	X3	7.8-kb	conjugative	bla_{NDM-1}	_	_	[180]
		C. sakazakii	_	B/O	80-kb	conjugative	bla_{NDM-9}	MCR-1, CTX-M-9, CTX-M-1,	IS	[199]
		R. planticola	_	X3	53 134-bp	conjugative	bla_{NDM-1}	SHV-12	IS26, IS*Aba125*, IS*5*	[200]
	2014	*K. pneumoniae*	ST889/966	A/C	245-kb	conjugative	bla_{NDM-1}	TEM-1, CTX-M-15	_	[177]
			ST113	N	55-kb	conjugative	bla_{NDM-1}	_	_	
		E. cloacae	ST40	FIB	310-kb	conjugative	bla_{NDM-1}	TEM-1, CMY-30, FosA3	-	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
China	2014	*E. cloacae*	ST410	I1	60-kb	conjugative	*bla*NDM-1	TEM-1, CTX-M-15, CMY-30	_	177
		C. freundii	_	A/C	170-kb	conjugative	*bla*NDM-1	FosA3, CMY-73	_	
United States	2018	*E. cloacae*	ST171	HI2	315-kb	-	*bla*KPC-4	-	Tn*4401b*	201
			ST171	FIA	141-kb	-	*bla*KPC-3	-	Tn*4401d*	
	2017	*K. pneumoniae*	ST111	N	69 888-bp	conjugative	*bla*KPC-2	aac(6')-Ib, aadA1,OXA-9, TEM-1, *strB, strA, sull2*	Tn*4401b*	49
		K. michiganensis	_	N	68 763-bp	conjugative	*bla*KPC-2	aac(6')-Ib, aadA1,OXA-9, TEM-1, strB, strA, sull2	Tn*4401b*	
		E. coli	ST218	Q1	10-kb	non-conjugative	*bla*IMP-27	CMY-2	_	178
		P. mirabilis	_	Q1	10-kb	non-conjugative	*bla*IMP-27	_	_	
		P. vulgaris	_	Q1	10-kb	non-conjugative	*bla*IMP-27	_	_	
		E. cloacae	_	Q1	10-kb	non-conjugative	*bla*IMP-27	_	_	
		C. farmeri	_	Q1	10-kb	non-conjugative	*bla*IMP-27	_	_	
	2016	*E. coli*	ST617	N	108-kb	conjugative	*bla*KPC-3	TEM-1	Tn*4401b*	84
			ST131	FII	116-kb	conjugative	*bla*KPC-2	TEM-1	Tn*4401a*	
			ST2289	FIA, A/C	99-kb	conjugative	*bla*KPC-2	TEM-1, OXA-9, FOX-5, PSE-1	Tn*4401d*	
			ST405	X3	39 520-bp	conjugative	*bla*NDM-5	*strA, strB, aac(6')-Ibcr*, OXA-1, *sul1*	_	147
			ST595	_	44-kb		*bla*KPC-3	OXA-9, TEM-1A, *aac(6')-Ib, aadA1, qnrB19*	Tn*4401b*	50
		E. xiangfangensis	ST114	F	_		*bla*KPC-3	*qnrS1*, TEM-1A	Tn*4401b*	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
United States	2016	*E. hormaechei*	ST594	Col	_		*bla*KPC-2	TEM-1B, SHV-12, *strB, strA, aadA2, aac(6')-lic, qnrB2, sul1, sul2, dfrA18*	Tn*4401*	[50]
			ST269	_	44-kb		*bla*KPC-2	TEM-1B, *qnrB2, sul1, dfrB3*	Tn*4401*	
			ST113	A/C	66-kb		*bla*KPC-4	TEM-1A, OXA-1, *aadA1, aac(3)-via, aph, mph* (A), *catB3, arr-3, qnrS1, sul1, dfrA14*, TEM-1B, *sul2, strA, strB*	Tn*4401*	
	2015	*E. cloacae*	ST171	FIA	63 481-bp	_	*bla*KPC-3	TEM-1, OXA-9	Tn*1331*/Tn*4401*	[202]
			ST171	HI2	_	_	*bla*KPC-4	TEM-1, OXA-1	Tn*4401b*	[168]
			ST78	N	_	_	*bla*KPC-4	_	Tn*4401b*	
		K. pneumoniae	ST101	L/M			*bla*OXA-48	_	Tn*1991*	
			ST258	R			*bla*KPC-2	_	Tn*4401a*	
			ST113	N			*bla*KPC-4	_	Tn*4401b*	
			ST258	I2			*bla*KPC-3	_	Tn*4401b*	
			ST258	A/C2			*bla*KPC-2	_	Tn*4401e*	
			ST16	X3			*bla*KPC-3	_	Tn*4401b*	
		E. coli	ST131	X3	116 803-bp	_	*bla*KPC-3	TEM-1, OXA-9, *sul2, strAB*	Tn*4401*	[202]
	2014	*K. pneumoniae*	ST258	FIA	73 635-bp	non-conjugative	*bla*KPC-3	TEM-1, OXA-9, *aacA4, aadA1, strB, sul1, dfrA14*	Tn*4401*/Tn*1331*	[129]
			ST963	FII	139 941-bp	conjugative	*bla*KPC-3	_	Tn*1331*/Tn*4401d*	
		E. cloacae	ST93/253/171	N	90-kb		*bla*KPC-3	SHV-5	_	[48]
				N	90-kb	_	*bla*KPC-2	CTX-M-15, SXT	_	
				FIB	30-kb		*bla*KPC-2	_	_	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene- mase gene	Other resistance	Genetic elements	References
Japan	2018	*K. pneumoniae*	ST1471	L	-	-	*bla*IMP-1	-	-	203
	2015	*K. pneumoniae*	ST5	N	47 236-bp	conjugative	*bla*IMP-6	CTX-M-2, *aacA4'*,*aadA2*, *tetR-tetA*	Class 1 integron (In*722*)	164
		K. oxytoca	ST37	N	_	conjugative	*bla*IMP-6	CTX-M-2	Class 1 integron (In*722*)	
		E. coli	ST37	N	_	conjugative	*bla*IMP-6	CTX-M-2	Class 1 integron (In*722*)	
Mexico	2017	*K. pneumoniae*	ST392	IIIk	130-kb	conjugation	*bla*NDM-1	_	_	183
			ST309	FII	130-kb	conjugation	*bla*NDM-1	_	_	
		E. cloacae	ST182	FII	150-kb	conjugation	*bla*NDM-1	_	_	
		E. coli	ST10	FII	130-kb	conjugation	*bla*NDM-1	_	_	
	2015	*K. pneumoniae*	ST22	FII	_	conjugation	*bla*NDM-1	CTX-M-15	_	179
		E. coli	ST617	FII	_	conjugation	*bla*NDM-1	CTX-M-15	_	
		E. cloacae	ST182	FII	_	conjugation	*bla*NDM-1	CTX-M-15	_	
Spain	2017	*E. coli*	ST1434	N	70-kb	conjugation	*bla*KPC-2	OXA-1, *aac(6')-Ib-cr*, *qnrB6*	_	174
			ST5001	R	48-kb	_	*bla*KPC-2	_	_	
			ST216	R	48-kb	non-conjugative	*bla*KPC-2	*aac(6')-Ib*	_	
			ST131	L/M	61 395-bp	conjugative	*bla*OXA-48	_	Tn*1991.2*	
		E. cloacae	ST822	FIB	170-kb	_	*bla*IMI-2	_	_	
			ST823	N	70-kb	conjugative	*bla*KPC-2	*aac(6')-Ib*, *qnrB6*	_	
		K. oxytoca	_	N	60-kb	_	*bla*KPC-2, *bla*VIM-1	OXA-1, *aac(6')-Ib*	_	174
		R. ornithinolytica	_	R	70-kb	_	*bla*VIM-1	OXA-1, *aac(6')-Ib*, *qnrB5*	_	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
Spain	2017	*R. ornithinolytica*	_	P6	_	_	bla_{KPC-2}	TEM-1	IS*Kpn6*-IS*pn27*	185
		C. freundii	_	P6	40-kb	conjugative	bla_{KPC-2}	TEM-1	IS*Kpn6*-IS*pn27*	
		E. cloacae	_	P6	_	_	bla_{KPC-2}	TEM-1	IS*Kpn6*-IS*pn27*	
		K. pneumoniae	_	N	_	_	bla_{KPC-2}	TEM-1	IS*Kpn6*-IS*pn27*	
		Kluyveraa sp.	_	U	_	_	bla_{KPC-2}	TEM-1	IS*Kpn6*-IS*pn27*	
Poland	2016	*K. pneumoniae*	ST11	R	90-kb	non-conjugative	bla_{NDM-1}	CTX-M-15, TEM-1, OXA-1	Tn*125*	54
			ST11	FII	100-kb	conjugative	bla_{NDM-1}	TEM-1	Tn*125*	
			ST11	R+FII	80-kb	non-conjugative	bla_{NDM-1}	TEM-1, OXA-1	Tn*125*	
Italy	2015	*K. pneumoniae*	ST101	FII	_	conjugative	bla_{KPC-2}	CTX-M-1	_	204
			ST1789	FII	_	conjugative	bla_{KPC-2}	CTX-M-1	_	
			ST512	FII	_	conjugative	bla_{KPC-3}	_	_	
			ST405	FII	_	conjugative	bla_{KPC-3}	_	_	
		E. coli	ST131	N	_	conjugative	bla_{VIM-1}	_	_	
			ST5	X3, FIB, colE			bla_{KPC-3}	SHV-11	Tn*4401a*	91
		C. freundii	ST91	X3	_		bla_{KPC-3}	SHV-11	Tn*4401a*	
			ST96	X3			bla_{KPC-3}, bla_{VIM-2}	SHV-11, TEM-1, CTX-M-9	Tn*4401a*	
				X3, N, HI1			bla_{KPC-3}, bla_{VIM-2}	SHV-11, TEM-1, CTX-M-9	Tn*4401a*	
Canada	2016	*K. pneumoniae*	ST258	FIA	_	_	bla_{KPC-3}	SHV, TEM	_	42
			ST512	FIA, FII	_	_	bla_{KPC-3}	SHV, TEM	_	
			ST15	N	-	-	bla_{KPC-3}	SHV, TEM, CTX-M, OXA-1, CMY-2	-	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance genes	Genetic elements	References
Canada	2016	*K. pneumoniae*	ST15	N	_	_	bla_{KPC-3}	SHV, TEM, CTX-M, OXA-1, CMY-2	_	[42]
			ST437	R	_	_	bla_{NDM-1}	SHV, CTX-M	_	
			ST11	A/C	_	_	bla_{NDM-1}	SHV, OXA-1	_	
			ST147	R	_	_	bla_{NDM-1}	SHV, CTX-M, OXA-1	_	
			ST15	R	_	_	bla_{NDM-1}	SHV, TEM, CTX-M	_	
			ST16	A/C	_	_	bla_{NDM-1}	SHV-1, CTX-15, OXA-1, CMY-6	_	
			ST101	N	_	_	bla_{OXA-48}	SHV, OXA-1	_	
		E. coli	cluster II	FIIA			bla_{KPC-3}	_	_	
			cluster VI	N			bla_{KPC-3}	_	_	
		E. cloacae	cluster IV	P, L/M	_	_	bla_{KPC-3}	_	_	
			_	L/M			bla_{KPC-3}	_	_	
			_	FIIA	_	_	bla_{KPC-3}	_	_	
			cluster VI	N			bla_{KPC-3}	_	_	
			_	Y	_	_	bla_{VIM-1}	_	_	
			_	R	_	_	bla_{VIM-1}	_	_	
		E. aerogenes	cluster VI	N			bla_{KPC-3}	_	_	
		C. freundii	cluster IV	P, L/M	_	_	bla_{KPC-3}	_	_	
		C. koseri	cluster IV	P, L/M	_	_	bla_{KPC-3}	_	_	
		C. youngae	cluster IV	P, L/M			bla_{KPC-3}	_	_	
		R. planticola	_	N	_	_	bla_{KPC-3}	_	_	
			cluster VI	N	_	_	bla_{KPC-3}	_	_	
			cluster IV	P, L/M	_	_	bla_{KPC-3}	_	_	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene- mase gene	Other resistance	Genetic elements	References
Canada	2014	*K. pneumoniae*	ST258	F, I2	120-kb, 80-kb	_	*bla*KPC-3	TEM-1, SHV-11	Tn*4401b*	[47]
			ST258	I2	70-kb	_	*bla*KPC-3	TEM-1, SHV-11	Tn*4401b*	
			ST258	A/C, FII	100-kb	_	*bla*KPC-2	TEM-1, SHV-11	Tn*4401a*	
			ST258	FII, I2	80-kb	_	*bla*KPC-2	TEM-1, SHV-11	Tn*4401a*	
			ST258	N, FII	50-kb	_	*bla*KPC-2	OXA-1, SHV-11	Tn*4401b*	
		E. cloacae	ST258	HI2	120-kb	_	*bla*KPC-3	TEM-1	Tn*4401b*	
		C. freundii	_	A/C	180-kb	_	*bla*KPC-2	TEM-1	Tn*4401b*	
		R. ornithinolytica	_	F	70-kb	_	*bla*KPC-2	_	Tn*4401b*	
Myanmar	2019	*E. coli*	ST167/101/410	FII	-	-	*bla*NDM-5	CTX-M-15	IS*Sba14*	[205]
			ST410	X3	50-kb	-	*bla*NDM-4/7	-	IS*sba14*	
	2017	*E. coli*	-	A/C		conjugative	*bla*NDM-1	CTY-4	Tn*125*, Tn*1548*	[134]
			_	X3	47-kb	conjugative	*bla*NDM-4	_	Tn*3*	
			_	X3		conjugative	*bla*NDM-7	_	Tn*3*	
			_	X3		conjugative	*bla*NDM-5	_	Tn*3*	
			_	FII		conjugative	*bla*NDM-4	_	IS*26*	
			_	FII		conjugative	*bla*NDM-5	_	IS*26*	
Germany	2018	*E. coli*	ST131	HI2	300-kb	-	*bla*VIM-1	*aac(6')-Ib-cr*, *aacA4*, *aadA1*, ACC-1, CMY-2, *catA1*, *strA/B*, *Sul*1	Tn*21*	[206]
		S. Infantis	ST32	HI2	300-kb	-	*bla*VIM-1	*aac(6')-Ib-cr*, *aacA4*, *aadA1*, ACC-1, CMY-2, *catA1*, *strA/B*, *Sul*1	Tn*21*	
Portugal	2018	*E. coli*	ST131	Q2	13-kb	non-conjugative	*bla*KPC-21	-	IS*kp6*	[207]
Denmark	2018	*E. coli*	ST410	F	-	-	*bla*OXA-181	CTX-M-15, TEM-30	-	[208]

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene- mase gene	Other resistance	Genetic elements	References
Denmark	2018	*E. coli*	-	X3	-	-	*bla*NDM-5	CMY-2	-	208
		K. pneumoniae	ST35	HI2	314-kb	conjugative	*bla*OXA-436	-	IS*91*/IS*CR1*	209
		C. freundii	ST22/65	HI2	314-kb	conjugative	*bla*OXA-436	-	IS*91*/IS*CR1*	
		E. asburiae	-	HI2	314-kb	conjugative	*bla*OXA-436	-	IS*91*/IS*CR1*	
Romania	2015	*K. pneumoniae*	ST258	FII		conjugative	*bla*KPC-2	CTX-M-15, TEM-1, OXA-1, OXA-9, AAC-6'-1b	Tn*4401*	53
			ST101	L/M		conjugative	*bla*OXA-48	CTX-M-15, TEM-1, OXA-9, AAC-6'-1b-cr	Tn*1999.2*	
		E. cloacae	ST93	FII		conjugative	*bla*VIM-4	CTX-M-15, TEM-1, OXA-1, AAC-6'-1b	class 1 integron	
Kuwait	2017	*K. pneumoniae*	ST1399	A/C	165-kb	conjugative	*bla*VIM-4	TEM-1, SHV-12, CTX-M-15, CMY-4, aac(6')-Ib-cr	In416	210
		E. aerogenes	_	FII		conjugative	*bla*KPC-3	TEM-1, OXA-30, CTX-M-15	Tn*4401b*	
		E. coli	ST58	FII		_	*bla*KPC-3	TEM-1	Tn*4401b*	
		K. pneumoniae	ST11	FII		conjugative	*bla*KPC-3	TEM-1, SHV-11, OXA-30, CTX-M-15	Tn*4401b*	
			ST147	FII		conjugative	*bla*KPC-3	TEM-1, SHV-11	Tn*4401b*	
			ST1138	FII		conjugative	*bla*KPC-3	TEM-1, SHV-36	Tn*4401b*	
Lebanon	2018	*E. coli*	ST354	L/M	-	-	*bla*OXA-48	CTX-M-15, CMY-42, TEM-1b, OXA-1	IS*1999*	211
			ST410	X3	-	-	*bla*OXA-181	CMY-2/4, CTX-M-15, TEM-1B, OXA-1	-	
Thailand	2018	*K. pneumoniae*	-	H1B	297-kb	-	*bla*NDM-1	*aadA2, armA, aph(3')*-Vla, *Sul*1, CTX-M-15, *qnrB1*	-	212
Pakistan	2018	*K. pneumoniae*	ST101	L/M	-	-	*bla*OXA-48	CTX-M-15, SHV-28, TEM-1, OXA-10	-	213
Czech Republic	2017	*E. coli*	ST4956/ ST216	L	64-Kb	_	*bla*OXA-48	_	-	153
		E. cloacae	ST109	L	64-kb	_	*bla*OXA-48	CTX-M-15, OXA-1, TEM-1	Tn*1999.2*	
		K. pneumoniae	ST101	L	64-kb	_	*bla*OXA-48	CTX-M-15, TEM-1	Tn*1999.2*	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
Czech Republic	2017	*K. pneumoniae*	ST18	X3	51-kb	_	*bla*OXA-181	CTX-M-15, OXA-1, TEM-1	IS26	
			ST15	colE2	13-kb	_	*bla*OXA-232, *bla*NDM-1	CTX-M-15, OXA-1	Tn1000	
			ST11	L	65-kb	_	*bla*OXA-48	CTX-M-15	Tn1999.5	
		P. stuartii	_	A/C	100-kb	conjugative	*bla*NDM-1	CTX-M-15	_	153
			ST1301	X3	>150-kb	non-conjugative	*bla*OXA-181	CTX-M-15, *qnr*S	ISEsp1-IS3000-ISKpn19	
			ST15	X3	120-kb	conjugative	*bla*NDM-1	CTX-M-15	_	
Taiwan	2018	*K. pneumoniae*	ST11	-	86-kb	-	*bla*KPC-2	CTX-M, SHV, TEM	IS1	214
Egypt	2016	*K. pneumoniae*	ST147	colE, R	60-97-kb	conjugative	*bla*NDM-1	CTX-M-15, SHV-11, *aac(3)-IIa*, *aph(30)-Ia*, *aac(60)-Ib-cr*, *rmtF*, *qnrB*	ISAba125	215
			ST11	colE, R, F	55-kb	conjugative	*bla*NDM-1	CTX-M-15, SHV-11, *aac(3)-IIa*, *aph(30)-Ia*, *aac(6')-Ib-cr*, *rmtF*, *qnrB*	ISAba125	
Gabon	2017	*K. pneumoniae*	ST307	X3	_	conjugative	*bla*NDM-7	CTX-M-15, SHV-28, OXA-9, *aac(6')-Ib*, *sul*, *fosA*	transposon	216
		E. cloacae	_	X3	_	conjugative	*bla*NDM-7	OXA-9, ampR, SHV-12, TEM-104	transposon	
India	2019	*K. pneumoniae*	ST347	FII	153-kb	conjugative	*bla*NDM-1	CTX-M-15, *qnr*S1, *qnr*B1, *oqxAB*, *aac(6')-Ib-cr*	ISAba125	217
			ST29	FII	115-kb	conjugative	*bla*NDM-1	CTX-M-15, *qnr*S1, *oqxAB*	ISEc33	
			ST1224	FII	270-kb	conjugative	*bla*NDM-1	CTX-M-15, *qnr*S1, *qnr*B1, *oqxAB*, *aac(6')-Ib-cr*	ISAba125	
			ST2558	FII	173-kb	conjuagtive	*bla*NDM-1	CTX-M-15, *qnr*S1, *aac(6')-Ib*	ISEc33	
	2016	*S. enterica*	_	A/C	146-kb	conjugative	*bla*NDM-1	CMY -4	IS26, IS4321	187
Vietnam	2015	*K. pneumoniae*		FII, A/C	_	_	*bla*NDM-1	TEM, CTX-M	_	173
		E. cloacae		FII, A/C	_	_	*bla*NDM-1	TEM, CTX-M, SHV	_	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene- mase gene	Other resistance	Genetic elements	References
Vietnam	2015	*E. coli*		FII	_	_	*bla*NDM-1	TEM, CTX-M, SHV	_	
		C. freundii		FII, A/C	_	_	*bla*NDM-1	TEM, CTX-M	_	173
		K. oxytoca		FII	_	_	*bla*NDM-1	TEM, CTX-M	_	
Brazil	2015	*E. hormaechei*		F	96 124-bp	conjugative	*bla*NDM-1	_	Tn*3000*	136
Korea	2018	*K. pneumoniae*	ST340	X3	-	-	*bla*NDM-1	-	IS	218
		E. coli	ST1642	X3	69 409-bp	conjugative	*bla*KPC-2	SHV-11	Tn*4401*	145
France	2018	*K. pneumoniae*	ST395	L	62-kb	-	*bla*OXA-48	CTX-M-15, *aac(6')-Ib-cr*, *qnr*S	-	219
			-	L/M	63-kb	conjugative	*bla*OXA-48	CTX-M-1	IS*9999*	
			-	L/M	167-kb	conjugative	*bla*OXA-48	-	IS*9999*	
Belgium	2016	*E. cloacae*	ST346	L/M	78 907-bp	conjugative	*bla*GES6/7	_	class 1 integron	192
Ireland	2014	*K. pneumoniae*	_	L/M	63 578-bp	conjugative	*bla*OXA-48	_	Tn*1999*	152
			_	FIB	63-kb	conjugative	*bla*OXA-48	_	Tn*1999*	
			_	FII	63-bp	conjugative	*bla*OXA-48	_	Tn*1999*	
			_	Y	_	_	*bla*OXA-48	_	_	
Tunisia	2018	*P. mirabilis*	-	P & A/C	-	-	*bla*NDM-1	CMY-4, *qnr*A6, *aph3* VIa, *aph3* Ia	-	220
Saudi Arabia	2018	*K. pneumoniae*	ST152	F, N	-	conjugative	*bla*NDM-1	-	IS*Aba125*	221
			ST37/974	L/M	-	conjugative	*bla*OXA-48	CTX-M-15, TEM-1, SHV-11	-	
South Africa	2018	*E. coli*	ST167	X3	46 25 3-bp	_	*bla*NDM-5	_	_	12
		K. pneumoniae	ST101	Col	6 141-bp	_	*bla*OXA-232	_	_	

Country	Year	Species	Clone	Plasmid type (Inc)	Size	Plasmid conjugation/ mobility	Carbapene-mase gene	Other resistance	Genetic elements	References
South Africa	2018	*K. pneumoniae*	ST101	FIB	223 4 34-bp	conjugative	*bla*NDM-1	qac/sul1, DHA-1	Tn*1548*-like	12
			ST2017	R, FIB, FII	212 3 26-bp	conjugative	*bla*NDM-1	qac/sul1, DHA-1	Tn*1548*-like	
			ST101	Q	8 201-bp	_	*bla*GES-5	aacA4	Class 1 integron	
Sao Tome and Principe	2018	*E. coli*	ST1163	X3	66-kb	conjugative	*bla*OXA-181	TEM-1	IS*kpn19*	222
			ST410	X3	60-kb	conjugative	*bla*OXA-181	CTX-M-15, TEM-1	IS*kpn19*	
		K. pneumoniae	-	X3	64-kb	conjugative	*bla*OXA-181	TEM-1	IS*kpn19*	
Croatia	2018	*K. pneumoniae*	-	L/M	70-kb	conjugative	*bla*OXA-48	CTX-M-15, TEM-1, OXA-1, *qnr*A/B	IS*1999*/IS *1R*	223
		E. coli	-	L/M	70-kb	conjugative	*bla*OXA-48	TEM-1	IS*1999*	
		E. cloacae	-	L/M	70-kb	conjugative	*bla*OXA-48	CTX-M, TEM-1	IS*1999*	
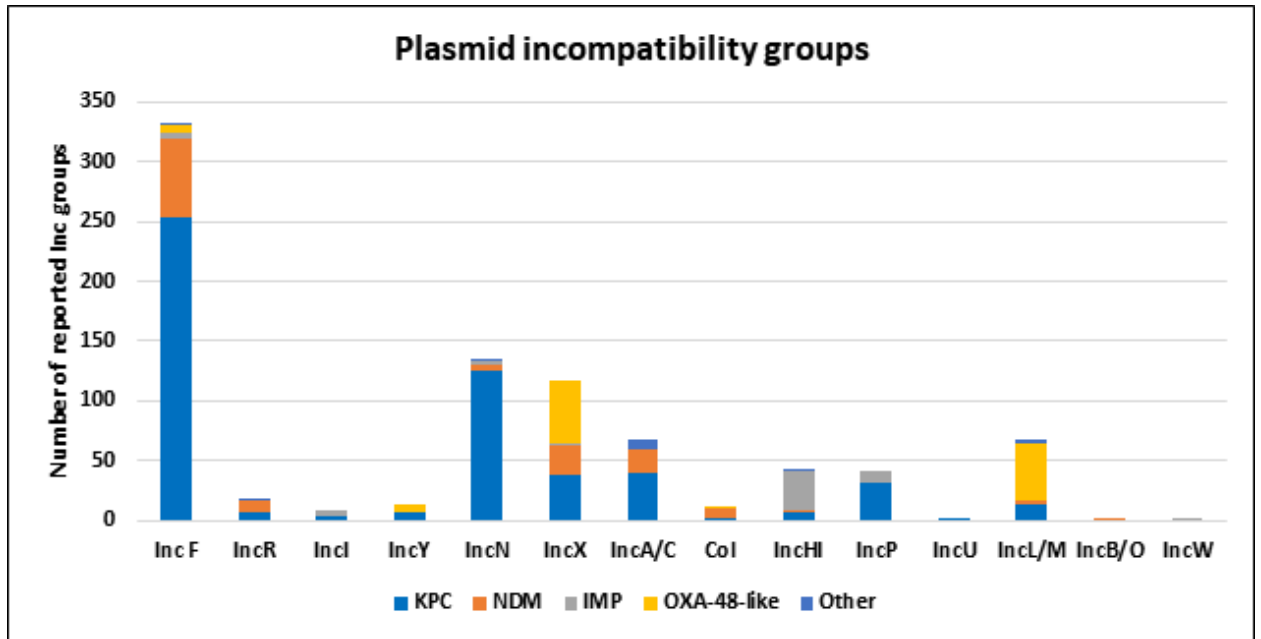
Figure 3. Frequency of plasmid incompatibility groups associated with different carbapenemase genes reported in *Enterobacteriaceae* in 23 countries. The commonest of these is the IncF types, followed by IncN, IncX, IncL/M, IncA/C, IncHI, and IncP, which are mostly associated with KPC, NDM, and OXA-48-like carbapenemases.
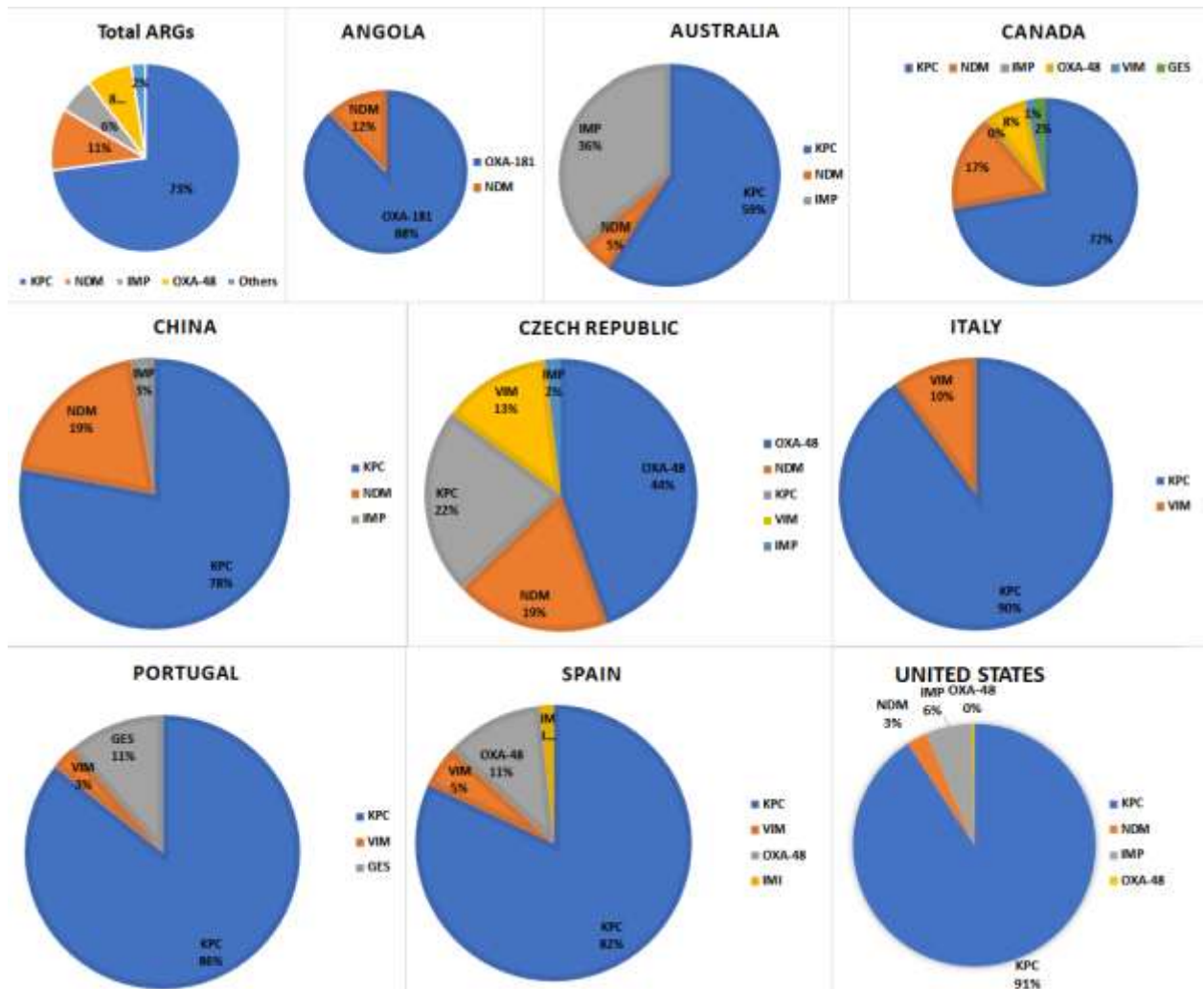
Figure 4. Charts showing the frequency of carbapenemase genes per country reported in the set of papers included in this review. KPC has been reported as the most prevalent in almost all shown countries, except the Czech Republic and Angola, where OXA-48-like were the most prevalent. KPC, *Klebsiella pneumoniae* carbapenemase; VIM, Verona-Integron metallo-β-lactamase; NDM, New-Delhi metallo-β-lactamase; GES, Guiana extended-spectrum β-lactamase; IMP, imipenemase; OXA-48, oxacillinase-48.

5.1.1. IncF plasmids

IncF plasmids are narrow-host-range plasmids that rely on both host-encoded and self-encoded factors for replication [123]. They are usually large in size (>100 kb), but with low copy number and often carry an additional replicon type to initiate replication [127]. This a strategy used by narrow-host range plasmids to obtain broad-host range replication. An example of this was seen in plasmid pKPX-1 from NDM-producing *K. pneumoniae* clinical isolates, which contains a narrow-host range (IncFIB) and a broad-host range (IncR) replicons, assisting with broad-host range replication [128]. This is an important characteristic of IncF plasmids, but these plasmids still encode regions essential for conjugative transfer, replication, and segregational stability [117]. Moreover, the plasmid's multi-replicon state can allow for acquisition of a plasmid carrying an incompatible replicon when replication is controlled by a compatible replicon, allowing the replicon not responsible for replication to undergo genetic alteration [123,129].

IncF plasmids are mostly associated with extended-spectrum beta-lactamases (ESBLs), particularly the $bla_{CTX-M-15}$ gene. A major IncF plasmid carrying the $bla_{CTX-M-15}$ gene was reported by Coque et al. (2008) to contain an MDR region containing bla_{TEM-1}, bla_{OXA-1}, and *aac(6')-Ib-Cr* genes, and other determinants of aminoglycoside and tetracycline resistance [130]. Moreover, these plasmids have been recently associated with carbapenemases in *Enterobacteriaceae*. Their great intracellular versatility and rapid evolution of their replicons' regulatory sequences allow them to succeed in their spread in *Enterobacteriaceae* [123]. This has been shown in most studies focusing on KPC- and NDM-producing *E. coli* and *K. pneumoniae* in different countries [128,131–134].

The first occurrence of an IncF plasmid (pKpQIL) in *K. pneumoniae* ST258 was reported by Villa and colleagues (2010), which was a 113-kb plasmid belonging to the IncFII replicon group [123]. Since then, IncF plasmids have been reported in other countries where they mediate the spread

of bla_{KPC}. Examples of IncF plasmids in *K. pneumoniae* carrying KPC include pBK30683 (140-kb) and pBK30661 (73,6-kb) plasmids, which were reported in US hospitals from patients with urinary tract infections[135]. pBK30661 was identified as an IncFIA plasmid harboring nine ARGs such as β-lactam resistance (*bla*KPC-3, *bla*TEM-1, *bla*OXA-9), aminoglycoside resistance (*aacA4*, *aadA1*, *strA*, *strB*), sulfonamide resistance (*sul2*), and trimethoprim resistance (*dfrA14*) genes [135]. Other IncF types such as pKP1504-KPC and pGR-1780 have also been reported to spread *bla*KPC-2 in *K. pneumoniae* clinical isolates, specifically ST258 and ST147 [136].

These narrow-host range (IncF) plasmids are not only responsible for disseminating KPC, but also NDM in *E. coli* and *K. pneumoniae* [137–139]. Multiple plasmids have been reported, since 2012 and until recently, to carry NDM variants particularly on IncFIB and IncFII plasmid types in *K. pneumoniae* and *E. coli*, respectively (Table 1, Figure 3) [128,131–133,140]. Bigger plasmids such as pPMK1-NDM (304,5-kb) and pNDM-EcoGN568 (166,7-kb), are examples of NDM-1-containing plasmids, which contained other resistance determinants including β-lactamases, with pPMK1-NDM containing a large conjugative transfer module [132,141]. Other IncF plasmids including pEh1A, pNDM-Ec1GN574, pKOX-NDM-1, and pCRCB-101_1 are also responsible for the dissemination of *bla*NDM-1 in other species such as *Citrobacter freundii*, *Enterobacter hormaechei*, and *Klebsiella michiganensis* [128,134,141,142]. pNDM-Ec1GN574 and pKOX-NDM1 plasmids were similar in size (110,8-kb), with the NDM region being flanked by 256-bp direct repeats, which are suggested to be responsible for the acquisition of the *bla*NDM-1 gene [141]. An IncFII plasmid was also reported in an isolate carrying both NDM-5 and MCR-1 genes in China [143].

Although IncF plasmids are the most prevalent, other narrow-host range incompatibility types such as IncI, L/M, and IncX are widely distributed and are associated with multiple

carbapenemases, ESBLs and MBLs. Only in a few instances have they been associated with the class D carbapenemases, specifically the OXA-181 gene (Table 1).

5.1.2. IncX plasmids

IncX plasmids were previously described as less predominant in *Enterobacteriaceae*, because of underestimations by PBRT. The first plasmids in this group, R6K, were discovered by Kontomichalou and colleagues in 1970, during the pre-antibiotic era in a *Salmonella* spp. isolate. This was a 39,8-kb self-transmissible low copy number (10-15 replicons) plasmid, containing ampicillin and streptomycin resistance determinants [144]. Comparison studies looking at plasmid R6K and modern plasmids revealed that this plasmid is different from other plasmids in the IncX group, suggesting that subdivisions are required in the IncX group [145]. Firstly, only two subgroups (IncX1 and IncX2) were characterized based on restriction analysis [145]. The expansion of this plasmid family to IncX3 and IncX4 was proposed by Johnson *et al*. (2012) based on a phylogeny deduced from polymorphisms of all conserved regions of sequenced IncX plasmids [146]. Another subgroup, IncX5, was further added shortly after this expansion in a KPC-5-producing *K. pneumoniae* isolate [147]. Since these expansions, IncX plasmids have been found to play a major role in the dissemination of β-lactamases, including carbapenemases. IncX1 was previously described as more predominant than IncX2 in environmental isolates [145]. However, Dobiasova and Dolejska (2016) reported a high prevalence of IncX1 and IncX4 in environmental isolates and none in human isolates in Africa [148].

IncX plasmids are usually associated with carbapenemase genes in *Enterobacteriaceae*, particularly *bla*KPC, *bla*OXA-181 and *bla*NDM (Table 1). According to recent studies, IncX3 is the predominant subgroup reported to harbour both *bla*KPC and *bla*NDM genes [149–152]. These studies reported this subgroup as predominantly associated with *bla*NDM variants than with *bla*KPC variants.

Further, bla_{NDM-1} and bla_{NDM-5} were frequently associated with IncX3 than any other bla_{NDM} variant. Only in a few instances have IncX4 and IncX5 plasmids been associated with carbapenemase genes (Table 1)[150,153]. Furthermore, an IncX5 plasmid, encoding bla_{IMP-4} was reported in Australia from an *E. coli* of animal origin[154]. These suggest that an essential role is played by IncX3 in the acquisition, emergence and dissemination of bla_{NDM}. IncX3 plasmids that have been associated with the spread of bla_{NDM} are the following: pEc2A (74,8-kb), pM213_X3 (43,5-kb), pNDM-NJ-IncX3 (39,5-kb), and pKW53T-NDM (46,1-kb) [140,142,153]. Other IncX3 plasmids have been reported in *E. cloacae* isolates producing bla_{NDM-4} recovered from Czech hospitals, in Europe[155]. An IncX plasmid was reported in an *E. coli* isolate co-producing both bla_{NDM-5} and *mcr-1* genes in china [143]. Occurrence of IncX6 was reported in 2016 in *E. cloacae*. Moreover, the dissemination of this plasmid type has been shown in at least six *Enterobacteriaceae* species in China [51,156]. IncX6 was reported to carry both bla_{KPC-2} and bla_{KPC-3} in China (Table 1)[51,156]. In bla_{KPC}-producing *Enterobacter* spp., another subgroup, IncX7, has also been reported in the United States [53]. All these findings suggest the wide dissemination of IncX subgroups in *Enterobacteriaceae* in China and the United states.

5.1.3. L/M plasmids

L/M plasmids have been considered an emerging threat due to their increasing prevalence in MDR clinical and environmental isolates [157]. L/M plasmids are broad host-range plasmids with an average size of 50 to 80-kb and a low copy number [98]. Foster *et al*. (2004) reported that the pEL60 plasmid in *Erwinia amylovora* has a basic L/M plasmid backbone but it lacks genetic elements and resistance determinants [158]. Moreover, genomic analysis of L/M plasmids have shown backbone genes such as replication and stability modules, conjugative transfer system and a *mucAB*-like mutagenic DNA repair system [158].

Separation of this group into IncL and IncM was suggested by Carattoli *et al.* (2015) due to differences in their ExcA, TraY and TraX proteins. This separation has been considered and the PBRT scheme has been updated to incorporate these separate plasmids. This incompatibility group has been associated with multiple ESBLs, AmpCs, and carbapenemases, specifically class B and D genes [157,159]. Several IncL plasmids in *bla*NDM-1- and *bla*OXA-48-producing clinical isolates have been widely reported, some of which include: pNDM-OM (87,1-kb), pNDM-HK (88,8-kb), E71T (63,5-kb), and pOXA-48-4963 (63,5-kb) [157,160,161]. Although L/M plasmids usually harbour *bla*NDM and *bla*OXA-48 genes, they are also reportedly associated with *bla*IMP in *Enterobacteriaceae* (Table 1, Figure 3). A few studies have identified these plasmids in *bla*IMP-4-producing isolates [162]. In animal origin, Dolejska and colleagues reported the presence of *bla*IMP-4 in *E. aerogenes* on an IncM plasmid (pEa1631, 85-kb)[154]. Bryant *et al.* (2013) have also reported L/M plasmids (pNE1280, 66,5-kb) in *bla*KPC-producing isolates from a female with a medical history of mitral and aortic valve stenosis, pulmonary hypertension, restrictive lung disease and diabetes [163].

5.1.4. A/C plasmids

Another important broad-host range incompatibility type is the A/C plasmids, which harbour various carbapenemase genes. These plasmid types are different from other plasmid types in that they contain an integron with the theta replicon, three integrative hotspots, putative transcriptional regulators, and hypothetical genes [164,165]. These plasmids are large with low copy numbers. The A/C plasmid types are usually associated with cephalosporinases eg. *bla*CMY and MBLs eg. *bla*NDM (Table 1)[166,167]. However, these plasmids have also been associated with the dissemination of carbapenemases such as *bla*NDM, *bla*VIM and *bla*KPC[155,168,169]. Two A/C groups have been identified and are named A/C$_1$ and A/C$_2$, with A/C$_2$ being the most predominant [170]. However, all the A/C plasmid types share most of the conserved regions such as the genes responsible for conjugative

transfer (*tra* genes) and replication (*repA*), as well as other genes with unknown functions [171]. A/C plasmids have been thoroughly reviewed previously [172]. Only a few plasmids belong to the first A/C plasmid types, including plasmids pRA1 and pIncAC-KP4898 [173,174]; pIncAC-KP4898, encoding bla_{VIM-1}, is a recently isolated 156,2-kb plasmid [174]. bla_{NDM-1} has been associated with A/C$_2$ plasmid types in different *Enterobacteriaceae* species; recently, bla_{NDM-4} was detected on A/C$_2$ plasmids [140]. Reported A/C$_2$ plasmids carrying bla_{NDM-1} include pM214_AC2 (176-kb), pNDM-EcoGN568 (166,7-kb), pNDM-KN (162,7-kb), and pNDM-PstGN576 (147,8-kb) [140,141,167]. pNDM-EcoGN568 is a multi-replicon (IncF and A/C) circular plasmid, which was reported to be identical to pNDM10-0505, an A/C plasmid with the same size as the pNDM-EcoGN576 plasmid [141]. All these three plasmids shared similar conserved sequences and genes, suggesting a lateral transfer between different species, albeit their independent acquisition of genes cannot be ruled out [141].

5.1.5. IncN plasmids

IncN plasmid types are also of broad-host range, with high transmission efficiency. They are also important in the dissemination of carbapenemase genes including bla_{KPC}, bla_{NDM}, bla_{IMP}, and bla_{VIM} (Table 1)[74,147,175,176]. Within this group of plasmids, three subgroups with similar plasmid scaffolds and less similarity in backbone sequences have been described: IncN1 (R46), IncN2 (p271A), and IncN3 (pN-Cit) [85,177]. These characteristics might be the reason for their stability and success in disseminating multiple carbapenemases. IncN plasmids are usually medium-sized conjugative plasmids and have been documented to be associated with bla_{VIM}-producing *Enterobacteriaceae* and bla_{KPC}-producing *K. pneumoniae* isolates[178]. Plasmids including p9 (70,6-kb), p12 (75,6-kb), pKPC-629 (80,1-kb), pBK31551 (83,7-kb), pKO6 (65,5-kb), and pKp58-N (69,8-kb) have been documented as carriers of bla_{KPC} [52,135,179]. Most of these plasmids have been

deposited into GenBank without a corresponding published article (accession numbers in Table S1).

The pKOX105 (54,6-kb) plasmid carried regions encoding genes conferring resistance to carbapenems (*bla*~VIM-1~), cephalosporins (*bla*~SHV-12~), aminoglycosides (*aacA4*), trimethoprim (*dfrA14*) and quinolones (*qnrS1*) [180]. This plasmid was compared with other previously reported IncN plasmid types i.e., plasmids 9 (70,6-kb) and 12 (75,6-kb) that carried a *bla*~KPC~ gene [179]. The scaffolds between these IncN plasmids were found to be the same, but the MDR regions were different in all the plasmids[180]. The major differences that are usually reported among IncN plasmids are related to their acquired genes[180].

Other plasmid groups

Other incompatibility groups such IncI, ColE, IncB/O, IncH and IncP have also been reported to be associated with carbapenemases in *Enterobacteriaceae*, albeit they are reported in few species and are limited to a few carbapenemase genes (Table 1).

5.2   Molecular epidemiology of plasmids in *Enterobacteriaceae*

K. pneumoniae

In the United States, KPC is the major carbapenemase associated with antibiotic resistance (Figures 2, 3 & 4)[24,49,135,181]. KPC variants, such as KPC-2, KPC-3, and KPC-4, were reported in several studies in the US and were associated with multiple plasmid replicon groups, facilitating their spread. KPC-2 was commonly associated with multiple STs, but ST258 was the most prevalent in the US. The IncF groups dominate in the spread of KPC-2 and KPC-3 in the US and other countries including Australia, Canada, China, Italy, Romania, and Spain [42,50,52,56,152,153,156,175] Only one study has reported on IncF groups in KPC-3 in Portugal and Romania [56,182]. Few

occurrences were also reported in other countries including USA, Mexico and Spain. This plasmid replicon group is commonly reported in KPC-producing *K. pneumoniae* species [183].

Other plasmid replicon groups such as IncN, IncP, IncX, IncU, IncI, A/C, IncR, and L/M are also occasionally reported in KPC-producing *K. pneumoniae* [48,52,53,57,176,181,184,185]. Moreover, these plasmid replicon groups were also associated with *K. pneumoniae* strains producing other carbapenemases. VIM was only reported by two studies in Italy and Kuwait to be hosted by IncN and A/C [175,178].

OXA variants in *K. pneumoniae* are usually spread by IncF and L/M replicon groups. L/M has been reported to spread OXA-48 in different countries including the US, Czech Republic, Romania, and Australia (Table 1)[56,161,162,181]. OXA-181 was reported in Angola and Australia on IncF, A/C, and IncX plasmid replicon groups (Figure 4)[138,162].

IncX is commonly associated with the spread of NDM variants and has been mostly described in China [186,187]. Other plasmid replicon groups including IncF, IncR, IncCol, L/M, and A/C have also been described in NDM-producing *K. pneumoniae* in Australia, China, Mexico, and Vietnam (Figure 3)[139,143,162,185,186,188]. Furthermore, other *Klebsiella spp*. such as *K. oxytoca,* do not have a wide distribution of replicon groups as only the IncN and IncF groups have been described in them in few countries (Table 1). IncN plasmids have been reported in *K. oxytoca* strains producing VIM-1 and IMP-6 β-lactamases [176,178]. These plasmid types were occasionally reported in VIM-1-producing *K. oxytoca* isolated from river samples [178]. Moreover, IncN plasmids have been found with *bla*IMP genes in Japan [176].

E. coli

Similar to *K. pneumoniae* species, *E. coli* strains have a wide distribution of plasmid replicon groups that have been reported worldwide. Most carbapenemase-producing *E. coli* usually harbor

IncF plasmids, which is also dominant in *K. pneumoniae* species. IMP variants in *E. coli* are spread by multiple plasmid replicon groups such as IncHI, IncN, IncQ, IncX, IncI, and IncW [162,176,189]. Most NDM variants in *E. coli* were detected in China, except NDM-4, which is mostly reported in Australia [162]. NDM-1 has been disseminated worldwide through various plasmid replicon groups including IncF, IncI, IncX, and A/C; however, IncX is the most prevalent replicon facilitating the spread of bla_{NDM} genes [138,141,142,190,191]. Among these plasmid types, IncX-3 was mostly associated with the dissemination of bla_{NDM-1} in China, a finding different from other countries [191–193].

Enterobacter spp.

In the United States, *Enterobacter spp.* are ranked 8th among all other pathogens causing health-care-associated infections [49]. MDR *E. cloacae* isolates have been associated with bloodstream infections, resulting in bacteremia and mortality as high as 40% [162]. bla_{NDM} and bla_{IMP} are the most predominant carbapenemases isolated from *Enterobacter* spp. in the United States, Australia, China and Vietnam [162,190,194]. In Vietnam, bla_{NDM-1} was disseminated by IncF (IncFII and IncFIB) plasmids and, in a few cases, by the A/C plasmids (Table 1). Similar results were reported in other countries including the United Kingdom, Canada and the United States (Table 1). A/C plasmids have been identified in unrelated *E. cloacae* clinical isolates in China. As well, IncHI2 and IncN have been also implicated in the dissemination of bla_{NDM-1} [195]. IncHI2, L/M and IncP are usually associated with bla_{IMP} gene in countries such as Australia, China and the United States in *Enterobacter spp.* [162,189,195]. However, IncP plasmids have so far been identified with IMP-27-producing *Enterobacter spp.* in the United States [189]. These plasmids are also present in Spain and carry the bla_{KPC-2} gene in sewage [196]. Chavda *et al*. (2016) reported a wide distribution of plasmid Inc groups in KPC-producing *Enterobacter spp.* in New York City, which included IncN and IncX7 (bla_{KPC-2}), IncF and L/M (bla_{KPC-3}), and A/C (bla_{KPC-4}) [53]. The complexity, diversity and

wide geographical distribution of these Inc groups disseminating major groups of classes A and B carbapenemases pose a major challenge to the control of MDR *Enterobacter spp*.

Providencia, Proteus, Citrobacter and Salmonella spp.

Other *Enterobacteriaceae* species including *Proteus* spp., *Providencia* spp., *Citrobacter* spp. and *Salmonella* spp. have been only reported in a relatively few cases, with few carbapenemases being identified in them (Table 1). Two major carbapenemases, bla_{IMP} and bla_{NDM}, are predominantly detected in these species, with IncHI2, A/C, IncP and IncX3 being the plasmid types responsible for their dissemination between species [138,141,162,186,189,197,198]. The isolates in these reports were recovered from both clinical and environmental samples, including river water and domestic cats in Angola, Australia, Canada, China, India, Spain and the United States (Table 1)[138,139,141,162,189,197,198]. *Citrobacter* spp. also harbour IncX, IncR, IncHI2, IncP and IncN plasmids, which host bla_{NDM}, bla_{KPC}, and bla_{IMP} carbapenemase genes [97,199,200]. A hospital sewage in China contained *C. freundii* that carried an IncX3 plasmid harbouring the bla_{NDM-1} gene[200]. Another study in China reported IncX3 plasmids in NDM-1-producing isolates collected from ready-to-eat vegetables[201]. Other countries such as Australia, Canada and Italy reported other plasmid types such IncFII, IncR, IncP and L/M in *Citrobacter* spp[41,50,97,162]. Recent studies are reporting on the increased isolation of A/C plasmids in *Enterobacteriaceae* species, including *E. coli*, *K. pneumoniae* and *Salmonella spp*. [202]. Most isolates reported in this review were clinical isolates from human, and only few studies addressed carbapenemases and plasmid replicon groups in animal and Environmental isolates (Figure 5).
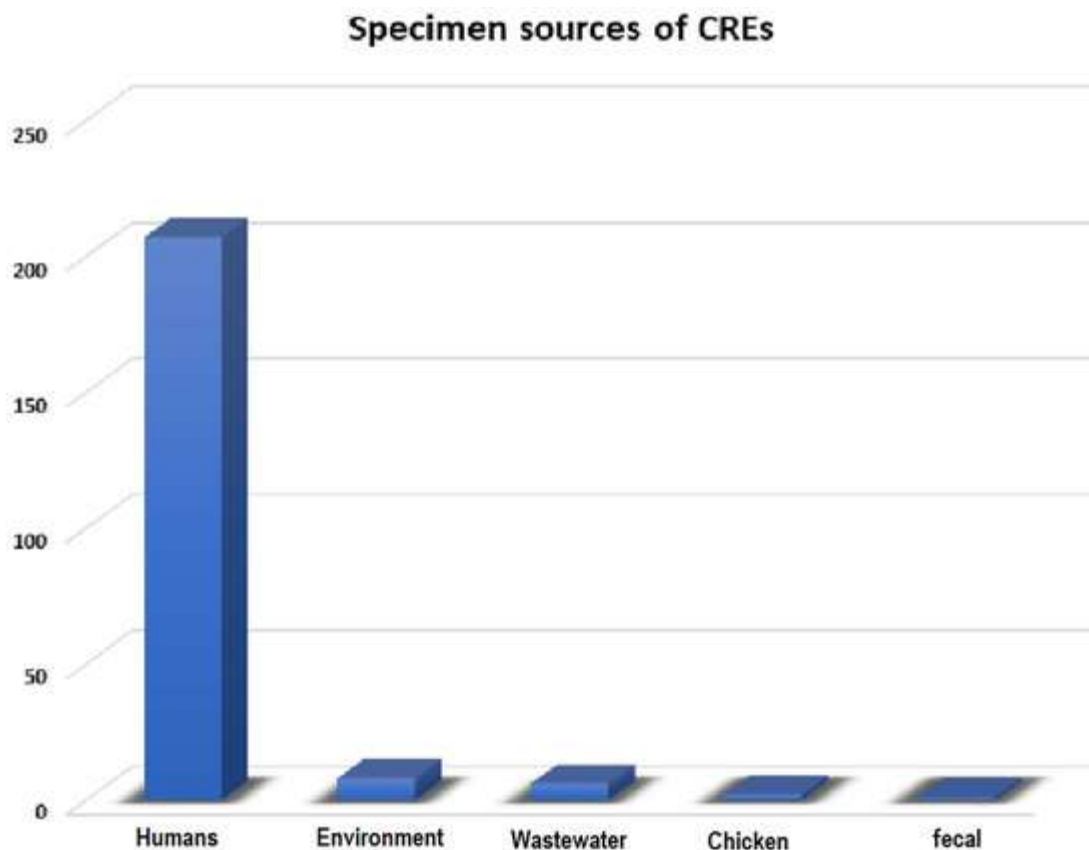
Specimen sources of CREs

Figure 5. Specimen sources of CREs identified in the included articles reported in this review. Most of the specimens were obtained from humans with a small number being obtained from environmental and animal sources. Frequencies were manually calculated and graphically represented using Microsoft Excel.

5.2. MGEs associated with plasmid incompatibility types

Most MGEs are commonly found on plasmids and play an important role in disseminating antimicrobial resistance determinants. MGEs such as integrons, transposons and insertions sequences, may be associated with specific incompatibility groups and carbapenemases.

In most A/C plasmids, the antimicrobial resistance island is usually embedded in or upstream of the *rhs*1 gene, and also contain an integron, multiple transposons, a Tn*21*-tnp module and a Tn*21*-mer module, which is interrupted by an insertion sequence IS*4321* [203]. Integrons,

particularly class 1 integrons, are usually associated with A/C plasmids and gene cassettes carrying ARGs, specifically bla_{NDM} [141]. This was shown in multiple A/C plasmids, and one IncF (pNDM-EcoGN568) plasmid, which was identical to A/C plasmids; the IncF plasmid only differed from the A/C ones by the number of ARG cassettes on the class 1 integron [141]. Other NDM-carrying plasmids, such as pM109-FII and pGUE-NDM, carry a 12-kb ARG region that surrounds the bla_{NDM} gene [140]. An additional gene cassette bracketed by two IS26 elements and carrying bla_{TEM-1} was found downstream the *rmtB* gene (an aminoglycoside resistance determinant) [140].

bla_{NDM} variants such as NDM-4/5/6 have been reported on IncX3 plasmids. The genetic structure of IncX3 plasmids is usually highly similar in almost all plasmids. A study performed in Myanmar (Burma) found NDM-4 and NDM-7 on IncX3 plasmids that were highly similar to previously reported IncX3 plasmids; suggesting a common ancestor[140]. NDM-4 was carried on a Tn*3* transposon unit and flanked by insertion sequences, with no other resistance gene being reported on this plasmid. The bla_{KPC} region of IncX6 plasmids are highly similar with Tn*6296* derivatives and an IS*Kpn19* element, However one plasmid reported by Li *et al* (2018) contained a Tn*6296* derivative and an IS*Kpn19*-containing Tn*6292* derivative [51,156].

The MGEs in A/C plasmids carrying other carbapenemases, such as $bla_{KPC,}$ are usually different from those carrying bla_{NDM}. Transposons are mostly associated with the acquisition of bla_{KPC} genes. The Tn*4401* transposon, which is approximately 10-kb in size and delimited by two 39-bp inverted repeat (IR) sequences, are associated with a 5-bp target-site duplications (TSDs) on both site adjacent to the IR sequences [204]. The 5-bp TSDs adjacent to the IR sequences are the target-site sequences for the Tn*4401* transposons. This is an important characteristic identified in plasmid p9 and p12, which contained a functional conjugative apparatus with a ~10-kb region

carrying the Tn*4401b* element with *bla*KPC and other ARGs [179]. The Tn*4401b* element in plasmid p9 was inserted in an inverted orientation downstream the EcoRII restriction/antirestriction system and the *uvp1* gene [179].

Similar characteristics are seen in IncF plasmids carrying *bla*KPC with additional elements. pBK30661, an IncF plasmid whose backbone genes are separated by multiple insertion sequence elements (IS*3*, IS*26*, IS*1294* and IS*66*), had a Tn*4401d* variant with a 68-bp deletion upstream of the *bla*KPC gene [135]. The region upstream the Tn*1331* was truncated by an 8-kb nickel resistance operon (*nic* operon), which resulted in a deletion of the corresponding 5-bp sequence and leaving a unique 5-bp sequence adjacent to the upstream IR sequence [135]. pNE1280, an L/M plasmid carrying the *bla*KPC gene, contained a major insertion of a 13-kb Tn*3* family transposon, the Tn*4401f*, with the *bla*KPC-4 flanked by IS*kpn6* on the left and IS*kpn7* on the right [163].

The genetic structure of the *bla*OXA-48 gene in L/M plasmids is different from that of other carbapenemases. This gene is usually part of the Tn*1999* transposon (Tn*1999* − Tn*1991.4*), with Tn*1991.2* being the most prevalent [157,205]. In 2016, Cuzon and colleagues reported an L/M plasmid carrying GES-5 and GES-6 on the same plasmid (Table 1). This plasmid harboured additional ARGs, including *aadA1* and *sul*1 [206]. *bla*GES-5 and *bla*GES-6 genes were located on a class 1 integron, and both sides were flanked by IS*26* and IS*6100*. This pEB-1 plasmid was compared to other L/M plasmids, pEL60 and pNDM-OM, and similar characteristics were observed, except that the integration site of the ARGs array was different [206]. In South Africa, a *bla*GES-5 gene ;was reported on an IncQ plasmid, but it was still harboured on a class 1 integron, with an additional *aadA4* on an integron mobilization unit (IMU) [12].

6. Conclusion

Our review showed a high frequency of bla_{KPC} (n=956, 73%) genes in almost all the countries reported in this review, with China, Canada, Greece and United States having the highest percentages. These genes are associated with multiple plasmid groups including IncF (n=254, 48%), IncN (n=125, 24%), IncX (n=38, 7%), A/C (n=39, 7%), and L/M (n=14, 3%) in different *Enterobacteriaceae* species. Furthermore, specific plasmid type such as IncF, L/M and IncX3 have been reported to be associated with the dissemination of bla_{KPC}, bla_{OXA-48}, and bla_{NDM}, respectively. We also showed the frequency of carbapenemases and plasmid replicon groups in the articles used for our review. Other countries, such the United States and China, had high frequencies due to more research being performed in these countries. Our review has also shown the important role played by MGEs, such as plasmids, transposon and insertion sequences, in acquisition and dissemination of ARGs among *Enterobacteriaceae* species, increasing the need for new antibiotics and antibiotic stewardship strategies. We also found that a major limitation to effective characterization of plasmid evolution was the use of PCR-based instead of WPS-based plasmid typing.

WGS has proven to give enough data for plasmid characterization, albeit PBRT still forms the basis of most plasmid characterization studies, particularly in low-income countries. Obviously, long-read WPS and WGS hold the key to an efficient characterization of plasmid types, epidemiology and evolution, towards an efficient description of antibiotic resistance dissemination and expansion among *Enterobacteriaceae*. By overriding the deficiencies of PBRT, WPS and WGS shall increase the effective identification and control of resistant bacteria, reducing mortalities, morbidities and healthcare-associated expenses involved in long-term hospitalization of infected patients.

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Supplementary Table S1. Metadata of plasmids deposited at GenBank and included in this study

Supplementary dataset. Nucleotide sequences of plasmids included in this study and obtained from Genbank