Carbapenemase-producing *Klebsiella pneumoniae*: a key pathogen set for global nosocomial dominance

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Abstract

The management of infections due to *Klebsiella pneumoniae* has been complicated by the emergence of antimicrobial resistance, especially to the carbapenems. Resistance to the carbapenems in *K. pneumoniae* involves multiple mechanisms, including the production of carbapenemases (e.g. KPC, NDM, VIM, OXA-48-like), as well as alterations in outer membrane permeability mediated by the loss of porins, and the up regulation of efflux systems. The latter two mechanisms are often combined with high levels of other types of β-lactamases (e.g.

AmpC). *K. pneumonaie* ST258 emerged during the early to mid-2000s as important human pathogens and has spread extensively throughout the world. ST258 comprises of 2 distinct lineages namely clade I and clade II and it seems that ST258 is a hybrid clone that was created by a large recombination event between ST11 and ST442. Incompatiblity group F plasmids with bla_{KPC} have contributed significantly to the success of ST258. The optimal treatment of infections due to carbapenemase-producing *K. pneumoniae* remains unknown. Some newer agents show promise for treating infections due to KPC-producers, however effective options for the treatment of NDM-producers remain elusive.

Keywords; *K. pneumoniae*, carbapenem resistance, ß-lactamases, clones, treatment

Introduction

The genus *Klebsiella* spp. belongs to the family Enterobacteriaceae that include saprophytes often isolated from the environment. *Klebsiella pneumoniae* is the most clinically relevant *Klebsiella* species and is responsible for over 70% of human infections due to this genus (1). In humans, *K. pneumoniae* most often colonizes the gastrointestinal tract, skin, and nasopharynx and is an important cause of serious community-onset infections such as necrotizing pneumonia, pyogenic liver abscesses, endogenous endophthalmitis (2, 3). During the 1970's, *K. pneumoniae* became an important cause of nosocomial infections, especially urinary tract infections (UTIs), respiratory tract infections and bloodstream-associated infections (BSIs) (1, 2, 4). A recent report from the CANWARD surveillance program showed that *K. pneumoniae*

was the 5th most common bacteria isolated from Canadian hospitals during the period of 2007-2011 (5).

The management of infections due to *K. pneumoniae* has been complicated by the emergence of antimicrobial resistance, especially since the 1980's. The cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole are often used to treat infections due to *K. pneumoniae* and resistance to these agents generates delays in appropriate empiric therapy with subsequent increased morbidity and mortality in patients (6). Therefore, clinical therapeutic choices for treating nosocomial infections due to *K. pneumoniae* have become challenging (6-8). Several global surveillance studies during the 2000's have shown that between 20-80% of *K. pneumoniae* were resistant to 1st line antibiotics including the cephalosporins, fluoroquinolones, and aminoglycosides (9-11). Of special concern is the emerging resistance to the carbapenems, since these agents are often the last line of effective therapy available for the treatment of infections caused by multidrug-resistant (MDR) *K. pneumoniae* (12).

Recently, the World Health Organization (WHO) released a report entitled: "Antimicrobial resistance: global report on surveillance 2014" (13). The report focused on antibiotic resistance in seven different bacteria responsible for common, serious diseases such as bloodstream infections, diarrhea, pneumonia, urinary tract infections and gonorrhoea. Specifically for *K. pneumoniae* the WHO report states the following (13): "Resistance to the treatment of last resort for life-threatening infections caused by a common intestinal bacteria, *K. pneumoniae* (i.e. carbapenem antibiotics) has spread to all regions of the world. *K. pneumoniae* is a major cause of hospital-acquired infections such as pneumonia, bloodstream infections, infections in newborns and intensive-care unit patients. In some countries, because of

resistance, carbapenem antibiotics would not work in more than half of people treated for *K. pneumoniae* infections".

The aim of this article is to provide a brief overview of the mechanisms responsible for carbapenem resistance in this specie, highlighting recent developments in the clonal expansion of certain high-risk sequence types or clones, describe the role of epidemic plasmids in the global dissemination and success of carbapenem-resistant *K. pneumoniae*. Sections on virulence and treatment are also included.

Mechanisms of resistance to carbapenems

Resistance to the carbapenems in *K. pneumoniae* are linked to different mechanisms (14). The co-occurrence of permeability defects together with production of ß-lactamases that possess very weak carbapenemase activity may lead to reduced susceptibility to carbapenems, particular to ertapenem (15). Such enzymes may be either Ambler class A extended-spectrum ß-lactamases (ESBLs) or Ambler class C AmpC cephalosporinases, and some of them (i.e. CTX-M-15, CMY-2) are more likely to contribute to reduced carbapenem susceptibility when combined with permeability defects (16).

Apart from those mechanisms involving ß-lactamases (e.g. ESBLs, AmpC) which are not considered as significant carbapenem-hydrolyzing enzymes, true carbapenemases are responsible for non-susceptibility to carbapenems without additional permeability defects in *K. pneumoniae*. Those carbapenemases either belong to Ambler class A, B, or D molecular classes (17).

The class A KPC-type ß-lactamases have been extensively and almost exclusively reported in *K. pneumoniae* (18). KPC-1 (it was later shown to be identical to KPC-2) was reported in the late 1990's from a *K. pneumoniae* isolated in North Carolina, USA. To date more

than 20 different KPC variants have been described even though KPC-2 and -3 remains the most commonly identified variants (19). These enzymes provide resistance to the penicillins, carbapenems, cephalosporins, cephamycins and monobactams and are inhibited by β-lactamase inhibitors such as clavulanic acid (weakly), tazobactam (weakly), boronic acid and avibactam. KPC β-lactamases (especially KPC-2 and –3) have been described in several enterobacterial species, especially *Klebsiella* spp. and to a lesser extend in *Enterobacter spp.* (20). Several nosocomial outbreaks most often due to *K. pneumoniae,* have been reported from North America (especially the USA), South America (Colombia, Argentina), Europe (Greece, Italy, Poland), Asia (China) and Middle East (Israel) (19, 21, 22). KPC-producing bacteria are considered to be endemic in certain parts of the world, such as the North Eastern USA, Puerto Rico, Colombia, Greece, Italy, Israel and China and are important causes of nosocomially-acquired infections in some parts of these countries (22). *K. pneumoniae* ST258 with KPC-2 and KPC-3 had significantly contributed to the world-wide distribution of this resistance trait (more details are provided in the high risk clone section) (22). In addition there are some scattered reports of GES-5, another class A carbapenemase that is a point mutant derivative of GES-1 (23).

The class B ß-lactamases or metallo-ß-lactamases (MBLs) identified in *K. pneumoniae* have also been identified in various enterobacterial species (17). They are mainly NDM-, VIM-, and IMP-type enzymes, with the first group being the most commonly identified worldwide. Although IMP producers are mainly identified in China, Japan, and Australia, VIM-producing *K. pneumoniae* isolates are mainly found in Italy and Greece (17). NDM-1 shares very little identity with other MBLs, the most similar being VIM-1/VIM-2 with only 32.4% amino acid identity. Since the first description of NDM-1, more than 10 variants of this enzyme has been described, the majority of them originated from Asia (24). Bacteria with MBLs are often resistant to the

penicillins, carbapenems, cephalosporins, and cephamycins but remain susceptible to the monobactams and their activity is inhibited by metal chelators such as EDTA, dipicolinic acid (Table 1). The majority of NDM-1-producing bacteria also carry a diversity of other resistance mechanisms (17). These additional mechanisms include the following: plasmid-mediated AmpC β-lactamases (especially CMY types), ESBLs (especially CTX-M-15), different carbapenemases (e.g. OXA-48-, VIM-, KPC-types), 16S ribosomal RNA methyltransferases, plasmid-mediated quinolone resistance determinants, macrolide modifying esterases, and rifampicin-modifying enzymes. Consequently, Enterobacteriaceae with NDMs remain mostly susceptible to agents such colistin, fosfomycin and tigecycline (24).

The only class D carbapenem-hydrolyzing ß-lactamase (CHDL) found in *K. pneumoniae* isolates is OXA-48 (and derivatives) that was firstly reported from a MDR *K. pneumoniae* isolate from Turkish isolates in Paris (25). OXA-48 hydrolyses efficiently narrow-spectrum ß-lactams such as penicillins, weakly hydrolyses carbapenems, and spares broad-spectrum cephalosporins (26). It has been found among all Enterobacteriaceae however it is mostly identified in *K. pneumoniae* (mostly from nosocomial origin) and *E. coli* (mostly from community origin) isolates. OXA-48-producing *K. pneumoniae* is endemic in Turkey and certain North African and European countries (e.g. Morocco, Tunisia, Spain, Belgium) showing a wide range of susceptibility profiles (25). Indeed MICs of carbapenems may significantly vary from isolate to isolate, depending on the host permeability background. Similarly, susceptibilities to broad-spectrum cephalosporins can also significantly vary, depending on the co-production of other ß-lactamases such as the ESBLs. Some OXA-48 derivatives have also been identified in *K. pneumoniae*, being OXA-181, OXA-204, and OXA-232, all sharing similar hydrolytic properties (27). These enzymes have been identified in North Africa, Australia, New Zealand, but one of

Table 1. Characteristics of *Klebsiella pneumoniae* that produce Carbapenemases

Enzymes	Classification	Examples	Spectrum of activity	Inhibition	Endemic areas	Molecular epidemiology
Metallo-β-lactamases	Class B	NDM-1 IMP, VIM,	Penicillins Cephalosporins Cephamycins Carbapenems	Metal chelators e.g. EDTA, dipicolinic acid	Japan (IMP), Taiwan (IMP), Indian subcontinent (NDM), Balkan states (NDM) Greece (VIM)	IncA/C, N plasmids (NDM) Class I integrons (VIM, IMP)
KPC	Class A	KPC-2, 3 others	Penicillins Cephalosporins Cephamycins Carbapenems	Clavulanic acid (weak) Tazobactam (weak) Boronic acid Avibactam	USA , Greece, Italy, Israel, China, Brazil, Colombia, Argentina	Tn*4401* IncFII plasmids CC258
OXA-β-lactamases	Class D	OXA-48, OXA-181 OXA-204 OXA-232	Penicillins Temocillin β-lactamase inhibitor combinations Carbapenems (weak)	NaCl	Turkey North Africa (Morocco, Tunisia) Europe (Spain, Belgium)	Tn*1999* IncL/M plasmids

the main sources of OXA-181 (which is the second most common OXA-48 derivative) is the Indian subcontinent. Finally, a different isoenzyme of OXA-48 named OXA-163, differing by a single amino acid substitution combined with four amino-acid deletions, has been identified in Argentina (28). This variant shows specific hydrolytic features since it strongly hydrolyzes broad-spectrum cephalosporins with weak activity against the carbapenems.

Carbapenemases possess variable hydrolytic activities, with the MBLs and KPC enzymes hydrolyzing the carbapenems more efficiently than OXA-48-like enzymes. However, high-level carbapenem resistance among *K. pneumoniae* with carbapenemases requires additional permeability deficiencies, regardless of the type of carbapenemase produced (24). Conversely, isolates exhibiting low carbapenem MICs have been identified for *K. pneumoniae* with all types of carbapenemases. This might explain the initial successful spread of *K. pneumoniae* with bla_{KPC} in the USA during the 1990's and the initial spread of *K. pneumoniae* with bla_{VIM} in Greece (some isolates with VIMs have MICs to imipenem lower than 0.5 µg/ml) (29). It is possible that the initial spread of *K. pneumoniae* with carbapenemases was due to isolates with low MICs to the carbapenems without permeability modifications.

Genetic supports of carbapenemase genes

The different carbapenemase genes circulating within *K. pneumoniae* are often carried by mobile structures including plasmids and transposons and therefore can spread efficiently to different members of the Enterobacteriaeacae. Transposon Tn*4401* has been shown to be the main genetic structures enhancing the spread of bla_{KPC}-type genes onto different plasmid scaffolds, but its transposition is not very efficient and the frequency of transmission has been quantified at 4.4×10^{-6} (30, 31). Tn*4401* is 10 kb in length, delimited by two 39-bp imperfect inverted repeat (IR) sequences, and contains Tn3 transposase gene, a Tn3 resolvase gene, and

two insertion sequences, IS*Kpn6* and IS*Kpn7*. The association of Tn*4401*with bla_{KPC} and other antibiotic resistance determinants provides an easy way for carbapenemases to effectively spread as a hitchhiker gene, even in the absence of carbapenem selection (32).

The bla_{OXA-48} gene is located into the Tn*1999* composite transposon that was shown to transpose at a very low frequency ($<1.0 \times 10^{-7}$) (33). The current dissemination of bla_{OXA-48} is therefore mainly due to the epidemic IncL/M-type plasmid (pOXA-48a) that was shown to be highly transferable (34, 35). The MBL genes (e.g. bla_{IMP}, bla_{VIM}, and bla_{NDM}) are found onto different broad host range plasmid types (e.g. IncA/C, IncN) with various different genetic features (36); bla_{IMP}, bla_{VIM} are usually found into class 1 integron structures which are located within transposon structures that enhance their dissemination. Conversely, the bla_{NDM} genes are associated to mosaic genetic structures including insertion sequences (e.g. IS*Aba1*) but the exact mechanism leading to their acquisition onto plasmid scaffolds remains unknown (37).

High risk clones among other carbapenemase producers

High-risk clones are defined as clones with a global distribution and showing enhanced ability to colonize, spread and persist in a variety of niches (38). High risk clones have acquired certain adaptive traits that increase their pathogenicity and survival skills accompanied with the acquisition of antibiotic resistance determinants. They have the tenacity and flexibility to accumulate and exchange resistance and virulence genes with other bacteria. High risk clones have contributed to the spread of different plasmids, genetic platforms, and resistance genes among Gram negative bacteria and have played a very important role in global spread of antibiotic resistance (39). Such clones are powerful source for the propagation of genetic antimicrobial resistant components (i.e. genes, integrons, transposons and plasmids) (39). Drug resistant determinants are provided to the offspring in a vertical fashion and such eminent or high

risk clones increase the prevalence of antibiotic resistance by its enhanced ability to survive and reproduce efficiently. The habitat of *K. pneumoniae* is not limited to humans but is ubiquitous to the ecological environment that includes surface water, sewage, and soil (2). Moreover, due to the ability of some isolates, including *K. pneumoniae* with carbapenemases (e.g. bla_{KPC} and $bla_{NDM)}$, to survive for long periods of time in the environment with temperature extremes, they play import roles in the horizontal transfer of dug resistance determinants to other bacteria, acting as efficient donors and recipients (40, 41).

Clonal complex (CC) 258: ST258

The rate and consequence with which carbapenem resistance had disseminated globally in *K. pneumoniae* had raised cause for alarm among the medical community at large. To date, bla_{KPC} has been found in more than 100 different sequence types (STs), but this pandemic is primarily driven by the spread of KPC-producing *K. pneumoniae* isolates that are members of clonal complex (CC) 258 (18). CC258 (founder member is ST292) consists of one predominant ST namely ST258, and to a lesser extent ST11, ST340 and ST512 that are single locus variants (SLVs) of ST258 (18, 32). *K. pneumoniae* ST258 is a prototype of a high risk clone and recent information about the epidemiology, genetic rearrangement and evolution of this successful clone had provided insights into the global spread of antimicrobial drug resistance.

K. pneumoniae with bla_{KPC} was first identified in a non-ST258 isolate during 1996 in the Southern United States (42). During the late 1990s to early 2000s there were sporadic reports of *K. pneumoniae* with bla_{KPC} from the North Eastern USA, however, large outbreaks due to related isolates were not described (43). In 2009, the Centers for Disease and Prevention from the USA in collaboration with investigators from Israel, performed MLST on *K. pneumoniae* with bla_{KPC} and they identified ST258 among isolates from the New York area collected during 2005 (44).

As time progressed, ST258 was detected in geographically diverse regions of the USA and in 2009 it became apparent that ST258 was the predominant clone in this country being responsible for 70% of *K. pneumoniae* with bla_{KPC} obtained from different parts of this country (45). During the mid-2000s Israel experienced several nosocomial outbreaks of infections due to *K. pneumoniae* with bla_{KPC} that was caused by a clone (identified with PFGE) and named clone Q (44). Interestingly, clone Q has a similar pulsotype than ST258 present in the USA. This was followed by global reports of ST258 among *K. pneumoniae* with bla_{KPC} from countries such Greece (46), Norway, Sweden (47), Italy (48), Poland (49), Canada (50), Brazil (51) and Korea (52) suggesting that this ST had characteristics of international multidrug resistant high risk clones. Recent reports from Israel (53) and Italy (54) demonstrated the endemicity and persistence of CC258 over time while remaining the predominant clone among *K. pneumoniae* with bla_{KPC}. Interestingly, Israel has seen an overall dramatic decrease in the incidence of KPCs among *K. pneumoniae* but ST258 still remain the most predominant clone (53).

Kreiswirth and colleagues recently performed whole genome sequencing on two *K. pneumoniae* ST258 urinary isolates from New Jersey and then did supplementary sequencing on a different global collection of just over eighty CC258 clinical isolates (55). The phylogenetic single nucleotide polymorphism (SNP) analysis of the core genomes of these isolates showed that ST258 *K. pneumoniae* belonged to two well defined lineages named clade I and clade II. Clade I was associated with KPC-2 and clade II was associated with KPC-3. The genetic divergence between these two clades occurred in a 215-kb area that included the genetic material used for capsule polysaccharide biosynthesis (*cps*), an important virulence factor for *K. pneumoniae*.

The same group then compared the genetic structures of the *cps* regions and disperse of SNPs in the core genomes of the ST258 clades I and II with other *K. pneumoniae* sequence types (i.e. ST11, ST442, and ST42) (56). Kreiswirth and colleagues found a 1.1-Mbp area in ST258 clade II that is identical to that of ST442, while the remainder part of the ST258 genome was homologous to that of ST11. This indicated that ST258 clade II is a hybrid or cross-breed clone that was created by a large recombination event between sequence types ST11 and ST442. The investigators then identified the same *cps* regions in ST42 and ST258 clade I. The likeness of the areas surrounding the *cps* regions from ST42, ST258 clade I and ST258 clade II indicated that the ST258 clade I evolved from ST258 clade II due to the replacement of the *cps* region from ST42.

Clonal complex (CC) 258: Other STs

ST11 which is closely related to ST258 is the major ST among *K. pneumoniae* harboring bla_{KPC} from Asia (especially China) (57), has also been described in Latin America (18) and have been associated with NDMs (58, 59) from the Czech Republic (60), Switzerland (61), Thailand (62), Australia (63), USA (64), the United Arab Emirates (65) and Greece (66), being responsible for nosocomial outbreaks in those two latter countries. ST11 with $bla_{\text{OXA-48}}$ have recently been identified in Spain (67). Other STs also belonging to CG258 with bla_{KPC} have been reported from Colombia (ST512), Italy (ST512), Israel (ST512), Spain (ST512), Brazil (ST340) and Greece (ST340) (18, 68).

Other sequence types

K. pneumoniae ST147 is an emerging high risk clone that was first identified in Greece (69) and have been associated with bla_{VIM} and bla_{KPC} in that country (46). This global sequence type has also been associated with bla_{NDM} (70), and $bla_{\text{OXA-181}}$ (71) from various countries

including Switzerland, Iraq, Canada, UK, India and Italy. ST14, ST25 and ST340 with $bla_{\text{NDM-1}}$ had been identified in India, Kenya, and Oman (72) and ST405 with OXA-48 from Spain (67).

The importance of epidemic plasmids in spreading carbapenemase genes

Plasmids are extra chromosomal elements of double stranded DNA present in bacteria, which replicate independently of the host genome (73). Plasmids can undergo horizontal transfer through conjugation thereby transferring the encoded genetic elements from one bacterium to another. This movement of plasmid-borne antibiotic resistance genes has been central to the recent and rapid increase in global antimicrobial resistance (17). DNA on plasmids used for replication purposes needs to be conserved and therefore is utilized for the classification of plasmids. This "incompatibility group typing" scheme is based on unique replication areas identified in different plasmids to demonstrate relatedness and behavior of particular plasmids groups (74, 75). Antimicrobial resistance plasmids can broadly be divided into two main groups namely the narrow-host range group that most often belongs to the incompatibility (Inc) group F and the broad-host range group that belongs to the IncA/C and IncN. They have recently been termed "epidemic resistance plasmids" due to their propensity to acquire resistance genes and rapid dissemination among Enterobacteriaceae (76). Antimicrobial resistance determinants on epidemic plasmids provide a selective advantage to high risk clones and are likely central to their success (77, 78).

Plasmids associated with *K. pneumoniae* ST258 with bla_{KPC}

Several different KPC-containing plasmids have been identified in ST258. They belong to IncF (with FIIk1, FIIk2, and FIA replicons), IncI2, IncX, IncA/C, IncR, and ColE1 and these plasmids often contain various genes encoding for non-susceptibility to different antimicrobial drugs (32). However, the most predominant bla_{KPC} -plasmid types associated with *K.*

pneumoniae ST258 is IncF with FIIk replicons (79). The first *bla*$_{KPC}$-plasmid identified in ST258 (named clone Q at that time) was obtained in 2006 from Israel and named pKpQIL (80). This was a 113-kb IncF plasmid with FII$_{K2}$ replicon containing Tn*4401a*, with a backbone very similar to the pKPN4 plasmid first characterized in 1994 from non-KPC antimicrobial resistant *K. pneumoniae* obtained in Massachusetts (80).

Retrospective plasmid analysis of *K. pneumoniae* with *bla*$_{KPC}$ from the New York and New Jersey areas isolated during the early 2000s showed that ST258 contained *bla*$_{KPC-2}$ and *bla*$_{KPC-3}$ on pKpQIL-like plasmids which were nearly identical to the Israeli pKpQIL plasmid described in 2006 (81). PKpQIL-like plasmids from the New York and New Jersey isolates were mostly associated with *bla*$_{KPC-2}$ and to a lesser extent with *bla*$_{KPC-3,}$ whereas the pKpQIL plasmids from Israel were mainly associated with *bla*$_{KPC-3}$ (81, 82). This suggests that ST258 with *bla*$_{KPC-3}$ on KpQIL plasmids were introduced during the mid-2000s from the USA into Israel (a Founder effect) followed by clonal expansion in Israel. The IncFIIk plasmids are also the most common plasmid identified among ST258 with *bla*$_{KPC}$ from several different geographically diverse areas including Canada, Poland, United States, Israel, Brazil, Italy, and Norway (51, 79, 83). There also appears to be an association between different plasmid Inc groups and the ST258 clades I and II. The *bla*$_{KPC-3}$-associated IncI2 plasmids and *bla*$_{KPC-3}$-associated IncFIA plasmids were found exclusively in clade II while the pKpQIL- associated IncFIIk2 plasmids with *bla*$_{KPC-2}$ were detected in both clades I and II (55). pKpQIL plasmids were not only restricted to ST258 and were present in 33% of non-ST258 *K. pneumoniae* in the New York area (81).

The complete sequencing of plasmids associated with ST258 from large collections are revealing that they are evolving over time through large genetic rearrangements (79, 84, 85).

This process is creating hybrid plasmids as was previously described in Italy with ST258 containing two different IncF plasmids namely pKpQIL-IT and pKPN-IT as well as a ColE-like plasmid with bla_{KPC-2} (86). Both pKpQIL-IT and pKPN-IT have a very high degree of homology to the historic plasmids pKPN4 and pKPN3 from a non-KPC producing *K. pneumoniae* isolated in 1994 (86). This suggests that certain ancestral plasmids are particularly well suited to Enterobacteriaceae such as *K. pneumoniae* and are good candidates for sustaining the presence of bla_{KPC} through multiple independent insertions and transposition events. This is further supported by a recent study from Korea that demonstrated that ancestral plasmids were present among ST258 isolates from various geographical regions and were obtained as early as 2002 (87).

It seems that the presence of plasmids with bla_{KPC} is central to the success of ST258. The loss of pKpQIL by ST258 has limited the ability of these isolates to successfully disseminate when compared to other *K. pneumoniae* without bla_{KPC} and ST258 with pKpQIL (78). This would suggest that the bla_{KPC} in combination with other virulence or persistence factors on the pKpQiL-like plasmids promoted the fitness and survival of ST258. This is further supported by the epidemiological observation that non-ST258 *K. pneumoniae* with bla_{KPC} did not demonstrate the same global success as ST258 with bla_{KPC}. It appears that the successful global dissemination and survival of *K. pneumoniae* ST258 is in part dependent on the combination of bla_{KPC} on IncF plasmids with factors inherently present on the chromosome of this high risk clone (77).

IncI2 with bla_{KPC-3} can also successfully pair with ST258 and was recently detected in 23% of ST258 from the New York and New Jersey areas (88). Interestingly, this IncI2 plasmid also contained type IV pili which may contribute to successful dissemination of *K. pneumoniae* ST258.

Plasmids associated with *bla*$_{NDM}$ and *bla*$_{OXA-48}$

The current global dissemination of NDM-1-producing *K. pneumoniae* is linked to the dissemination of epidemic broad-host range plasmids. Several epidemiological studies showed a high diversity of plasmid backbones bearing the *bla*$_{NDM}$ genes. Molecular epidemiology indicated that the IncA/C-type plasmids are the main backbones responsible for spreading *bla*$_{NDM-1}$ among Enterobacteriaceae (72, 89), but IncFII, IncN, IncH and IncL/M types have also been identified in association with *bla*$_{NDM}$ (90-92). Noteworthy, IncA/C plasmids with the *bla*$_{NDM}$ often contain various clinically-relevant antibiotic resistance genes, such as those encoding RmtA or RmtC (16S rRNA methylases encoding high level resistance to aminoglycosides), QnrA (quinolone resistance), and CMY-type ß-lactamases (broad-spectrum cephalosporin resistance).

By contrast to what has been observed with *bla*$_{NDM}$ genes, the current emergence of OXA-48-producing isolates in many geographical areas is mainly explained by the success of one specific plasmid (pOXA-48a). This plasmid is 62-kb in-size and belongs to the IncL/M incompatibility group (34). Noteworthy, it possesses *bla*$_{OXA-48}$ as a unique antibiotic resistance gene, in contrast again with *bla*$_{NDM}$-positive plasmids that often contain several antibiotic resistance genes. Plasmid pOXA-48a is self-conjugative and it has been demonstrated that its *tir* gene, known to encode a transfer inhibition protein, was truncated. This inactivation was shown to be responsible for a 50- to 100-fold increase in the efficiency of transfer of pOXA-48a, and therefore explains the very high conjugation rate of that latter plasmid which was estimated to be around 1×10^{-1} (35). Therefore, it is considered that these specific features of plasmid pOXA-48a do explain in large part the current spread of the OXA-48 encoding gene.

The success and virulence factors of *K. pneumoniae* ST258

K. pneumoniae is responsible for human and animal infections and has also been implicated in causing diseases in certain plants such as spinach, rice and pineapples (93). It remains unclear how one bacterium are successful in causing infections in plants and human. *K. pneumoniae* also has the ability to survive from long periods in the hospital environment (83). Recently Lerner and colleagues identified super-spreaders among carbapenemase-producing *K. pneumoniae* isolated from rectal and environmental specimens (94). These super-spreaders were more likely to have high rectal concentrations and more likely to have high concentrations in the immediate environment may play a central role in transmission of carbapenemase-producing *K. pneumoniae*. Reservoirs in the patient or health care worker populations and the environment represent principle modes of spread in nosocomial outbreaks with the patient population being the most important reservoir in high-frequent outbreaks (83, 94).

The global molecular epidemiology of KPC-producing bacteria shows that *K. pneumoniae* is the most common species and ST258 is the predominant clone, suggesting a unique fitness and selective advantage beyond merely antimicrobial resistance. The reasons for the particular success of ST258 and its association with certain resistance plasmids are uncertain. However, its ability to spread swiftly is beyond dispute.

It is unclear if ST258 has increased virulence when compared to other *K. pneumoniae* isolates. A recent study demonstrated that ST258 is non-virulent in animal models, highly susceptible to serum killing and rapidly undergoing phagocytosis (95). Another study showed that not all ST258 behaved the same in a mouse lethality model but consistency did exist in a moth (*Galleria mellonella*) virulence model (96). ST258 also lacks well-characterized *K. pneumoniae* virulence factors, including K1, K2, and K5 capsular antigen genes, the aerobactin

genes, and regulator of mucoid phenotype gene *rmpA* (95). Lavigne and colleagues using the *Caenorhabditis elegans* model have shown that the plasmid with bla_{KPC} is not necessarily associated with increase virulence (97).

Capsular polysaccharide is a recognized virulence factor enabling *K. pneumoniae* to evade phagocytosis. The in-depth molecular epidemiologic examination of the genome region from different clades of ST258 which have independently acquired bla_{KPC} revealed that capsule polysaccharide biosynthesis region *cps*-1 and *cps*-2 is likely involved in the global success of these clades (56, 98). This region of diversification could be advantageous for *K. pneumoniae* isolates to change polysaccharide as a mechanism to evade host defenses. Capsule switching is a species-specific mechanism used by bacteria to escape the host immune response. DNA exchange in-and-around the *cps* regions may be an important mechanism used by *K. pneumoniae* to rapidly diversify and evolve (99).

Adler and colleagues investigated the association of the integrated conjugative element ICEKp258.2 with ST258 by testing 160 *K. pneumoniae* strains with diverse sequence types for the presence of *pilV*, a gene carried on ICEKp258.2 (100). They found that *pilV* was present only in ST258 and genetically related STs such as ST512. Based on sequence analysis, ICEKp258.2 harbors a type IV pilus gene cluster and a type III restriction-modification system. A type IV pilus could increase the uptake and exchange of DNA, such as plasmids, as well as facilitate adherence to living and nonliving surface which may in part explain the high transmissibility of ST258. Additionally, a type III restriction-modification system could serve in "host specificity" regarding the exchange of certain compatible plasmids and other mobile elements (56). The restriction of plasmids and specific mobile elements may explain the differences observed between ST11 (which lacks ICEKp258.2) and ST258, as the former is

associated with a broad range of plasmids and carbapenemases (KPC, VIM, IMP, NDM, and OXA-48), whereas ST258 strains predominantly harbor KPC. Taken together, the association of ICEKp258.2 with ST258 *K. pneumoniae* strains raises the possibility that this element may contribute to epidemiological success of this sequence type (56).

So far no specific virulence factor has been identified in those widespread clones producing NDM- or OXA-48-type enzymes, the main driving factor of those disseminated clones apparently being resistance to antibiotics only.

Treatment of infections due to *K. pneumoniae* with carbapenemases

Infections due to *K. pneumoniae* with carbapenemases often reach mortality rates ranging between 23 and 75%, which is attributed to the lack of active antimicrobial agents and underlying co-morbidities in patients (101). A delay in the appropriate antibiotic therapy for severe infections is strongly associated with impaired outcome and increased mortality for patients with severe sepsis and septic shock and is also relevant for patients with infections due to *K. pneumoniae* with carbapenemases (101). The optimal treatment of infections due to carbapenemase-producing *K. pneumoniae* is unknown and none of the currently available antibiotics used as single therapy may be effective for treating infections with all types of carbapenemase producers. Source control in addition to antimicrobial therapy, is essential for the effective management of these infections and is especially significant for the successful treatment of urinary tract and intra-abdominal infections. Empirical combination therapy including colistin, carbapenem or aminoglycoside, based on the local resistance epidemiology, might be justified for severely ill patients with suspected infection due to *K. pneumoniae* with carbapenemases (102).

Most clinical data on the efficacy of antibiotics for treating carbapenemase producers are from retrospective case series and anectodal case reports and mostly due to KPC-producing *K. pneumoniae* (103, 104). It seems logical to tailor the antimicrobial therapy to the *in-vitro* antimicrobial susceptibility patterns of tested antibiotic molecules and definitive therapy should always be guided by susceptibility testing. Often the polymyxins (e.g. colistin or polymyxin B), tigecycline, fosfomycin and sometimes select aminoglycosides are the only agents with *in-vitro* activity. Other antimicrobials, such as fosfomycin and nitrofurantoin, can be used if found to be active, but their use as monotherapy is generally limited to lower urinary tract infections (102). Since carbapenemase producers are mostly resistant to various other important antibiotic classes such as fluoroquinolones and the aminoglycosides, it is important to test for last resort agents such as polymyxins (e.g. colistin), fosfomycin, tigecycline, and rifampicin.

The antibiotic susceptibility patterns in particular to ß-lactam drugs depend of the carbapenemase type. KPC producers are usually resistant to all ß-lactams, however, temocillin does retain activity against some isolates and this drug is a treatment option in lower UTIs due to *K. pneumoniae* with bla_{KPC} (105). NDM, VIM and IMP producers remain susceptible to aztreonam while OXA-48-like producers may test susceptible to the expanded-spectrum cephalosporins in approximately 20% of the cases (14). Combined mechanisms of resistance to ß-lactams are often observed among the carbapenemase-producing *K. pneumoniae* (22, 25, 37).

Combined therapy may maximize bacterial killing (synergistic effect) and minimize bacterial resistance. The best antibiotic associations contain two molecules that show *in-vitro* activities against carbapenemase producers (103, 106). Several studies indicated that mortality was significantly lower in patients when given combination therapy (106, 107) while other studies indicated that the superiority of the combined therapy as compared to monotherapy was

not significant (103). A recent review article recommends using combination therapy for treating bloodstream infections when multidrug-resistant bacteria are suspected (108). Doi and Paterson, based on an extended analysis of the *in-vivo* efficacy data, recommend combination therapy that includes a carbapenem with the second agent such as colistin, tigecylcine and gentamicin depending of the results of *in-vitro* susceptibility testing (109).

Options for the treatment of infections with carbapenemase-producing *K. pneumoniae* are limited. Some studies suggest that for infections due to KPC producers, the use of combination therapy that includes a carbapenem (eg, polymyxin-carbapenem or aminoglycoside-carbapenem), may have a mortality benefit (101). Clinical data are scant for treatment of infections due to OXA-48 and NDM infections; a recent retrospective, observational study suggested that for bacteremia from OXA-48 producers, combination therapy that included colistin showed a mortality benefit (110).

Colistin (polymyxin E) was discovered more than 60 years ago while polymyxin B is available only in a limited number of countries. The major side effect of these molecules is nephrotoxicity, while the optimal dosage is unknown. Colistin has become the most popular agent for the treatment of infections due to *K. pneumoniae* with carbapenemases (101, 102). Colistin monotherapy has been associated with mortality rates exceeding 50% when used for severe infections (111) and one Brazilian study shows that combination therapy was not superior to monotherapy (112). Recent understanding of the pharmacokinetics of colistin has resulted in the use of higher doses than those used in the early studies. The current recommendations include a loading dose and a total standard dose of 9–10 million international units daily divided into two or three doses (113). This molecule has significant activity against various carbapenemase-producing isolates and is often used in combined therapy (e.g. aminoglycoside,

aztreonam, carbapenems, rifampicin, tigecycline or fosfomycin) (103, 104). Unfortunately, due to the increase use of this agent, colistin-resistant *K pneumonaie* are increasingly being reported (114).

Intravenous Fosfomycin is available in Europe where it has been used in combination with other antibiotics for treating severe infections due to multiple drug resistant bacteria (with tigecycline and colistin) (115). *In-vitro* analysis indicated synergies between colistin and fosfomycin for some NDM producers (116).

Tigecycline is a tetracycline derivative and has been available since 2005. This molecule does not diffuse sufficiently into the urinary tract where many of the infections due to carbapenemase-producing *K. pneumoniae* originate (104). The FDA issued in 2013 a warning indicating an increase rate of death when tigecycline has been used (2.5%) when compared with other antibiotics (1.8%) that were related to treatment failures (104). In addition, acquired tigecycline resistance has been reported in patients infected by KPC-producing *K. pneumoniae* (117). A recent report suggest that high dose tigecycline (100 mg every 12 hours following a 200 mg loading dose) may improve outcome when compared to conventional doses (118).

Rifampicin has a very broad spectrum of activity including the Enterobacteriaceae. Several reports show some *in vitro* synergy in the killing of carbapenemase-producing *K. pneumoniae* between rifampicin and tigecycline or colistin (119, 120). However definitive clinical data are lacking to advocate the routine use of rifampicin for the treatment of infections due to carbapenemase-producing *K. pneumoniae*.

Several aminoglycosides molecules may retain activity against carbapenemase producing *K. pneumoniae*. Some KPC and OXA-48 producers remain susceptible to gentamicin while this is rare for NDM producers (121). Aminoglycosides have been used with some clinical success

either alone or in combined therapy for treating infections due to KPC producers (106). A recent report suggested better outcomes when gentamycin (as monotherapy or in combination with tigecycline) was used for colistin-resistant, KPC-producing *K. pneumoniae* (122). Side effects of aminoglycosides include nephrotoxicity especially when used in combination with colistin.

The carbapenems, despite being hydrolyzed by carbapenemases (hence the definition of those enzymes) may retain some activity against carbapenemase-producing *K. pneumoniae* (106, 123). Treatment regimens using a carbapenems may be an option when the MICs of the carbapenems are below or equal to 8 mg/L when a second antibiotic is added or when prolonged intravenous infusion regimen is given (123, 124). Encouraging results with VIM and OXA-48 producers in humans and with NDM producers in animal models had been obtained (106, 125, 126). Studies performed with an animal model of infection (i.e. pneumonia model in mice) suggested that dual-carbapenem therapy (i.e. meropenem + ertapenem) may be effective (126). Ertapenem most likely acts as a "suicide" molecule for carbapenemase activity whereas the more active drug, meropenem retains its efficacy. Efficacy of this double-carbapenem therapy has been shown in humans infected with KPC producers (127). Among other ß-lactams, the extended-spectrum cephalosporins (i.e. 3rd and 4th generation) may be effective against OXA-48 producers without ESBLs (128) while aztreonam remains an option for treating infections due to MBL producers that test susceptible to this agent (37).

Several antibiotics in development may have significant activity against carbapenemase producers in *K. pneumoniae* (104). One of the most promising drugs is the combination of avibactam with ceftazidime. Avibactam is an efficient ß-lactam inhibitor that inhibits *in vitro* the activity of serine- ß-lactamases such as KPC and OXA-48. Ongoing phase III studies show the efficacy of this inhibitor combination against KPC producers (104). The combination of

avibactam with other agents such as ceftaroline and aztreonam are in developing stages (phase I and phase II studies) (104). The advantage of the combination of avibactam and aztreonam would be treating infections due to isolates with MBLs (129). Another potent serine-ß-lactamase inhibitor is MK7655 in combination with imipenen that sufficiently inhibit various KPC producers (104). Some promising molecules include the following: the aminoglycoside, plazomicin (ACHN-490), which has significant activity against all types of carbapenemase producers except the NDM producers that often produce 16S ribosomal RNA methylases conferring resistance to all aminoglycoside molecules (130); the tetracycline analogue, eravacylcline for treating KPC producers (104); and the novel polymyxins under development such as NAB 739, 4061, 741 with a lower nephrotoxicity (131).

Within the next 24 months, is likely that the combination of avibactam and ceftazidime will be available in clinical medicine and may represent an important additional value for treating the increasing number of difficult-to-treat infections due to carbapenemase producers. Implementation of hygiene measures, rapid detection of carbapenemase producers and the use of the combination of avibactam and ceftazidime might be the cornerstones to treat and control infections due to *K. pneumoniae* with KPCs or OXA-48. However, the efficient treatment of MBL producers (i.e. VIM, IMP and NDM) will remain to be determined.

Recent recommendations

Rodríguez-Baño and colleagues from Spain (102) and Giamarellou (101) from Greece recently published excellent recommendations regarding the treatment of infections with carbapenemase-producing Enterobacteriaceae. These recommendations or guidelines contained pertinent and detailed information on this important topic and we urged interested readers to securitize these articles.

Summary

The management of infections due to *K. pneumoniae* has been complicated by the emergence of antimicrobial resistance. Of special concern is the emerging resistance to the carbapenems, since these agents are often the last line of effective therapy available for the treatment of infections caused by multidrug-resistant (MDR) *K. pneumoniae.* Resistance to carbapenems in *K. pneumoniae* may be linked to different mechanisms and the co-occurrence of permeability defects together with production of certain ß-lactamases (e.g. AmpC cephalosporinases) possessing a very weak carbapenemase activity may lead to reduced susceptibility to carbapenems. True carbapenemases are responsible for non- susceptibility to carbapenems without additional permeability defects in *K. pneumoniae*. Those carbapenemases either belong to Ambler class A (i.e. KPC, GES), B (i.e. NDM, VIM, IMP), or D (i.e. OXA-48-like) molecular classes. For a summary on the classification, spectrum of activity, inhibition properties, types, endemic regions and molecular epidemiology of carbapenemasese in K. pneumoniae, please refer to Table 1.

K. pneumoniae ST258 is an important human pathogen, have spread extensively throughout the world and are responsible for the rapid increase in the prevalence of antimicrobial *K. pneumoniae*. This clone is known to cause UTIs, respiratory tract infections, BSIs and is associated with carbapenemase production most often to KPC-2 and KPC-3. Recent molecular studies have shown that ST258 consists of 2 distinct lineages namely clade I and clade II. Clade I was specifically associated with KPC-2 and clade II with KPC-3. The genetic differentiation between the two clades resulted from a 215-kb region of divergence that includes genes involved in capsule polysaccharide biosynthesis indicating that these 2 clades had distinct evolutionary pathways. Additional investigation showed that ST258 clade II is a hybrid clone that was created

by a recombination event between ST11 and ST442. Moreover, it seems that ST258 clade I strains evolved from a clade II strain as a result of *cps* region replacement from ST42.

The integrative conjugative element ICEKp258.2 contains gene clusters for a type IV pilus (i.e. *pilV*) and a type III restriction-modification system. The *pilV* on ICEKp258.2 may in part be responsible the high transmissibility and durability of ST258 on foreign surfaces and it seems that this integrative conjugative element contribute significantly to the epidemiological success of *K. pneumonaie* ST258. Different KPC-encoding plasmids have been identified in ST258 with IncFIIk1, FIIk2 being the most common. These plasmids often contain several genes encoding for resistance to other antimicrobial agents, such as the aminoglycosides, quinolones, trimethoprim, sulphonamides, and tetracyclines and have played an important role in the success of ST258.

The optimal treatment of infections due to carbapenemase-producing *K. pneumoniae* is unknown and none of the currently available antibiotics used as single therapy may be efficient for treating all types of carbapenemase producers. Various agents, most often as part of combination therapy, such as polymyxins, fosfomycin, tigecycline, rifampicin and carbapenems have been used with varying success rates to treat infections due to MDR-*K. pneumoniae*. Several antibiotics (e.g. avibactam with ceftazidime) in development have significant activity against carbapenemase-producing *K. pneumoniae*, especially those with KPCs. However effective options for the treatment of infections due to NDM-producers remain elusive.

Infection control measures that had been shown to be effective in successfully decreasing the acquisition of carbapenemase-producing *K. pneumoniae* include combined interventions of increased compliance with hand hygiene, contact precautions, environmental cleaning, early identification of asymptomatic carriers, and the physical separation of carbapenemase-producing

K. pneumoniae -positive patients and their staff (132). Prompt and appropriate infection control measures should be implemented upon isolation of carbapenemase-producing *K. pneumoniae*. Expert guidelines on infection control measures have been provided by the Centers for Disease Control and Prevention and the European Society of Clinical Microbiology and Infectious Diseases (133). Colonized or infected patients should be isolated individually or in groups and treated in accordance with strict infection control directives, including hand disinfection, use of gowns and disposable aprons as well as proper cleaning (111) .

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