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In vivo adaptive antimicrobial resistance in Klebsiella pneumoniae during antibiotic therapy

Shuangshuang Li[1], Xudong Feng[2], Min Li[3]* and Zhen Shen[3]*

[1]Department of Laboratory Medicine, Ningbo Hospital, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Ningbo, China, [2]State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China, [3]Department of Laboratory Medicine, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Klebsiella pneumoniae is one of the leading pathogens contributing to antimicrobial resistance. The emergence of carbapenem-resistant K. pneumoniae (CRKP) has put the use of clinical antimicrobial agents in a dilemma. In particular, CRKP exhibiting resistance to ceftazidime/avibactam, tigecycline and colistin have raised great clinical concern, as these are the last-resort antibiotics for the treatment of CRKP infections. Within-host evolution is a survival strategy closely related to the emergence of antimicrobial resistance, while little attention has been paid to the in vivo genetic process of conversion from antibiotic-susceptible to resistant K. pneumoniae. Here we have a literature review regarding the in vivo evolution of resistance to carbapenems, ceftazidime/avibactam, tigecycline, and colistin in K. pneumoniae during antibacterial therapy, and summarized the detailed resistance mechanisms. In general, acquiring bla_{KPC} and bla_{NDM} harboring-plasmid, specific mutations in bla_{KPC}, and porin genes, such as ompK35 and ompK36, upregulation of bla_{KPC}, contribute to the development of carbapenem and ceftazidime/avibactam resistance in vivo. Overexpression of efflux pumps, acquiring plasmid-carrying tet (A) variants, and ribosomal protein change can lead to the adaptive evolution of tigecycline resistance. Specific mutations in chromosomes result in the cationic substitution of the phosphate groups of lipid A, thus contributing to colistin resistance. The resistant plasmid might be acquired from the co-infecting or co-colonizing strains, and the internal environment and antibiotic selection pressure contribute to the emergence of resistant mutants. The internal environment within the human host could serve as an important source of resistant K. pneumoniae strains.

Introduction

Klebsiella pneumoniae is a common pathogen for community-and hospital-acquired infections, such as pneumonia, urinary tract infection, and bacteremia (Podschun and Ullmann, 1998; Bengoechea and Sa Pessoa, 2019). The emergence of antimicrobial resistance in K. pneumoniae poses a serious threat to public health, particularly with the rise of carbapenem-resistant K. pneumoniae (CRKP). According to China Antimicrobial Surveillance

Network, carbapenem resistance in *K. pneumoniae* increased rapidly from 3% in 2005 to more than 25% in 2019 (Hu et al., 2020). The irrational use of antibiotics in clinical practice has fostered the occurrence and spread of resistance to "old class antimicrobials." Ceftazidime/avibactam, tigecycline, and colistin are now considered the last resort for the treatment of CRKP infections (Pournaras et al., 2016; Rabanal and Cajal, 2017; Shields et al., 2017b; Xu et al., 2022). However, the emergence of resistance to the last-resort antibiotics in CRKP has been repeatedly reported (Ah et al., 2014; van Duin et al., 2014; Shields et al., 2016, 2017a). More attention should be paid to the resistance of these types of antimicrobials. Besides, it is nerve-wracking to find that the antimicrobial resistance in *K. pneumoniae* is changing rapidly in response to *in vivo* microenvironmental stress during antimicrobial therapy (MacKenzie et al., 1997; Gaibani et al., 2022).

The prevailing view is that the growing prevalence of antimicrobial resistance is largely attributable to selection pressure from antibacterial drugs. However, our understanding of the *in vivo* development of antimicrobial resistance in *K. pneumoniae* is limited. While the majority of studies have focused on the epidemiology, risk factors, and treatment outcome of CRKP and other multi-drug resistant *K. pneumoniae* infections, relatively little attention has been paid to the *in vivo* genetic process underlying the conversion of a bacterial strain from antibiotic-susceptible to resistant. Within-host evolution is an important survival strategy, often associated with persistent or recurrent infections. Understanding the mechanisms that drive the *de novo* development of antimicrobial resistance in *K. pneumoniae* in patients during treatment is crucial for optimizing infection treatment and preventing the emergence of resistance. In this review, we focused on the antimicrobial drugs currently used in clinical practice, and summarized the adaptive evolution of antimicrobial resistance in *K. pneumoniae* under internal pressures. Our aim is to provide valuable insights into the emergence of antimicrobial resistance in *K. pneumoniae* during antibacterial therapy.

In vivo adaptive resistance to carbapenem

Acquisition of carbapenemase encoding genes

The production of carbapenemase is the leading cause of carbapenem resistance in *K. pneumoniae*, with *K. pneumoniae carbapenemase* (KPC) being the most prevalent in several countries, including China (Hu et al., 2012; Giani et al., 2013; Pollett et al., 2014; Zhang et al., 2020). Ding et al. reported that the *in vivo* acquisition of bla_{KPC-2} led to carbapenem resistance in *K. pneumoniae* during antimicrobial therapy, and bla_{KPC-2} was acquired through horizontal transfer of an insertion sequence containing IS*Kpn6*-like, bla_{KPC-2} and IS*Kpn8* (Ding et al., 2016). Duplicative transposition might involve in the mobilization of this insertion sequence, since the transposase gene *tnpA* was located upstream of bla_{KPC-2} in KPC plasmids of the CRKP strains. Moreover, *in vivo* horizontal dissemination of the bla_{KPC-2} gene carried on IncL/M type conjugative plasmids has been observed among diverse Enterobacteriaceae clinical isolates with different genetic backgrounds, including

K. pneumoniae, *E. coli*, and *E. cloacae complex* (Anchordoqui et al., 2015). These studies demonstrated that *in vivo* carbapenem resistance in *K. pneumoniae* can result from the horizontal transfer of a resistance plasmid or an insertion sequence (Figure 1).
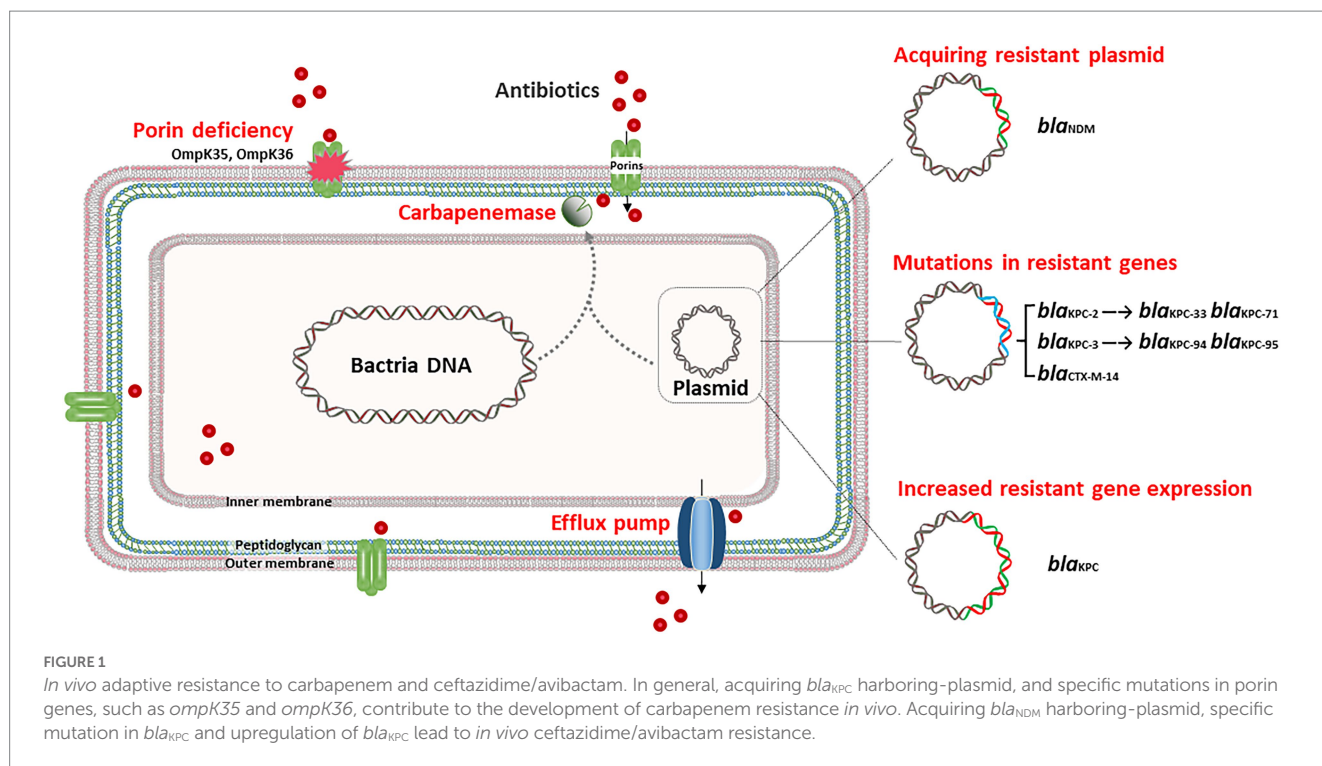
Hypervirulent *K. pneumoniae* (hvKP) can also evolve into carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKP) under long-term antibiotic treatment by acquiring a resistance plasmid. Recently, a report showed that a sequence type (ST) 218 hvKP strain developed into CR-hvKP in a patient by acquiring an IncFIIk bla_{KPC}-harboring plasmid donated by a ST585 CRKP strain (Tang et al., 2021). *K. pneumoniae* ST218 is a single-locus variant of ST23 and belongs to clonal group 23, which comprises hypervirulent clones harboring the virulence factors of *iuc* locus, *iro* locus, and *rmpA/rmpA2* at high frequencies. The IncFIIk bla_{KPC}-harboring plasmid is conjugative and carries five antibiotic resistance genes, bla_{KPC-2}, $bla_{CTX-M-24}$, *tet (A)*, *arr*-3, and *floR* (Table 1).

Porin deficiency

Outer membrane protein (OMP) is the main component of the outer membrane of gram-negative bacteria, including porin and lipoprotein. Porins typically aggregate to form pores, enabling the passage of small hydrophilic molecules, such as β-lactams, across the membrane (Pagès et al., 2008). The porins of *K. pneumoniae* mainly include OmpK35 and OmpK36 (Hernández-Allés et al., 1999). The deficiency of OmpK35, known as OmpF, is reported widely reported in Enterobacteriaceae strains (Gravey et al., 2020). While OmpK35 is not thought to be the primary pathway for *K. pneumoniae* resistance (Shields et al., 2015; Pagès et al., 2016). The function of OmpK35 should be further studied *in vivo* circumstance of *K. pneumoniae*. OmpK36 is classified as a member of the OmpC porin group, and functions as a non-specific, passive diffusion pore (Ye Y. et al., 2017). The loss of OmpK36, coupled with extended-spectrum β-lactamases (ESBLs) and/or AmpC production, can result in carbapenem resistance in *K. pneumoniae* (Hamzaoui et al., 2018). Tian et al. (2020) reported three *K. pneumoniae* strains successively isolated from one patient during hospitalization and found that the final strain developed carbapenem resistance after 14-day of imipenem treatment. This particular CRKP strain exhibited OmpK36 deficiency due to a premature stop codon in the *ompK36* gene. This study highlights that the alteration of outer membrane porins due to the 14-day use of imipenem plays a potential role in leading to clinical presentation of carbapenem resistance (Figure 1). In addition, some researchers have gradually discovered that porins are also associated with ceftazidime/avibactam resistance of *K. pneumoniae* (Xu et al., 2022), which reflects the important role of porins deficiency in the adaptive evolution of *K. pneumoniae*.

In vivo adaptive resistance to ceftazidime/avibactam

Ceftazidime/avibactam is a novel β-lactam/β-lactamase inhibitor combination, which has been approved for the treatment of complicated intra-abdominal infections and urinary tract infections in 2015 (Zasowski et al., 2015; Shirley, 2018). Avibactam exhibits

FIGURE 1

In vivo adaptive resistance to carbapenem and ceftazidime/avibactam. In general, acquiring *bla*$_{KPC}$ harboring-plasmid, and specific mutations in porin genes, such as *ompK35* and *ompK36*, contribute to the development of carbapenem resistance *in vivo*. Acquiring *bla*$_{NDM}$ harboring-plasmid, specific mutation in *bla*$_{KPC}$ and upregulation of *bla*$_{KPC}$ lead to *in vivo* ceftazidime/avibactam resistance.

activity against Ambler class A enzymes (including ESBLs and KPC) and some Ambler class C and D (e.g., OXA-48) enzymes, but it is ineffective against class B enzymes like New Delhi metallo-β-lactamase (NDM) (Shirley, 2018). Though ceftazidime/avibactam displayed potent activity against KPC-producing *K. pneumoniae* (KPC-KP), the emergence of ceftazidime/avibactam resistance in clinical strains during antimicrobial treatment has been repeatedly reported (Humphries et al., 2015; Shields et al., 2016; Both et al., 2017).

Specific mutations of *bla*$_{KPC}$

The major *in vivo* adaptive ceftazidime/avibactam resistance mechanism is the emergence of specific mutations in *bla*$_{KPC}$ (Figure 1). A report from China revealed that following a 6-day course of ceftazidime/avibactam treatment, a ST11 *K. pneumoniae* strain developed ceftazidime/avibactam resistance, owing to the mutation of KPC-2 to KPC-33, with a substitution of D179Y within the Ω loop (Li D. et al., 2021). The Ω loop is located at residues 164–179, which is an essential domain for class A β-lactamases, and the substitution of D179Y may be detrimental to the binding of avibactam (Gaibani et al., 2021; Wang et al., 2021). Another study conducted in Italy showed that *in vivo* development of ceftazidime/avibactam resistance in *K. pneumoniae* was also linked to the D179Y substitution in KPC-3, which emerged after 17-day of ceftazidime/avibactam treatment (Gaibani et al., 2018). Similarly, *in vivo* development of KPC-71, KPC-76, KPC-94, and KPC-95-mediated ceftazidime/avibactam resistance during antimicrobial treatment has also been reported in ST11 and ST512 KPC-KP (Li X. et al., 2021; Guzmán-Puche et al., 2022; Shen et al., 2022). Of note, the low antibiotic pressure may have selected hybrid subpopulations of KPC-KP due to the high adaptability of KPC to ceftazidime/avibactam. For example, a study reported that KPC-KP strains isolated from bronchoalveolar lavage harbor *bla*$_{KPC-3}$ and T243M mutations, while those isolated from the blood have

D179Y mutation (Gaibani et al., 2021). Except for KPC, the *in vivo* emerging P170S exchange in CTX-M-14 has also been associated with elevated ceftazidime/avibactam MICs for independent *K. pneumoniae* isolates, but this substitution was not sufficient for full resistance (Both et al., 2017).

Interestingly, KPC mutations mediating ceftazidime/avibactam resistance are generally associated with the restoration of carbapenem susceptibility (Haidar et al., 2017; Shields et al., 2017a), and this kind of reversion may be dynamic. As illustrated in a recent study, the infection began with a KPC-2-producing *K. pneumoniae*. After treatment with ceftazidime/avibactam, the strain switched to a KPC-33 mutant (D179Y), which restored carbapenem susceptibility. However, the restored carbapenem susceptibility *in vivo* was not stable and the subsequent use of imipenem against KPC-33-producing *K. pneumoniae* infection resulted in a reversion of KPC-2 producers (Wang et al., 2021). The selective pressure of antibiotics in the mutation and reversion of *bla*$_{KPC}$ genes may lead to the dynamic change of KPC enzymes and the emergence of resistance to ceftazidime/avibactam and carbapenems.

Increased *bla*$_{KPC}$ gene expression

Increased gene expression and copy number of *bla*$_{KPC}$ can lead to ceftazidime/avibactam resistance in *K. pneumoniae*. A study reported that a novel ST4496 strain, which is a novel ST closely related to ST11, displayed ceftazidime/avibactam resistance after 1 month of ceftazidime/avibactam treatment. Sequencing analysis showed that there was duplication of *bla*$_{KPC-2}$ on a 108 kb IncFII KPC plasmid due to unequal crossover of the IS26 composite transposon, resulting in elevated levels of *bla*$_{KPC-2}$ expression (Han et al., 2021). The increased expression of KPC carbapenemase could lead to enhanced hydrolysis of ceftazidime, as avibactam may not be able to completely inhibit the higher amounts of KPC. Besides,

TABLE 1 Examples of studies on *in vivo* adaptive resistance in *Klebsiella pneumoniae*.

Resistant types	Initial strain types	Interval between S and NS isolates (days)	Sequence type (ST)	Underlying mechanisms	Region/Country
Carbapenem	KP	23	11	Acquisition of bla_{KPC-2}-harboring Plasmid	Shanghai, China (Ding et al., 2016)
		7	11		
	KP	NA	37	Acquisition of bla_{OXA-1}-harboring plasmid	Hunan, China (Li et al., 2017)
	hvKP	5	218	Acquisition of bla_{KPC}-harboring plasmid	Hebei, China (Tang et al., 2021)
	KP	14	660	Porin deficiency	Zhejiang, China (Tian et al., 2020)
Ceftazidime/avibactam	KP	15/14	11	Mutation and reversion of bla_{KPC} genes	Beijing, China (Wang et al., 2021)
	CRKP	6	11	Mutations in bla_{KPC}	Henan, China (Li D. et al., 2021)
	CRKP	10	101	Mutations in bla_{KPC}	Italy (Gaibani et al., 2018)
	CRKP	21	NA	Mutations in bla_{KPC}	Shanghai, China (Shen et al., 2022)
	MDR KP	23	383	Mutations in $bla_{CTX-M-14}$	German (Both et al., 2017)
	CRKP	30	4496	bla_{KPC-2} duplication	Zhejiang, China (Han et al., 2021)
	CRKP	13–22	11	Increased gene expression of mutated bla_{KPC}	Beijing, China (Sun et al., 2021)
	CRKP	11	11	Acquisition of bla_{NDM-5}-harboring plasmid	Fujian, China (Huang et al., 2021)
Tigecycline	CRKP	41	11	Deletion of the *ramR* RBS	Shanghai, China (Ye M. et al., 2017)
	CRKP	340	1544	Upregulation of RamA and/or RarA	Taiwan, China (Lin et al., 2016)
		37	23		
		147	1526		
		7	660		
		5	45		
	hv-CRKP	50	11	Mutations of *ramR* and *lon*	Zhejiang, China (Jin et al., 2021)
	MDR KP	4	NA	*kpgABC* overexpression.	United States (Nielsen et al., 2014)
	CRKP	13	11	The *tet(A)* variant (S251A)	Zhejiang, China (Du et al., 2018)
	CRKP	NA	11	Plasmid harbors the bla_{KPC-2} and *tet(A)* variant genes	Zhejiang, China (Zhang et al., 2018)
	CRKP	29	11	The *rpsJ* variant (V57L)	Zhejiang, China (He et al., 2018)
Colistin	CRKP	30	258	Inactivation or deletion of the *mgrB* gene	Italy (Cannatelli et al., 2013)
	CRKP	12	512	Mutations of *pmrB*	Italy (Cannatelli et al., 2014)
	hv-CRKP	50	11	Mutations of *pmrB*, *phoQ*, and *mgrB* genes	Zhejiang, China (Jin et al., 2021)

KP, Klebsiella pneumoniae; CRKP, carbapenem resistant Klebsiella pneumoniae; hvKP, hypervirulent Klebsiella pneumoniae; ST, sequence type; S, susceptible; NS, not susceptible; RBS, ribosomal binding site; MDR, multi-drug resistance; BSI, bloodstream infection; NA, not available.

the increased gene expression of bla_{KPC-51} also leads to high-level ceftazidime/avibactam resistance (Sun et al., 2021).

Acquiring bla_{NDM-5}-harboring plasmid

Acquiring bla_{NDM-5}-harboring plasmid could also lead to ceftazidime/avibactam resistance, since avibactam could not inhibit NDM. One study observed that a ST11 CRKP strain displayed ceftazidime/avibactam resistance after 11-day of treatment. This strain harbored bla_{KPC-2}, $bla_{SHV-182}$, and bla_{TEM-1B}, and it acquired an additional IncX3 bla_{NDM-5}-harboring plasmid when compared with the corresponding susceptible isolate. This bla_{NDM-5} plasmid is conjugative and could be successfully transferred into *E. coli* J53 with high frequency (Huang et al., 2021).

In vivo evolution of tigecycline resistance

Tigecycline is a derivative of the semi-synthetic tetracycline minocycline, which was approved in China in 2012 (He et al., 2015; Alhashem et al., 2017). It has a broad antibacterial spectrum and displays potent antibacterial activity against gram-positive cocci, gram-negative bacilli (excluding *Pseudomonas aeruginosa* and some *Proteus*), anaerobic bacteria, and atypical pathogens (Society of Clinical Microbiology and Infection of China International Exchange and Promotion Association for Medical and Healthcare, Clinical Microbiology Group of the Laboratory Medicine Society of the Chinese Medical Association, Clinical Microbiology G.I.S. ofthe C. M, 2020). Tigecycline mainly binds to the bacterial ribosome 30S subunit, preventing aminoacyl-tRNA from entering the ribosome A site, thus exerting an antibacterial effect by inhibiting bacterial protein synthesis (Velkov et al., 2010). Recently, tigecycline-resistant *K. pneumoniae* strains have been frequently reported (Sun et al., 2013). Overall, the most commonly reported mechanisms of tigecycline resistance include overexpression of efflux pumps, acquisition of plasmid-carrying *tet (A)* variants and ribosomal protein change (Figure 2).

Increased efflux pumps activity

Efflux pumps can actively squeeze drugs out of cells, and resistance nodulation-cell division (RND) type efflux pump, including AcrAB-TolC and OqxAB, is the dominant drug-associated efflux pump in gram-negative bacteria (Nikaido, 2018). AcrAB-TolC is predominantly related to tigecycline resistance in CRKP (Masi et al., 2019). AcrAB-TolC is mainly composed of three parts: membrane fusion protein (AcrA), efflux transporter (AcrB), and outer membrane channel protein (TolC). Meanwhile, the expression of the AcrAB-TolC is regulated by a variety of regulatory factors, including *acrR*, *ramA*, and *marA* (Wang et al., 2001; Keeney et al., 2007). Studies have shown that bacteria can sense flux rate and regulate efflux pumps to survive the environmental antibiotic challenge, suggesting that resistance mechanisms *in vivo* may differ from those *in vitro* (Fritz et al., 2015). Understanding the mechanisms underlying the *de novo* development of tigecycline resistance in patients is challenging. One strategy is to perform a longitudinal study by isolating *K. pneumoniae* strains from patients

before, during and after tigecycline therapy. Ye et al. reported that the deletion of *ramR* ribosomal binding site (RBS) could lead to *in vivo* development of tigecycline resistance in *K. pneumoniae* after more than 40 days of tigecycline therapy (Ye M. et al., 2017, 2022). RamR exerted negative regulation on *acrAB* gene expression, and this 12-bp deletion abolished RamR protein production, resulting in high levels of *acrAB* expression and tigecycline resistance.
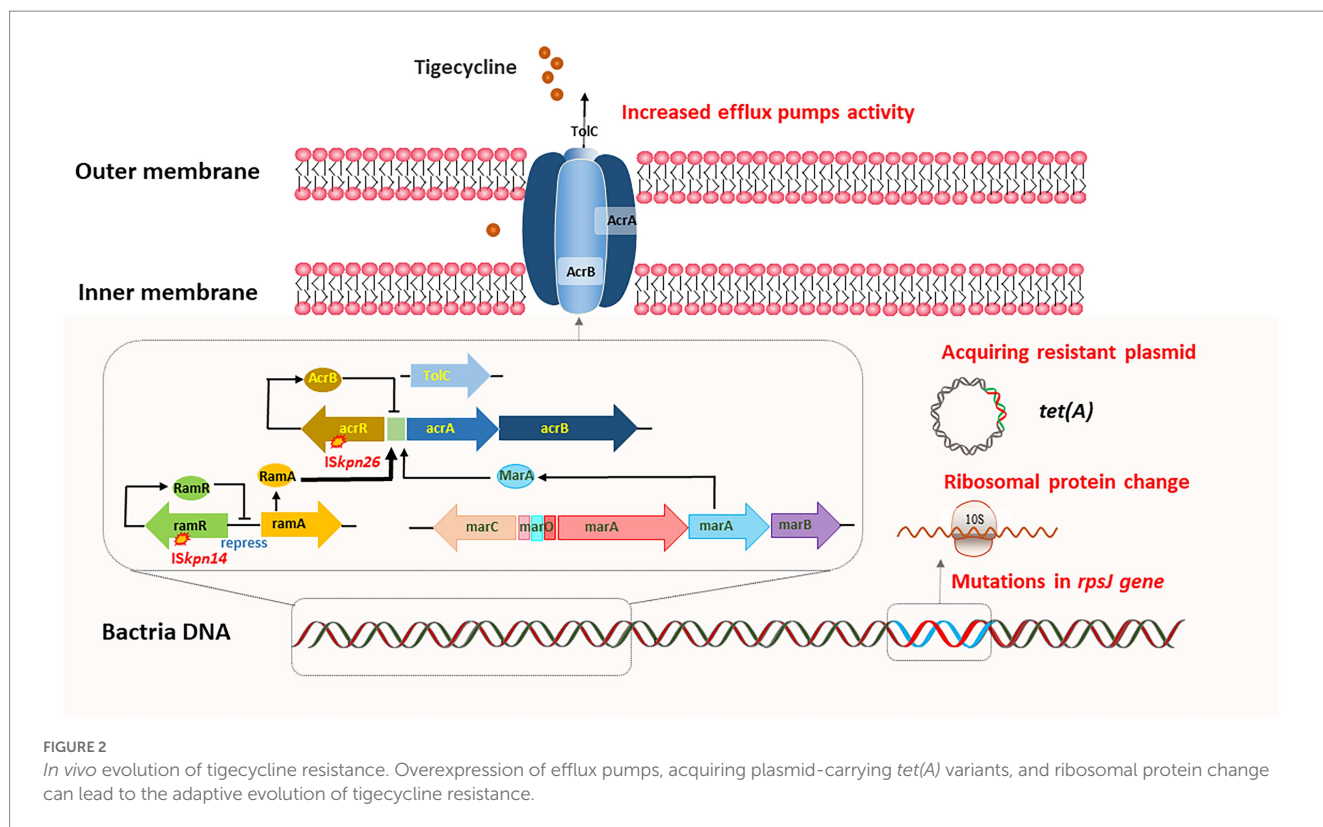
In another study, researchers identified five paired clinical isolates of *K. pneumoniae* that were initially tigecycline-susceptible, but later developed into tigecycline-non-susceptible (Lin et al., 2016). They found that tigecycline-non-susceptibility was associated with upregulation of RamA and/or RarA and the corresponding AcrAB-TolC and/or OqxAB efflux pump (s), respectively. Furthermore, various mutations in *ramR* and *oqxR* lead to *ramA* and *rarA* overexpression. Meanwhile, AcrAB-TolC efflux pump-mediated tigecycline resistance has also been confirmed in CR-hvKP strains. ST11-KL64 CR-hvKP developed tigecycline resistance due to the mutation of *ramR* during tigecycline therapy, and a novel frameshift mutation of *lon* was identified in the high-level tigecycline-resistant strain (Jin et al., 2021). In addition, Nielsen et al. also reported a new efflux pump operon, *kpgABC*. An insertion sequence (IS5) was correlated with an elevated *kpgABC* expression, which led to the *in vivo* development of tigecycline nonsusceptibility in *K. pneumoniae* (Nielsen et al., 2014).

Acquiring plasmid-carrying *tet (A)* variants

The widely disseminated plasmid-carrying *tet (A)* variants in *K. pneumoniae* have greatly contributed to tigecycline resistance (Chiu et al., 2017). Tet (A) is one of the most common major facilitator superfamilies (MFS) efflux pumps. Tigecycline therapy could upregulate the expression of *tet (A)* in tigecycline-susceptible CRKP, leading to the development of tigecycline resistance. Du et al. (2018) reported tigecycline-resistant ST11 CRKP isolates from a 56-year-old female patient during tigecycline therapy. One amino acid substitution S251A in TetA was identified in the tigecycline-resistant isolates. Subsequent transformation experiments confirmed the contribution of this TetA variant (S251A) to tigecycline resistance and the *tetA* gene was located on a transferable plasmid. Another study reported that clinical CRKP strains carrying a conjugative plasmid harboring the bla_{KPC-2} and *tet (A)* variant genes readily evolved into tigecycline-resistant CRKP upon treatment and persisted in the human gastrointestinal tract (Zhang et al., 2018).

Ribosomal protein change

Mutations in *rpsJ* encoding ribosomal protein S10, the target site of tigecycline, have also been associated with tigecycline resistance (Villa et al., 2014). He et al. (2018) monitored a 59-year-old male patient infected with ST11 KPC-producing *K. pneumoniae*. They identified the V57L amino acid substitution in *rpsJ*, and confirmed that this mutation was the main cause of tigecycline resistance through transformational complementation assay. This study demonstrated that the evolution of the *rpsJ* gene could lead to tigecycline resistance in CRKP during tigecycline therapy. Since this gene is located on the chromosome, it provides a clinical warning that under the selective pressure of tigecycline, *rpsJ* mutations may occur, resulting in drug resistance and treatment failure.

FIGURE 2
In vivo evolution of tigecycline resistance. Overexpression of efflux pumps, acquiring plasmid-carrying *tet(A)* variants, and ribosomal protein change can lead to the adaptive evolution of tigecycline resistance.

In vivo evolution of colistin resistance

Polymyxins (polymyxin B and colistin) are the key drugs for the treatment of infections caused by CRKP (Livermore et al., 2011; Petrosillo et al., 2013). However, the emergence of colistin-resistant *K. pneumoniae* has been increasingly reported (Giani et al., 2013; Ah et al., 2014). There are some reports of *K. pneumoniae* acquiring colistin resistance under *in vivo* selective pressure.

The outer membrane of gram-negative bacteria is the action site of polymyxins due to its affinity for the phosphate group of lipid A. Lipid A is the hydrophobic part located in the outer monolayer of the outer membrane and is synthesized by a series of enzymes encoded by the *lpx* gene cluster. Lipid A has a negative charge due to the presence of free phosphate groups, while polymyxin has a high affinity for the negative charge of lipid A (Li et al., 2019). As shown in Figure 3, two ionic groups, pEtN and L-Ara4N, can impact the affinity between polymyxin and the outer membrane and lead to resistance. The expression of these two ionic groups is controlled by LPS-modifying enzyme genes *pmrC*, *pmrE*, and *pmrHFIJKLM*, which is then controlled by PmrAB and PhoPQ two-component system (Kox, 2000; Lee et al., 2004; Olaitan et al., 2014). In addition, CrrAB and MgrB are also involved in this regulatory axis. CrrAB could regulate the expression of PmrAB and PhoPQ (Olaitan et al., 2014), while MgrB is a negative regulator of the PhoPQ signaling system. The mutations in certain genes are involved in the regulation of these signaling pathways, such as *pmrAB*, *phoPQ*, *mgrB*, *crrB*, which can lead to colistin resistance (Cheng et al., 2010; Cannatelli et al., 2013; Chen and Groisman, 2013; Xie et al., 2022).

Comparative genomic analysis of a pair of sequential KPC-KP isolates from the same patient, including a colistin-susceptible isolate

and a colistin-resistant isolate selected after colistin exposure, revealed that insertional inactivation of the *mgrB* gene is a genetic mechanism for acquired colistin resistance (Cannatelli et al., 2013). Another study found a non-synonymous nucleotide substitution in the *pmrB* gene that resulted in a leucine-to-arginine substitution at amino acid position 82 in CRKP-infected patients treated with low-dose colistin (Cannatelli et al., 2014). This substation upregulated transcription of *pmrA* and *pmrK*, which is part of the *pmrHFIJKLM* operon responsible for modification of the colistin lipopolysaccharide target. Besides, the mutations of *pmrB*, *phoQ* and *mgrB* genes also account for the in-host evolution of CR-hvKP to colistin resistance (Jin et al., 2021).

Conclusion

Our work summarized *in vivo* adaptive evolution of antimicrobial resistance in *K. pneumoniae* during antimicrobial therapy in currently clinical practice. We further described the underlying mechanisms of evolved resistance to carbapenems, ceftazidime/avibactam, tigecycline, and colistin within human hosts. In general, acquiring bla_{KPC} and bla_{NDM} harboring-plasmid, specific mutations in bla_{KPC}, and porin genes, *ompK35* and *ompK36*, upregulation of bla_{KPC}, contribute to the development of carbapenems and ceftazidime/avibactam resistance *in vivo*. Overexpression of efflux pumps, acquiring plasmid-carrying *tet (A)* variants, and ribosomal protein change can lead to the adaptive evolution of tigecycline resistance. Specific mutations in chromosomes result in a variety of modifications of LPS, contributing to colistin resistance.

The adaptive evolution of *K. pneumoniae* can be attributed to the impact of the human host's internal environment and antibiotic
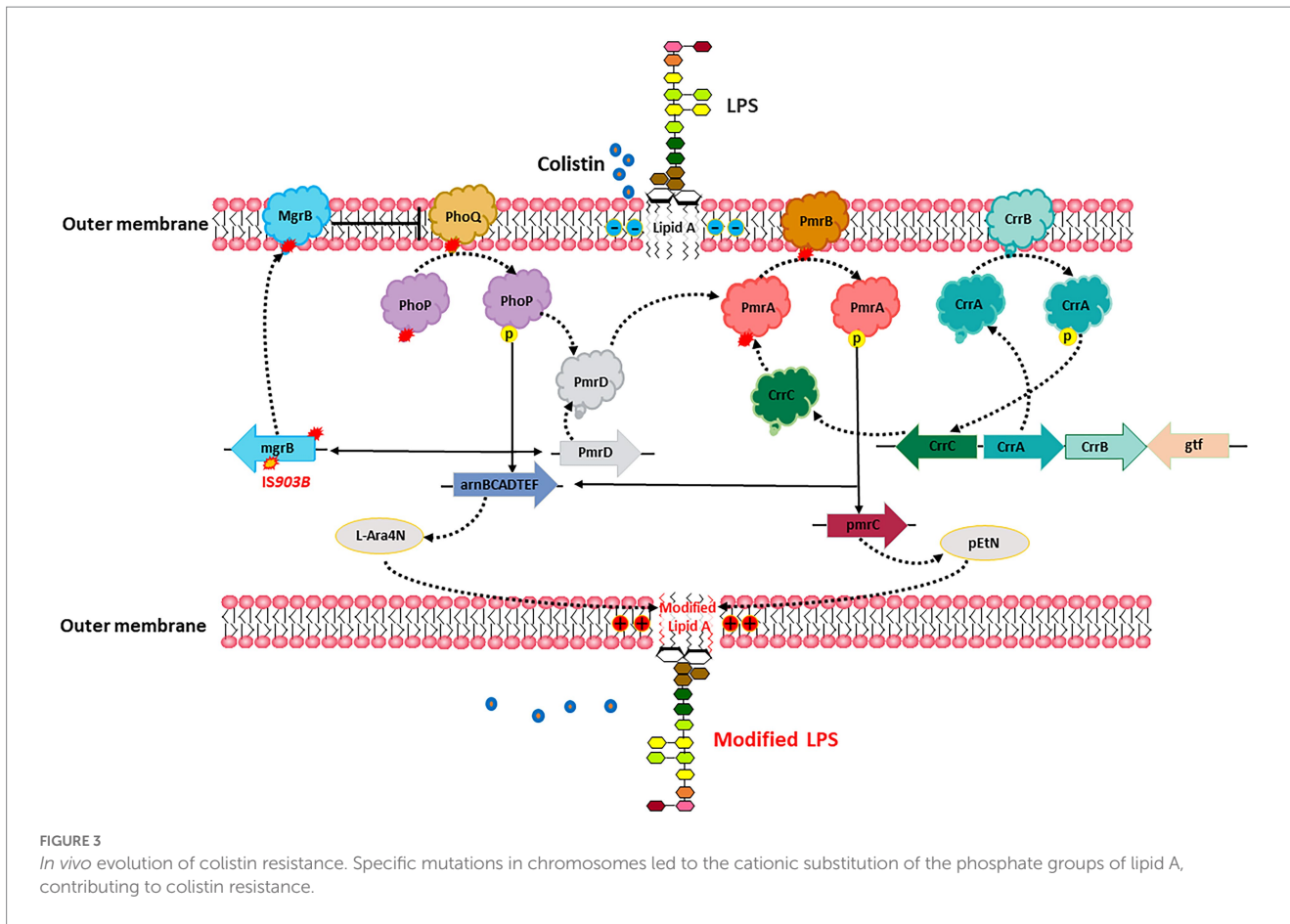
FIGURE 3
In vivo evolution of colistin resistance. Specific mutations in chromosomes led to the cationic substitution of the phosphate groups of lipid A, contributing to colistin resistance.

selection pressure. The *in vivo* development of antimicrobial resistance in *K. pneumoniae* was established mainly through the acquisition of a resistant plasmid and the emergence of specific mutations. Plasmid-carried resistant genes have been proven to disseminate ubiquitously, such as bla_{KPC} and *tet (A)*. Moreover, acquired antimicrobial resistance in hvKP clones demonstrated that *in vivo* adaptive evolution also promotes the convergence of hypervirulence and resistance.

The resistant plasmid might be acquired from the co-infecting or co-colonizing strains, and the internal selection factors may contribute to the horizontal transfer of plasmid within the human host. The emerging resistant *K. pneumoniae* strains *in vivo* could then disseminate through nosocomial settings and be screened by sectional molecular epidemiology studies. It is possible that the internal environment within the human host could serve as an important source of resistant *K. pneumoniae* strains. In the future, more attention should be paid to the *in vivo* genetic process of conversion from antibiotic-susceptible to resistant *K. pneumoniae*, and the high possibility of convergence of hypervirulence and resistance. The evolution of resistant strains could be effectively reduced by blocking this conversion process.

Author contributions

SL and ML: conceptualization. SL and XF: methodology and writing-original draft preparation. ZS: validation and supervision. SL and ZS: writing-review and editing. ML: project administration. SL:

funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Ah, Y.-M., Kim, A.-J., and Lee, J.-Y. (2014). Colistin resistance in Klebsiella pneumoniae. *Int. J. Antimicrob. Agents* 44, 8–15. doi: 10.1016/j.ijantimicag.2014.02.016

Alhashem, F., Tiren-Verbeet, N. L., Alp, E., and Doganay, M. (2017). Treatment of sepsis: what is the antibiotic choice in bacteremia due to carbapenem resistant Enterobacteriaceae? *World J. Clin. Cases* 5, 324–332. doi: 10.12998/wjcc.v5.i8.324

Anchordoqui, M. S., De Belder, D., Lucero, C., Rapoport, M., Faccone, D., Rodriguez, A., et al. (2015). *In vivo* horizontal dissemination of the blaKPC-2 gene carried on diverse genetic platforms among clinical isolates of Enterobacteriaceae. *J. Glob. Antimicrob. Resist.* 3, 210–213. doi: 10.1016/j.jgar.2015.05.001

Bengoechea, J. A., and Sa Pessoa, J. (2019). Klebsiella pneumoniae infection biology: living to counteract host defences. *FEMS Microbiol. Rev.* 43, 123–144. doi: 10.1093/femsre/fuy043

Both, A., Büttner, H., Huang, J., Perbandt, M., Belmar Campos, C., Christner, M., et al. (2017). Emergence of ceftazidime/avibactam non-susceptibility in an MDR Klebsiella pneumoniae isolate. *J. Antimicrob. Chemother.* 72, 2483–2488. doi: 10.1093/jac/dkx179

Cannatelli, A., D'Andrea, M. M., Giani, T., Di Pilato, V., Arena, F., Ambretti, S., et al. (2013). In vivo emergence of Colistin resistance in Klebsiella pneumoniae producing KPC-type Carbapenemases mediated by insertional inactivation of the PhoQ/PhoP mgrB regulator. *Antimicrob. Agents Chemother.* 57, 5521–5526. doi: 10.1128/AAC.01480-13

Cannatelli, A., Di Pilato, V., Giani, T., Arena, F., Ambretti, S., Gaibani, P., et al. (2014). In vivo evolution to Colistin resistance by PmrB sensor kinase mutation in KPC-producing Klebsiella pneumoniae is associated with low-dosage colistin treatment. *Antimicrob. Agents Chemother.* 58, 4399–4403. doi: 10.1128/AAC.02555-14

Chen, H. D., and Groisman, E. A. (2013). The biology of the PmrA/PmrB two-component system: the major regulator of lipopolysaccharide modifications. *Annu. Rev. Microbiol.* 67, 83–112. doi: 10.1146/annurev-micro-092412-155751

Cheng, H.-Y., Chen, Y.-F., and Peng, H.-L. (2010). Molecular characterization of the PhoPQ-PmrD-PmrAB mediated pathway regulating polymyxin B resistance in Klebsiella pneumoniae CG43. *J. Biomed. Sci.* 17:60. doi: 10.1186/1423-0127-17-60

Chiu, S.-K., Huang, L.-Y., Chen, H., Tsai, Y.-K., Liou, C.-H., Lin, J.-C., et al. (2017). Roles of ramR and tet (a) mutations in conferring Tigecycline resistance in Carbapenem-resistant Klebsiella pneumoniae clinical isolates. *Antimicrob. Agents Chemother.* 61:e00391-17. doi: 10.1128/AAC.00391-17

Ding, B., Shen, Z., Hu, F., Ye, M., Xu, X., Guo, Q., et al. (2016). *In vivo* acquisition of carbapenemase gene blakpc-2 in multiple species of enterobacteriaceae through horizontal transfer of insertion sequence or plasmid. *Front. Microbiol.* 7, 1–8. doi: 10.3389/fmicb.2016.01651

Du, X., He, F., Shi, Q., Zhao, F., Xu, J., Fu, Y., et al. (2018). The rapid emergence of tigecycline resistance in blaKPC-2 harboring Klebsiella pneumoniae, as mediated in vivo by mutation in tetA during tigecycline treatment. *Front. Microbiol.* 9, 1–7. doi: 10.3389/fmicb.2018.00648

Fritz, G., Dintner, S., Treichel, N. S., Radeck, J., Gerland, U., Mascher, T., et al. (2015). A new way of sensing: need-based activation of antibiotic resistance by a flux-sensing mechanism. *MBio* 6:e00975. doi: 10.1128/mBio.00975-15

Gaibani, P., Bovo, F., Bussini, L., Lazzarotto, T., Amadesi, S., Bartoletti, M., et al. (2022). Dynamic evolution of imipenem/relebactam resistance in a KPC-producing Klebsiella pneumoniae from a single patient during ceftazidime/avibactam-based treatments. *J. Antimicrob. Chemother.* 77, 1570–1577. doi: 10.1093/jac/dkac100

Gaibani, P., Campoli, C., Lewis, R. E., Volpe, S. L., Scaltriti, E., Giannella, M., et al. (2018). In vivo evolution of resistant subpopulations of KPC-producing Klebsiella pneumoniae during ceftazidime/avibactam treatment. *J. Antimicrob. Chemother.* 73, 1525–1529. doi: 10.1093/jac/dky082

Gaibani, P., Gatti, M., Rinaldi, M., Crovara Pesce, C., Lazzarotto, T., Giannella, M., et al. (2021). Suboptimal drug exposure leads to selection of different subpopulations of ceftazidime-avibactam-resistant Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae in a critically ill patient. *Int. J. Infect. Dis.* 113, 213–217. doi: 10.1016/j.ijid.2021.10.028

Giani, T., Pini, B., Arena, F., Conte, V., Bracco, S., Migliavacca, R., et al. (2013). Epidemic diffusion of KPC carbapenemase-producing Klebsiella pneumoniae in Italy: results of the first countrywide survey, 15 may to 30 June 2011. *Euro Surveill.* 18:20489.

Gravey, F., Cattoir, V., Ethuin, F., Fabre, L., Beyrouthy, R., Bonnet, R., et al. (2020). ramR deletion in an Enterobacter hormaechei isolate as a consequence of therapeutic failure of key antibiotics in a long-term hospitalized patient. *Antimicrob. Agents Chemother.* 64:e00962-20. doi: 10.1128/AAC.00962-20

Guzmán-Puche, J., Pérez-Nadales, E., Pérez-Vázquez, M., Causse, M., Gracia-Ahufinger, I., Mendez-Natera, A., et al. (2022). In vivo selection of KPC-94 and KPC-95 in Klebsiella pneumoniae isolates from patients treated with ceftazidime/avibactam. *Int. J. Antimicrob. Agents* 59:106524. doi: 10.1016/j.ijantimicag.2022.106524

Haidar, G., Clancy, C. J., Shields, R. K., Hao, B., Cheng, S., and Nguyen, M. H. (2017). Mutations in Bla KPC-3 that confer ceftazidime-avibactam resistance encode novel KPC-3 variants that function as extended-Spectrum β-lactamases. *Antimicrob. Agents Chemother.* 61:e02534-16. doi: 10.1128/AAC.02534-16

Hamzaoui, Z., Ocampo-Sosa, A., Fernandez Martinez, M., Landolsi, S., Ferjani, S., Maamar, E., et al. (2018). Role of association of OmpK35 and OmpK36 alteration and blaESBL and/or blaAmpC genes in conferring carbapenem resistance among non-carbapenemase-producing Klebsiella pneumoniae. *Int. J. Antimicrob. Agents* 52, 898–905. doi: 10.1016/j.ijantimicag.2018.03.020

Han, X., Shi, Q., Mao, Y., Quan, J., Zhang, P., Lan, P., et al. (2021). Emergence of ceftazidime/avibactam and Tigecycline resistance in Carbapenem-resistant Klebsiella pneumoniae due to in-host microevolution. *Front. Cell. Infect. Microbiol.* 11, 1–9. doi: 10.3389/fcimb.2021.757470

He, F., Fu, Y., Chen, Q., Ruan, Z., Hua, X., Zhou, H., et al. (2015). Tigecycline susceptibility and the role of efflux pumps in Tigecycline resistance in KPC-producing Klebsiella pneumoniae. *PLoS One* 10:e0119064. doi: 10.1371/journal.pone.0119064

He, F., Shi, Q., Fu, Y., Xu, J., Yu, Y., and Du, X. (2018). Tigecycline resistance caused by rpsJ evolution in a 59-year-old male patient infected with KPC-producing Klebsiella pneumoniae during tigecycline treatment. *Infect. Genet. Evol.* 66, 188–191. doi: 10.1016/j.meegid.2018.09.025

Hernández-Allés, S., Albertí, S., Álvarez, D., Doménech-Sánchez, A., Martínez-Martínez, L., Gil, J., et al. (1999). Porin expression in clinical isolates of Klebsiella pneumoniae. *Microbiology* 145, 673–679. doi: 10.1099/13500872-145-3-673

Hu, F., Chen, S., Xu, X., Guo, Y., Liu, Y., Zhu, D., et al. (2012). Emergence of carbapenem-resistant clinical Enterobacteriaceae isolates from a teaching hospital in Shanghai. *China. J. Med. Microbiol.* 61, 132–136. doi: 10.1099/jmm.0.036483-0

Hu, F., Guo, Y., and Zhu, D. (2020). CHINET surveillance of bacterial resistance across tertiary hospitals in 2019. Chin J infect Chemother. *Chin. J. Infect. Chemother.* 20, 233–243.

Huang, J., Zhang, S., Zhao, Z., Chen, M., Cao, Y., and Li, B. (2021). Acquisition of a Stable and Transferable Bla NDM-5-positive plasmid with low fitness cost leading to ceftazidime/avibactam resistance in KPC-2-producing Klebsiella pneumoniae during treatment. *Front. Cell. Infect. Microbiol.* 11, 1–8. doi: 10.3389/fcimb.2021.658070

Humphries, R. M., Yang, S., Hemarajata, P., Ward, K. W., Hindler, J. A., Miller, S. A., et al. (2015). First report of ceftazidime-avibactam resistance in a KPC-3-expressing Klebsiella pneumoniae isolate. *Antimicrob. Agents Chemother.* 59, 6605–6607. doi: 10.1128/AAC.01165-15

Jin, X., Chen, Q., Shen, F., Jiang, Y., Wu, X., Hua, X., et al. (2021). Resistance evolution of hypervirulent carbapenem-resistant Klebsiella pneumoniae ST11 during treatment with tigecycline and polymyxin. *Emerg. Microbes Infect.* 10, 1129–1136. doi: 10.1080/22221751.2021.1937327

Keeney, D., Ruzin, A., McAleese, F., Murphy, E., and Bradford, P. A. (2007). MarA-mediated overexpression of the AcrAB efflux pump results in decreased susceptibility to tigecycline in Escherichia coli. *J. Antimicrob. Chemother.* 61, 46–53. doi: 10.1093/jac/dkm397

Kox, L. F. F. (2000). A small protein that mediates the activation of a two-component system by another two-component system. *EMBO J.* 19, 1861–1872. doi: 10.1093/emboj/19.8.1861

Lee, H., Hsu, F.-F., Turk, J., and Groisman, E. A. (2004). The PmrA-regulated pmrC gene mediates Phosphoethanolamine modification of lipid a and Polymyxin resistance in salmonella enterica. *J. Bacteriol.* 186, 4124–4133. doi: 10.1128/JB.186.13.4124-4133.2004

Li, X., Ke, H., Wu, W., Tu, Y., Zhou, H., and Yu, Y. (2021). Molecular mechanisms driving the in vivo development of KPC-71-mediated resistance to ceftazidime-avibactam during treatment of Carbapenem-resistant Klebsiella pneumoniae infections. *mSphere* 6:e0085921. doi: 10.1128/mSphere.00859-21

Li, D., Li, K., Dong, H., Ren, D., Gong, D., Jiang, F., et al. (2021). Ceftazidime-avibactam resistance in klebsiella pneumoniae sequence type 11 due to a mutation in plasmid-borne blakpc-2 to blakpc-33, in Henan. *China. Infect. Drug Resist.* 14, 1725–1731. doi: 10.2147/IDR.S306095

Li, J., Nation, R. L., and Kaye, K. S. (2019). Polymyxin antibiotics: from laboratory bench to bedside. Berlin: Springer International Publishing.

Li, P., Wang, M., Li, X., Hu, F., Yang, M., Xie, Y., et al. (2017). ST37 Klebsiella pneumoniae: development of carbapenem resistance in vivo during antimicrobial therapy in neonates. *Future Microbiol.* 12, 891–904. doi: 10.2217/fmb-2016-0165

Lin, Y. T., Huang, Y. W., Huang, H. H., Yang, T. C., Wang, F., and Fung, C. P. (2016). In vivo evolution of tigecycline-non-susceptible Klebsiella pneumoniae strains in patients: relationship between virulence and resistance. *Int. J. Antimicrob. Agents* 48, 485–491. doi: 10.1016/j.ijantimicag.2016.07.008

Livermore, D. M., Warner, M., Mushtaq, S., Doumith, M., Zhang, J., and Woodford, N. (2011). What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int. J. Antimicrob. Agents* 37, 415–419. doi: 10.1016/j.ijantimicag.2011.01.012

MacKenzie, F., Forbes, K., Dorai-John, T., Amyes, S., and Gould, I. (1997). Emergence of a carbapenem-resistant Kiebsiella pneumoniae. *Lancet* 350:783. doi: 10.1016/S0140-6736(05)62567-6

Masi, M., Winterhalter, M., and Pagès, J.-M. (2019). "Outer membrane porins" in *Bacterial cell walls and membranes*. ed. A. Kuhn (Berlin: Springer International Publishing), 79–123.

Nielsen, L. E., Snesrud, E. C., Onmus-Leone, F., Kwak, Y. I., Avilés, R., Steele, E. D., et al. (2014). IS 5 element integration, a novel mechanism for rapid in vivo emergence of Tigecycline nonsusceptibility in Klebsiella pneumoniae. *Antimicrob. Agents Chemother.* 58, 6151–6156. doi: 10.1128/AAC.03053-14

Nikaido, H. (2018). RND transporters in the living world. *Res. Microbiol.* 169, 363–371. doi: 10.1016/j.resmic.2018.03.001

Olaitan, A. O., Morand, S., and Rolain, J.-M. (2014). Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. *Front. Microbiol.* 5:e00643. doi: 10.3389/fmicb.2014.00643

Pagès, J.-M., James, C. E., and Winterhalter, M. (2008). The porin and the permeating antibiotic: a selective diffusion barrier in gram-negative bacteria. *Nat. Rev. Microbiol.* 6, 893–903. doi: 10.1038/nrmicro1994

Pagès, J.-M., Peslier, S., Keating, T. A., Lavigne, J.-P., and Nichols, W. W. (2016). Role of the outer membrane and Porins in susceptibility of β-lactamase-producing Enterobacteriaceae to ceftazidime-avibactam. *Antimicrob. Agents Chemother.* 60, 1349–1359. doi: 10.1128/AAC.01585-15

Petrosillo, N., Giannella, M., Lewis, R., and Viale, P. (2013). Treatment of carbapenem-resistant Klebsiella pneumoniae: the state of the art. *Expert Rev. Anti-Infect. Ther.* 11, 159–177. doi: 10.1586/eri.12.162

Podschun, R., and Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev.* 11, 589–603. doi: 10.1128/CMR.11.4.589

Pollett, S., Miller, S., Hindler, J., Uslan, D., Carvalho, M., and Humphries, R. M. (2014). Phenotypic and molecular characteristics of Carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. *J. Clin. Microbiol.* 52, 4003–4009. doi: 10.1128/JCM.01397-14

Pournaras, S., Koumaki, V., Spanakis, N., Gennimata, V., and Tsakris, A. (2016). Current perspectives on tigecycline resistance in Enterobacteriaceae: susceptibility testing issues and mechanisms of resistance. *Int. J. Antimicrob. Agents* 48, 11–18. doi: 10.1016/j.ijantimicag.2016.04.017

Rabanal, F., and Cajal, Y. (2017). Recent advances and perspectives in the design and development of polymyxins. *Nat. Prod. Rep.* 34, 886–908. doi: 10.1039/C7NP00023E

Shen, S., Shi, Q., and Hu, F. (2022). The changing face of Klebsiella pneumoniae carbapenemase: *in-vivo* mutation in patient with chest infection. *Lancet* 399:2226. doi: 10.1016/S0140-6736(22)01011-X

Shields, R. K., Chen, L., Cheng, S., Chavda, K. D., Press, E. G., Snyder, A., et al. (2017a). Emergence of ceftazidime-avibactam resistance due to plasmid-borne Bla KPC-3 mutations during treatment of Carbapenem-resistant Klebsiella pneumoniae infections. *Antimicrob. Agents Chemother.* 61:e02097-16. doi: 10.1128/AAC.02097-16

Shields, R. K., Clancy, C. J., Hao, B., Chen, L., Press, E. G., Iovine, N. M., et al. (2015). Effects of Klebsiella pneumoniae Carbapenemase subtypes, extended-Spectrum β-lactamases, and Porin mutations on the in vitro activity of ceftazidime-avibactam against Carbapenem-Resistant K. pneumoniae. *Antimicrob. Agents Chemother.* 59, 5793–5797. doi: 10.1128/AAC.00548-15

Shields, R. K., Nguyen, M. H., Chen, L., Press, E. G., Potoski, B. A., Marini, R. V., et al. (2017b). Ceftazidime-avibactam is superior to other treatment regimens against Carbapenem-resistant Klebsiella pneumoniae bacteremia. *Antimicrob. Agents Chemother.* 61:e00883-17. doi: 10.1128/AAC.00883-17

Shields, R. K., Potoski, B. A., Haidar, G., Hao, B., Doi, Y., Chen, L., et al. (2016). Clinical outcomes, drug toxicity, and emergence of ceftazidime-avibactam resistance among patients treated for Carbapenem-resistant Enterobacteriaceae infections. *Clin. Infect. Dis.* 63, 1615–1618. doi: 10.1093/cid/ciw636

Shirley, M. (2018). Ceftazidime-avibactam: a review in the treatment of serious gram-negative bacterial infections. *Drugs* 78, 675–692. doi: 10.1007/s40265-018-0902-x

Society of Clinical Microbiology and Infection of China International Exchange and Promotion Association for Medical and Healthcare, Clinical Microbiology Group of the Laboratory Medicine Society of the Chinese Medical Association, Clinical Microbiology G.I.S. ofthe C. M (2020). Expert consensus on polymyxins, tigecycline and ceftazidime/avibactam susceptibility testing. *Chinese J. Lab. Med.* 43, 964–972. doi: 10.3760/cma.j.cn114452-20200719-00619

Sun, Y., Cai, Y., Liu, X., Bai, N., Liang, B., and Wang, R. (2013). The emergence of clinical resistance to tigecycline. *Int. J. Antimicrob. Agents* 41, 110–116. doi: 10.1016/j.ijantimicag.2012.09.005

Sun, L., Li, H., Wang, Q., Liu, Y., and Cao, B. (2021). Increased gene expression and copy number of mutated Bla KPC lead to high-level ceftazidime/avibactam resistance in Klebsiella pneumoniae. *BMC Microbiol.* 21, 1–10. doi: 10.1186/s12866-021-02293-0

Tang, N., Hu, J., Zhao, Y., Song, Y., Wang, C., Zhang, G., et al. (2021). *In vivo* evolution of carbapenem resistance in hypervirulent Klebsiella pneumoniae in a patient undergoing long-term treatment. *J. Antimicrob. Chemother.* 77, 531–533. doi: 10.1093/jac/dkab380

Tian, X., Wang, Q., Perlaza-Jiménez, L., Zheng, X., Zhao, Y., Dhanasekaran, V., et al. (2020). First description of antimicrobial resistance in carbapenem-susceptible Klebsiella pneumoniae after imipenem treatment, driven by outer membrane remodeling. *BMC Microbiol.* 20, 218–211. doi: 10.1186/s12866-020-01898-1

van Duin, D., Cober, E. D., Richter, S. S., Perez, F., Cline, M., Kaye, K. S., et al. (2014). Tigecycline therapy for carbapenem-resistant Klebsiella pneumoniae (CRKP) bacteriuria leads to tigecycline resistance. *Clin. Microbiol. Infect.* 20, O1117–O1120. doi: 10.1111/1469-0691.12714

Velkov, T., Thompson, P. E., Nation, R. L., and Li, J. (2010). Structure−activity relationships of Polymyxin antibiotics. *J. Med. Chem.* 53, 1898–1916. doi: 10.1021/jm900999h

Villa, L., Feudi, C., Fortini, D., García-Fernández, A., and Carattoli, A. (2014). Genomics of KPC-producing Klebsiella pneumoniae sequence type 512 clone highlights the role of RamR and ribosomal S10 protein mutations in conferring Tigecycline resistance. *Antimicrob. Agents Chemother.* 58, 1707–1712. doi: 10.1128/AAC.01803-13

Wang, H., Dzink-Fox, J. L., Chen, M., and Levy, S. B. (2001). Genetic characterization of highly fluoroquinolone-resistant clinical Escherichia coli strains from China: role of acrR mutations. *Antimicrob. Agents Chemother.* 45, 1515–1521. doi: 10.1128/AAC.45.5.1515-1521.2001

Wang, C., Zhao, J., Liu, Z., Sun, A., Sun, L., Li, B., et al. (2021). *In vivo* selection of imipenem resistance among ceftazidime-avibactam-resistant, imipenem-susceptible Klebsiella pneumoniae isolate with KPC-33 Carbapenemase. *Front. Microbiol.* 12:e727946. doi: 10.3389/fmicb.2021.727946

Xie, M., Chen, K., Dong, N., Xu, Q., Chan, E. W.-C., Zhang, R., et al. (2022). Phenotypic changes associated with in vivo evolution of Colistin resistance in ST11 Carbapenem-resistant Klebsiella pneumoniae. *Front. Cell. Infect. Microbiol.* 12:e841748. doi: 10.3389/fcimb.2022.841748

Xu, T., Guo, Y., Ji, Y., Wang, B., and Zhou, K. (2022). Epidemiology and mechanisms of Ceftazidime–Avibactam resistance in gram-negative bacteria. *Engineering* 11, 138–145. doi: 10.1016/j.eng.2020.11.004

Ye, M., Ding, B., Qian, H., Xu, Q., Jiang, J., Huang, J., et al. (2017). In vivo development of tigecycline resistance in Klebsiella pneumoniae owing to deletion of the ramR ribosomal binding site. *Int. J. Antimicrob. Agents* 50, 523–528. doi: 10.1016/j.ijantimicag.2017.04.024

Ye, M., Liao, C., Shang, M., Zou, D., Feng, X., Lu, X., et al. (2022). Reduced virulence and enhanced host adaption during antibiotics therapy: a story of a within-host Carbapenem-resistant Klebsiella pneumoniae sequence type 11 evolution in a patient with a serious scrotal abscess. *mSystems* 7:e0134221. doi: 10.1128/msystems.01342-21

Ye, Y., Xu, L., Han, Y., Chen, Z., Liu, C., and Ming, L. (2017). Mechanism for carbapenem resistance of clinical Enterobacteriaceae isolates. *Exp. Ther. Med.* 15, 1143–1149. doi: 10.3892/etm.2017.5485

Zasowski, E. J., Rybak, J. M., and Rybak, M. J. (2015). The β-lactams strike Back: ceftazidime-avibactam. *Pharmacother. J. Hum. Pharmacol. Drug Ther.* 35, 755–770. doi: 10.1002/phar.1622

Zhang, R., Dong, N., Huang, Y., Zhou, H., Xie, M., Chan, E. W. C., et al. (2018). Evolution of tigecycline-and colistin-resistant CRKP (carbapenem-resistant Klebsiella pneumoniae) *in vivo* and its persistence in the GI tract article. *Emerg. Microbes Infect.* 7, 1–11. doi: 10.1038/s41426-018-0129-7

Zhang, P., Shi, Q., Hu, H., Hong, B., Wu, X., Du, X., et al. (2020). Emergence of ceftazidime/avibactam resistance in carbapenem-resistant Klebsiella pneumoniae in China. *Clin. Microbiol. Infect.* 26, 124.e1–124.e4. doi: 10.1016/j.cmi.2019.08.020