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Klebsiella species: Taxonomy, hypervirulence and multidrug resistance

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Summary

Members of the genus *Klebsiella* have rapidly evolved within the past decade, generating organisms that simultaneously exhibit both multidrug resistance and hypervirulence (MDR-hv) phenotypes; such organisms are associated with severe hospital- and community-acquired infections. Carbapenem-resistant infections with unknown optimal treatment regime were of particular concern among the MDR-hv *Klebsiella* strains. Recent studies have revealed the molecular features and the mobile resistance elements they harbour, allowing identification of genetic loci responsible for transmission, stable inheritance, and expression of mobile resistance or virulence-encoding elements that confer the new phenotypic characteristics of MDR-hv *Klebsiella* spp. Here, we provide a comprehensive review on the taxonomic position, species composition and different phylotypes of *Klebsiella* spp., describing the diversity and worldwide distribution of the MDR-hv clones, the genetic mutation and horizontal gene transfer events that drive the evolution of such clones, and the potential impact of MDR-hv infections on human health.

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Introduction

Various strains in the genus *Klebsiella* have evolved to become a major clinical and public health threat worldwide.[1] *Klebsiella* spp. are opportunistic pathogens which are normally found in the flora of healthy individuals' nose, throat, skin, and intestinal tract, but can also cause a range of infections, including pneumonia, soft tissue and surgical wound infections, urinary tract infections, bloodstream infections and sepsis.[2] The *Klebsiella* genus comprises a wide diversity of species, including the *Klebsiella pneumoniae* species complex (KpSC) and several more genetically distant species.[3] A large proportion of infections caused by *Klebsiella* spp. are due to two major pathotypes, namely the multidrug-resistant (MDR) and hypervirulent (hv) clones.[4] Strains of the two branches were considered to be non-overlapping since they each exhibit different genetic backgrounds.[4] However, *Klebsiella* spp. has demonstrated the ability to acquire genetic elements and mutations that confer antimicrobial resistance and/or virulence traits, leading to the ultimate emergence of the convergent clones, termed multidrug-resistant and hypervirulent (MDR-hv) *Klebsiella* spp.[5,6] MDR-hv *Klebsiella* spp. are simultaneously hypervirulent and resistant to multiple antibiotics, and are known to be undergoing further evolution to produce phenotypically novel strains.[6,7] A broad diversity of MDR-hv strains of *Klebsiella* spp. that evolved through diverse mechanisms has been reported across different continents in the world.[8] The rise in number of severe infections and the increasing limitations in effective treatments rendered MDR-hv *Klebsiella* spp. real superbugs that pose serious challenges to public health.[9] In this review, we provide an overview of the taxonomic position and species composition of *Klebsiella* spp. Based on the current information we classify different *Klebsiella* sp. clones of diverse phenotypes, explored the genetic diversity and worldwide distribution of the MDR-hv *Klebsiella* spp., describe the genetic mutation and horizontal gene transfer (HGT) events driving the evolution of such clones, and discuss the clinical impact of infections caused by MDR-hv strains.

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Taxonomic position and species composition of the *Klebsiella* genus

The genus *Klebsiella* is a class of Gram-negative, encapsulated, nonmotile, rod-shaped and oxidase-negative bacteria.[10] Strains of this genus were first isolated in the late 19th century and named by Trevisan (1885) to honor the German microbiologist Edwin Klebs (1834-1913).[10] *Klebsiella* is classified under the *Enterobacteriaceae* family which contained a large array of biochemically distinct genus, including the model organism *Escherichia coli* and the notorious human pathogens *Salmonella, Yersinia, Serratia, Enterobacter, Citrobacter, Kluyvera, Leclercia, Raoultella, Cronobacter*, etc.[3] The *Klebsiella* genus currently comprises a wide diversity of species, including species belonging to the *K. pneumoniae* species complex (KpSC) and other *Klebsiella* species (*K. indica, K. terrigena, K. spallanzanii, K. huaxiensis, K. oxytoca, K. grimontii, K. pasteurii* and *K. michiganensis*) that share an average of only 90% nucleotide identity with KpSC.[3] KpSC has no formal taxonomic designation, and it commonly refers to closely related species that share 95%–96% average nucleotide identity with *K. pneumoniae sensu stricto*.[3] At the time of writing, seven phylogroups that belong to KpSC have been classified, including *K. pneumoniae* (Kp1), *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2), *K. variicola* subsp. *variicola* (Kp3), *K. quasipneumoniae* subsp. *similipneumoniae* (Kp4), *K. variicola* subsp. *tropica* (Kp5), *K. quasivariicola* (Kp6) and *K. africana* (Kp7) .[11] All taxa of the KpSC used to be misassigned by biochemical or proteomics assays as *K. pneumoniae*, since they possess overlapping features.[12] Modern species identification methods rely largely on sequence-based classifiers or mass spectrometry (MALDI-TOF) platforms, both of which perform comparative analysis with reference genomes or strains, and require updated databases to reflect an accurate taxonomy.[12,13] The multilocus sequence typing (MLST) scheme developed for typing *K. pneumoniae* isolates can be applied across the entire species complex.[11] Closely related sequence types (ST) could be further designed as clonal complexes (CC) using eBURST (http://eburst.mlst.net/).[14] The species *K. terrigena, K. planticola* and *K. ornithinolytica* have been transferred to the *Raoultella* genus based on the *rpoB* sequence.[15]

The type species of the *Klebsiella* genus, *K. pneumoniae*, is highly prevalent in clinical collections, accounting for an isolation rate of approximately 85%.[3] *K. pneumoniae* was classified as one of the ESKAPE organisms (*Enterococcus faecium*,*Staphylococcus aureus*,*K. pneumoniae*,*Acinetobacter baumannii*,*Pseudomonas aeruginosa* and *Enterobacter* species), which are well-known highly virulent and antimicrobial resistant clinical pathogens.[16] Two subspecies of *K. pneumoniae*, namely *K. pneumoniae* subsp. *ozaenae* and *K. pneumoniae* subsp. *rhinoscleromatis*, which are associated with a specific disease syndrome (atrophic rhinitis and rhinoscleroma, respectively), and one monomorphic clone, have been reported.[17] Both subspecies sit squarely within the general population of *K. pneumoniae* phylogenetically and are regarded as hypervirulent clones derived from *K. pneumoniae*.[11] Species including *K. variicola* and *K. oxytoca*, which are associated with clinical infections, have also emerged, yet their virulence profiles has not been fully characterized.[12,18,19] The genetic features and clinical relevance of *K. pneumoniae* virulence factors in distant species remain poorly understood.[11]

Defining classical, hypervirulent and MDR-hv *Klebsiella* spp

Klebsiella spp. have gained the ability to acquire external genetic materials that enable the organisms to undergo extensive evolution.[6] Two pathotypes, classical and hypervirulent *Klebsiella* spp., are reported to be associated with infections.[6] Currently, the majority of currently available data are concentrated on *K. pneumoniae*, which is also of highest clinical importance.[3] Classical *K. pneumoniae* (cKP) strains have gained increasing notoriety owing to its propensity to accumulate mutations and acquire determinants, leading to the emergence of multiple, extensive, or pan-drug resistant clones.[1,20] More than 100 acquired antimicrobial resistance genes have been identified in *K. pneumoniae*, which encodes different products that confer resistance towards distinct classes of antibiotics including β-lactams, aminoglycosides, quinolones, tigecycline and polymyxins.[16,20] The majority of *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* worldwide belong to the notorious CC258 clone (including ST258, ST11, ST340, ST437 and ST512).[21] Several other clonal groups (CG), including CG14/15, CG17/20, CG29, CG37, CG43, CG101, CG147, CG152, CG231, CG307 and CG490, are also globally distributed and associated with multidrug resistance.[20,22]

Hypervirulent *K. pneumoniae* (hvKp), initially reported in the mid-1980s from the Asian Pacific Rim, are increasingly reported worldwide.[23] Despite the lack of 100% specific and sensitive marker for hvKp, several phenotypic and clinical features have defined this *K. pneumoniae* variant.[24] The first is their ability to cause severe infections associated with high pathogenicity and mortality in both immunocompromised and healthy hosts, typically presenting as pyogenic liver abscesses.[6,25] Also, hvKp has been observed to trigger diseases at unusual sites, including endophthalmitis, necrotizing fasciitis, central nervous diseases (meningitis, bacteremia), etc.[26] A second trait is their propensity for metastatic spread to distinct sites, which is uncommon among enteric Gram-negative bacilli, including cKP.[9,23-25] Furthermore, a large proportion of hvKp

colonies present a hypermucoviscous phenotype on agar plates which could be semi-quantitatively defined by a "string test".[6] However, the degree of correlation between hypermucoviscosity, capsule gene expression and hypervirulence remains to be clarified.[26] Clinical hvKp clones are known to be less diverse than MDR *K. pneumoniae*.[22] The majority of reported hvKp clones belong to serotype K1 or K2, with CG23 being the dominant K1 hvKp clone, whereas several genetically unrelated groups (CG25, CG65, CG66, CG86 and CG380, etc.) constitute the K2 strains.[3,27] To date, hvKp belonging to serotypes K5, K16, K20, K54, K57, and KN1 have also been documented.[26] The hvKp strains rely on a battery of virulence factors for survival and infection, among which enhanced capsule production and the synthesis of siderophores are dominant.[26] Evidence from previous studies highlighted the importance of pLVPK-like virulence plasmids in the expression of the hypermucoid phenotype and siderophore production among hvKp isolates, since they harbor virulence-associated determinants that encode the regulators of the mucoid phenotype (*rmpADC* and *rmpA2*), production of siderophores (*iutAiucABCD* encoding aerobactin and *iroBCDN* clusters encoding salmochelin), expression of the metabolite transporter PEG344 and ABC-type transporter (*fepBC*) and the regulatory system for iron uptake (*fecIRA*).[28-30] Additionally, a "high pathogenicity island" (KPHP1208) harboring genes that encode colibactin, microcin E492, and the siderophore yersiniabactin is related to the hypervirulence phenotype.[27] A few candidate biomarkers were proposed for differentiation between hvKp from cKP, among which the genes *peg-344, iucA, iroB*, and plasmid-borne *rmpA* and *rmpA2*, and quantitative siderophore production of ≥30 μg/mL demonstrated >0.95 diagnostic accuracy, whereas the string test exhibited an accuracy of only 0.90.[31] Besides, a previous study has demonstrated that the murine model, but not the *Galleria mellonella* model, is appropriate for validating suspected hvKp strains.[32]

MDR *K. pneumoniae* and hvKp are generally associated with two distinct subsets of *K. pneumoniae* lineages distinguishable by the presence of acquired resistance genes and several key virulence loci.[22] The two lineages were considered to be non-overlapping over a period of 30 years after the discovery of hvKp.[33] Yet the last few years witnessed an increasing number of reports on the convergent *K. pneumonia* clones (MDR-hvKp) that are simultaneously multidrug resistant and hypervirulent.[5,34] MDR-hvKp isolates are highly diverse in genetic backgrounds and exhibit diversified antimicrobial resistance profiles.[8] Hypervirulent and MDR-hv *Klebsiella* spp. other than *K. penumoniae*, including *K. quasipneumoniae* subsp. *similipneumoniae, K. variicola*, etc., has been reported, posing a new challenge to public health.[12,35,36]

Worldwide distribution and genetic backgrounds of MDR-hv *Klebsiella* spp

Since the first report of MDR-hvKp in 2015 and the fatal outbreak of ST11 carbapenem-resistant hvKp (CR-hvKp) reported in 2017 in China, research on MDR-hv *Klebsiella* spp. has become a hot topic.[5,37] To date, MDR-hv *Klebsiella* spp. have been reported across several continents including Asia (China (China mainland, Hong Kong and Taiwan), India, Iran), Europe (France, Norway, the United Kingdom, Russia), Africa (Egypt), South (Brazil) and North (Canada, the USA) America.[8] Most MDR-hv *Klebsiella* spp.-related studies were from Asia, in particular China. Infections caused by such convergent clones could be dated back to as early as 2008, almost a decade before the first report of such clones, when ST2888 and ST1264 extended-spectrum-β-lactamase (ESBL)-producing hvKp strains were isolated from the elderly in China.[38] Results from previous retrospective studies suggested MDR-hv *Klebsiella* spp. is increasing in China over the recent years, with their resistance phenotypes expanding from ESBL-producing to non-susceptibility to last-line antibiotics including carbapenems, tigecycline and colistin.[38,39] The majority of MDR-hv *Klebsiella* spp. reported belonged to *K. pneumoniae sensu stricto*, which could potentially be associated with the inaccuracy in species identification. MDR-hvKp belonging to at least 51 diverse sequence types and 13 serotypes (K1, K2, K5, K16, K19, K20, K24, K47, K51, K54, K57, K62, K64, KL108) have been reported, with regional differences seen from the literature (Figure 1). Only a few cases of MDR-hvKp infections were reported from Europe, Africa, South and North America, most of which were ESBL- or carbapenemase-producing isolates belonging to the sequence and sero-types of ST15:K24, ST23:K1, ST29: K19, ST268:K20, ST661: K_{NA}, ST4415: K_{NA}, and ST4416:K_{NA} (NA, not available).[8] MDR-hvKp isolates circulating outside Asia was reported in as early as the year 2012, when an ESBL-producing ST86:K2 hvKP strain was isolated from an immunocompromised patient in France.[40] In India, infection cases caused by MDR-hvKp producing ESBLs or encoding resistance to carbapenems or colistin were reported, including those belonging to ST11: K_{NA}, ST23:K1, ST43:K_{NA}, ST231:K51, ST2318:K_{NA} and ST5235:K_{NA}.[41] Particularly, ST5235 hvKp isolates resistant to the last-line antibiotics carbapenems and colistin were isolated from a series of neonatal sepsis.[42] Infections caused by ST11:K_{NA}, ST893: K_{NA} and ST23:K1 CR-hvKp were reported from Iran from 2012-2018.[43,44] In Taiwan (China), ESBL-producing and/or tigecycline-resistant hvKp that belong to ST1049:K5, ST23:K1, ST380:K2, ST65:K2, ST660:K16, ST8:K1, and ST86:K2 were reported during the period 2015-2018.[45,46] Mainland China has reported the most cases of MDR-hvKp infections, covering at least 38 diverse sequence types and 11 serotypes.[5,34,47] ST11 and ST23 MDR-hvKp isolates were most frequently reported

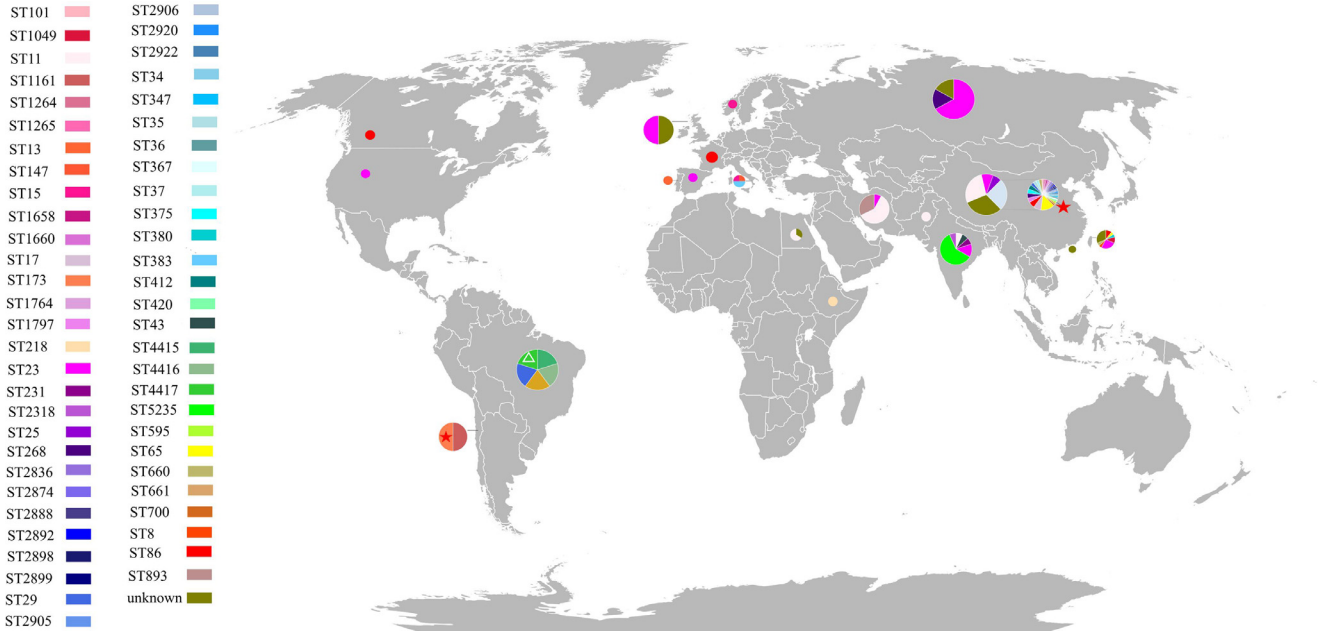

Figure 1. Worldwide distribution of MDR-hv *Klebsiella* spp. Different colours of sectors represent different sequence types of the MDR-hv *Klebsiella* spp. as shown in the left of the figure. Sectors with asterisk and triangle represent the existence of *K. variicola* and *K. quasipneumoniae subsp. similipneumoniae*, respectively, and sectors without symbols represent that of *K. pneumoniae*. All MDR-hv *Klebsiella* spp. reported up to February 20th, 2022, were included. Multidrug-resistant *Klebsiella* sp. strains were defined based on the resistance to at least one agent in three or more antimicrobial categories in addition to ampicillin. Pie graph areas are relatively proportional to the total number of MDR-hv strains reported in each country/region.

from China, which is consistent with the finding in a previous study that ST11 and ST23 accounted for the majority of MDR *K. pneumoniae* (MDR-KP, 51/66, ~77.27%) and hvKp (16/23, ~69.57%), respectively.[48] Species other than *K. pneumoniae* were reported, including carbapenem-resistant and colistin-resistant ST595:K16 *K. variicola* strains isolated in 2015 and 2016 in China, respectively, and strains of ST4417:K47 *K. quasipneumoniae* subsp. *similipneumoniae* isolated in Brazil in 2016 that were resistant to various antibiotics (fluoroquinolones, β-lactams, tetracyclines, trimethoprim, aminoglycosides, sulfonamides, macrolides, and fosfomycin) (**Supplementary Table S1**).[36,47,49] CR-hvKp isolates producing carbapenemases were of particular concern among all MDR-hvKp isolates. KPC-2 was the most frequently reported carbapenemase produced by CR-hvKp isolates, and the production of other carbapenemases including NDM (NDM-1, NDM-5, and NDM-7), IMP, SIM, VIM (VIM-1 and VIM-2), and OXA-48-like has also been described.[8]

Mutation and horizontal gene transfer events driving the formation of MDR-hv *Klebsiella* spp

Klebsiella spp. have gained the capacity to acquire both drug resistance and hypervirulence phenotype through accumulation of mutations and/or horizontal gene transfer (HGT).[50] MDR-hv *Klebsiella* spp. was commonly considered to have evolved as a result of acquisition of virulence determinants by MDR-KP or through development of MDR phenotypes by hvKp.[8] Accumulation of mutations, including mutations in the quinolone resistance-determining region (QRDR mutations in *gyrA* and *parC*), porin gene mutations (*ompK36*) and mutations leading to the over-expression of efflux pumps (OEP mutations: *acrAB, oqxAB, ramA* and *rarA*), was reported among hypervirulent clones (Table 1 g,h,i), leading to the emergence of MDR-hv *Klebsiella* spp. These mutations confer resistance to a diverse range of antibiotics including quinolones, carbapenems and tigecyclines. Taiwan (China) has reported the most cases of resistance-associated mutations among MDR-hvKp, covering isolates of ST23:K1, ST86:K2, ST268:K20, ST307:K1 and ST1049:K5 which often contain QRDR and OEP mutations.[46,51] An ST86:K2 hvKp isolate carrying the plasmid-borne $bla_{\text{CTX-M-15}}$ gene from France has developed carbapenem resistance due to deletion of a 11 bp region in the *ompK36* gene, which resulted in truncation of the outer membrane protein.[52] In China, QRDR mutations were reported among ST23:K1 hvKp strains carrying plasmid-mediated quinolone resistance determinants.[53]

HGT is mainly mediated by several well-recognized mechanisms including transduction, transformation and conjugation, among which conjugation is considered the most significant.[54,55] Acquired resistance and virulence-associated genes transferred among bacteria via mobile genetic elements include those located in conjugative and mobilizable plasmids, integrative conjugative elements (ICE), integrons, insertion sequences and transposons.[56] Acquisition of resistance genes by hvKp through HGT involves mechanisms including resistance gene capture by virulence plasmid via intermolecular replicative transposition, acquisition of conjugative resistance plasmid, acquisition of resistance plasmid with unknown mechanism of conjugal transfer and acquisition of resistance genes through other unknown mechanisms (Table 1 a,b,c,j). HGT of resistance genes was reported from China, France, India and Iran, and involved hvKp clones ST23:K1, ST29:K54, ST35:KL108, ST86:K2, ST307:K1, ST661:K1 and ST1265:K1.[8] Acquisition of resistance plasmids was the most commonly reported mechanism, followed by acquisition of resistance genes. Intermolecular transposition was an important mechanism responsible for integration of antibiotic resistance genes carried by the virulence plasmids. Mobile elements that mediate this transposition target the related hot spots on the virulence plasmids, leading to the integration of resistance genes and a duplicated hot spot sequence termed target site duplications (TSDs).[57] The $bla_{\text{CTX-M-24}}$, $bla_{\text{KPC-2}}$ and *dfrA14*, and *catA1* genes were integrated into the virulence backbones in ST23:K1 hvKp isolates upon mobilization of insertion sequences IS*903D*, IS*26* and IS*5075*, generating 8-bp (GCACAGAGA), 8-bp (CTAAAATT) and 10-bp (TACCGGGAAG) TSD sequences bordering the mobile elements, respectively.[41,58,59]

The acquisition of virulence-associated genes by MDR *Klebsiella* spp. involves three main mechanisms: (i) obtaining a virulence plasmid by forming a cointegrate with a helper conjugative plasmid mediated by homologous recombination, (ii) acquisition of conjugative plasmid that contains virulence genes by homologous recombination, and (iii) chromosomal integration of ICE elements carrying the virulence factors (Table 1 d,e,f). Homologous recombination therefore plays a pivotal role in dissemination of hypervirulence-associated genes. An ST15:K19 MDR-hvKp and an ST11:K64 CR-hvKp strain with a homologous fragment of unknown size and 241-bp, respectively, were both found to have evolved through the first mechanism.[60,61] An ST595:K16 CR-hv *K. variicola* strain which contained a 889-bp upstream and a 1,246-bp downstream homologous fragment, evolved from the second mechanisms.[62] Strains that evolved from the third mechanism, which involves chromosomal integration of genetic elements harboring virulence-associated factors have also been reported.[63] In addition, an ST35:KL108 CR-hvKp isolate was reported to have evolved through acquisition of a $bla_{\text{NDM-5}}$-carrying plasmid and integration of a ~76 Kb ICE*Kp1* element with the *iroBCDN* and *rmpA* genes in the chromosome.[64] Another potential mechanism of evolution for MDR-hv *Klebsiella* spp. is the acquisition

Country/region	Year of isolation	ST/serotype	No. of plasmids	Plasmid replicons and plasmid-borne resistance genes	gene mutation and overexpression *	formation mechanism **	Genetic marker	Reference
China mainland	2014	ST23/K1	1	*pvir-res*: IncHI1B/FIB (*bla*$_{CTX-M-24}$)	-	a	8-bp TSD bordering IS*903D* ***	59
China mainland	2013	ST23/K1	1	*pvir-res*: IncHI1B/FIB (*bla*$_{KPC-2}$, *dfrA14*)	-	a	8-bp TSD bordering IS*26*	58
China mainland	2015	ST23/K1	2	*pvir*: IncHI1B/FIB; *pres*: IncHI5 (*bla*$_{DHA-1}$, *sul1*, *qnrB4*)	-	b	-	69
China mainland	2018	ST23/K1	3	*pvir*: IncHI1B/FIB; *pres-A*: IncA (*bla*$_{VIM-1}$, *dfrA14b, aacA4, aphA15, aacA4, aphA15, aadA1b, catB2*); *pres-B*: IncFII (*qnrS, bla*$_{CTX-M-15}$, *bla*$_{TEM-1C}$)	-	b	-	70
China mainland	2017	ST23/K1	7	*pvir*: IncHI1B; *pres-A*: IncHI1B/FIB (*bla*$_{NDM-1}$, *aac(6′)-Ib-cr, bla*$_{OXA-1}$, *catB3, arr-3, sul1*); *pres-B*: IncFII (*bla*$_{CTX-M-14}$); *pres-C*: IncFII$_{k}$ (*aadA16, aph(3′)-Ia, qnrB2, qnrS1, aac(6′)-Ib-cr, mph*(A), *tet*(A), *dfrA27, sul1*); others: IncX4, IncP, ColRNAI	-	b	-	71
China mainland	2014	ST1265/K1	2	*pvir*: IncHI1B/FIB; *pres*: NA (*bla*$_{KPC-2}$)	-	b	-	72
China mainland	2017	ST23/K1	NA*****	NA (*qnrS1, acc(6′)-Ib-cr, bla*$_{CTX-M-14/15}$)	M: *gyrA, parC*	b+g	-	53
China mainland	2015	ST661/K1	NA	NA (*bla*$_{DHA-1}$, *mcr-1*)	-	b	-	73
China mainland	2018	ST29/K54	NA	*pvir*: IncHI1B/FIB; *pres*: IncX3 (*bla*$_{NDM-5}$)	-	b	-	74
China mainland	2016	ST35/KL108 ****	3	*pres*: IncX3 (*bla*$_{NDM-5}$); *others*: NA	-	c+d	-	64
China mainland	2017	ST86/K2	2	*pvir*: IncHI1B; *pres*: IncX6 (*bla*$_{KPC-2}$ and △*bla*$_{TEM-1}$)	-	b	-	75

Table 1 (Continued)

Country/region	Year of isolation	ST/serotype	No. of plasmids	Plasmid replicons and plasmid-borne resistance genes	gene mutation and overexpression *	formation mechanism **	Genetic marker	Reference
China mainland	2017	ST15/K19	3-4	*pvir*: IncHI1B/FIB; *pres-A*: IncFIB/FII (*bla*$_{OXA-1}$, *tet*(A), *aph (3')-Ia, mph*(A), *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *bla*$_{SHV-11}$, *mph*(E), *msr*(E), *armA, sul1, bla*$_{DHA-1}$, *qnrB4, dfrA12, aadA2, sul1*); *pres-B*: IncFII (*bla*$_{KPC-2}$, *aac(6')-Ib-cr, aac(3)-IId, mdf*(A), *catB4*); *other*: ColRNAI	M: *ramR, parC*	e	homologous fragment (unknown size)	[60]
China mainland	2016	ST11/K64	4	*pvir*: IncHI1B/FIB; *pres-A*: IncFII (*bla*$_{KPC-2}$, *bla*$_{SHV-12}$, *bla*$_{CTX-M-65}$, *bla*$_{TEM-1}$); *pres-B*: IncFII (*qnrS1, bla*$_{LAP-2}$, *tet*(A), *catA2, sul2*); *other*: IncFIA	-	e	homologous fragment (241-bp)	[61]
China mainland	2015	ST595/K16	3	*pvir*: IncFIB; *pres*: IncX5 (*bla*$_{KPC-2}$, *bla*$_{TEM-1}$); *other*: IncFIB/IncFII	-	f	homologous fragments (889-bp upstream and 1,246-bp downstream)	[62]
France	2017	ST86/K2	NA	*pvir*: IncHI1B/FIB; *pres*: IncL (*bla*$_{OXA-48}$)	-	c	-	[52]
France	2017	ST86/K2	NA	*pvir*: IncHI1B/FIB; *pres*: IncN (*bla*$_{CTX-M-15}$)	M: *ompK36, ramR*	c+h	-	[52]
India	2018	ST23/K1	7	*pvir*: IncFIB (*catA1*); *pres*: IncFIB (*qnrB1, catB, aac(6')-Ib3, rmtF, arr-2*); *others*: IncA/C2, IncFIB, IncX3, ColRNAI, Col440II	-	a+c	10-bp TSD bordering IS*5075*	[41]
India	2017	ST23/K1	8	*pvir*: IncFIB (*catA1*); *pres-A*: IncFIB(*qnrB1, catB, aac(6')-Ib3, rmtF, arr-2*); *pres-B*: IncX3 (*aac(6')-Ib-cr, armA, mph*(E), *msr*(E), *sul1*); *pres-C*: ColKP3 (*bla*$_{OXA-232}$); *others*: IncA/C2, IncFIB, ColRNAI, Col440II	-	a+c	10-bp TSD bordering IS*5075*	[41]

Table 1 *(Continued)*

Country/region	Year of isolation	ST/serotype	No. of plasmids	Plasmid replicons and plasmid-borne resistance genes	gene mutation and overexpression *	formation mechanism **	Genetic marker	Reference
Iran	2012	ST23/K1	NA	*pvir*: NA; *pres*: IncN (*aacA7*, bla_{VIM-2}, *dhfrI*)	-	b	-	44
Norway	2014	ST15/K24	7	*pvir*: IncHI1B/FIB ($bla_{CTX-M-15}$, *aph(3')-Ia*, *dfrA*, *sat2*, bla_{SHV-5}, *sul1*, *aadA1*); *pres*: IncFII (*aacA4*, bla_{OXA-1}, bla_{TEM}, *cat*, $bla_{CTX-M-15}$); *others*: IncFIB, Col440I, ColpVC and 2 nontypable plasmids	-	k	genes for conjugal transfer on fusion plasmid	65
Norway	2015	ST15/K24	4	*pvir*: IncHI1B/FIB (*aph(3')-Ia*, *dfrA*, *sat2*, bla_{SHV-5}, *sul1*, *aadA1*); *pres*: IncFII (*aacA4*, bla_{OXA-1}, bla_{TEM}, *cat*, *tet*(A), $bla_{CTX-M-15}$); *others*: IncFIB and a nontypable plasmid	-	k	genes for conjugal transfer on fusion plasmid	65
Taiwan, China	2015-2018	ST268/K20; ST307/K1; ST23/K1; ST86/K2; ST1049/K5	NA	NA	ramA upstream alterations, etc.	i	-	46
Taiwan, China	2013-2016	ST23/K1	NA	NA (bla_{DHA-1}, *qnrS*)	M: *oqxR*, *ramR*	i+j	-	51
Taiwan, China	2013-2016	ST307/K1	NA	NA (*qnrB*, *qnrS*, *aac(6')-Ib-cr*)	M: *ramR*, *gyrA*, *parC*; O: AcrAB efflux pump and RamA	g+i+j	-	51

***Table 1*: Mechanisms driving the convergence of multidrug resistance and hypervirulence in *Klebsiella* spp.**

*M, mutation; O, overexpression. ** Formation mechanisms: **a**, acquisition of resistance gene by virulence plasmid via intermolecular transposition; **b**, acquisition of conjugative resistance plasmid; **c**, acquisition of resistance plasmid with its conjugal transferability unknown; **d**, chromosomal integration of ICE element carrying virulence factors; **e**, transfer of virulence plasmid by forming a cointegrate with a helper conjugative plasmid mediated by homologous recombination; **f**, acquisition of conjugative plasmid with virulence genes acquired by homologous recombination; **g**, mutations in quinolone resistance-determining region; **h**, porin mutation; **i**, overexpression of efflux pump; **j**, acquisition of resistance gene (unknown mechanism); **k**, acquisition of conjugative fusion plasmid with virulence and resistance genes (hypothetical). Among these mechanisms, g, h, and i are accumulation of mutations, and others are horizontal gene transfer. *** TSD, target site duplication. **** KL108 is correspond to capsule loci defined on the basis of gene content, for which the corresponding serological capsule types are yet to be defined. ***** NA, not available. This table only covers strains with validated evolutionary mechanisms. Except the ST595:K16 isolate which belonged to *K. variicola*, all other strains belonged to *K. pneumoniae*.

of a conjugative fusion plasmid which contains both virulence and resistance genes; yet reports of this mechanism are scarce and not supported by experimentally validated data (Table 1> k).[65]

The evolution of MDR-hv *Klebsiella* spp. is complicated and involves the aforementioned or other unknown mechanisms. For example, evolutionary events including simultaneous acquisition of resistance genes or plasmids and development of resistance-associated mutations, as well as acquisition of resistance genes or plasmids and chromosomal integration of ICE element, have been reported (Table 1). The highly variable genetic features of *Klebsiella* spp. rendered it able to rapidly adapt to diverse environmental niches.

Clinical implications of MDR-hv *Klebsiella* infections

Management of MDR-hv *Klebsiella* sp. infections requires both active antibiotic therapy and adequate infection control.[66] No clinical trials assessing the ideal antibiotics for treating MDR-hv *Klebsiella* spp. infections has been conducted, hence empiric antibiotic therapy should take into account both the local antimicrobial resistance patterns and infection sites.[66] A previous comparative study on evolutionary genomics suggested that MDR *Klebsiella* sp. clones pose the greatest risk, since they are more likely to acquire virulence genes than hypervirulent clones are to acquire resistance genes.[22] *Klebsiella* spp. are intrinsically resistant to penicillin by producing different types of β-lactamases, such as SHV in *K. pneumoniae*, OKP in *K. quasipneumoniae* and LEN in K. *variicola*.[2] The acquisition of antimicrobial resistance genes and accumulation of resistance-associated mutations rendered MDR *Klebsiella* spp. unresponsive to antimicrobial drugs.[1] Infections caused by CR isolates are of key concern among all MDR *Klebsiella* strains, for which optimal treatment remains unavailable.[67] According to a recent retrospective study, ceftazidime-avibactam is superior to other treatment regimens in treatment of CR *K. pneumoniae* bacteremia.[68] Alternative antibiotic treatment options to consider include eravacycline, plazomicin, colistin, tigecycline, cefiderocol, meropenem/vaborbactam and imipenem/relebactam.[5,66] These antibiotics have not been systematically evaluated for their efficacy against MDR-hv *Klebsiella* sp., thus they should only be consumed upon advice by clinical microbiologists. Besides, MDR-hv *Klebsiella* spp. infections should be controlled in a timely manner, since their hypervirulent nature and ability to undergo metastatic transfer would cause extensive damage in various vital organs.

Outstanding questions

Klebsiella, a notorious bacterial pathogen, comprises a wide diversity of species. It has evolved into hypervirulent and/or multidrug resistant strains over the past few decades. The convergent clone, MDR-hv *Klebsiella* sp., has disseminated worldwide, posing a significant threat on human health. Such strains evolved through acquisition of virulence genes by MDR clones or through development of MDR phenotypes by hv clones. The evolutionary events driving the emergence of MDR-hv *Klebsiella* sp. isolates is complicated, which predominantly involve accumulation of mutations and horizontal gene transfer. No optimal treatment is currently available for infections caused by MDR-hv *Klebsiella* sp. More work needs to be done to better understand this 'superbug' and help design feasible approaches to eradicate or halt further evolution of the existing strains of MDR-hv *Klebsiella* sp., including setting up a standard pipeline for accurate and rapid species identification, devising methods for rapid detection of capsule gene expression, hypermucoviscosity and hypervirulence, worldwide surveillance and molecular typing, and design of optimal treatment regime for a diverse range of MDR-hv *Klebsiella* spp.

Search strategy and selection criteria

Articles for this review were identified using PubMed, Google Scholar, and references from relevant articles using the search terms: 'hypervirulence' or 'hypervirulent' or 'hypermucoviscous' and '*Klebsiella*', or 'resistance' or 'resistant' and '*Klebsiella*'. All papers reporting the case of multidrug resistant and hypervirulent *Klebsiella* sp. up to May 31st, 2021, were included in the MDR-hv *Klebsiella* spp. section and only the most impactful papers in other sections were considered.

Contributors

DN drafted the manuscript, XY, EWCC and RZ edited the manuscript, SC edited the manuscript and supervised the project. All authors read and approved the final version of the manuscript. All figures are original creations for this manuscript, and no additional permissions are required for inclusion into the manuscript.

Declaration of interests

All authors declare that they have no competing interests.

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Supplementary materials

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