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REVIEW

Correlation between antimicrobial resistance and virulence in *Klebsiella pneumoniae*

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Abstract *Klebsiella pneumoniae* is responsible for a wide range of infections, including urinary tract infections, pneumonia, bacteremia, and liver abscesses. In addition to susceptible clinical isolates involved in nosocomial infections, multidrug-resistant (MDR) and hypervirulent (hvKP) strains have evolved separately in distinct clonal groups. The rapid geographic spread of these isolates is of particular concern. However, we still know little about the virulence of *K. pneumoniae* except for hvKP, whose secrets are beginning to be revealed. The treatment of *K. pneumoniae* infections is threatened by the emergence of antimicrobial resistance. The dissemination of resistance is associated with genetic mobile elements, such as plasmids that may also carry virulence determinants. A proficient pathogen should be virulent, resistant to antibiotics, and epidemic. However, the interplay between resistance and virulence is poorly understood. Here, we review current knowledge on the topic.

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Introduction

Klebsiella pneumoniae ssp. *pneumoniae* is the causative agent of a variety of diseases, including urinary tract and soft tissue infections, bacteremia, and pneumonia. In developed countries, *K. pneumoniae* has traditionally been considered as an opportunistic pathogen responsible for nosocomial infections [1]. However, over the past three decades, a distinctive syndrome of community-acquired invasive infections, primarily in the form of pyogenic liver abscesses, has emerged [2]. This invasive syndrome has been reported mostly in Asia, but an increasing number of cases have appeared worldwide [3]. These infections are caused by hypervirulent (hvKP) isolates, mainly of serotypes K1 and K2. Serotype K1 strains belong to particular clones such as clonal complex 23 (CC23), comprising ST23 and ST57 [4]. Serotype K2 strains belong to several sequence types (STs), some of which are linked to hypervirulence, such as ST86, ST375, and ST380. Isolates belonging to ST57, ST65, and ST375 have been involved in invasive infections [5].

In parallel, *K. pneumoniae* clinical isolates have acquired increasingly high levels of antimicrobial drug resistance. For example, the rates of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) isolates of *K. pneumoniae* were 61.4 %, 22 %, and 1.8 %, respectively, for a period of 14 months between 2010 and 2011 in hospitals in Beijing, China [6]. This makes them difficult to eradicate and has led to their rapid spread in hospitals. *Klebsiella pneumoniae* belongs to the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which causes most nosocomial infections in US hospitals [7]. *Klebsiella pneumoniae* "escapes" antibiotic treatment by becoming resistant. Most MDR *K. pneumoniae* isolates, which produce carbapenemases

(KPC) and/or extended-spectrum β-lactamases (ESBLs) in combination with quinolone and aminoglycoside resistance, belong to particular clones (e.g., CC258 comprising ST258, ST11, ST512, ST340,…, CC15, CC14) [5]. MDR and hypervirulent populations of this species were, for a long time, non-overlapping [5], but a few cases of MDR hvKP have recently been reported [8]. ESBL-producing organisms were first detected in Europe, almost all in France [9]. The prevalence of ESBLs in *Klebsiella* ranged from as low as 3 % in Sweden to as high as 34 % in Portugal in intensive care units (ICUs). In North America, 6.1 % of *K. pneumoniae* isolates from ICUs were resistant to third-generation cephalosporins. ESBLs were found in 30–60 % of *Klebsiella* from ICUs in Brazil, Colombia, and Venezuela. 36.1 % of *K. pneumoniae* isolates collected in a single South African hospital were ESBL producers. The proportion of these isolates in Australian hospitals is about 5 %. Rates of ESBL production by *K. pneumoniae* are as low as 5 % in Japan, compared with 20–50 % elsewhere in Asia [9]. KPC-positive isolates have also spread worldwide. In some countries, such as Israel, Greece, and Colombia, cases are endemic, while in others, such as Australia, New Zealand, and Canada, they are only imported [10].

The main virulence factors of *K. pneumoniae* are capsule, fimbriae, lipopolysaccharides (LPS), and siderophores (enterobactin, aerobactin, salmochelin, yersiniabactin), and efflux [1]. Some factors, such as fimbriae, capsule, enterobactins, and biofilm formation, are found in almost all isolates and seem to be at the origin of classical pathogenesis. A number of putative virulence factors have been associated with hvKP, while CC258 is almost entirely devoid of virulence genes [5]. hvKP strains are characterized by the presence of RmpA (a regulator of the mucoid phenotype) and aerobactin, which are both encoded by a large virulence plasmid [11]. Additional iron acquisition systems such as yersiniabactin, which is encoded by an integrative and conjugative element (ICE) ICEKp1 [12], and a region associated with allantoin metabolism [13] have also been associated with specific hvKP strains. Generally, the acquisition of any one of the siderophore clusters by *K. pneumoniae* isolates increases the risk of severe infection in humans [14].

Bacteria can acquire antimicrobial resistance by DNA mutation or by horizontal gene transfer [15]. However, acquisition of antibiotic resistance can carry a fitness cost, which reduces the competitive ability of the bacteria in the absence of antibiotics. Deletion or mutations in chromosomic genes involved in antimicrobial resistance (e.g., porins) lead to a fitness cost. This cost is lower for plasmid acquisition [16]. Plasmids play a central role in the dissemination and acquisition of resistant determinants and virulent genes in *K. pneumoniae*. They are responsible for so many particular properties of bacteria that certain authors consider them as independent organisms [17]. *Klebsiella pneumoniae* is particularly permeable to plasmids. The strains usually harbor more than one, including small high-copy-number plasmids and low-copy-number plasmids that are usually large [17]. Owing to the diversity of the acquisition of antimicrobial resistance and of the virulence factors and phylogenetic background of the strains, the relation between resistance and virulence is a complex issue. Like *Escherichia coli*, *K. pneumoniae* has a high degree of genomic plasticity, with gene loss or gain of genomic segments by lateral gene transfer [15, 17].

The main aim of this review is to discuss the association between antimicrobial resistance and virulence in *K. pneumoniae* strains.

Resistance to β-lactams

β-Lactam antibiotics are a large class of antibiotics, including penicillins, cephalosporins, monobactams, carbapenems, and β-lactamase inhibitors. They have a β-lactam ring in their molecular structure and are the most widely used antibiotics. The first mechanism in resistance to β-lactams in *K. pneumoniae* is the production of β-lactamase, followed by alterations in permeability and extrusion by efflux pumps.

Association between β-lactamase expression and virulence

Extended-spectrum β-lactamases

Klebsiella pneumoniae is naturally resistant to ampicillin and carbenicillin by the production of SHV-1 β-lactamase encoded on the chromosome. In the early 1980s, the first ESBL able to hydrolyze oxyimino-cephalosporins was identified [18]. Since then, third-generation cephalosporin resistance has been mainly due to the production of ESBLs. These enzymes are mostly plasmid-mediated and confer resistance against penicillins, first-, second-, and third-generation cephalosporins, and aztreonam by hydrolyzing these antibiotics. They are inhibited by β-lactamase inhibitors and remain inactive against cefoxitin and carbapenems. Their plasmids frequently encode other resistance mechanisms involving resistance to fluoroquinolones, cotrimoxazole, and aminoglycosides. In the early 1980s, and for more than two decades, genetic variants of the classic SHV-1 and TEM-2 were predominantly ESBLs. At the beginning of the 1990s, a new ESBL family, named the CTX-M group, emerged. CTX-M enzymes are now the dominant ESBL type, with CTX-M-15 being the main enzyme currently observed in *K. pneumoniae* [19].

Correlation with adhesins

Klebsiella pneumoniae is present in the gastrointestinal tract of patients. Interaction with cells is, therefore, a crucial step in its colonization process. Colonization occurs by intestine cell adhesion via bacterial surface adhesins. Several studies of this species were made in our laboratory in the 1990s and 2000s to investigate the link between cell adhesion capabilities and resistance to β-lactams by ESBL production. At this time, SHV- and TEM-type ESBLs were predominant. They were encoded by large conjugative plasmids called R-plasmids [17]. It was firstly reported that some strains adhered to the microvilli of the Caco-2 cell lines though a non-fimbrial protein CF29K [20, 21]. The gene encoding this protein was located on an R-plasmid together with genes encoding the ESBL TEM-5, aerobactin, and its ferric aerobactin receptor. The prevalence of the *cf29k* gene was low among the ESBL-positive strains tested (3.5 %). At the same time, Vernet et al. showed that only 3.7 % and 7 % of 190 ESBL-positive *K. pneumoniae* strains produced aerobactin and mucoid phenotypes, respectively, unrelated to the type of β-lactamase, and that only 2 % had both factors [22]. The presence of the *cf29k* gene was not looked for in these strains. Thereafter, this adhesin was no longer found in nosocomial strains. Interestingly, Brisse et al. recently showed that CF29K was particularly prevalent in the CC23 clone (80 %) and suggested that this protein could be either directly implicated in the pathogenesis of pyogenic liver abscess or linked to another virulence factor on the same plasmid [23]. A recent publication from South Korea also reported the presence of *cf29k* in only one CTX-M-14-producing, ST11 isolate and in 14 non-ESBL-producing strains [24], suggesting an Asian diffusion for this gene. However, Struve et al. found no *cf29k* genes in the CC23 strains that they studied [25]. Another study showed the role of a 28-kDa fimbrial protein in the adhesion of ESBL-producing *K. pneumoniae* to Caco-2 cells [26]. The gene encoding this protein, called KPF-28, was also located on the R-plasmid in SHV-4-producing strains. However, it has not been found subsequently. Sahly et al. also observed an increase in the adhesion and/or invasion process upon acquisition of ESBL (SHV-12)-encoding plasmids in parallel with an upregulation of type 3 fimbriae expression [27], suggesting that an element of the plasmid acted as a transcriptional regulator. In another study, it was shown that some isolates of *K. pneumoniae* producing SHV-4 β-lactamase were associated with a localized pattern of adhesion [28]. Since 45 % of such localized-adhesion isolates in the same study were involved in severe infections [28], this phenotype could also be considered as a marker of pathogenicity.

Comments Adhesins or potential transcriptional regulators are carried upon ESBL acquisition. Interestingly, three virulence factors associated with ESBL-producing *K. pneumoniae* strains (adhesin CF29K, aerobactin, and mucoid phenotype, now associated with RmpA) have been found in hvKP strains and correlated with a syndrome of community-acquired invasive infections [25]. Unfortunately, although these three virulence factors were found associated on a single plasmid, none of the studies clearly identified them in ESBL-producing isolates. Sequencing of these plasmids would bring us new insights into the virulence of these strains.

Correlation with capsule production

Another work showed that the frequency of serum-resistant isolates was higher among ESBL-producing strains (SHV- and TEM-types) than among non-ESBL-producing strains [29]. The property of serum resistance depends on the capsule synthesis that protects the bacteria from phagocytosis. These strains are, therefore, more likely to be responsible for blood infections. However, curing ESBL-coding plasmid did not influence the serum resistance of the bacteria. On the contrary, Shin and Ko related that non-ESBL-producing *K. pneumoniae* showed higher serum resistance than CTX-M-producing isolates [24]. Indeed, the hypermucoviscosity phenotype was more frequently identified in non-ESBL-producing isolates in this study. However, plasmids with the $bla_{\text{CTX-M-15}}$ gene confer higher serum resistance to transconjugants than that observed in their original host. Although there was no difference in serum resistance between CTX-M-producing and non-ESBL-producing ST11 *K. pneumoniae* isolates, acquisition of the plasmid by conjugation increased the ability of the strain to survive against serum. The authors suggested that the *traT* gene of the plasmid may have contributed to serum resistance in this case. In another study, we showed that clinical isolates that produced ESBL TEM-47 or TEM-68 was responsible for infections in newborns, despite the presence of few genes of virulence [30]. However, these strains over-expressed their capsule, which is a major virulence factor in *K. pneumoniae* because of its role in protecting the bacteria from the immune system.

Comments These results strengthen the hypothesis that hypermucoviscosity is more associated with SHV- and TEM-type than with CTX-M-type ESBLs. However, hypermucoviscosity is sometimes related, whereas the strains were negative for RmpA or RmpA2 [31]. There might, therefore, exist an unknown regulator of mucoid phenotype. RmpA is usually located on plasmids of virulence, but it has also been found in genomic islands associated with an ICE [25], which could explain why the curing of plasmids did not modify serum resistance.

These studies suggest that acquisition of ESBL-encoding plasmids increase the virulence potential of the strains because, in these few cases, the gene encoding ESBLs were located on plasmids also encoding virulence factors. However, their prevalence was low among the ESBL-producing strains, and the former plasmids that also carried

these virulence genes seemed to have disappeared, at least in their original form. In these studies, acquisition of ESBL-encoding plasmids did not evidence biological cost. However, a fitness cost could have existed and would explain why the plasmids did not disseminate and persist. This was illustrated by another study from our laboratory in which we showed that the presence of ESBL-encoding plasmids altered the basal adhesion capacity of the strains, since cured strains adhered more than parental strains [32].

Correlation with non-virulence

At the end of the 2000s, a clinical isolate of *K. pneumoniae* producing a CTX-M-15 was responsible for an outbreak at the teaching hospital of Clermont-Ferrand (France) [33]. The patients were infected though an endoscope in which the bacteria were in a biofilm state. The strain harbored few virulence genes, despite belonging to the virulent capsular serotype K2 and, accordingly, there was no evidence of virulence in affected patients. This strain has a great ability to transfer its plasmid to other bacteria and to survive in a hospital environment. There is growing evidence that capsular type is not the predominant marker of virulence. For example, Wand et al. showed that *K. pneumoniae* strains from the Murray collection (Enterobacteriaceae isolated between 1917 and 1949) were mainly of the K1 serotype but presented little or no virulence [34]. Another outbreak with a strain of *K. pneumoniae* producing the CTX-M-15 ESBL occurred at the Uppsala University Hospital (Sweden) during 2005–2007 [35]. A total of 248 patients were either infected or colonized by the isolate. The CTX-M-15-encoding plasmid carried no virulent genes but was perfectly adapted to its host and, therefore, created no fitness cost. In contrast, fitness cost was observed for an *E. coli* recipient of this plasmid, in which it was unstable. The authors concluded that this plasmid ended in a strain particularly prone to dissemination and that it could have genetic elements to compensate for the fitness cost of the plasmid.

Comments These two examples show that the plasmids producing CTX-M-15 ended in strains with different genetic backgrounds and, therefore, of variable virulence. Both had a propensity to disseminate and to cause outbreaks. These strains were adapted to the plasmids and did not seem to present fitness cost. Plasmids encoding CTX-M-15 were recently found in CC23 strains [36], which is worrying because this clone associates hypervirulent traits and multidrug resistance. To date, ESBL *K. pneumoniae* isolates have been less hypermucoviscous and less virulent than non-ESBL *K. pneumoniae* isolates, mostly because of concurrently lower carriage and higher mutation rates of the *rmpA* and *rmpA2* genes [37].

Plasmid-encoded cephalosporinases

This group of β-lactamases was formally identified at the end of the 1980s [38]. They hydrolyze all penicillins and first-, second-, and third-generation cephalosporins, but remain inactive against cefepime and carbapenems and are resistant to clavulanic acid and tazobactam. Various enzymes have been classified among this group (CMY, DHA, ACC, EBC, FOX), but DHA enzymes are the main cephalosporinases observed in *K. pneumoniae*. Since the 1980s, plasmid-mediated DHA-type cephalosporinases have been reported in clinical strains of *K. pneumoniae*. bla_{DHA} expression is regulated by the transcriptional regulator AmpR [39]. DHA-producing strains have been frequently observed in nosocomial spreads [40]. AmpR was also shown to be involved in the upregulation of capsule synthesis and resistance to killing by serum, in the modulation of biofilm formation and type 3 expression, and in adhesion to HT-29 intestinal cells and the murine gastrointestinal tract [41]. We showed that the virulence of clinical strains could be increased by the plasmid acquisition of transcriptional factors under antibiotic pressure.

Carbapenemases

Carbapenemases have been observed since the beginning of the 1980s. While they all hydrolyze penicillins and carbapenems, their activity against cephalosporins differs according to their family. Metallo-carbapenemases (VIM, IMP, NDM) hydrolyze all cephalosporins but remain inactive against aztreonam. Carbapenemases belonging to class A of the Ambler classification hydrolyze all cephalosporins and aztreonam. Finally, OXA-type carbapenemases are only active against first-generation cephalosporins, cefotaxime, ceftriaxone, and cefepime, and remain inactive against ceftazidime and aztreonam. KPC, whose gene was plasmid-encoded, was first isolated in 1996 [42]. Since then, these strains have disseminated worldwide owing to the rapid spread of broad host-range conjugative plasmids. Eleven KPC variants (KPC-2 to KPC-12) have been identified that differ by a few amino acids changes, and of which KPC-2 is the most frequent [43]. Additional carbapenemases have now been found. bla_{NMD} (New Delhi metallo-beta-lactamase-1) was isolated from a patient who acquired the bacterium in New Delhi, India [44] and has rapidly spread. bla_{OXA48} (OXA-48) was first identified from a *K. pneumoniae* isolate in Turkey [45]) and has gradually disseminated in Europe [46]. The explanation for the epidemiological success of KPC remains unclear. No specific virulence factor has been associated with KPC-producing strains [47]. In 2012, in the north-west of Italy (the second place in Europe after Greece in terms of resistance), of all the isolates of *K. pneumoniae*, 17.5 % were KPC-producing strains. KPC was more frequently isolated in tertiary care

referral hospitals and from urine samples (50 %), and 31 % of KPC were identified in patients admitted to medical wards, followed by ICUs (15 %), surgical wards (13 %), and emergency departments (14 %). Risk factors for KPC colonization were identified, such as duration of hospitalization, number of antimicrobials administered, number of comorbidities, and number of invasive catheters [48]. KPC enzymes are frequently associated with resistance to other antibiotics, such as fluoroquinolones, aminoglycosides, and cotrimoxazoles. All these factors could explain why a 20 % absolute increase in hospital mortality for patients with KPC was observed [49]. In these conditions, it is difficult to link the epidemiological success of these strains to their own virulence or to the best environmental conditions for their development that were inadvertently created. De Rosa et al. concluded their review on a particularly interesting but not easily applicable strategy for limiting the expansion of these isolates, even though it seems currently difficult to apply. *Klebsiella pneumoniae* is very well adapted to the gastrointestinal tract, especially when the protective bacteria are eliminated by a broad-spectrum antimicrobial treatment. The authors suggested, therefore, that, to limit the multiplication of KPC, there is a great need to restore the integrity of the gut by improving the specificity of diagnosis and limiting the duration of treatments [48].

There have been few studies on the fitness cost of carbapenemase production in *K. pneumoniae*. Beyrouthy et al. studied the virulence of six OXA-48-producing *K. pneumoniae* strains and showed that they had no particular trait of virulence [50]. Fuursted et al. compared the virulence of five strains of *K. pneumoniae*, including one carrying NDM-1 [51]. In a murine sepsis model, they showed that the NDM-1-producing strain was the most virulent strain and possessed several virulence factors (capsular serotype K2, strong biofilm production, and resistance to killing in human and murine serum). However, to draw any definite conclusions, this study needs to be extended to other NDM-1-producing strains. In contrast, Lavigne et al. showed that the presence of bla_{KPC-2} gene decreased the virulence of strains in a *Caenorhabditis elegans* model [43]. This last report had the advantage of studying the effect of the gene alone on bacterial virulence, whereas that of Beyrouthy et al. studied the virulence of the entire bacterium, including the plasmids. Another report showed a decreased virulence for KPC isolates compared to non-producing carbapenemase in a *Galleria mellonella* model. Opposite findings were observed in patients [49]. However, we saw above that the virulence of these strains could be linked to factors external to the strains, such as the antibiotic treatment and the number of comorbidities. In the light of these observations, the success of these strains does not seem to be linked to their particular virulence.

Association between outer membrane proteins and virulence

The outer membrane of Gram-negative bacteria serves as a channel to regulate the influx and efflux of substances such as ions, nutrients, and antibiotics [52]. As they are surface-exposed, they could be highly immunogenic and used for vaccination trials [53]. High-level carbapenem resistance may develop via the loss of the OmpK36 porin coupled with the expression of various β-lactamases [54]. The two major porins, OmpK35 and OmpK36, are often missing in MDR strains (especially the ESBL-positive strains). The loss of OmpK36 resulted in decreased resistance to the neutrophil phagocytosis and increased mice DL50 compared to parental strains [52, 54], thereby impairing the virulence potential of the strain. This result was confirmed in another study, where it was shown that the loss of non-specific trimeric porins such as OmpK35 and OmpK36 was associated with a decrease in virulence in a *C. elegans* model [55]. *Klebsiella pneumoniae* outer membrane proteins (OMPs) contribute to phagocytosis resistance. OmpA thwarts the innate system, but March et al. showed that OmpK36 also contributes to phagocytosis resistance by *Klebsiella*. In contrast to OmpA, OmpK36 does not play any role in resistance to antimicrobial peptides and, unlike OmpK36, OmpA does not play any role in resistance to antibiotics [56]. OmpA is a multifunctional OMP, one of the major proteins in the outer membrane of many members of the Enterobacteriaceae.

We also showed that isolates resistant to cefoxitin, chloramphenicol, and quinolones had a significantly lower adhesion index to Int-407 cells compared to a median adhesion index made with all the tested isolates [57]. The isolates resistant to cefoxitin, chloramphenicol, and tetracycline showed a greater ability to mutate. Resistance to cefoxitin could be due to impermeability resulting from the loss or modification of porins or, more generally, bacterial surface components following mutations. These proteins could also be involved in the adhesion process. To survive the presence of antibiotics, bacteria sacrifice one or several proteins, thereby losing certain abilities. These results illustrate the biological cost of acquiring antibiotic resistance via mutations.

Association between efflux pumps and virulence

The *K. pneumoniae* genome harbors several operons encoding efflux systems. These pumps export not only antibiotics but also other substances, such as dyes and detergents [58]. *Klebsiella pneumoniae* encodes an AcrAB multidrug efflux system, which is involved in resistance to quinolones (nalidixic acid, ciprofloxacin) and other antibiotics (cefoxitin, chloramphenicol, erythromycin, tigecycline). This system is also involved in virulence by mediating resistance against antimicrobial peptides present in the lung [59]. The

overexpression of this efflux pump was correlated with an increase in virulence in a *C. elegans* model [55]. Another efflux system, OqxAB, involved in resistance to nalidixic acid, ciprofloxacin, chloramphenicol, and cefoxitin, was recently described [60]. It belongs to the *rarA-oqxABR* locus, with RarA acting as a transcriptional regulator of *oqxAB* and OqxR acting as a transcriptional repressor of *oqxAB* and *rarA* [61]. A mutation in this latter repressor was responsible for the multidrug resistance and the increased virulence of the strain observed in a *C. elegans* model. KexD, a resistance–nodulation–cell division (RND)-type efflux pump from *K. pneumoniae*, was shown to contribute to multidrug resistance, but its role in virulence has not been studied [62]. Other pumps such as EefABC, although involved in colonization of the murine digestive tract, were not linked to any antimicrobial drug resistance phenotype [63]. The multidrug and toxic compound extrusion (MATE) KetM was also not significantly correlated with resistance to antibiotics [64].

Resistance to other antibiotics

Resistance to fluoroquinolones

Fluoroquinolone resistance has been associated with mutations in the quinolone resistance-determining regions (QRDRs) of the *gyrA* (gyrase) and *parC* (topoisomerase IV) genes, plasmid-mediated resistance to quinolones, altered permeability (porin loss), and also with a lower uptake of quinolones because of efflux overexpression [65]. The prevalence of *qnr* genes was 3.9 % in *K. pneumoniae* strains isolated from patients' blood in Taiwan [66]. Tóth et al. suggested that there was a strong link between fluoroquinolone resistance and fitness [67]. However, this phenomenon seems related to an enhancing activity of efflux rather than to amino acid substitutions in the quinolone resistance regions.

Resistance to colistin

Colistin acts by interacting with lipid A, leading to outer membrane disruption [68]. Colistin resistance in *K. pneumoniae* is mainly related to LPS modification following the addition of 4-amino-4-deoxy-L-arabinose to lipid A. This modification is associated with the *pbgPE* operon, which is regulated by PmrAB and PhoPQ. Insertional activation of the PhoQ/PhoP MgrB regulator has also been proposed as a determinant of colistin resistance [69]. Colistin resistance was related to mutations in three different genes, *mgrB*, *phoQ*, and *ccrAB*, a two-component regulatory system; *ccrAB*, however, is present in only some strains [70]. Choi and Ko showed that resistance to colistin in hypervirulent *K. pneumoniae* ST23 strains may result in an in vitro fitness defect and in defects in hypermucoviscous, CPS production, and serum resistance [71]. Recently, Liu et al. characterized the plasmid-encoded phosphoethanolamine transferase MCR-1, which confers resistance to colistin. This enzyme is rare in *K. pneumoniae* strains (0.7 %) and its role in virulence remains unknown [72].

The contribution of whole-genome sequencing

Whole-genome sequencing allows an in-depth characterization of bacterial strains and will serve as a powerful tool to study and compare strains in nosocomial infections and outbreaks. This method is still in its infancy but will greatly contribute to the better understanding of the virulence and epidemiology of *K. pneumoniae* strains. For this purpose, Brisse et al. have developed a freely accessible BIGSdb-Kp database. A few studies have already used high-throughput sequencing, in particular to obtain the genomes of hypervirulent (CC23) and MDR strains (CC258), which are endemic. Bialek-Davenet et al. showed that CC258 was entirely devoid of virulence genes and that MDR and hypervirulent strains are largely non-overlapping [5]. Struve et al. suggested that CC23 isolates are highly effective colonizers of the human intestinal tract. Homologs of a large virulence plasmid encoding two siderophores, aerobactin and salmochelin, and RmpA were detected in all hvKP. They possessed additional siderophores, such as yersiniabactin, colibactin, and microcin E492 associated with an ICE. These strains, which have remarkable genome plasticity, seem to be characterized by recombination events with gain/loss of genomic segments [25, 73].

Conclusion

In *Klebsiella pneumoniae*, the most common mechanism of resistance consists of enzyme synthesis and, more particularly, that of β-lactamases: extended-spectrum β-lactamases (ESBLs), cephalosporinases, and carbapenemases. Currently, the large class of antibiotics called β-lactams is the most frequently used in human therapeutic situations. The genes encoding these enzymes are carried by plasmids that also carry other genes, including genes of virulence factors. Thus, it is difficult to appreciate the real effect of the mechanism of resistance on the fitness cost for the bacteria. In the 1980s and 1990s, ESBL (SHV- and TEM-types) encoding genes were observed on plasmids of virulence. Consequently, acquisition of these plasmids by the bacterium increased its virulence potential. Currently, the epidemiology has shifted to a majority of CTX-M-type ESBLs and the spread of *K. pneumoniae* carbapenemases (KPC). The plasmids that carried these enzymes do not seem to have fitness cost, but the strains are less virulent. At present, clonal complexes of hypervirulent (hvKP) and multidrug-resistant (MDR) strains are non-overlapping. Let us hope that MDR plasmids will not be able to

maintain stability in hvKP, because this would lead to the emergence of a "super" bug. Much work remains to be done to fully understand the relationship between virulence and resistance in *K. pneumoniae*. In particular, little is known about the influence of the acquisition of several antibiotic mechanisms by the bacteria on fitness cost.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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