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# ***Klebsiella pneumoniae* Population Genomics and Antimicrobial Resistant Clones**

Kelly L. Wyres<sup>1,2</sup> and Kathryn E. Holt<sup>1,2</sup>

<sup>1</sup>Centre for Systems Genomics, University of Melbourne, Parkville, Victoria 3010, Australia

<sup>2</sup>Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville, Victoria 3010, Australia

\*Correspondence: kholt@unimelb.edu.au (K.E. Holt).

## **Keywords**

*Klebsiella pneumoniae*, genomics, antimicrobial resistance, population structure

1 **Abstract**

2

3 Antimicrobial resistant *Klebsiella pneumoniae* (*Kp*) has emerged as a major global  
4 public health problem. While resistance can occur across a broad range of *Kp* clones,  
5 a small number have become globally distributed and commonly cause outbreaks in  
6 hospital settings. Here we describe recent comparative genomics investigations that  
7 have shed light on *Kp* population structure and the evolution of antimicrobial resistant  
8 clones. These studies provide the basic framework within which genomic  
9 epidemiology and evolution can be understood, but have merely scratched the surface  
10 of what can and should be explored. We assert that further large-scale comparative  
11 and functional genomics studies are urgently needed to better understand the biology  
12 of this clinically important bacterium.

13

## 14 ***Klebsiella pneumoniae* Is a Major Public Health Threat**

15

16 *K. pneumoniae* (*Kp*) is a Gram-negative bacteria belonging to the family  
17 Enterobacteriaceae. Closely related to the well-known pathogens *Salmonella enterica*  
18 and *Escherichia coli*, *Kp* can colonise a similarly wide range of animal hosts, but can  
19 also be found in association with plants; in soil, water and drains; and colonising a  
20 diversity of body sites including the respiratory tract, gut, nasopharynx, oropharynx  
21 and skin [1,2]. *Kp* is considered an opportunistic pathogen, with the majority of  
22 infections occurring in neonates, the elderly and the immunocompromised [2].  
23 Urinary tract infection (UTI), pneumonia and wound or soft tissue infections are the  
24 most common disease syndromes. *Kp* has been amongst the most frequent agents  
25 causing hospital-acquired infections in all settings for many decades [2,3]. It is the  
26 ‘K’ in the ESKAPE pathogens, the six most significant and dangerous causes of drug  
27 resistant hospital infections identified by the Infectious Diseases Society of America  
28 [4]. More recently, *Kp* has been recognised by the World Health Organization,  
29 Centers for Disease Control and Prevention, European Union and other organisations  
30 as a significant threat to global public health due to its high rates of antimicrobial  
31 resistance (AMR) (see [5] and [www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf](http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf)). This increased attention is largely due to the increasing occurrence of high-  
32 profile hospital outbreaks and deaths associated with a particular AMR clone  
33 producing the *Kp* carbapenemase (KPC). However it is also associated with the role  
34 of *Kp* as the ‘canary in the coalmine’ – the organism in which most new AMR genes  
35 to be discovered in the last two decades were first detected, before becoming  
36 widespread in Gram-negative bacterial pathogens [including the extended spectrum  
37 beta-lactamase (ESBL) forms of SHV [6] and CTX-M [7]; the carbapenemases KPC  
38 [8] and NDM [9]; and most recently MCR-1 [10], the first plasmid-borne gene to be  
39 associated with colistin resistance]  
40

41

42 The emergence of AMR *Kp* as a major global health problem has coincided with the  
43 establishment of whole genome sequencing as a viable tool for investigating and  
44 tracking bacterial pathogens, thanks to the development of cost-effective high  
45 throughput sequencing. Genomic comparisons can offer a high-resolution view of  
46 genetic variation at whole-genome scale and can be applied to explore the diversity of  
47 pathogen populations, the evolution of clinically important traits such as AMR, and

48 patterns of disease transmission and dissemination. Here we review recent insights  
49 into the population structure of *Kp* and the evolution of AMR clones gleaned from  
50 genomic studies; outline current tools available for genomic investigation of *Kp*; and  
51 identify outstanding questions concerning the problem of AMR *Kp* that would benefit  
52 from further application of genomics.

53

#### 54 **Population Structure and Genome Variation**

55 The population structure of *Kp* has been elucidated using various DNA sequencing  
56 approaches. A *Kp* multi-locus sequence typing (MLST) scheme, targeting seven  
57 chromosomally encoded housekeeping genes, was established in 2005 [11,12]. MLST  
58 provides a standardised reproducible system for strain identification and nomenclature  
59 for a given species [13]. The *Kp* MLST scheme has been widely adopted and has been  
60 centrally important to the identification and investigation of clinically important  
61 phylogenetic lineages, which are typically referenced by their sequence type (ST; e.g.  
62 ST258). The availability of high throughput whole genome sequencing has since  
63 afforded much deeper resolution of the *Kp* population. In 2014, the MLST approach  
64 was extended to a core gene MLST (cgMLST) scheme targeting 694 core genes,  
65 which can be used to define high-resolution STs and their aggregation into clonal  
66 groups (CGs) [14]. The publicly available cgMLST database for *Kp* is hosted at the  
67 Institut Pasteur using the BIGSdb platform [15]. It now includes the seven-locus  
68 MLST scheme, which still forms the basis for the nomenclature of clinically  
69 important *Kp* CGs (e.g. CG258 designates the clonal group that includes ST258). *Kp*  
70 genome data can also be interrogated using phylogenetic analysis of single nucleotide  
71 polymorphisms (SNPs) across the whole genome [16,17]. In addition to identifying  
72 phylogenetic lineages or CGs, this approach can provide a very high-resolution view  
73 of recent evolution within CGs, which can be particularly useful for investigating  
74 local *Kp* outbreaks and global dissemination patterns [14,17–24].

75

76 Isolates identified as *K. pneumoniae* using standard biochemical or proteomics tests  
77 typically include three phylogenetically distinct groups or phylogroups that were  
78 originally designated KpI, KpII and KpIII but have now been designated as distinct  
79 species *K. pneumoniae*, *Klebsiella quasipneumoniae* and *Klebsiella variicola*,  
80 respectively [16,25,26]. All three are covered by the same MLST and cgMLST

81 schemes, which can be used to differentiate the species [11,12]. Whole genome  
82 sequence comparison has shown that these groups are distinguished by 3-4% average  
83 nucleotide divergence across the core genome, hardly ever recombine, and can be  
84 differentiated on the basis of gene content, indicating that they represent distinct  
85 independently-evolving populations and supporting their recognition as distinct  
86 species [16]. For the remainder of this review, the term *K. pneumoniae* (*Kp*) will be  
87 used to refer strictly to *K. pneumoniae* (i.e. the KpI phylogroup).

88

89 The *Kp* population is comprised of numerous deep-rooted phylogenetic lineages  
90 radiating from a single common ancestor (**Figure 1a**), with approximately 0.5%  
91 average nucleotide divergence between lineages [12,16]. These lineages show  
92 evidence of occasional homologous recombination [11,12,16,27,28] but estimates of  
93  $r/m$  (the relative probability that a nucleotide change resulted from recombination vs  
94 point mutation) based on limited MLST data have yielded conflicting results [12,29].  
95 Further investigation of recombination dynamics based on whole genome data is  
96 warranted, however the overall population structure appears to be relatively clonal.

97

98 A total of 157 lineages were reported based on whole genome analysis of a diverse  
99 collection of 289 *Kp* genomes [16] and 155 CGs are currently defined in the public  
100 cgMLST database [14], however the rate of discovery of new lineages suggests that  
101 the total number in existence far exceeds this, likely reaching the thousands (**Figure**  
102 **1b**). The long-term persistence of so many distinct *Kp* lineages has yet to be  
103 explained. *Kp* occupies a wide range of ecological niches including many non-host  
104 associated environments [1,2,16,26]. Extensive exopolysaccharide diversity has been  
105 described, but this is not generally associated with phylogenetic lineage. Only 12 O  
106 antigen serotypes have been identified in *Kp*, each of which are shared by diverse  
107 lineages [30]. *Kp* capsular variation is more extensive: 77 phenotypically defined  
108 capsular serotypes are recognised [31–33], and genetic studies of capsule biosynthesis  
109 (K) loci indicate the existence of twice this number [18,27,28,30,34,35]. A single  
110 capsular serotype can be found in numerous distinct *Kp* lineages and extensive  
111 capsular diversity has been identified within lineages, resulting from horizontal  
112 transfer and recombination of K locus genes [12,14,16,28,30].

113

114 The average *Kp* genome is 5.5 Mbp in size and encodes ~5,500 genes. Whole genome  
115 comparisons of hundreds of isolates indicate that the core genome, that is the set of  
116 genes that are common to all *Kp*, includes fewer than 2,000 genes [14,16]. The  
117 additional 3,500 ‘accessory’ genes in each genome are drawn from a pool of more  
118 than 30,000 protein-coding genes (using a cut-off of >30% amino acid divergence to  
119 define a new gene; or >70,000 using a cut-off of >10% amino acid divergence) [16].  
120 The rate of accumulation of *Kp* accessory genes with increasing genome sequences  
121 indicates the *Kp* population has an open pan genome [36], meaning that *Kp* has access  
122 to a vast gene pool (**Figure 2a**). Assignment of *Kp* accessory genes to functional  
123 groups identified common functions including carbohydrate metabolism (19%), other  
124 metabolic pathways (18%), membrane transport (13%), exopolysaccharide capsule  
125 (11%), iron resistance and metabolism (2%) and resistance to antibiotics, heavy  
126 metals and stress (1%); a third of protein-coding genes found in *Kp* have as-yet  
127 unknown functions [16]. Although there is evidence that individual accessory genes  
128 can be distributed across multiple phylogenetic lineages, each lineage is associated  
129 with a distinct complement of genes that differs from that of other lineages (see  
130 **Figure 2b**) [16]. It is therefore likely that different *Kp* strains vary substantially in  
131 their metabolic capacity, which may account for the wide array of ecological niches in  
132 which *Kp* is found and also the persistence of distinct chromosomal lineages, which  
133 could potentially differ quite substantially from one another in terms of the range of  
134 niches that they can readily inhabit. Furthermore, there is evidence that the circulation  
135 of highly mobile accessory genes within the *Kp* population, via plasmids and other  
136 conjugative elements, may contribute to survival of *Kp* in different niches [16,37–39].  
137 A recent genomic analysis found the presence of a plasmid-encoded *lac* (lactose  
138 utilisation) operon, identified in ~50% of sequenced *Kp* isolates, was significantly  
139 associated with *Kp* isolated from dairy cows with mastitis, while the presence of  
140 plasmid-encoded aerobactin, a siderophore that promotes growth in blood by  
141 removing iron from high affinity sites on human transferrin [40], was associated with  
142 *Kp* isolated from bacteraemia and other invasive infections in humans [16].

143

#### 144 **AMR Determinants**

145 *Kp* is intrinsically resistant to ampicillin due to the presence of the SHV beta-  
146 lactamase in the core genome (note *K. quasipneumoniae* and *K. variicola* carry highly  
147 divergent forms of this beta-lactamase known as OKP and LEN [16]). Comparative

148 genomic analysis indicates that *fosA* and the efflux pump *oqxAB*, which confer low-  
149 level resistance to fosfomycin and the quinolone nalidixic acid, are also core genes in  
150 *K. pneumoniae*, *K. quasipneumoniae* and *K. variicola* [16]. However the majority of  
151 AMR in *Kp* results from the acquisition of AMR genes via horizontal transfer, mainly  
152 carried by plasmids [41]. More than 100 distinct acquired AMR genes have been  
153 identified in *Kp* [16] (**Table 1**), and hundreds of AMR-associated plasmids belonging  
154 to dozens of distinct *rep* types (plasmid replication machinery types) have been  
155 reported [16,37,41]. It is not uncommon for individual *Kp* strains to carry multiple  
156 plasmids, and for several of these to contain distinct sets of AMR genes, resulting in  
157 resistance to nearly all available antimicrobials [21,23,37,42]. Direct transfer of AMR  
158 plasmids between distinct *Kp* strains, and between *Kp* and other Enterobacteriaceae,  
159 has been detected in whole genome sequencing studies of hospitalised patients and in  
160 hospital environments, presumably driven by selection from exposure to a range of  
161 antimicrobials [42–44].

162

163 Of particular clinical concern are the dissemination of carbapenemase genes KPC,  
164 OXA-48 and NDM-1, and the ESBL gene CTX-M-15. Each of these genes is  
165 associated with a specific transposon that mobilises it between different plasmid  
166 backbones (which can then spread to other strains and species) and sometimes into the  
167 *Kp* chromosome itself [45–47]. All four genes have been reported in diverse *Kp*  
168 lineages. KPC is associated with a broad range of plasmids and is mobilised by  
169 Tn4401, a 10 kbp Tn3-like transposon, for which there are five known isoforms  
170 [48,49]. KPC was intimately linked with the emergence of ST258 and its derivative  
171 ST512 (see below), but has become more widely disseminated [45,50,51]. OXA-48 is  
172 mobilised by Tn1999 and is most commonly, but not exclusively, associated with  
173 IncL/M plasmids [52–55]. NDM-1 is found in a broad range of plasmids of distinct  
174 *rep* types but its mechanism of mobilisation is less certain [9]. Complete or truncated  
175 IS*Aba1* is often found upstream of NDM-1, suggesting at least an historical role for  
176 this insertion sequence (IS) [9,54]. However, there is also evidence of alternative  
177 mobilisation e.g. via IS26 or IS*CR1* [56,57]. CTX-M-15 is mobilised by IS*Ecp1* and  
178 in *Kp* is most commonly associated with IncFII plasmids that simultaneously carry  
179 other AMR genes [20,21,58–60].

180



181 Mutational resistance can also occur in *Kp*. Induced expression of intrinsic efflux  
182 pumps such as those encoded by *acrAB* and *oqxAB* have been associated with reduced  
183 susceptibility to tigecycline, fluoroquinolones and other antimicrobials [61,62].  
184 Reduced permeability of the outer membrane via functional loss of the outer  
185 membrane porins encoded by *ompK35* and *ompK36* can cause resistance to extended  
186 spectrum cephalosporins and reduced susceptibility to carbapenems and  
187 fluoroquinolones [63]. Fluoroquinolone resistance is often conferred by a combination  
188 of substitutions in the genes encoding the topoisomerase targets, GyrA and ParC  
189 [64,65]. The presence of these mutations and of acquired AMR plasmids do not  
190 necessarily reduce fitness in terms of competitive growth or efficiency of transmission  
191 between patients [39,66,67], consequently both are often encountered on first  
192 isolation rather than evolving *in vivo* during treatment. In areas where fluoroquinolone  
193 and carbapenem resistance is common, treatment of *Kp* infections generally relies on  
194 tigecycline or colistin [68]. Colistin resistance is rare upon first isolation but often  
195 arises during treatment via mutations that upregulate the PhoQ/PhoP system and  
196 *pmrHFIJKLM* operon, most commonly by inactivation of *mgrB* via IS insertions, but  
197 also occasionally by deletions or nonsense mutations in this gene or others involved in  
198 the same pathway [69–71]. Additional mechanisms of colistin resistance have  
199 recently been reported, including mutations in the chromosomal *crrB* gene [72] and  
200 acquisition of the plasmid-borne genes *mcr-1* or *mcr-1.2* [10,73]. It was initially  
201 hoped that *mgrB* inactivation would compromise the ability of *Kp* to transmit and  
202 cause infections in new hosts. However studies to date have found no fitness cost  
203 during *in vitro* competitive growth [74] or animal models [75] and sustained  
204 outbreaks of *mgrB*-mutant colistin resistant strains have been reported [76].  
205 Tigecycline resistance in *Kp* is usually caused by increased activity of the AcrAB  
206 efflux pump via interruption of the regulators *ramA*, *ramR* or *acrR* [77–79]. A non-  
207 synonymous substitution in the *rpsJ* gene (encoding the S10 30S ribosomal subunit)  
208 has also been implicated in tigecycline resistance [80].

209

### 210 **Genomic Insights Into the Emergence of Antibiotic Resistant Clones**

211 AMR has emerged within many distinct *Kp* and some *K. variicola* CGs [14,16,19,81],  
212 however a small number have become widely disseminated and commonly cause  
213 infections in a range of settings, despite the fact that they are not generally associated  
214 with any of the known *Klebsiella* virulence determinants [14,16]. **Figure 3** shows the

215 geographical distribution of *Kp* outbreaks reported in the literature and associated  
216 with a CG identified by MLST, as of 24<sup>th</sup> June 2016. These represent just the tip of  
217 the iceberg of the global burden of *Kp* outbreaks, since most outbreaks are not  
218 reported in the literature and MLST data are not ubiquitously generated. Notably, of  
219 all reported outbreaks where MLST was performed, 72% identified one of five  
220 common CGs (CG258, CG14/15, CG17/20, CG43, CG147, **Figure 3**). Twenty-two of  
221 the remaining 24 outbreaks were associated with *Kp* STs, one was associated with *K.*  
222 *variicola* (ST48 and its single locus variant, ST1236) and one was associated with *K.*  
223 *quasipneumoniae* (ST334). Genomic investigations of some of these common CGs, or  
224 ‘clones’ are beginning to provide specific insights into their evolution.  
225  
226

## 227 **CG258**

228 Undoubtedly the most widely recognised and globally distributed clone is CG258  
229 (ST258, ST11, their single locus variants and other close relatives, e.g ST340, ST512,  
230 ST437, ST833, ST855 and ST1199). ST258 is widely acknowledged as the major  
231 cause of carbapenem-resistant *Kp* infections [48,82,83] and is predominantly  
232 associated with the KPC-2 and KPC-3 carbapenemases. In contrast, other members of  
233 this CG have been associated with a more diverse selection of carbapenemases and  
234 ESBLs, including NDM-1, OXA-48 and CTX-M-15 [19,81,84–86]. The  
235 epidemiology of CG258 has been well reviewed previously [48,49,82,83] so here we  
236 focus on the most recent evolutionary insights from comparative genomic studies.

237

238 An analysis of 319 *Kp* genomes, including 203 CG258 (predominantly ST258 and  
239 ST11) suggested that a large genomic recombination event of ~1.3 Mbp length  
240 distinguishes CG258 from its closest relatives [81] (**Figure 4**). This event was dated  
241 to ~1985, suggesting that the most-recent common ancestor of CG258 was circulating  
242 in the population at that time. ST258, ST340 and ST437 each form a single  
243 monophyletic sub-clade within CG258, while ST11 is a paraphyletic group [19,28].  
244 ST258 arose from an ST11-like ancestor following a second large-scale genomic  
245 recombination event, in which a ~1.1 Mbp genomic region was acquired from an  
246 ST442 *Kp* [27,28]. The recombinant region included the K locus, which was distinct  
247 from the ST11-like ancestor and presumably associated with a change of capsule  
248 phenotype (**Figure 4**). Subsequently ST258 also acquired an integrative conjugative  
249 element known as ICE258.2, which encoded a type IV pilus and a type III restriction  
250 modification system [23,27]. It was speculated that the former may facilitate  
251 improved adherence, while the latter may play a role in determining which plasmids  
252 can be maintained within ST258 [23].

253

254 Early studies had suggested that ST258 was further divided into two distinct sub-  
255 lineages (I and II), distinguished by a third large-scale genomic recombination event  
256 of ~215 kbp [23,87] (**Figure 4**). Again the recombinant region, which was acquired  
257 from an ST42 *Kp*, included a distinct K locus [23,28]. Subsequently, Bowers and  
258 colleagues showed that sub-lineages I and II actually form a monophyletic sub-clade  
259 within ST258, and the remainder of the clade is paraphyletic [19]. Isolates from the  
260 United States were distributed throughout; supporting the hypothesis that ST258 arose

261 in that country, where it was first identified and remains highly prevalent [19,88].  
262 Further molecular dating analyses suggested the origin of ST258 circa 1995-1997  
263 [19,81], just a few years before the first clinical reports [88,89].

264

265 A total of 22 distinct K loci have now been associated with CG258, each of which  
266 presumably imported by an independent recombination event [19,28]. The extensive  
267 variability of this locus suggests that it is subject to strong diversifying selection,  
268 although the drivers are as yet unclear. CG258 is also highly diverse in terms of  
269 acquired AMR genes and chromosomal AMR-conferring variants, suggesting that  
270 AMR has arisen independently multiple times, largely driven by the acquisition of a  
271 diverse array of plasmids [19,22,23,42]. ST258 isolates typically harbour between two  
272 and five plasmids of 10.9 kbp to 142.7 kbp [23,42]. The majority, although not all  
273 [19,90], ST258 harbour at least one plasmid containing either KPC-2 or KPC-3.  
274 pKpQIL is one such plasmid that is common among sub-lineages I and II [19], but  
275 rare among the rest of the clade [22,23,42]. In fact, sub-lineages I and II are generally  
276 associated with greater conservation of plasmids compared to the rest of the CG,  
277 which is highly diverse [19]. Taken together, these genomic studies unravel a story of  
278 a rapidly evolving, highly adaptive epidemic clone.

279

### 280 ***CG14/15***

281 CG14/15 is another globally distributed MDR clone [18,20,91–93]. Similar to  
282 CG258, it has also been associated with a diverse array of AMR genes, including  
283 those encoding ESBLs (in particular CTX-M-15 [18,20,94]) and carbapenemases  
284 such as KPC [95], NDM-1 [18], OXA-48 [91], OXA-181 [93] and VIM-1 [92].  
285 Colistin resistance has been reported both with and without concomitant ESBL and/or  
286 carbapenemase production [70,96].

287

288 Genomic analyses of ST15 isolates from The Netherlands and Nepal showed that they  
289 can be divided into at least two sub-lineages, each associated with a distinct K locus  
290 [18,20]. All of the Nepalese isolates harboured CTX-M-15, while 42 also harboured  
291 NDM-1. The latter isolates were part of an outbreak from which nine NDM-1  
292 negative isolates were also identified [18,21]. Long read SMRT sequencing of a  
293 representative outbreak isolate identified four distinct plasmid replicons ranging from  
294 69 kbp to 305 kbp. Three of the four plasmids contained AMR genes and/or heavy

295 metal resistance genes. The fourth plasmid contained a tellurite resistance cassette.  
296 The largest plasmid, pMK1-NDM, harboured NDM-1 in combination with CTX-M-  
297 15, OXA-1, *aac(6')-Ib-cr*, *aadA2*, *folP*, *catA1*, *dfrA12* and *armA* [21]. Short read  
298 Illumina sequencing data suggested that all of the outbreak isolates harboured pMK1-  
299 NDM-like plasmids, including those that were NDM-1 negative due to deletion of the  
300 NDM-1 region [18,21].

301

### 302 ***Other Clonal Groups***

303 Several other globally distributed MDR clones including CG17/20, CG43 and CG147  
304 have been associated with a number of disease outbreaks (**Figure 3**). All were first  
305 recognised in the mid-late 2000s and are associated with a range of different AMR  
306 genes. Of note, ST101 from CG43 seems to be widely distributed in Europe and is  
307 commonly associated with CTX-M-15, largely through plasmid acquisition  
308 [46,70,97–100]. However, a genome sequence from a representative isolate of an  
309 ST101 outbreak in Germany showed that this strain harboured a chromosomal copy of  
310 the *ISEcpI*-CTX-M-15 transposon [46]. Isolates from this outbreak were resistant to  
311 extended spectrum beta-lactams, gentamicin, tetracycline, ciprofloxacin and  
312 sulphamethoxazole/trimethoprim and harboured CTX-M-15, TEM-1, and plasmid  
313 replicons FIA and FIB. Aside from CTX-M-15, the location of the remaining AMR  
314 genes was unclear [46]. This finding is potentially of concern given that the fitness  
315 cost of chromosomal CTX-M-15 is likely much reduced compared to the cost of  
316 maintenance of an entire CTX-M-15 plasmid. Consequently, it is more likely that the  
317 host will retain the gene even in the absence of antimicrobial selective pressure.  
318 Unfortunately, CG43 is not the only *Kp* AMR clone within which chromosomal CTX-  
319 M-15 has been reported. More worryingly, the genome of an ST147 isolate from the  
320 United Arab Emirates contained a chromosomal *ISEcpI*-CTX-M-15 plus three  
321 chromosomal copies of *ISEcpI*-OXA-181, which conferred resistance to the  
322 carbapenems [47]. The situation was worsened by the fact that one of the *ISEcpI*-  
323 OXA-181 transposons had interrupted the *mgrB* gene, resulting in colistin resistance  
324 and generating a truly pan-resistant strain [47].

325

326

## 327 **Concluding Remarks and Future Perspectives**

328 There is now widespread recognition of the immense potential for genomics to  
329 enhance surveillance and tracking of specific pathogens and of AMR more generally,  
330 and to aid infection control and outbreak investigations. Several studies have reported  
331 the use of genomics to aid investigations of AMR *Kp* outbreaks in hospitals, with  
332 emerging themes being the detection of persistent polyclonal outbreaks resulting from  
333 transmission of AMR plasmids as well as AMR clones; asymptomatic colonisation of  
334 healthcare workers and patients with AMR clones; and sinks, taps and drains as  
335 persistent reservoirs of infection [17,22,42,43]. We contend that analysis and  
336 interpretation of genome data generated in such studies will be greatly assisted in the  
337 future by the emerging genomic framework for *Kp*, which helps investigators to  
338 readily extract the most useful information and place it in the context of the existing  
339 knowledge base. Currently the key elements of the *Kp* genomic framework are  
340 identification of CGs; AMR determinants including acquired genes and common  
341 mutations; known virulence genes and alleles; plasmids; and capsular and O antigen  
342 loci. Details of current data sources and tools for extracting these elements from *Kp*  
343 genome data are given in **Box 1**.

344

345 While the availability of thousands of *Kp* genomes may sound ample to some, we  
346 believe there is a pressing need to dramatically expand our current understanding of  
347 the *Kp* population through further functional, clinical and ecological genomics  
348 studies. Understanding of *Kp* disease, transmission and evolution is arguably decades  
349 behind that of other human pathogens, but genomics can help scientists and clinicians  
350 to rapidly advance our knowledge of this important threat to global health. Studies to  
351 date show population structure of *Kp* is complex and intriguing, and raises important  
352 questions about the functional and ecological differences between lineages, which are  
353 highly relevant to understanding why certain *Kp* lineages appear to pose greater  
354 clinical problems than others (see **Outstanding Questions**). Functional genomics  
355 studies are needed to identify factors involved in environmental persistence of *Kp*, as  
356 well as transmission, colonisation, and pathogenicity in humans [101]. Functional  
357 genomics can also be used to search for lineage-specific factors that might explain  
358 why certain AMR determinants appear to be maintained in some CGs but transient in  
359 others [67,102], which could be novel targets for inhibition of the seemingly never-  
360 ending accumulation of AMR in the problem clones. Analysis of the available

361 genome data indicates that the *Kp* sequenced so far represent the tip of the iceberg of  
362 a much larger *Kp* population (**Figure 1b, 2a**). Much deeper sampling will be required  
363 in order to begin to understand the ecology of *Kp*, which could identify important  
364 reservoirs of bacterial diversity and help to understand why *Kp* appears to have so  
365 often been the first step in the trafficking of AMR genes from environmental bacteria  
366 into human-associated bacterial populations.

367

368 After simmering away for decades, the problem of AMR *Kp* has become too  
369 important to ignore and the international medical, public health and scientific  
370 communities now need to play catch-up. Genomics has played a key role in the past  
371 few years and has plenty more to offer in tackling the global threat of AMR *Kp*.  
372 Given the scale of the challenge, it will be important to continue to build a deeper  
373 understanding of the underlying population out of which problem clones emerge and  
374 to share genomic data together with associated source and phenotypic data, in order to  
375 maximize the potential benefits of genomic approaches.

376 **Figure Legends**

377

378 **Figure 1. Lineage Diversity in *Klebsiella pneumoniae*.** (a) Core gene phylogeny for  
379 *K. pneumoniae*. Unrooted maximum likelihood phylogenetic tree for 283 isolates  
380 sampled from diverse sources and locations, tips are coloured by country as indicated  
381 in panel b. (b) Discovery of novel *K. pneumoniae* lineages with increasing sampling  
382 of isolates in different locations. Curves show the discovery rate for new *K.*  
383 *pneumoniae* lineages as more isolates were sampled for whole genome sequencing;  
384 Simpson's diversity index is shown in parentheses. Plots are reproduced from [16];  
385 tree and source information are available for interactive viewing at  
386 <https://microreact.org/project/BJC1Qz9H>.

387

388 **Figure 2. Gene Content Diversity in *Klebsiella pneumoniae*.** (a) *K. pneumoniae* pan  
389 genome. Curves show the discovery rate for new *K. pneumoniae* protein-coding genes  
390 as more isolates were sampled for whole genome sequencing (mean and 95%  
391 confidence interval for each sample size). Different absolute numbers are obtained  
392 depending on the level of amino acid (aa) identity used to define a new protein-coding  
393 gene, however both curves show that the *K. pneumoniae* population has an open pan  
394 genome, indicating there is no upper limit to the number of accessory genes that the  
395 population can sustain. (b) Differences in gene content within and between *K.*  
396 *pneumoniae* lineages. Boxplots show the distribution of gene content distances  
397 (measured using Jaccard distance) for pairs of *K. pneumoniae* genomes that belong to  
398 the same (blue) or different (green) lineages. Plots are reproduced from data in [16].

399

400



401 **Figure 3. Distribution of *Klebsiella pneumoniae* Outbreaks by Clonal Group**  
402 **(CG) and Region.** Outbreak reports as of June 2016 were identified in the literature  
403 by PubMed search using the following search terms; “*Klebsiella pneumoniae*” AND  
404 “outbreak” AND (one of “MLST” OR “multilocus sequence typing”); “*Klebsiella*  
405 *pneumoniae*” AND “outbreak” AND (one of “ST1\*” ... “ST9\*” OR “CG1\*” ...  
406 “CG9\*” OR “CC1\*” ... “CC9\*”). Pie graph areas are proportional to the total number  
407 of outbreaks reported in each World Health Organization region (each region is  
408 indicated by a different shade of grey), slices indicate frequency of each CG. CG 258  
409 is divided into two categories; ST258 and its derivative ST512; and the remaining  
410 sequence types (STs) identified in the literature search (ST11, ST340 and ST437). CG  
411 14/15 includes ST14 and ST15; CG 17/20 includes ST16, ST17 and ST20; CG 43  
412 includes ST101; CG 147 includes ST147 and ST273; other indicates outbreaks caused  
413 by 22 different *Kp* STs that are not part of any named CG, one *K. variicola* ST and its  
414 derivative (ST48 and ST1236, respectively) and one *K. quasipneumoniae* ST334. Red  
415 stars indicate the locations of the earliest recorded ST258 outbreaks in the United  
416 States and Israel, for which MLST was not applied. Blue star indicates the location of  
417 the Nepalese ST15 outbreak, which did not meet the search criteria but is described in  
418 the main text.

419

420 **Figure 4. Genomic Evolution of *Klebsiella pneumoniae* Clonal Group (CG) 258.**

421 A schematic cladogram of the relationships within CG258 is shown alongside colour  
422 bars that represent the bacterial chromosome. Coloured blocks represent regions of  
423 the genome acquired through horizontal transfer from a *K. pneumoniae* that is not part  
424 of CG258, as indicated by the arrows. The relative positions of the seven *K.*  
425 *pneumoniae* multi-locus sequence typing loci are indicated by grey pointers. The  
426 position of the K locus is indicated by an orange pointer. ST258 lineage I and II are  
427 labelled ST258-I and ST258-II, respectively.

428 **Table 1. Genetic Determinants of AMR in *Klebsiella pneumoniae* Genomes.**

429

<b>Beta-lactamases</b>	<b><i>bla</i> genes conferring resistance (*intrinsic)</b>	
Class A	CARB-3, PSE-1, SCO-1, SHV-1*, TEM-1	
- ESBL	CTX-M, SHV-5, TEM-10, VEB	
- Carbapenemase	KPC, GES-5	
Class B (Metallo-beta-lactamase)	CphA, IMP, NDM, SIM, VIM	
Class C (Cephalosporinase)	AmpC, CMY, DHA, FOX, MIR	
Class D	OXA-1, OXA-2, OXA-7, OXA-9, OXA-10, OXA-12	
- ESBL	OXA-11, OXA-15	
- Carbapenemase	OXA-48, OXA-51, OXA-181, OXA-237	
<b>Other AMR</b>	<b>Genes conferring resistance (*intrinsic)</b>	<b>Mutations</b>
Aminoglycosides	<i>aac, aadA, aadB, aph, armA, rmt, strAB</i>	-
Carbapenems	(see carbapenemase <i>bla</i> genes, class A & D above)	Mutations in <i>ompK35, ompK36</i>
Colistin	<i>mcr-1, mcr1.2</i>	Inactivation of <i>pmrB, mgrB</i> ; mutations in <i>crrB</i>
Fluoroquinolones	<i>qepA, qnrA, qnrB, qnrD, qnrS, qepA</i>	SNPs in <i>gyrA, parC</i> ; Upregulation of <i>acrAB</i> or <i>oqxAB</i> efflux
Macrolides	<i>ereA, ereB, ermB, mef, mph, msrE</i>	-
Phenicol	<i>catA, catB, cml, floR</i>	-
Rifampin	<i>arr</i>	-
Sulfonamides	<i>folP, sul1, sul2, sul3</i>	-
Tetracycline	<i>tet</i> genes	-
Tigecycline	-	Upregulation of <i>acrAB</i> or <i>oqxAB</i> efflux; mutation in <i>rpsJ</i>
Trimethoprim	<i>dfr</i> genes	-

430

431 **Box 1. Tools and Databases for *K. pneumoniae* Genomic Analyses.**

- 432 • ***Klebsiella pneumoniae* BIGSdb:** An online database and integrated set of tools for  
433 analysis of genome assemblies [14]. The *K. pneumoniae* MLST database,  
434 cgMLST, virulence and resistance gene databases are available through this single  
435 resource, which also hosts a searchable repository of *K. pneumoniae*, *K. variicola*  
436 and *K. quasipneumoniae* genomes. As of June 2016 the database includes 2328  
437 distinct STs. Available at [bigsdb.pasteur.fr/klebsiella](http://bigsdb.pasteur.fr/klebsiella).
- 438 • **Centre for Genomic Epidemiology:** A suite of online tools for analysis of  
439 genome assemblies or short read data. *K. pneumoniae* MLST analysis [103],  
440 virulence and AMR gene screening [104,105], and plasmid screening [106] are all  
441 available. The AMR screening protocol uses the ResFinder database [105].  
442 Available at [www.genomicepidemiology.org](http://www.genomicepidemiology.org).
- 443 • **SRST2:** An offline tool for allelic typing from short read sequence data [107].  
444 MLST, virulence and resistance gene screening can be achieved directly from  
445 sequence reads. In fact, SRST2 can be used in conjunction with any appropriately  
446 formatted gene or allelic database. Available at [github.com/katholt/srst2](https://github.com/katholt/srst2).
- 447 • **ISmapper:** An offline tool for determination of insertion sequences (IS), copy  
448 number and insertion sites within genomes [108]. ISmapper takes as input paired-  
449 end short read sequence data, a genome assembly or reference genome and a set of  
450 IS references. Available at [github.com/jhawkey/IS\\_mapper](https://github.com/jhawkey/IS_mapper).
- 451 • **ISfinder:** A searchable online database of bacterial IS. Users can access and/or  
452 download IS nucleotide sequences and relevant information, including general  
453 features, direct and inverted repeat sequences and predicted protein sequences.  
454 There is a BLASTn query function, a list of IS annotated bacterial genomes and a  
455 browser for visualisation of IS within genomes. Available at [www-is.biotoul.fr](http://www-is.biotoul.fr).
- 456 • **Kaptive:** A database of complete sequences of *Klebsiella* capsule loci and  
457 accompanying tool for identification and typing of capsule loci from genome  
458 assemblies. Available at [github.com/katholt/kaptive](https://github.com/katholt/kaptive).
- 459 • **NCBI Pathogen Detection resources:** Curated databases of AMR genes and  
460 genomes of antimicrobial resistant bacterial pathogens. As at June 2016 the  
461 databases include 3,275 AMR gene nucleotide sequences and 2,391 annotated  
462 genomes drawn from Genbank. Genome-wide phylogenetic analyses, pre-

463 computed at the species level, can also be accessed. Available at  
464 [www.ncbi.nlm.nih.gov/pathogens](http://www.ncbi.nlm.nih.gov/pathogens).

465 • **PATRIC database:** An integrated resource for analysis and exploration of  
466 pathogen genomes including *Klebsiella*. Users can access and download hundreds  
467 of *Klebsiella* genome assemblies with accompanying annotation and source  
468 information. Protein sorting and metabolic pathway comparison tools are also  
469 included. Available at [www.patricbrc.org](http://www.patricbrc.org).

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