*Klebsiella pneumoniae* is an opportunistic bacterial pathogen known for its high frequency and diversity of antimicrobial resistance (AMR) genes. In addition to being a significant clinical problem in its own right, *K. pneumoniae* is the species within which several new AMR genes were first discovered before spreading to other pathogens (e.g., carbapenem-resistance genes KPC, OXA-48 and NDM-1). Whilst *K. pneumoniae*’s contribution to the overall AMR crisis is impossible to quantify, current evidence suggests it has a wider ecological distribution, significantly more varied DNA composition, greater AMR gene diversity and a higher plasmid burden than other Gram negative opportunists. Hence we propose it plays a key role in disseminating AMR genes from environmental microbes to clinically important pathogens.

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Antimicrobial resistance in Gram negative opportunistic pathogens
The antimicrobial resistance (AMR) crisis facing hospitals globally is driven by the ESKAPE pathogens (Gram positives *Enterococcus faecium*, *Staphylococcus aureus*; and Gram negatives *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter*), which are responsible for the majority of infections in hospital patients that are difficult to manage with antimicrobial therapy [1]. Notably the ESKAPE pathogens are environmental or commensal bacteria that cause opportunistic infections in hospitalised or immunocompromised patients, but are generally not pathogenic otherwise. Each of these species has intrinsic resistance to one or more antibiotics, and individual strains have accumulated resistance to many additional drugs [1]. The Gram negative ESKAPE pathogens are considered the greatest threat, due to the emergence of strains that are resistant to all or most available antibiotics [2**]. Accumulation of AMR in these organisms is primarily due to horizontal gene transfer (HGT) aided by plasmids and mobile genetic elements [1]. The catalogue of known mobile AMR genes subject to HGT amongst Gram negative pathogens numbers in the hundreds [3]. The origins of the AMR genes themselves are environmental bacteria (particularly soil bacteria), assumed to be those which have co-evolved with the relevant antimicrobial producing organisms for millennia [4–6]; however there is typically a lag of several years between the clinical use of a drug and the arrival of relevant mobile AMR genes in human pathogen populations [7]. Hundreds of mobile AMR genes have been found in *K. pneumoniae* [8,9], the species associated with the earliest reports of many AMR genes before their dispersal amongst other clinically relevant Gram negatives. Here we discuss this phenomenon in detail, and then explore what is currently known about *K. pneumoniae* ecology and its genome plasticity, arguing that these characteristics position the species as a key amplifier and spreader of AMR genes from environmental sources to human pathogen populations.

The canary in the coalmine
*K. pneumoniae* are intrinsically resistant to ampicillin due to the presence of the SHV-1 penicillinase in their chromosome [8,10]. Resistance to additional drugs occasionally arises through chromosomal mutations [11], however most AMR in *K. pneumoniae* results from acquisition of AMR genes via HGT, mainly via large conjugative plasmids [9,11,12]. The accumulation of resistance determinants in a single strain can result in pan-resistant strains that are untreatable with all available antibiotics [2**]. The earliest mobile ampicillin resistance genes identified in Gram negative bacterial populations were TEM (present in the first described plasmids in the 1960s), and the *K. pneumoniae* chromosomal SHV-1 gene which was first detected in mobile, plasmid borne form in other Enterobacteriaceae in 1973 [13,14] (Figure 1). Following the introduction of third generation cephalosporins for clinical use in the early 1980s, extended spectrum beta-lactamase (ESBL) genes conferring resistance to these drugs began to be detected and...
characterised. The earliest forms include ESBL variants of mobile SHV (SHV-2; 1985) [15], TEM (1984) and CMY (1989) [16], which were first identified in *K. pneumoniae* (Figure 1) and are now widespread amongst Enterobacteriaceae [17], and in some cases have also spread to *Acinetobacter* [18] and *Pseudomonas* [19]. The most widely dispersed ESBL gene is CTX-M, variants of which were detected in *Escherichia coli* and *K. pneumoniae* in the late 1980s and early 1990s, having been mobilised out of environmental Enterobacteriaceae (*Kluyvera*) [20,21]. CTX-M is now intimately associated with the *E. coli* ST131 pandemic clone [22] and several *K. pneumoniae* clones [11], and is present in diverse plasmid backgrounds, resulting in broad dissemination amongst hospital, commensal, and animal associated microbial populations [22,23].

The 1990s introduction of carbapenems and fluoroquinolones were met by rapid appearance of associated resistance genes, with *K. pneumoniae* often playing a key role (Figure 1). Mobile quinolone resistance genes *qnrA* and *qnrB* were first identified in *K. pneumoniae* [24,25], following mobilisation from marine bacterium *Shewanella* [26], and are now common amongst Enterobacteriaceae plasmids. The *K. pneumoniae* carbapenemase (KPC) appeared in the mid-1990s in the USA and drove the spread of pandemic hospital outbreak clone *K. pneumoniae* ST258, which is now globally disseminated [22]. The KPC gene has been transferred to many different plasmids, is now widely dispersed amongst Enterobacteriaceae and has also found its way into *Pseudomonas* [27] and *Acinetobacter* [28]. The OXA-48 carbapenemase originates from *Shewanella* [29] and was first detected in *K. pneumoniae* in Turkey in 2003 [30]. It was initially associated with hospital outbreaks across Europe and is now reported worldwide [31], although not as widely dispersed as KPC. The NDM-1 metallo-beta-lactamase was first detected in 2008 in *K. pneumoniae* from a patient who had recently travelled to India [32]. The gene was plasmid-borne and shortly thereafter was reported in different *K. pneumoniae* strains isolated from patients with and without recent travel [33]; by 2010 NDM-1 had spread to numerous plasmids and Enterobacteriaceae species, was detected within the chromosome of *E. coli* and *Procidicidae stuartii* [34], and was spreading amongst *Acinetobacter* and *Pseudomonas* [35,36]. The first mobile colistin resistance gene MCR-1 was reported in China in 2015 in *E. coli* and *K. pneumoniae* [37]; by 2016 it had been detected across five continents, among *Enterobacter* and numerous other species, and in association with over a dozen distinct plasmids [38**].

It is impossible to accurately reconstruct the precise flow of genes, plasmids and bacteria involved in the capture of AMR genes from environmental microbes and their dissemination among human-associated bacterial pathogen populations, although *qnrA* and OXA-48 provide compelling examples of AMR gene mobilisation from marine bacteria to *K. pneumoniae*, and onwards to other ESRAPE pathogens. Regardless of precise HGT flow, the dominance of *K. pneumoniae* amongst early clinical reports of new AMR genes is notable, and indicates *K. pneumoniae* to be a prime target for sentinel surveillance of new AMR genes entering Gram negative pathogen populations.

**Means and opportunity: genome plasticity, plasmid diversity and ecology**

Figure 2a shows the total number of distinct acquired AMR genes and load per strain for all genome sequences of *K. pneumoniae* and fellow Gram negative opportunists (*A. baumannii*, *P. aeruginosa*, *E. cloacae* and *E. coli*) currently in the NCBI Pathogen Genomes portal (>1400 genomes for each species). Over 400 acquired AMR genes are present in the *K. pneumoniae* genomes, double the number in *E. coli* and 50% more than that of the other species (Figure 2a), suggesting that *K. pneumoniae*
receives and/or amplifies a wider range of AMR genes from their ultimate source in environmental microbes [5,6]. Below we consider the genomic and ecological characteristics of *K. pneumoniae* in comparison to the other ESKAPE pathogens and *E. coli*, highlighting factors that may enhance exposure to environmental AMR genes and the ability to pass these genes on to other clinically important pathogens.

**Ecological range**

Most HGT occurs between cells residing in the same habitat [39], hence bacteria adapted to survive in environmental and animal/human-associated microbial communities can be predicted to contribute most to the trafficking of AMR genes between these niches. Reports in the 1970s–1980s highlighted the ubiquitous distribution of *K. pneumoniae* among diverse fresh and salt water environments, plants, and soil [40]. *K. pneumoniae* causes infections in cows, horses and other wild and domestic animals [8,41,42,43,44–48]. However as an opportunistic pathogen, it is likely that *K. pneumoniae* is more often a component of the normal animal gut microbiota. In humans the rate of intestinal *K. pneumoniae* colonisation has been estimated at ~6% [49,50], while in dairy cows the rate may be much higher (~44% among herds in New York, USA, [51]). *K. pneumoniae* has also been cultured from the faeces of other agricultural and domestic animals, from the cloacae of birds, and from fish, shellfish, insects and earthworms [42,48,52–56]. It is a common contaminant of animal and plant-based foods, which likely plays a key role in introducing environmental strains into the human gut [48].

Enterobacteriaceae are well known gut colonisers, and all the ESKAPE pathogens can be isolated from environmental sources [57], however systematic comparisons of
isolation rates across environmental and animal sources are lacking. We used the IMNGS website [58**] to query the growing body of publicly available 16S taxonomic profiling data for the presence of Gram negative opportunists across a wide range of sample types (Figure 3a). These data confirm the three Enterobacteriaceae species are frequent commensals of humans and animals, with E. coli the most commonly detected; however K. pneumoniae and E. cloacae showed equivalent or even greater prevalence amongst plant and environmental samples (7–14%), whereas E. coli was significantly less common in these niches (3–4%, p = 0.01). Notably these data indicate that K. pneumoniae and E. cloacae have similarly broad ecological distributions, but K. pneumoniae was more prevalent in human and other animal microbiomes (6.8% versus 3.6%), likely increasing its exposure to antimicrobial use and its contact with other clinically important pathogens, therefore enhancing amplification and onward dissemination of acquired AMR genes.

It is difficult to directly assess movement of individual K. pneumoniae strains between niches, however there is evidence that isolates from human, animal and environmental sources do not represent distinct subpopulations..

**Figure 3**

(a) Ecological distribution from 16S rRNA studies

(b) Clinically important AMR K. pneumoniae sequence types

| Samples with >0.1% relative abundance |
|---------------------------------------|
| humans                               |
| agricultural animals                  |
| aquatic animals                       |
| insects                               |
| plants                                |
| aquatic environment                   |
| other environment                     |

| ST11 | ST258 | ST340 | ST14 | ST15 | ST17 | ST37 | ST101 | ST147 |
|------|-------|-------|------|------|------|------|-------|-------|
| CG258 || || || || || || ||

| n = 2 | n = 4 | n = 6 |
|-------|-------|-------|

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Ecological distribution of species and sequence types based on public sequence data. (a) The IMNGS database (http://imngs.org, [58**]) was queried in March 2018 using one 16S rRNA sequence each from Klebsiella pneumoniae (NUST258), Acinetobacter baumannii (A1), Pseudomonas aeruginosa (PA01), Enterobacter cloacae (ATCC 13047) and E. coli (K12), for hits with ≥99% identity and ≥200 bp. Bars indicate mean frequency of detection (defined as relative abundance >0.1%) for each species in category descriptors containing >100 samples; error bars indicate standard error of the mean. IMNGS output and code to generate plots is available in FigShare (https://doi.org/10.4225/49/5ac3670f83717). (b) Top: globally distributed AMR STs reported from at least 2 non-human sources; CG258 = KPC-associated clonal group 258. Below: bar heights indicate number of isolates for which STs were reported in the literature (March 2018) and/or inferred from published genome data. Data represent published STs from reports identified by PubMed search for ‘Klebsiella pneumoniae’ AND ‘ST’ AND one of ‘cat’, ‘feline’, ‘dog’, ‘canine’, ‘cow’, ‘bovine’, ‘pig’, ‘porcine’, ‘water’, ‘aquatic’ (‘horse’ and ‘equine’ were also searched but yielded no reports of globally distributed AMR STs); additional STs were compiled from two genomic studies which had each reported >10 isolates from non-human sources [8,64].
Clinically important K. pneumoniae lineages have been isolated from specific non-human sources (Figure 3b); for example, ESBL ST15 in cats and dogs [59–61], and hypervirulent ST23 or ST25 in horses, non-human primates and pigs [43*,62,63]. Genomic comparisons of K. pneumoniae from diverse sources are rare but show little evidence of segregation between niches: in a global diversity study, 59 bovine isolates were distributed around the species phylogeny comprising mostly human isolates [8]; and a comparison of ESBL isolates from Thai hospitals and a local canal system indicated phylogenetic intermingling [64*]. These data and others [48] confirm that at least some strains, including those recognised as globally distributed hospital pathogens [11], can move between and proliferate in multiple niches, providing opportunity for genetic exchange with a broad range of bacterial species (Figure 3b).

**Genome composition and HGT**

K. pneumoniae genomes are highly diverse [8], comprising hundreds of distinct phylogenetic lineages that differ from each other by ~0.5% nucleotide divergence. Individual strains harbour ~2000 ‘core’ (shared) genes, plus a further ~3500 accessory genes that differ between strains and are drawn from a large pool of ~30 000 [8]. The ~2000 core genes likely facilitate K. pneumoniae’s broad ecological range by providing metabolic and other capabilities enabling survival in a wide range of niches. A substantial proportion of the total pan-genome (core + accessory genes, [65]) is predicted to encode proteins with metabolic functions; 19% associated with carbohydrate metabolism, 18% with other metabolic pathways and 13% with membrane transport [8]. This extensive diversity results in variable metabolic capacity [66*], potentially supplementing individual strains with additional ecological range and providing even more opportunities for genetic exchange. Direct comparison of population structures are difficult due to different sampling and analysis strategies [65]; nonetheless it is clear that the other Gram negative opportunists also have many deep branching lineages and large pan-genomes [8,67–70].

Coding capacity and genome size are easy to compare using public genome data (Figure 2c): K. pneumoniae has a significantly larger genome than the other Enterobacteriaceae species considered here (mean 5.7 Mbp, 5455 protein coding genes, versus 5.1 Mbp/4915 genes in E. coli and 5.0 Mbp/4680 genes in E. cloacae, p < 1 × 10\(^{-15}\) using two-sided t-test), which may help equip K. pneumoniae for survival in a broader range of niches.

DNA base composition varies widely between taxa, and can be used as a signature of bacterial species [71]. The mean G + C content of K. pneumoniae core genes is 58%, whereas that of accessory genes ranges from 20% to >70%, suggesting they originate from a taxonomically diverse array of donors [8]. Figure 2d shows that genes annotated in complete genomes of K. pneumoniae display significantly more variability in their G + C content than those of the other species considered here, with 50% greater variance in G + C content than E. coli and E. cloacae (p < 1 × 10\(^{-15}\) using F-test), and more than double the variance of P. aeruginosa and A. baumanii. This suggests K. pneumoniae receives DNA from a wider diversity of HGT partners; indeed lowest common ancestor analysis of K. pneumoniae accessory genes has implicated >20 distinct genera as DNA donors, including numerous other members of the Enterobacteriaceae but also diverse groups such as Acinetobacter, Burkholderia, Streptomyces, Vibrio, Xanthomonas and Xyella [8]. More direct evidence for K. pneumoniae engaging in inter-species HGT can be found in recent genomic comparisons of carbapenem-resistant Enterobacteriaceae in hospitals, which captured identical or highly similar carbapenemase encoding plasmids and/or transposons from K. pneumoniae and other species originating from the same ward and/or patient (E. coli, E. cloacae, Enterobacter asburiae, Citrobacter freundii, Klebsiella oxytoca, Raoultella ornithinolytica) [72*,73**,74].

**Plasmid load**

The vast majority of AMR genes in K. pneumoniae are plasmid-borne [9,11], hence the ability to amplify and spread AMR genes across ecological niches is likely linked to plasmid-permissive traits. Highly diverse environmental microbial communities, especially soils, are considered hotspots for gene transfer [75], and Enterobacteriaceae have been identified as a component of the ‘super-permissive community’ that supports the spread of plasmids across diverse soil communities [76]. The specific role of K. pneumoniae in such activities remains to be explored, however the species has been associated with hundreds of distinct plasmids spanning many plasmid replicon types [8,9,12,77], which suggests it acts as a recipient for plasmids originating from a wide array of HGT donors. The median number of plasmids per complete K. pneumoniae genome currently in NCBI GenBank is three (interquartile range, 2–5; range 0–10), significantly higher than that for the other species of interest (p < 1 × 10\(^{-5}\); see Figure 2b). This is consistent with numerous reports of K. pneumoniae strains carrying multiple AMR plasmids; for example, the ST11 reference genome, H511286, harbours six plasmids (1.3–123 kbp in size), three of which carry AMR genes [78]; and there are many examples in other lineages [9,79,80*].

Its elevated plasmid load (Figure 2b) suggests K. pneumoniae is particularly permissive for plasmids, meaning it may be more likely to capture plasmid-borne material from diverse donors in varied niches, and to hold on to this material long enough to transmit it to new recipients in human and animal-associated niches (Figure 4). This enhanced permissiveness may reflect a comparatively lower fitness burden of plasmid carriage in K. pneumoniae,
a hypothesis supported by a small number of studies showing lower fitness costs for specific AMR plasmids in *K. pneumoniae* versus *E. coli* in vitro [81**,82**, and reports of long-term plasmid maintenance in *K. pneumoniae* in vivo [63,72**,82**]. Plasmid–host interactions are complex, and there are many reported examples of specific adaptations of hosts to plasmids (and vice versa) [83,84**]. It is has recently been shown that some chromosomal adaptations (such as helicase and RNA polymerase mutations) can increase general plasmid permissiveness of a host bacterium [84**]. Intriguingly, it is clear that AMR genes and plasmids are not randomly distributed amongst *K. pneumoniae* lineages [8,9], suggesting that there may be significant variation in plasmid permissiveness between lineages. Related species and strains can vary substantially in their ability to act as plasmid donors [76,85], however variation in plasmid-donor potential between *K. pneumoniae* and other bacteria, or between strains of *K. pneumoniae*, remains to be investigated.

**Conclusions**

*K. pneumoniae* have the means and opportunity to capture plasmids from environmental microbial populations; to survive within and move between multiple environmental and animal-associated niches; to maintain AMR plasmids for prolonged periods; and to pass plasmids on to other clinically important Gram negative bacteria (Figure 4). Whilst the contribution of *K. pneumoniae* to the AMR crisis is impossible to quantify, the available evidence suggests it is unique amongst the Gram negative ESKAPE pathogens and *E. coli* in a few key respects including its high diversity of acquired AMR genes, high plasmid load, wide variability of G + C content reflecting diverse HGT partners and broad ecological range — whilst systematic studies of comparative ecology are lacking, the available 16S data suggests *K. pneumoniae* is equally likely to be found living in human, animal and environmental niches. Combined these factors may position *K. pneumoniae* as a key amplifier and spreader of clinically important AMR genes. Better understanding and monitoring of this highway of AMR gene transfer could potentially help limit the spread of AMR and prolong the life of new antibiotics.

**Conflict of interest statement**

Nothing declared.

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**References and recommended reading**

Papers of particular interest, published during the period of review, have been highlighted as:

- of special interest
- **of outstanding interest**

1. Pendleton JN, Gorman SP, Gilmore BF: Clinical relevance of the ESKAPE pathogens. Expert Rev Anti Infect Ther 2013, 11:297-308.
Drug resistance gene dissemination by Klebsiella Wyres and Holt

2. de Man TJB, Lutgring JD, Lonsway DR, Anderson KF, Kiehlbauch JA, Chen L, Walters MS, Sjölund-Karlsson M, Rasheed JK, Kallen A et al.: Genomic analysis of a pan-resistant isolate of Klebsiella pneumoniae, United States 2016. MBio 2018, 9(4):e00440-00408.

3. Jiang B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN et al.: CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 2017, 45:D566-D573.

4. Doostan VM, King CE, Kalam L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R et al.: Antibiotic resistance is ancient. Nature 2011, 477:457-461.

5. Neve J, Cécillon S, Belmont TO, Monier JM, Vogel TM, Simonet P: Large-scale metagenomic-based study of antibiotic resistance in the environment. Curr Biol 2014, 24:1096-1100.

6. Benveniste R, Davies J: Aminoglycoside antibiotic-inactivating enzymes in Actinomycetes similar to those present in human isolates of antibiotic-resistant bacteria. Proc Natl Acad Sci U S A 1973, 70:2276-2280.

7. Lewis K: Platforms for antibiotic discovery. Nat Rev Drug Discov 2013, 12:371-387.

8. Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Husy L, Severin J et al.: Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in Klebsiella pneumoniae, an urgent threat to public health. Proc Natl Acad Sci U S A 2015, 112: E3574-E3581.

9. Navon-Venezia S, Kondratyeva K, Carattoli A: Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev 2017, 41:252-275.

10. Wand ME, Baker KS, Benthall G, McGregor H, McCowen JWI, Deheer-Graham A, Sutton JM: Characterization of pre-antibiotic era Klebsiella pneumoniae isolates with respect to antibiotic/disinfectant susceptibility and virulence in Galleria mellonella. Antimicrob Agents Chemother 2015, 59:3966-3972.

11. Wyres KL, Holt KE: Klebsiella pneumoniae population genomics and antimicrobial-resistant clones. Trends Microbiol 2016, 24:944-956.

12. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J: Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. Antimicrob Agents Chemother 2018 http://dx.doi.org/10.1093/jac/ddx488.

13. Matthew M, Hedges RW, Smith JT: Types of beta-lactamase determined by plasmids in gram-negative bacteria. J Bacteriol 1979, 138:657-662.

14. Roupas A, Pitton JS: R factor-mediated and chromosomal resistance to ampicillin in Escherichia coli. Antimicrob Agents Chemother 1974, 5:186-191.

15. Labia R, Morand A, Tiwari K, Pitton JS, Sirot D, Sirot J, Ben Yaghlane H, Boujenah A: The kinetics of SHV-2 plasmid-mediated beta-lactamase compared to those of the parent enzyme from which it is derived. Drugs Exp Clin Res 1988, 14:335-339.

16. Jacoby GA, Medeiros AA: More extended-spectrum beta-lactamases. Antimicrob Agents Chemother 1991, 35:1697-1704.

17. Liakopoulos A, Mavis M, Deccarelli D: A review of SHV extended-spectrum beta-lactamases: neglected yet ubiquitous. Front Microbiol 2016, 6:1374.

18. Dai N, Li DZ, Chen JC, Chen YS, Geng R, Hu YH, Yang JP, Du J, Hu CP, Zhang W et al.: Drug-resistant genes carried by Acinetobacter baumanii isolated from patients with lower respiratory tract infection. Chin Med J (Engl) 2010, 123:2571-2575.

19. Naas T, Philippou L, Poirel L, Ronco E, Nordmann P: An SHV-derived extended-spectrum beta-lactamase in Pseudomonas aeruginosa. Antimicrob Agents Chemother 1999, 43:1281-1284.

20. D’Andrea MM, Arena F, Pallecchi L, Rossolini GM: CTX-M-type beta-lactamases: a successful story of antibiotic resistance. Int J Med Microbiol 2013, 303:305-317.

21. Cantón R, González-Alba JM, Galán JC: CTX-M enzymes: origins and diffusion. Front Microbiol 2012, 3:110.

22. Mathers AJ, Peirano G, Pitout JJD: The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev 2015, 28:565-591.

23. Woetker PL, Burdet C, Chachaty E, Andremont A: Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: toward the globalization of CTX-M. Clin Microbiol Rev 2013, 26:744-758.

24. Martinez-Martinez L, Pascual A, Jacoby GA: Quinolone resistance from a transferable plasmid. Lancet 1998, 351:797-799.

25. Jacoby GA, Walsh KE, Mills DM, Walker VJ, Oh H, Robicsek A, Hooper DC: qnrB, another plasmid-mediated gene for quinolone resistance. Antimicrob Agents Chemother 2006, 50:1178-1182.

26. Poirel L, Rodriguez-Martinez J-M, Mammeri H, Liard A, Nordmann P: Origin of plasmid-mediated quinolone resistance determinant QnrA. Antimicrob Agents Chemother 2005, 49:3523-3525.

27. Villegas MV, Lolan K, Correa A, Kattan JN, Lopez JA, Quinn JP: First identification of Pseudomonas aeruginosa isolates producing a KPC-type carbapenem-hydrolyzing beta-lactamase. Antimicrob Agents Chemother 2007, 51:1553-1555.

28. Martinez T, Vazquez GJ, Aquino EE, Martinez I, Robledo IE: IS6100-mediated transposition of blaKPC into the chromosome of a clinical isolate of Acinetobacter baumanii from Puerto Rico. J Med Microbiol 2014, 63:1644-1648.

29. Taciá M, Araújo S, Vendras M, Alves A, Henriques I: Shewanella species as the origin of blaOXA-48 genes: insights into gene diversity, associated phenotypes and possible transfer mechanisms. Int J Antimicrob Agents 2018, 51:340-348.

30. Poirel L, Heritier C, Tolu V, Nordmann P: Emergence of oxacillin-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother 2004, 48:15-22.

31. Maini A, Pantel A, Sotto A, Lavigne JP, Touati A: OXA-48-like carbapenemases producing Enterobacteriaceae in different niches. Eur J Clin Microbiol Infect Dis 2018, 37:587-604.

32. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR: Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 2000, 53:5046-5054.

33. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, BalaKrishnan R, Chaudhury HS, Domsch M, Giske CG, Irfan S et al.: Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis 2010, 10:597-602.

34. Poirel L, Dortet L, Bernabeu S, Nordmann P: Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother 2011, 55:5403-5407.

35. Walsh TR, Weeks J, Livermore DM, Toleman MA: Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis 2011, 11:355-362.
36. Karthikeyan K, Thirunavukkarasun MA, Krishnan P: Coexistence of blaOXA-23 with blaNDM-1 and arnA in clinical isolates of Acinetobacter baumannii in India. J Antimicrob Chemother 2010, 65:2253-2254.

37. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X et al.: Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016, 16:161-168.

Discovery of the first mobile gene conferring resistance to colistin, MCR-1, encoding a phosphoethylenamine transferase enzyme. The gene was first identified in an E. coli isolated from an intensive pig farm in China; retrospective surveillance of E. coli and K. pneumoniae clinical isolates in China identified the gene in both species.

38. Wang R, Van Dorp L, Shaw LP, Bradley P, Wang Q, Wang X, Jin L, Zhang Q, Liu Y, Rieux A et al.: The global distribution and spread of the mobilized colistin resistance gene mcr-1. Nat Commun 2015, 6:9117.

The authors describe the first systematic phylogenetic and comparative sequence analysis of the emergence and spread of MCR-1 using 457 whole-genome sequenced MCR-1 positive isolates. They confirm that MCR-1 has been mobilised by the transposon ISAP1 into at least 13 different plasmid backgrounds, which have carried the gene between at least 8 different Enterobacteriaceae species.

39. Popa O, Dagan T: Trends and barriers to lateral gene transfer in prokaryotes. Curr Opin Microbiol 2011, 14:615-623.

40. Podschun R, Ullmann U: Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998, 11:589-603.

41. Brisse S, Van Duijkeren E: Identification and antimicrobial susceptibility of 100 Klebsiella animal clinical isolates. Vet Microbiol 2005, 105:307-312.

42. Zhang PLC, Shena X, Chalmers G, Reid-Smith RJ, Slavidc D, Dick H, Boerlin P: Prevalence and mechanisms of extended-spectrum cephalosporin resistance in clinical andecal Enterobacteriaceae isolates from dogs in Ontario, Canada. Vet Microbiol 2012, 163:82-88.

43. Rowling BG, Fathy VA, Morris A, Collins AM: An unusual culprit: Klebsiella pneumoniae causing septicemia outbreaks in neonatal pigs? Vet Microbiol 2017, 203:267-270.

Characterising K. pneumoniae causing septicaemia and pneumonia in Australian pigs, this is one of the first reports to describe both antimicrobial susceptibility and STs for K. pneumoniae causing infections in pigs. This work highlights the contribution of clones commonly isolated from human clinical infections (hypervirulent clone ST25 and AMR clones ST14 and ST17) to disease in these animals.

44. He T, Wang Y, Sun L, Pang M, Zhang L, Wang R: Occurrence and characterization of blaNDM-5-positive Klebsiella pneumoniae isolates from dairy cows in Jiangsu, China. J Antimicrob Chemother 2017, 72:90-94.

45. Sasaki E, Tokiwa T, Tsugo K, Higashi Y, Hori H, Une Y: Peracetic bacterial meningitis due to infection with Klebsiella pneumoniae in captive-bred ruffled lemur (Varecia variegata). J Comp Pathol 2017, 156:281-285.

46. Bagley ST: Habitat association of Klebsiella species. Infect Control 1985, 6:52-58.

47. Schadich E: Skin peptide activities against opportunistic bacterial pathogens of the African Clawed Frog ( Xenopus laevis ) and three Litoria frogs. J Herpetol 2009, 43:173-183.

48. Davis GS, Price LB: Recent research examining links among Klebsiella pneumoniae from food, food animals, and human extraintestinal infections. Curr Environ Health Rep 2016 http://dx.doi.org/10.1007/s40502-016-0089-9.

49. Gorrie C, Wick R, Edwards D, Strugnell R, Pratt N, Garlick J, Watson K, Pilcher D, McGloughlin S, Spelman D et al.: Gastrointestinal carriage is a major reservoir of K. pneumoniae infection in intensive care patients. Clin Infect Dis 2017, 65:208-215.

Culture based study of K. pneumoniae carriage, estimating colonisation prevalence in human following admission to an intensive care unit. Whole genome sequence analyses showed extensive diversity of STs, and implicated gut-colonising K. pneumoniae strains as a common cause of infection in hospitalised patients.

50. Conlan S, Kong HH, Segre JA: Species-level analysis of DNA sequence data from the NIH human microbiome project. PLOS ONE 2012, 7:e47075.

51. Zadoks RN, Griffiths HM, Munoz MA, Ahtabrom C, Bennett GJ, Thomas E, Schukken YH: Sources of Klebsiella and RaoultellaM species on dairy farms: be careful where you walk. J Dairy Sci 2011, 94:1045-1051.

52. Founou LL, Founou RC, Allam M, Ismail A, Djoko CF, Essack SY: Genome sequencing of extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae isolated from pigs and abattoir workers in Cameroon. Front Microbiol 2018, 9:1-12.

53. Stenkat J, Krautwald-Junghans ME, Schmitz Ornès A, Eilers A, Schmidt V: Aerobic cloacal and pharyngeal bacterial flora in six species of free-living birds. J Appl Microbiol 2014, 115:1564-1571.

54. Brahim S, Touati A, Dnyach-remy C, Sotto A: High prevalence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in wild fish from the Mediterranean Sea in Algeria. Microb Drug Res 2017:1-9.

55. Raknjar B, Izadi M, Hafshejani TT, Khamsempour F: Molecular detection and antimicrobial resistance of Klebsiella pneumoniae from house flies (Musca domestica) in kitchens, farms, hospitals and slaughterhouses. J Infect Public Health 2016, 9:499-505.

56. Parthasarathi K, Ranganathan LS, Anandi V, Zeyer J: Diversity of microflora in the gut and casts of tropical composting earthworms reared on different substrates. J Environ Biol 2007, 28:87-97.

57. Singh SK, Ekka R, Mishra M, Mohapatra H: Association study of multiple antibiotic resistance and virulence: a strategy to assess the extent of risk posed by bacterial population in aquatic environment. Environ Mont Asses 2017, 199:390.

58. Lagkouvardos I, Joseph D, Kapflhammer M, Giritli S, Horn M, Haller D, Clavel T: IMNGS: a comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. Sci Rep 2016, 6:33721.

This paper describes a database of public 16S RNA profile data curated from published studies of diverse microbial communities including human, animal and environmental samples. The database is a valuable resource for the research community because it allows methodologically consistent querying by sequence to obtain ecological distributions of taxa of interest.

59. Stolle I, Prenger-bemighoff E, Stamn I, Scheuten S, Hassdenteufel E, Guenther S, Betha A, Pfister Y, Ewers C, Med V et al.: Emergence of OXA-48 carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in dogs. J Antimicrob Chemother 2013, 68:2802-2808.

60. Donati V, Feltrin F, Hendriksen RS, Svendsen CA, Cordaro G, Lorenzetti S, Lorenzetti R, Battisti A, Franco A, Garcia A: Extended-spectrum-beta-lactamases and plasmid mediated quinolone resistance in Klebsiella spp. from companion animals in Italy. PLOS ONE 2014, 9:1-7.

61. Sato T, Harada K, Usui M, Tsuwayi Y, Shirashita T, Tamura Y, Yokota S: Tigecycline susceptibility of Klebsiella pneumoniae complex and Escherichia coli isolates from companion animals: the prevalence of tigecycline-nonsusceptible K. pneumoniae complex, including internationally expanding human pathogenic. Microb Drug Res 2017:1-8.

62. Anzai EK, de Souza Júnior JC, Peruchi AR, Fonseca JM, Gumpel EK, Pignatari ACC, Hirano ZMB, de Silveira ACO: First case report of non-human primates ( Alouatta clamitans ) with the hypervirulent Klebsiella pneumoniae serotype K1 strain ST23: a possible emerging wildlife pathogen. J Med Primatol 2016, 45:337-342.

63. Lam MMC, Wyres KL, Duchêne S, Wick RR, Judd LM, Gan Y, Hoh C, Achuleta S, Molton JS, Kalimuddin S et al.: Population genomics of hypervirulent Klebsiella pneumoniae clonal group 23 reveals early emergence and rapid global dissemination. bioRxiv 2018.
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64. Ruchanzen C, Moradagharan D, Blane B, Paksanont S, Thammanochote J, Arun S, Parkhill J, Chantrillita N, Peacock SJ: Whole genome sequencing reveals high-resolution epidemiological links between clinical and environmental Klebsiella pneumoniae. Genome Med 2017, 9:6.

This report describes one of the first whole-genome comparative studies to include a significant number of contemporary K. pneumoniae isolates from clinical and environmental sources (primarily local canals). Isolates from both sources displayed significant diversity of lineages and AMR genes, but more closely related strains were identified in both clinical and environmental isolates.

65. Vernikos G, Medini D, Riley DR, Tettelin H: Ten years of pan-genome analyses. Curr Opin Microbiol 2015, 23:148-154.

66. Blin C, Passet V, Touchon M, Rocha EPC, Brisse S: Metabolic diversity of the emerging pathogenic lineages of Klebsiella pneumoniae. Environ Microbiol 2017, 19:1881-1898.

This study compares comprehensive metabolic phenotype profiles across a diverse set of >30 K. pneumoniae isolates, representing dozens of distinct phylogenetic lineages including both global AMR and hyper-virulent clones. The data show that K. pneumoniae strains differ substantially in their ability to utilise distinct substrates (carbon and nitrogen sources were tested), and provide support for associations with ecology and epidemiology.

67. Gordienko EN, Kazanov MD, Gelfand MS: Evolution of pan-genomes of Escherichia coli, Shigella spp., and Salmonella enterica. J Bacteriol 2013, 195:2786-2792.

68. Liu W-Y, Wong C-F, Chung KM-K, Jiang J-W, Leung FC-C: Comparative genome analysis of Enterobacter cloacae. PLOS ONE 2013, 8:e74487.

69. Chan AP, Sutton G, DePew J, Krishnakumar R, Choi Y, Huang XZ, Beck E, Hartkins DM, Kim M, Lesho EP et al.: A novel method of consensus genome dissemination and large-scale comparative analysis reveal the highly flexible pan-genome of Acinetobacter baumannii. Genome Biol 2015, 16:143.

70. Mosquera-Rendón J, Rada-Bravo AM, Cárdenas-Brito S, Corredor M, Restrepo-Pineda E, Benitez-Paz A: Pan-genome-wide and molecular evolution analyses of the Pseudomonas aeruginosa species. BMC Genomics 2016, 17:45.

71. Mann S, Chen YPP: Bacterial genomic G + C composition eliciting environmental adaptation. Genomics 2010, 95:7-15.

72. Conlan S, Park M, Deming C, Thomas PJ, Young AC, Coleman H, Sison C, Progam NCS, Weingarten RA, Lau AF et al.: Plasmid dynamics in KPC-positive Klebsiella pneumoniae during long-term patient colonization. MBio 2016, 7:e00742-816.

This paper describes the first use of PacBio long read sequencing to explore the long-term carriage and evolution of K. pneumoniae plasmids within patients. The data show that AMR plasmids can be carried by gut-colonising K. pneumoniae for over a year without significant nucleotide sequence variation.

73. Sheppard AE, Stoesser N, Wilson DJ, Sebra R, Kasarskis A, Anson LW, Giess A, Pankhurst LJ, Vaughan A, Grim CJ et al.: Nested Russian doll-like genetic mobility drives rapid dissemination of the carbapenem resistance gene blaKPC. Antimicrob Agents Chemother 2016, 60:3767-3778.

One of the first high-resolution explorations of wide-spread KPC dissemination within a single hospital over several years. Using whole-genome sequence analyses, the authors show spread of the KPC gene within the hospital via three distinct mechanisms; by dissemination of KPC-positive strains, by transfer of KPC plasmids between strains and species, and by movement of KPC transposons between diverse plasmid backbones within and between diverse species.

74. Martin J, Phan HTT, Findlay J, Stoesser N, Pankhurst L, Navickaitė I, De Maio N, Eyre DW, Toogood G, Orsi NM et al.: Covert dissemination of carbapenemase-producing Klebsiella pneumoniae (KPC) in a successfully controlled outbreak: long- and short-read whole-genome sequencing demonstrate multiple genetic modes of transmission. J Antimicrob Chemother 2017, 72:3025-3034.

75. Popa O, Hazzani-Covo E, Landan G, Martin W, Dagan T: Directed networks reveal genomic barriers and DNA repair bypasses to lateral gene transfer among prokaryotes. Genome Res 2011, 21:599-609.

76. Klümpner U, Riber L, Dechesne A, Sannazzaro A, Hansen LH, Sørensen SJ, Smets BF: Broad host range plasmids can invade an unexpectedly diverse fraction of a soil bacterial community. ISME J 2015, 9:934-945.

77. Ramirez MS, Traglia GM, Lin DL, Tran T, Tolmasky ME: Plasmid-mediated antibiotic resistance and virulence in gram-negatives: the Klebsiella pneumoniae paradigm. Microbiol Spectr 2014, 2:1-15.

78. Liu P, Li P, Jiang X, Bi D, Xie Y, Tai C, Deng Z, Rajakumar K, Ou HY: Complete genome sequence of Klebsiella pneumoniae subsp. pneumoniae HS11286, a multidrug-resistant strain isolated from human sputum. J Bacteriol 2012, 194:1841-1842.

79. Gorrie CL, Mirceta M, Wick RR, Judd LM, Wyres KL, Thomson NR, Strugnell RA, Pratt NF, Garlick JC, Watson KM et al.: Antimicrobial resistant Klebsiella pneumoniae carriage and infection in specialized geriatric care wards linked to acquisition in the referring hospital. Clin Infect Dis 2018 http://dx.doi.org/10.1093/cid/ciy027.

80. Wick RR, Judd LM, Gorrie CL, Holt KE: Completing bacterial genome assemblies with multiplex MiniON sequencing. MBio 2017, 8.

This paper describes a protocol for efficiently resolving bacterial genome sequences into circularised chromosome and plasmid sequences, by supplementing short read Illumina data with small amounts of long read Nanopore sequences. Twelve novel K. pneumoniae genomes were completed by this protocol, harbouring between 0 and 10 plasmids. This study also demonstrates the potential for cost-effective high throughput plasmid finishing, which could facilitate large-scale comparative analyses and tracking plasmid movements between host strains.

81. Göttig S, Riedel-Christ S, Saleh A, Kemppi VAJ, Hamprecha A: Impact of blaNDM-1 on fitness and pathogenicity of Escherichia coli and Klebsiella pneumoniae. Int J Antimicrob Agents 2016, 47:430-435.

An NDM-1 containing plasmid was transferred into naïve E. coli and K. pneumoniae strains and the resultant transconjugants were characterised in growth, in vitro competition and virulence assays. Competition assays indicated a fitness cost to NDM-1 plasmid acquisition for both the E. coli and K. pneumoniae transconjugants compared to their isogenic plasmid-negative counterparts, however the cost to the E. coli strain was significantly greater than that for the K. pneumoniae strain.

82. Lörh IH, Hüffer N, Bernhoff E, Johnsen PJ, Sundsfjord A, Nasseur U: Persistence of a pKPN3-like CTX-M-15-encoding IncFIIK plasmid in a Klebsiella pneumoniae ST17 host during two years of intestinal colonization. PLOS ONE 2015, 10:e0116516.

83. Bouma JE, Lenski RE: Evolution of a bacteria/plasmid association. Nature 1988, 335:351-352.

84. Lootie-Eaton W, Bashford K, Quinn H, Dong K, Millstein J, Hunter S, Thomason MK, Merrick H, Ponciano JM, Top EM: Compensatory mutations improve general permissiveness to antibiotic resistance plasmids. Nat Ecol Evol 2017 http://dx.doi.org/10.1038/s41559-017-0243-2.

The authors used a combination of experimental evolution and genomics approaches to identify chromosomal mutations in a Pseudomonas that compensated the fitness cost associated with AMR plasmid acquisition. Key mutations were identified in two helicase genes and the RNA polymerase beta-subunit; notably isolates experimentally evolved with the AMR plasmid also showed enhanced permissiveness to additional diverse plasmids.

85. De Gelder L, Vandeveerelle FPJ, Brown CJ, Forney LJ, Top EM: Plasmid donor affects host range of promiscuous IncP-1β plasmid pB10 in an activated-sludge microbial community. Appl Environ Microbiol 2005, 71:5309-5317.