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Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria

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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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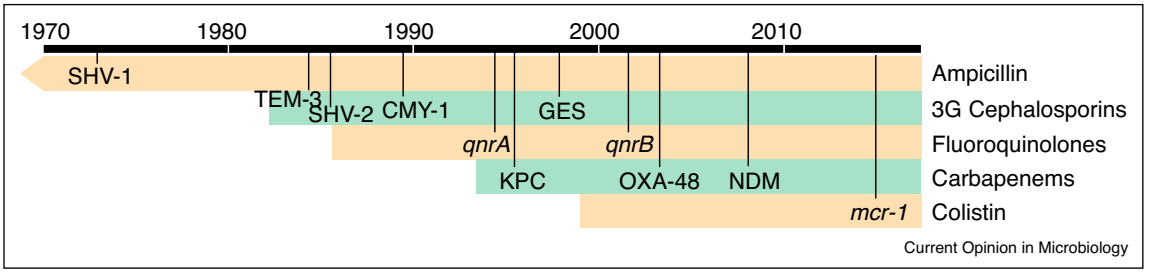
Antimicrobial resistance in Gram negative opportunistic pathogens

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

Figure 1



Timeline of mobile AMR genes first detected in *Klebsiella pneumoniae*. Shading indicates the period since which isolates of *K. pneumoniae* resistant to each drug class have been reported (regardless of mechanism). Selected mobile AMR genes that were first detected in *K. pneumoniae* are labelled on the timeline, within the row corresponding to the relevant class; all have since been reported in clinically important Enterobacteriaceae and other Gram negative bacteria. Note ampicillin resistance is intrinsic to *K. pneumoniae* due to the chromosomal beta-lactamase gene SHV-1, and this gene was shown to be mobilised by plasmids in *E. coli* and *K. pneumoniae* in the 1970s. The other genes shown did not originate in *K. pneumoniae*, but they were first detected in mobile form (i.e. within mobile genetic elements on plasmids) in *K. pneumoniae* isolates, as detailed in the 'The canary in the coalmine' section.

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, human 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 *Providencia 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 ESKAPE 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. cloaceae* 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*