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The evolution and transmission of multi-drug resistant *Escherichia coli* and *Klebsiella pneumoniae*: the complexity of clones and plasmids

Steven J Dunn, Christopher Connor and Alan McNally

The vast majority of *Escherichia coli* and *Klebsiella pneumoniae* isolated from human clinical extra-intestinal infections are now multi-drug resistant (MDR). Extended Spectrum Beta Lactamase (ESBL) carriage in clinical isolates of these bacteria is now commonplace, and carriage of carbapenemases is continuing to increase. MDR is primarily concentrated in a small number of globally disseminated clones, which generally differ between ESBL and carbapenemase carrying-clones in *E. coli*, but seem to converge in *K. pneumoniae*. In both species MDR is mediated by acquisition and maintenance of MDR plasmids. The plasmids associated with ESBL and carbapenemases also differ, and when both resistances are present in the same strain they are generally on distinct plasmids. Recent research is attempting to provide clues as to why some lineages appear better suited to acquisition and maintenance of these plasmids without a fitness cost. Central to this is the appearance of adaptive mutations in intergenic regions, and selection on genes involved in anaerobic metabolism, hinting at a process whereby these clones can outcompete commensal strains of the same species to initiate long-term intestinal colonization.

Address
Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom

Corresponding author: McNally, Alan (a.mcnally.1@bham.ac.uk)

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Introduction

The term antimicrobial resistance (AMR) has become a byword to encapsulate one of the most pressing issues to global human health; the emergence of common human pathogens that are resistant to the majority of, or all, frontline antibiotics clinically prescribed. However, AMR is an almost infinitely complex area, and one could argue an entire scientific research discipline in its own right. The functions that underpin the various antimicrobial resistances seen in nature are vast (Figure 1) and beyond the scope of a single review article. As such we have chosen to focus on what we consider to be one of the most relevant AMR issues to global public health, the evolution and emergence of common gram-negative pathogens that are multi-drug resistant (MDR). Specifically, we look at the rise of MDR clones of *Escherichia coli* and *Klebsiella pneumoniae* over the past 10 years, offering insights from the most recent papers into what the commonalities may be in the evolutionary processes leading to their emergence and successful transmission.

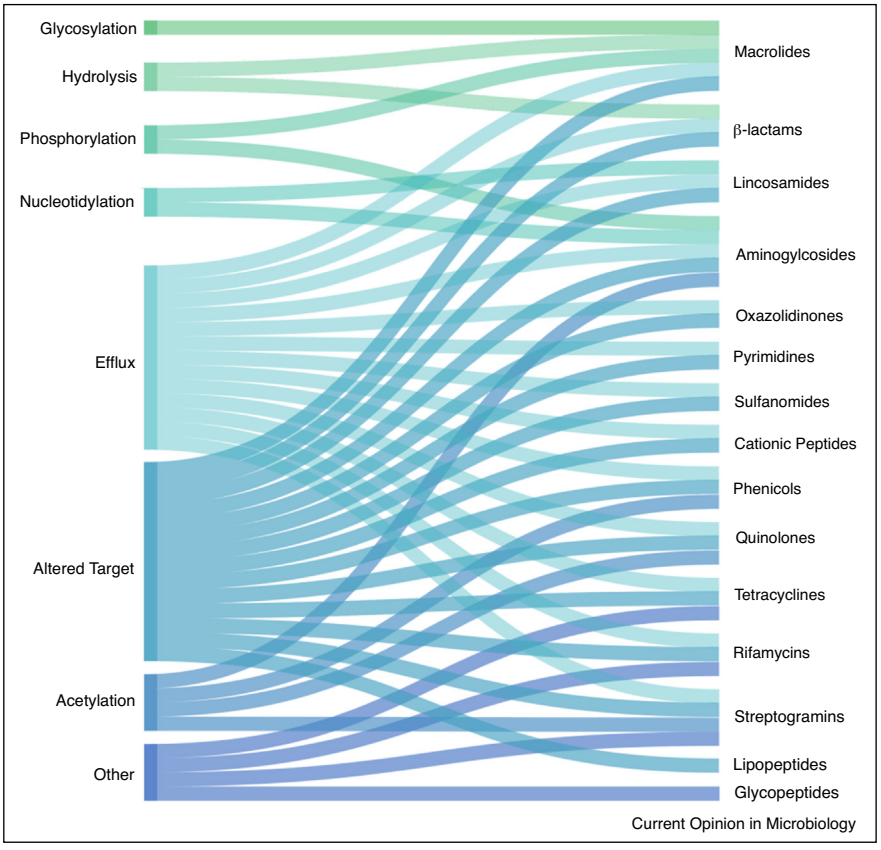
Evolution and emergence of AMR in gram-negative pathogens

AMR in gram-negative bacteria is not a new event. The MDR clone of *Salmonella* Typhimurium DT104 caused a public health panic in the late 20th century [1]. And clinical isolates of *E. coli* and *K. pneumoniae* resistant to clinically relevant antibiotics have been isolated since the 1970s. However, the 21st century has seen the rise of particular clones that dominate the human clinical landscape. The emergence of these clones has been driven by the acquisition of extended-spectrum beta-lactamase (ESBL) genes, conferring resistance to the third generation cephalosporins such as ceftazidime and ceftriaxone [2]. These genes are found on plasmids which also carry genes conferring resistance to other clinically important non-beta-lactam classes of antibiotics such as aminoglycosides and trimethoprim-sulfamethoxazole [2]. As such ESBL carrying strains of *E. coli* and *K. pneumoniae* are often only susceptible to carbapenems and colistin.

Dominant clones

The emergence of ESBL positive *E. coli* is dominated by the ST131 lineage, first described in 2008 as a common cause of hospital infections in multiple countries [2]. *E. coli* ST131 is particularly associated with the $bla_{\text{CTX-M-15}}$ ESBL gene, though isolates from South East Asia commonly carry $bla_{\text{CTX-M-14}}$ or $bla_{\text{CTX-M-27}}$ [3]. Phylogenomic studies have shown that an MDR clone exists within the ST131 lineage (clade C, which is subdivided into subclades C1 and C2, the latter of which is also known as H30-Rx and is most associated with CTX-M-15) which emerged in the 1980s and rapidly spread world-wide [4•,5,6]. The emergence of ST131 clade C as a human clinical pathogen was rapid (Figure 2), quickly overtaking other lineages of *E. coli* such as ST73 and ST95 as the

Figure 1



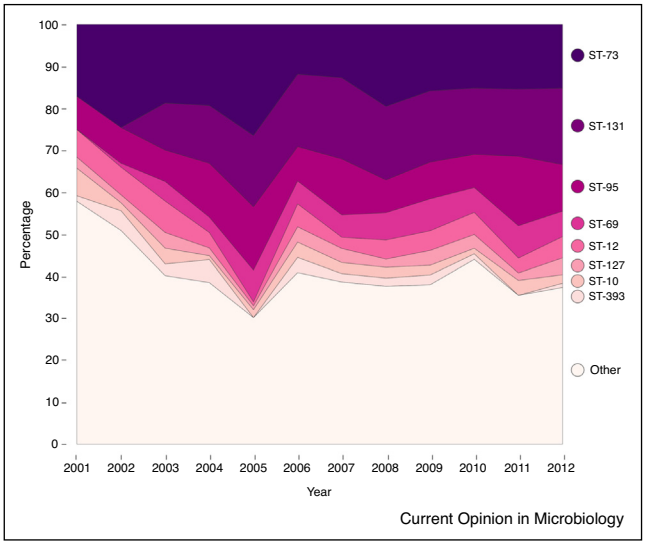
Graphical representation of the known mechanisms by which resistance can occur to the most commonly prescribed antibiotic classes.

most common cause of extra-intestinal clinical infections [7•]. Other globally disseminated ESBL positive clones of *E.coli* have since emerged including ST410 and ST648 [8,9], as well as localized reports of ESBL positive ST73 [10,11], though none to the same extent as ST131.

ESBL positive *E. coli* are now relatively commonplace in the clinical setting, meaning there is a heavy reliance on the use of carbapenems in front line treatment of extra-intestinal *E. coli* infections. Predictably, this has led to the emergence of carbapenem resistant clones of *E. coli*. Evidence suggests that *E. coli* ST131 is perfectly adept at acquiring and maintaining carbapenem resistance genes and plasmids [12]. Thankfully however, carbapenemase gene carriage has not occurred extensively in the ST131 clade C clone [13]. Rather carbapenemase carriage appears to be concentrated in lineages of *E. coli* not previously associated with MDR dissemination. These include lineages belonging to the ST10 complex within phylogroup A, a phylogenetic lineage of *E. coli* more associated with asymptomatic intestinal carriage in humans [14]. Recent work has described ST167 and ST617 as globally disseminated carbapenem resistant *E. coli* (CREC). Other common CREC lineages include ST410 which was previously described as an ESBL-associated clone [8,15], and ST38 which is associated with carriage and dissemination of the OXA-48 carbapenemase gene [16]. Of concern is that many of these new CREC clones also carry ESBL genes, and many isolated from Asia also carry the mobile colistin resistance gene *mcr*, rendering them resistant to all current front-line clinical antibiotics [17].

The emergence of *K. pneumoniae* as a significant MDR human pathogen is also associated with a number of clones. However, the picture differs from *E. coli* in that there seems to be geographical boundaries to the dissemination and establishment of *K. pneumoniae* clones. The increase in MDR *K. pneumoniae* in North America was driven by the ST258 complex [18], responsible for multiple hospital outbreaks [19]. However in Asia, ST11 is by far the predominant MDR *K. pneumoniae* clone, whilst in the UK and Europe multiple clones seem to circulate with ST147 a common clinical isolate type [20,21], as well as the newly emerging globally disseminated ST307 clone [22]. Also different from *E. coli* is that in many cases it is the same dominant clones of *K. pneumoniae*, such as ST11, that are becoming dominant

Figure 2



Graphical representation of the temporal prevalence of different *E. coli* clones isolated from clinical cases of bacteraemia in the United Kingdom. The graph shows the rapid increase and establishment of the MDR clone ST131 in 2002. The figure is adapted from Figure 2 of Kallonen *et al.* [7].

carbapenem resistant *K. pneumoniae* (CRKP), and are acquiring colistin resistance, making totally drug resistant *K. pneumoniae* a very real threat [23]. Global phylogenomic studies have shown that hypervirulent *K. pneumoniae* clones are also emerging in the community setting, but that thankfully clones appear to be either MDR or hypervirulent [24•]. However, increasing reports of hypervirulent MDR *K. penumoniae* and other species such as *Klebsiella variicola* are of significant public health concern [25,26].

The role of plasmids in the emergence of dominant MDR clones

One common theme in the emergence of all of these dominant clones of MDR *E. coli* and *K. pnuemoniae* is the pivotal role of plasmids in their evolution. The dissemination of ESBLs such as $bla_{\text{CTX-M}}$ and carbapenemases such as bla_{NDM} and bla_{KPC} is completely contingent upon MDR plasmids. In *E. coli* these are dominated by plasmids of the IncF family, which are almost exclusively responsible for the global dissemination of $bla_{\text{CTX-M}}$ [3]. CREC are predominantly associated with bla_{NDM} carried on plasmids of the IncX3 or IncFII family [27], and $bla_{\text{OXA-48}}$ carried on IncL plasmids [28]. CRKP are dominated by carriage of bla_{KPC} on IncR family plasmids [27] and IncFII plasmids [29] suggesting parallel evolutionary events. The commonplace implementation of long-read sequencing is giving us unparalleled insights into the complexity of MDR plasmids. It is common for single MDR plasmids to have multiple copies of the same resistance gene, despite the perception that such carriage would impose fitness costs on the host bacteria [30,31]. These gene multiplications result from the fact that individual resistance genes reside in their own mobile gene cassettes [31] which usually have identical genetic context suggesting these insertion sequence (IS) elements are the true currency of mobile genetic exchange with respect to AMR. What is also striking from long-read sequence data is that rather than these AMR cassettes piggy-backing on existing plasmids, as one might imagine in light of perceived plasmid fitness costs, MDR strains usually carry multiple plasmids, with ESBL and carbapenemase genes on distinct plasmids [32].

Evolutionary events in the emergence of a dominant MDR lineage

A number of key questions exist in our attempts to understand the dynamics of evolution of AMR in *E. coli* and *Klebsiella*. If all *E. coli* and *Klebsiella* share core metabolic traits and ecology, then all lineages of these bacteria are equally as likely to be exposed to MDR plasmids, and indeed antimicrobials, and so evolve into MDR lineages. Since plasmids are the drivers of AMR in these species why is their prevalence so lineage restricted, and indeed the range of plasmids on which the MDR genes reside so restricted?

Large scale population genomic analyses on the MDR *E. coli* ST131 clade C have proposed a defined series of events in the formation of this clone. Clone-specific mutations in the key type I fimbriae adhesin, and fixation of fluoroquinolone resistance have been suggested to have led to enhanced infection of humans, and then acquisition of the $bla_{\text{CTX-M}}$ plasmid led to expansion and global dissemination of a successful clone [5,6]. However recent analyses of genomic data suggest that fluoroquinolone resistance played no significant role in the successful emergence of ST131 clade C, and that the level to which ST131 emerged as a globally dominant clinical isolate is restricted by negative frequency-dependent selection [7•].

Rather it appears that there are common signatures of evolution in MDR lineages of *E. coli*. Among these are MDR clone-specific alterations in intergenic sequences, which perfectly mirror-specific plasmid types and sequences in those strains [15,33•]. Given the high levels of evolutionary constraint on intergenic sequences, these mutations must be significant and could play a role in adaptation to plasmids and transcriptional rewiring to offset fitness costs [33•,34]. Experimental data have shown this offsetting is critical in early stages of adaptation to MDR plasmids in *K. pneumoniae* [35] and the identification of MDR clone-specific intergenic mutations across MDR *E. coli* lineages is significant [15,33•]. It would therefore seem that a key determinant of which lineages evolve to be MDR clones is their ability to rapidly and successfully adapt to the acquisition of an

MDR plasmid, via intergenic adaptations and transcriptional rewiring (Figure 3).

Alongside these intergenic adaptations, recent work has also shown that MDR clones contain a number of unique mutations in genes involved in anaerobic metabolism and mammalian colonization factors [36]. Rather than being mutations that fix within the population, these are alleles of genes that move into clones via recombination with other *E. coli* [36], and once again negative frequency-dependent selection appears to play a role in preventing any single set of beneficial alleles sweeping to fixation in the bacterial population. It has been shown that evolutionary events that enhance colonization in bacterial pathogens are likely to also lead to enhanced levels of AMR [37••], and it appears that MDR clones of *E. coli* emerge first from their evolution towards outcompeting other *E. coli* in the intestinal tract, followed by subsequent acquisition of plasmids and evolution of AMR.

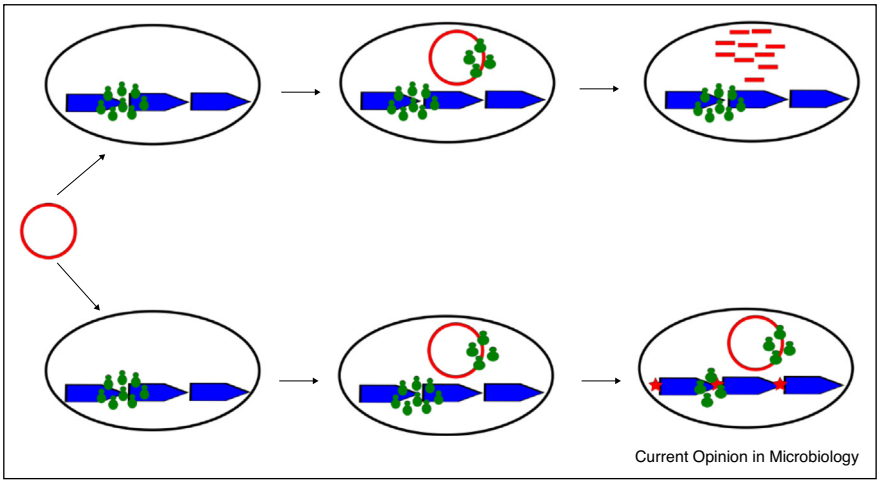
The picture in *K. pneumoniae* is once again different, with MDR lineages of the species more likely to be low-virulence, hospital-associated clones. This is in contrast to community-associated infectious clones which are hypervirulent but not MDR and rarely associated with hospital infections [24•].

Acquisition and transmission of MDR clones

The idea that the evolution of MDR clones of *E. coli* and *K. pneumoniae* is underpinned by mutations in anaerobic metabolism genes ties in with key findings on how such clones are acquired and disseminated. Studies of volunteers from Europe travelling to South and East Asia have shown that during travel to MDR endemic areas, travellers are very quickly colonized with an exogenous MDR strain [38••,39]. Furthermore this colonization displaces the resident commensal *E. coli* strain of the traveller, and can colonize for up to eight months [38••,39]. This fits with a hypothesis whereby MDR bacteria quickly outcompete commensal strains of the same species via anaerobic metabolic processes leading to prolonged colonization and dissemination (Figure 4). This is analogous to the mechanism by which enteric pathogens gain a foothold in the human intestine [40]. The wider impact of this colonization on the microbiome is yet to be elucidated, and is an area that merits substantial further study.

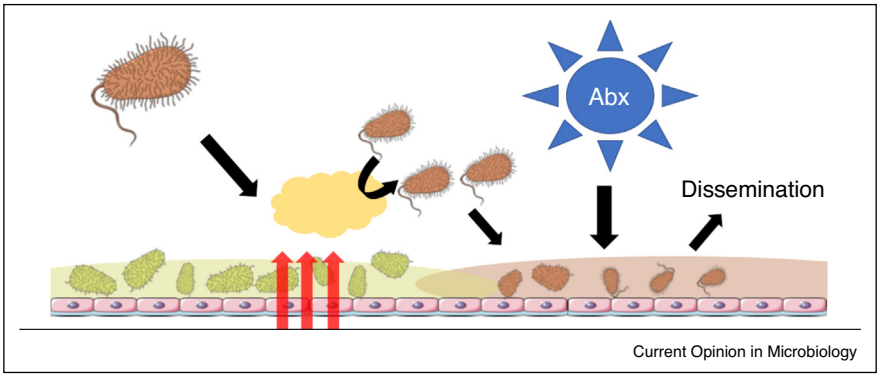
With respect to localized transmission of MDR *E. coli* and *K. pneumoniae*, this is an area that is still greatly underinvestigated, despite the clear importance to public health. Clinical studies do exist showing likely endogenous sources of clinical MDR *Klebsiella* infections [41], but the fact is that there is a distinct paucity of empirical evidence on whether or not clinical infections of MDR *E. coli* and *K. pneumoniae* are exogenous or endogenous. Recent data have shown persistent colonization of hospital patients with strains, but the exact reservoirs and transmission vehicles are not definitively known. If we are to successfully decrease the prevalence of both MDR infections and gram-negative bacteraemia infections then both of these open questions need to be urgently addressed.

Figure 3



Graphical representation of the process that occurs within *E. coli* upon acquisition of an MDR plasmid. The plasmid (red circle) is acquired, upon which it recruits transcription/translation machinery (green) from the host cell to allow plasmid gene expression. This takes transcription/translation machinery away from essential chromosomal operons (blue) resulting in altered host cell gene expression and a loss in fitness. In the majority of *E. coli* lineages this will result in plasmid degradation to maintain fitness (top panel), but in a small number of lineages, adaptive mutations will occur in intergenic regions (red stars) allowing a re-balancing of the transcription/translation machinery and maintenance of the plasmid without a loss in fitness (bottom panel).

Figure 4



Graphical representation of the interplay between an invading MDR strain of *E. coli* (brown) and the host commensal *E. coli* (green). Upon entering the intestinal tract the MDR strain will induce a small inflammatory response, resulting in a number of by-products that can be used in anaerobic metabolism (yellow). The invading MDR strain is capable of utilising these substrates more efficiently than that host commensal, resulting in a competitive growth advantage and displacement of the host strain by the invader. Prolonged colonization then leads to a higher probability of exposure to antibiotics (Abx) and acquisition of resistance genes.

Conclusions

The last few years have led to an enormous increase in our knowledge of how AMR emerged in *E. coli* and *K. pneumoniae*. However much of what we know is still lacking strong biological evidence. The extent to which different bacterial lineages can acquire and adapt to MDR plasmids requires more experimental evidence. The precise mechanisms by which MDR bacteria displace commensal strains to initiate colonization needs to be elucidated, and whether these processes can be circumvented. Finally, despite enormous advances in genomic epidemiology, we still need properly designed and sufficiently broad studies to determine exactly how infections with these pathogens occur in hospitals, and what their reservoirs are in the hospital environment.

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Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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