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Eco-evolutionary mechanisms driving within-patient emergence of bacterial antimicrobial resistance

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Abstract: The eco-evolutionary mechanisms of AMR emergence within patients and how these vary across bacterial infections are poorly understood. Increasingly widespread use of pathogen genome sequencing in the clinic now enables deeper understanding of these processes. Here, we review the clinical evidence supporting four major mechanisms of within-patient AMR emergence in bacteria: (i) spontaneous resistance mutations, (ii) *in situ* horizontal gene transfer of resistance genes, (iii) selection of pre-existing resistance, and (iv) immigration of resistant lineages. Within-patient AMR emergence occurs across a wide range of host niches and bacterial species, but the importance of each mechanism varies between bacterial species and infection sites within the body. We identify potential drivers of such differences and discuss how eco-evolutionary analysis could be embedded within clinical trials of antimicrobials, which are powerful but underutilised tools for understanding why eco-evolutionary mechanisms vary between pathogens, infections and individuals. Ultimately, improving understanding of how host niche, bacterial species, and antibiotic mode of action combine to govern the eco-evolutionary mechanism of AMR emergence in patients will enable more predictive and personalised diagnosis and antimicrobial therapies.

1.0 - Introduction

The antimicrobial resistance (AMR) crisis threatens to endanger modern medicine within the next 20-30 years[1], with an estimated 4.95 million deaths associated with bacterial AMR in 2019[2]. Whilst the transmission of antibiotic resistant infections between patients, animals and their environment has received much attention[3–7], a key part of the problem that is less well studied is the evolutionary emergence of bacterial AMR within patients during treatment[8,9]. Low-cost pathogen genome sequencing is increasingly revealing instances of within-patient emergence of AMR across a wide variety of infection types, therapeutic regimens, and important bacterial pathogens (including *Mycobacterium tuberculosis*[10], *Pseudomonas aeruginosa*[11,12], *Staphylococcus aureus*[13,14], and *Klebsiella pneumoniae*[15]). Moreover, the emergence of AMR within-patients has been associated with treatment failure, increasing morbidity, and increased numbers of deaths[16].

The emergence of AMR within patients is driven by antibiotic-mediated selection for resistant genotypes, promoting the dominance of resistant lineages over their susceptible competitors[9]. Indeed, at the population level increased outpatient antibiotic use correlates with increased antibiotic resistance prevalence[17,18], in part due to increasing selection for resistance within treated patients. Less clear, however, are the precise ecological and evolutionary mechanisms (termed collectively eco-evolutionary mechanisms here for brevity) through which AMR emerges within patients, their relative importance, and how this varies among bacterial pathogens, infection sites and with host ecological factors (such as, nutrient supply, turnover, or immune responses, among other factors explored further in the third section of this review). This is of clinical importance, as the speed of resistance emergence will likely vary depending on which eco-evolutionary mechanism predominates in a particular host niche and infection: for example, the presence of hypermutable strains, pre-existing resistant lineages, or mobile elements capable of transferring resistance genes *in situ* can all theoretically accelerate resistance emergence within patients[19]. Different eco-evolutionary modes of resistance emergence can additionally have contrasting effects upon the long-term stability and persistence of resistance: on average, horizontally transferred resistance genes impose lower fitness costs compared to spontaneous resistance mutations[20], meanwhile the magnitude of fitness costs can vary according to the antibiotic target and/or between bacterial pathogen species[21]. Consequently, understanding which evolutionary mechanism dominates in a particular infection setting could guide the optimal treatment strategy. For example, informing the optimal choice of antibiotic and/or other interventions, such as decolonisation procedures to remove pre-existing resistant lineages or stricter infection control measures to limit opportunities for exogenous resistant lineages to superinfect.

While there is a rich body of theory and lab experiments investigating the molecular and eco-evolutionary mechanisms of AMR, such approaches inevitably do not capture many key features of the complexity of within-host environments likely to mediate AMR emergence *in vivo*, including the physiological heterogeneity of infection sites as well as the host's immune responses. Although evidence for the clinical importance of within-patient bacterial AMR evolution is rapidly growing, there is little crosstalk between these fields limiting the translation of eco-evolutionary concepts into the clinic. Here, we review the fast-growing body of clinical case reports describing within-patient AMR emergence and synthesise this clinical literature with eco-evolutionary theory to classify the underlying causal mechanisms driving within-patient AMR emergence in the clinic. We focus on bacterial infections, contrasting a wide range of pathogens and infection sites, and using studies documenting emergence of resistance within patients, where individuals were sampled before and after antibiotic treatment and the genetic mechanisms of resistance responsible were identified. Our focus is distinct from previous AMR reviews, which nonetheless are highly complementary to ours, including those focusing on a within-patient dynamics of AMR in single-species[10,15], population genetics/genomics of AMR[22], molecular mechanisms of AMR[23], or One-Health[24] and environmental dimensions[25,26] of AMR. We highlight gaps in current understanding and identify how current practices, particularly pertaining to clinical trials for new antibiotic treatments, could be augmented to deliver greater understanding of eco-evolutionary mechanisms of AMR emergence, which will ultimately improve prediction and control of AMR. More accurate prediction of AMR emergence may allow for more informed and personalised antimicrobial treatment plans, to slow the rate of resistance emergence, increase the efficacy of existing treatments, and facilitate the development of novel therapies.

2.0 - The eco-evolutionary mechanisms of within-patient AMR emergence

We highlight four major eco-evolutionary mechanisms by which AMR can emerge: (i) spontaneous resistance mutations, (ii) *in situ* horizontal gene transfer of resistance genes, (iii) selection of pre-existing resistant lineages, and (iv) immigration of resistant lineages. Broadly, these can be defined as either *de novo* mechanisms driven by the formation of new resistant genotypes (i and ii), or mechanisms selecting on pre-existing (or standing) genetic variation for resistance (iii and iv) (Fig. 1). In this section, we summarise the clinical evidence for these mechanisms occurring within-patients treated with antibiotics and discuss how these mechanisms may modulate the response of an infection to antimicrobial therapy. It is important to note that these mechanisms are not necessarily mutually exclusive, they may co-occur and interact, and in many clinical situations the boundaries between mechanisms may be blurred and/or the necessary evidence may not exist to definitively distinguish

between them. Key challenges of identifying and distinguishing these mechanisms are explored further in supplementary Box S1.

2.1 - Spontaneous resistance mutations

Spontaneous resistance mutations include point mutations, insertions/deletions, and other genetic rearrangements that can increase resistance to an antimicrobial. They occur randomly within a previously susceptible population and are positively selected during antimicrobial treatment (also referred to as *de novo* mutation). Case reports of spontaneous mutations occurring within-patient during treatment are relatively common, with large numbers of single-patient case studies (e.g., for example 21 out of 27 clinical studies cited in the paragraph below are single-patient reports). This literature covers a wide range of infection sites throughout the body (Fig. 2), particularly in the lung[27–34] and bloodstream infections[35–43], but also including urinary tract infections[44–46], infections of burns[47], the endocardium[48,49], gut[50], liver cysts[51], biliary tract[36], cerebrospinal fluid[52] and implant associated bone infections[53].

Spontaneous resistance mutations occurring within patients can be grouped into three functional categories: i) Target modifying mutations that reduce antibiotic efficacy for example by reducing drug-binding. These include mutations in *gyrAB* (DNA gyrase enzyme) or *parCDE* (DNA topoisomerase IV enzyme) associated with ciprofloxacin resistance[29,39], *rpoB* (RNA polymerase β-subunit) conferring rifampicin resistance[29], *rpsJ* (Ribosomal protein subunit S10) conferring tetracycline resistance[33], or mutations affecting penicillin-binding-protein (PBP) genes for resistance to β-lactams[32,47,54,55]. Such mutations sometimes confer cross-resistance, typically to drugs of the same class or those with related targets[41]. ii) Mutations modifying pre-existing resistance genes to enhance or modify the spectrum or level of resistance they confer, e.g., modification of the *tetA* efflux pump gene resulting in enhance tigecycline export[31], and mutation of the PDC cephalosporinase gene conferring both ceftolozane-tazobactam and ceftazidime-avibactam resistance[36,56] (again, likely due to the shared mechanism of these drugs); iii) regulatory mutations affecting resistance gene expression, for example mutation of the regulator *ampD* resulting in upregulation of AmpC β-lactamase[53], loss of repression from repressors RamR or AdeS conferring tigecycline resistance through increased AcrAB[43,46] and AdeABC[57] efflux pumps, respectively, and loss of MexT regulator function derepressing *mexAB* efflux pump expression to confer ciprofloxacin and other resistances[58]. Since efflux systems commonly export multiple antibiotics, including those belonging distinct classes such as ciprofloxacin and meropenem resistance efflux by AdeABC[47], increased expression of efflux can lead to multidrug resistance.

Provided that they escape genetic drift[59], genotypes carrying new spontaneous resistance mutations are expected to increase in frequency driven by antibiotic selection, displacing their susceptible neighbours[60]. Direct evidence for such selective sweeps within patients is limited due to requiring intensive longitudinal sampling of multiple bacterial isolates to capture these evolutionary dynamics. However, within-patient selective sweeps have been observed, principally in chronic lung infections: For example, in *P. aeruginosa* lung infections, *pbpB* (penicillin-binding-protein) mutation during aztreonam therapy of one patient[55], *ampD* (β-lactamase) and *oprD* (outer membrane porin) mutation during meropenem and ceftazidime therapy[27], and *oprD*, *wbpM* (O-antigen biosynthesis enzyme) and *mexAB/oprM* (efflux pump and porin) during carbapenem and colistin combined therapy[61], have all been observed to occur by spontaneous mutation and sweep to high frequency within-patients driven by antibiotic treatment. Similarly, in a report of *M. tuberculosis* infection, resistance to multiple antibiotics emerged by spontaneous mutation and swept to high frequency in a single patient across 9 years of therapy, with rises in frequencies of mutations to the *katG*, (catalase-peroxidase enzyme), *embB* (Mycobacterial cell wall synthesis enzyme), *rpoB* (RNA polymerase β-subunit), *rpsL*/*rrs* (Ribosomal protein S12 / 16S rRNA) and *ethA* (drug-activating monooxygenase enzyme) genes[29], observed during antibiotic treatment with isoniazide, ethambutol, rifampicin, streptomycin, and ethionamide, respectively. Interestingly, in the *M. tuberculosis* study, fluctuations in prevalence of particular resistance mutations occurred during the course of therapy, with the predominant streptomycin resistance mutation switching from an *rpsL* to an *rss* mutant. Fitness costs associated with spontaneous resistance mutations can also drive their loss after treatment is withdrawn[57,62]. More complex selection dynamics among competing spontaneous resistance mutations have been observed in lung infections caused by these organisms: For example, replacement of an original *rpsL* mutation conferring streptomycin resistance by a different mutation in the same gene was observed in an *M. tuberculosis* lung infection during treatment[62]. In a *P. aeruginosa* lung infection, two coexisting *pbpB* mutations providing resistance to aztreonam fluctuated in frequency during long-term treatment with combinations of 10 different antibiotics. These *pbpB* mutations provided differing levels of cross-resistance against ceftazidime and piperacillin[55], drugs that were also used periodically during treatment of this patient.

Supply of spontaneous resistance mutations is determined by the pathogenic bacterium's population size and mutation rate, but can also be affected through stress-induced mutagenesis[63] or exposure to chemical or physical mutagens (e.g., reactive oxygen species produced by host inflammatory immune responses[64], or the antibiotic treatment itself[65,66]) which can vary with infection site. Mutation frequency may also vary during different stages of an infection, for example an elevated mutation rate

ten times higher during the initial infection phase compared to established infection has been reported for *Helicobacter pylori* infection. This study implicated elevated production of mutagenic reactive oxygen species by the host inflammatory response[67]. Note, however, other *H. pylori* studies have not reported such variation in mutation supply with infection stage[68]. Alternatively, infecting populations may evolve elevated mutation rates through gaining mutations in mismatch repair systems that substantially increase the supply of all mutations including those involved in AMR, and thus potentially accelerating the emergence of AMR[69]. Such hypermutator lineages have frequently been observed to arise in chronic lung infections, particularly in cystic fibrosis patients[54,70]. Hypermutators have been reported to contribute to within-patient AMR emergence by increasing supply of spontaneous resistance mutations in lung infections caused by *P. aeruginosa*[54,71]*, Achromobacter spp.*[30,72], *Burkholderia pseudomallei*[73]*,* and *Stenotrophomonas maltophilia*[74,75]. Hypermutable *S. aureus* genotypes with mutations affecting recombinational repair protein RecQ have also been implicated in driving within-patient AMR emergence[76]. However, beyond individual case reports, the link between hypermutation and accelerated AMR evolution is debated, with conflicting evidence on whether hypermutators really are more likely to show higher AMR than non-hypermutators on average[77].

2.2 – *In situ* horizontal gene transfer

Within-patient AMR emergence in a focal pathogen has been observed in a range of clinical studies to occur through *in situ* horizontal gene transfer (HGT)[78–83]. This evolutionary process, whereby resistance determinants are exchanged between bacterial cells, is often mediated by mobile genetic elements (MGEs), such as plasmids, transposons and bacteriophages, but can also occur via uptake of DNA from the extracellular environment (natural competence)[84]. Despite long-standing interest in HGT of AMR, reports of this process within-host are almost entirely confined to the gut[78,80–83], with some limited reports of MGEs aiding AMR evolution through transposons increasing resistance gene copy number in bloodstream infection[85]. It is unclear whether this pattern represents a fundamental difference between the gut and other niches (e.g., higher microbial diversity or the species causing infections, explored further in the third section of this review) or a bias in the literature.

Of particular concern are cases where harmful bacterial pathogens can gain new resistance genes by HGT from coexisting commensals within the patient's microflora, which has most commonly been observed in the gut where high diversity microbial communities coexist at high densities[86,87]. Plasmid transmission in the gut is likely highly frequent. For example, *Clostridium difficile* gained a 46 Kbp conjugative plasmid with the *ermB* clindamycin resistance gene from enteric commensal

Faecalibacterium prausnitzii during therapy with clindamycin[80]. *K. pneumoniae* isolated from the gut of a patient undergoing treatment for bacteraemia gained a 36 Kbp plasmid, enabling duplication of several resistance genes[81] during therapy with a variety of antimicrobials. A *Salmonella enterica* infection treated with ceftriaxone has also been reported gaining a 309 Kbp plasmid within-patient containing three β-lactamase genes ($bla_{\text{CTX-M15}}$, $bla_{\text{TEM-1b}}$, $bla_{\text{OXA-30}}$) conferring ceftriaxone resistance, transferred from an unknown donor species, but likely one co-existing in the gut microflora[88]. Additionally, extensive within-patient transfer of a $bla_{\text{OXA-48}}$ β-lactamase carrying plasmid pOXA-48 has been shown to occur in the gut microbiomes of intensive care patients. The same plasmid was isolated from 8 different bacterial species across 105 different patients and transfer of the plasmid was directly observed in at least five different patients[82]. Transfer of this plasmid has also been reported within the gut of a patient from *K. pneumoniae* to *Escherichia coli* during amoxicillin treatment[78]. This pOXA-48 plasmid acted not only as a vehicle for AMR transfer, but also as a catalyst for the evolution of altered levels of resistance: In one patient receiving meropenem treatment, pOXA-48 evolved a higher plasmid copy number conferring enhanced meropenem resistance, whereas in other patients, the bla_{OXA-48} gene was lost from the plasmid, reducing the plasmid fitness cost in the absence of antibiotic[83].

Transposable elements also play an important role mobilizing AMR genes between bacterial chromosomes, plasmids and other MGEs, and have been shown to contribute to within-patient AMR emergence. For example, the bla_{KPC-53} carrying transposon Tn4401 providing resistance to imipenem/relebactam expanded in copy number during therapy with ceftazidime/avibactam by transposing to multiple plasmids in the *K. pneumoniae* genome leading to enhanced resistance[85]. Transposable elements are also important vectors for transferring resistance mechanisms between lineages, for example IS26-mediated transfer of the bla_{NDM-1} metallo-β-lactamase drove a multi-species hospital outbreak of carbapenem resistant infections, with spread of the bla_{NDM-1} gene across *E. coli*, *K. pneumoniae*, *Citrobacter freundii*, *Morganella morganii* and *Enterobacter cloacae* isolates, the majority of which were isolated from rectal swabs[89].

The importance of bacteriophages in driving *in situ* HGT of AMR within patients is debated. Analysis of phage genomes from human microbiomes found that these rarely encode AMR genes[90], a pattern supported by data from pigs[91]. However, a study of human faecal phages found higher frequency of AMR genes in phage particles following ciprofloxacin treatment[92], suggesting phage transduction may more commonly transfer AMR in patients exposed to antibiotics and in particular those known to induce phage lysis, such as ciprofloxacin[93]. For some organisms, transduction is well known to play an important role in adaptation to the human host. This is the case for *S. aureus*[94], for which efficient

transduction of a β-lactamase-containing plasmid has been demonstrated *in vitro*[95], indicating this mechanism of AMR transfer may be likely to also act within-patient.

2.3 - Selection of pre-existing resistant genotypes

Resistant genotypes are sometimes stably present prior to treatment at an appreciable frequency detectable by standard culturing within an infection, for example due to prior history of treatment with the same antibiotic. Selection of such pre-existing resistant genotypes can accelerate AMR emergence by negating any waiting time for new spontaneous mutations to arise[96], and can display lower fitness costs[97]. Moreover, by being present at appreciable frequencies, pre-existing resistant genotypes are less likely lost to drift and can more rapidly increase in frequency upon commencement of treatment than any spontaneous mutant[60]. Mixed-strain lung infections of *P. aeruginosa* evolve resistance faster due to selection of pre-existing resistant strains, with mixed-strain infections showing a ~20% greater increase in resistance after treatment than single-strain infections[98]. In the same study, patients with pre-existing resistant strains ranging in their pre-treatment frequency from ~5% to ~60% displayed rapid increases in the frequency of these resistant strains upon treatment. Examples of pre-existing resistant lineages contributing to the evolution of AMR within patients have been reported for both acute and chronic infections of the lung[27,98,99], the stomach[100,101], and some evidence for this effect in the bowel[102]. As the global prevalence of resistant infections increases[103], this mechanism may be increasingly common as infection or colonisation with pre-existing resistant bacterial lineages becomes more likely.

Antibiotic treatment has been shown to trigger rapid increases in pre-existing resistance allele frequency within an infecting population: during acute *P. aeruginosa* infection of a single patient, *nalD* (*mexAB-oprM* efflux pump repressor)*, anmK* and *sltB1* (both peptidoglycan metabolism enzymes) resistance alleles at 7-8% frequency pre-treatment increased to 44-49% frequency after 12 days of combination therapy which started with piperacillin-tazobactam, followed by ciprofloxacin and cefepime[27]. Such selection works both ways however: the same study documented a separate patient for which two independent alleles of *mexR* (repressor of *mexAB-oprM* efflux pump) conferring levofloxacin resistance went extinct within 5 days of treatment with piperacillin-tazobactam only, indicating purging of pre-existing resistance alleles that provided no selective advantage under the prevailing treatment regime.

Clinical studies further suggest that the frequency of a pre-existing resistant lineage can offer an indication of the likelihood of resistance evolution upon treatment: for example, across 200 *M.*

tuberculosis lung infections, the presence of pre-existing resistant lineages at frequencies of ≥19% was found to be highly predictive of subsequent fixation of resistance following antibiotic treatment[104]. Complete fixation of pre-existing resistant lineages following treatment does not always occur however, and can be highly localised to specific host niches. For example, in *H. pylori* stomach infections, pre-existing resistance against clarithromycin, ciprofloxacin, and metronidazole were rarely fixed following antibiotic treatment, with 20-60% of isolates remaining drug sensitive[100]. Moreover, from the same study, all isolates from one patient's stomach corpus were resistant to ciprofloxacin, but all isolates from the stomach antrum remained susceptible. This example highlights the potential complexities of resistance mutation dynamics along with the variable impacts of infection biogeography within hosts.

The probability of pre-existing resistant lineages being present can depend on a variety of factors. Prior antibiotic therapy is a probable driver as this can result in dynamic shifts in microbiome community structure and stable increases in the frequency of AMR lineages after treatment ceases[105,106], which in turn can increase the future risk of both antibiotic resistant and sensitive infection for a particular patient[107]. Another likely cause, especially in the context of chronic infection, is the duration of the patient's infection because multiple generations of replication during establishment of infection can enable diversification and the accumulation of resistance mutations before any antibiotic is given: for example, up to 4% of patients colonised by *H. pylori* gain resistance mutations during the infection establishment phase but before antibiotic treatment, which subsequently drove treatment failure even for sequential multi-drug treatment regimes[101]. The longer evolutionary history of pre-existing resistant lineages within a patient may enable compensatory evolution, reducing fitness costs of resistance and enhancing their long-term persistence.

2.4 - Immigration of resistant genotypes from elsewhere in the body

Even if antibiotic treatment is successful at clearing the focal bacterial pathogen infection from a particular body site, an AMR infection can still potentially occur by immigration of resistant genotypes from elsewhere in the body. This ecological process, also referred to as strain translocation or strain replacement, has received less attention than the other eco-evolutionary mechanisms, but appears to be important for certain kinds of infections and body sites, including the urinary tract[108], lungs[109], skin and wounds[110,111], and bloodstream infections[112]. Exogenous sources of resistant strains, for example from the wider hospital environment, can also play a role in AMR transmission distinct from within-host processes, but are not our focus here and have been reviewed elsewhere[113].

The most robust evidence to date for immigration of resistant genotypes from other sites within the patient's body driving resistance emergence is a recent study investigating ~140,000 urinary tract infection (predominantly caused by *E. coli*) and ~7000 wound infections (predominantly caused by *S. aureus*). Here, the predominant mode of AMR emergence was immigration of resistant lineages to the site of infection from within the patient's own microflora[108]. This included immigration of resistant lineages belonging to other species (*K. pneumoniae* and *Proteus mirabilis*) when patients had been treated with antibiotics against which resistance in *E. coli* is rarely seen (fosfomycin and nitrofurantoin), or invasion by other *E. coli* lineages already resistant to ciprofloxacin (*gyrB* and *parC* mutations) even though resistance evolution by spontaneous mutations of these genes in *E. coli* is well documented. Similarly, in a study of four patients with MRSA soft tissue infections, in two patients strain replacement by MRSA lineages resistant to aminoglycoside and fluoroquinolone antibiotics drove emergence of resistance upon treatment with antibiotics from these drug classes[110]. Strain replacement has also been reported for *K. pneumoniae* lung infections, where carbapenem resistant lineages of a different sequence type were observed to replace drug sensitive lineages in 36 of 44 patients who received carbapenem therapy[114] - however this study does not conclusively rule out that the invading sequence types were not present prior to treatment. A vancomycin sensitive *Enterococcus faecium* lineage from a bloodstream infection was replaced during vancomycin therapy by resistant lineages that were present alongside the sensitive strain in the patient's gut, from where the bloodstream infection had arisen[112]. Similarly, immigration of resistant genotypes from within the patient's own gut microbiota has also been observed in pneumonia[115], sepsis, and acute respiratory distress syndrome[116]. A key challenge here is that few studies definitively identify the source of the immigrating resistant strain, in part because this requires in depth study of the patient's microbiome and potentially the wider hospital environment, for example using metagenomics, which is costly and raises ethical considerations.

2.5 - Mixed and interacting mechanisms

Although it may often be challenging to distinguish between different eco-evolutionary mechanisms due to the practical limitations of clinical studies (explored further in supplementary Box S1), in other cases, multiple mechanisms could be at play and potentially interact, complicating interpretation. There are several clinical reports where immigration of strains from another body site is followed by the evolution of resistance by spontaneous mutation. These include a chronic MRSA wound and bloodstream infection, which was treated with multiple Gram positive-targeting antibiotics (daptomycin, clindamycin and vancomycin) driving replacement of MRSA by a succession of three genetically distinct lineages of *Enterobacter hormaechei,* one of which then evolved meropenem resistance by spontaneous mutation[111]. Similarly, in a lower respiratory tract infection, immigration

of a *P. aeruginosa* strain from the gut to the lung was followed by evolution of enhanced resistance through spontaneous mutation at both body sites[115]. These and other studies highlight the risk that systemic antibiotic treatments are likely to enrich for resistance at non-targeted body-sites, potentiating subsequent immigration of resistant lineages from this reservoir[112].

3.0 - Genetic, ecological, and host factors shaping within-patient AMR emergence

Our review of the clinical literature highlights that each eco-evolutionary mechanism occurs within patients. In the following section, we explore the factors driving variation in the predominance of these mechanisms across infections, including taxonomic/genetic, ecological, and host factors, and synthesize the evidence for their action within-patients. The probability of each eco-evolutionary mechanism is likely to vary with each of these factors, bearing implications for any attempts to predict which mode is likely to predominate (Box 1). In addition, since AMR emergence has been subject of intensive theoretical and experimental evolution investigation[117–121], we also highlight other factors known to influence AMR emergence in these *in silico* and *in vitro* studies, and consider how these fundamental discoveries could be translated to improve understanding of clinical AMR emergence.

3.1– Variation between bacterial species and strains

It is unlikely that any bacterial species evolves AMR exclusively through a single eco-evolutionary mode, but some species appear to preferentially use certain modes more than others. This is evident in contrasting pangenome structures among species, wherein higher proportions of accessory (genes variable among strains) versus core (genes common to all strains) genome compartments indicates greater propensity for HGT. For example, whereas the accessory genome makes up ~80% of the *E. coli* pangenome, the accessory genome is ~0-1% of the *M. tuberculosis*[122] pangenome. Accordingly, *M. tuberculosis* is rarely reported engaging in HGT[122], perhaps owing in part to limited interactions with other bacteria due to its intracellular infection lifestyle. Thus, HGT is a relatively unimportant mode of AMR emergence for this organism, and *M. tuberculosis* is primarily reported to evolve AMR through spontaneous mutation[99,123,124]. Similarly, an epidemiological study of *Streptococcus pneumoniae* carriage found that the rate of HGT was unimportant for determining the frequency of AMR, which was better explained by selection for pre-existing resistance[125]. Conversely, HGT of resistance likely plays an important role in pathogens with larger accessory genomes, such as *S. aureus, E. coli,* and *K. pneumoniae,* where MGEs make up substantial fractions of genomes and commonly encode resistance genes[94,126,127]. The drivers of taxonomic differences in rates of HGT are not well-understood but are likely to have complex ecological and genomic causes, for example intracellular pathogens (like many symbionts) are cut-off from the supply of mobile genetic elements,

whereas genome defence systems, including restriction modification or CRISPR-Cas, can act as barriers to gene exchange.

Mutation supply rates can vary extensively between species[128], among strains within species[128], and even for specific resistance-conferring genes[129], within a genome. Such differences are due to a variety of molecular and genetic factors, which can additionally vary with prevailing physiological and environmental conditions[130]. Additionally, the frequency of hypermutator lineages varies extensively, ranging from 0.5-2% in *E. coli* and *Haemophilus influenzae*, up to >50% in *Neisseria meningitidis* and *P. aeruginosa* infections[131]. Higher mutation supply can potentiate spontaneous resistance mutations and/or promote the accumulation of pre-existing standing genetic variation, potentially enhancing the contribution of either of these modes of AMR emergence. Note, however, that hypermutation can be detrimental after the initial gain of resistance due to subsequent accumulation of deleterious mutations reducing fitness, particularly in highly competitive polymicrobial communities[132]. Accordingly, there are extensive case reports of *P. aeruginosa* evolving AMR by spontaneous mutation within lung infectios[28,36,54,133–135], as well as bloodstream infection[71] and infections of the gut[115]. Nonetheless, both *M. tuberculosis*[62,104] and *P. aeruginosa*[27,98] have also been reported to evolve AMR via selection upon pre-existing resistant lineages, and for *P. aeruginosa* immigration of resistant genotypes from other body sites also plays a less well-studied role[115]. Differences in evolvability between lineages can arise for a range of other reasons besides mutation supply. Gain of resistance determinants by HGT can potentiate subsequent evolution of resistance by spontaneous mutation[136], leading to differences in AMR evolvability attributable to the presence/absence of key genes, such as efflux systems[137], per lineage. Similarly, allelic variation in genes not directly related to resistance, such as metabolic genes that alter antibiotic tolerance[138] can potentiate subsequent gain of resistance[139], as well as influence the ability of MDR strains displace commensal bacteria in competitive host niches[140]. Indeed, bacterial persistence, the tolerance of antibiotics through cellular dormancy, has been shown in lab studies to enable subsequent evolution of resistance[141], although such dynamics have not yet been demonstrated in patients.

More comparative studies focusing on a wider range of pathogens are required to quantitatively determine what drives variation in the relative contribution of the different eco-evolutionary modes of AMR emergence between taxa.

3.2 - Variation between patient body sites

The human body constitutes a diverse range of niches, differing in moisture, pH, salinity, nutrient availability, and commensal microflora abundance and diversity[142]. These factors are all likely to

influence the predominant eco-evolutionary modes of AMR emergence. Ecological factors, such as transmission bottlenecks, the pathogen population size present at a body site, and the rate of population turnover are likely to influence key evolutionary parameters and vary between infection sites. For example, smaller transmission bottlenecks can favour more frequent but costlier resistance mutations, the supply of spontaneous resistance mutations will scale with population size[60,143], whereas higher rates of population turnover may strengthen selection against more costly resistance mechanisms[144]. Crucially, the properties of these parameters are rarely well-understood within patients, except perhaps at the coarse-grained level of differences of magnitude between infection sites.

Whereas some host niches contain abundant, diverse microbial communities (the gut, oral cavity, upper respiratory tract, skin and vagina), others may be transiently colonised or contain far lower microbial abundances (lower respiratory tract, urinary tract) or are typically sterile (blood, cerebrospinal fluid, brain, bone, liver, kidneys, spleen)[145]. Accordingly, the presence and abundance of AMR genes will vary between body sites[146], and this may promote or constrain particular eco-evolutionary modes of AMR emergence. For example, the gastrointestinal tract supports high bacterial abundance and diversity, and consequently high AMR gene diversity[87,147], and as such is associated with high levels of HGT of AMR, particularly mediated by conjugative plasmids[86,148]. By contrast, HGT of AMR is rarely reported in body sites with low bacterial abundance and/or diversity, including the bloodstream, urinary tract, lower respiratory tract, and normally sterile sites including internal vital organs. However, it must be noted that due to low accessibility and fewer studies of such sites, this apparent pattern could in part be caused by under-sampling. Diverse microbial communities have been shown to limit the invasion of costly resistance or hypermutators across a variety of studies[132,149,150], whereas species interactions within diverse communities can promote increased resistance, tolerance or protection of susceptible strains against antibiotics through a variety of ecological mechanisms[151].

Body sites also vary extensively in their "openness" to immigration of bacteria from other body sites. Immigration of bacteria from the gastrointestinal tract to the urinary tract is particularly common owing to the physical proximity of these niches. Accordingly, AMR evolution in *E. coli* urinary tract infections is dominated by immigration of resistant lineages from the gut[108], a common reservoir for drug resistant *E. coli*[152], with only sporadic reports of *E. coli* evolving AMR by spontaneous mutation in the urinary tract[44,45,153]. Other clinical examples of immigration of resistant lineages include ventilator associated pneumonia and wound infections, both of which are also relatively "open" body sites where immigration of bacteria from other body sites is probable in hospital settings. It should

also be noted that the "openness" of a body site can change, particularly during critical illness, wherein gut permeability can increase, leading to greater spreading of commensal bacteria and opportunistic pathogens to other body sites[154]. Conversely, for more sheltered body sites that offer significant isolation from microbial communities inhabiting other body sites, reports of within-patient AMR emergence most commonly involve spontaneous mutation. For example, an infection of liver cysts by *E. coli*[51], and an implant-associated *Cedecea davisae* bone infection[53] both evolved β-lactam resistance through spontaneous mutations in *ampC* and *ampD* (both β-lactamases) respectively. Additionally, a cerebrospinal fluid infection by *Staphylococcus capitis* evolved rifampicin resistance through *rpoB* (RNA polymerase β-subunit) mutation[52], and infections of the endocardium by *Staphylococcus* spp. evolved daptomycin[49], rifampicin[48], and vancomycin[42] resistance through *mprF/ yycH* (phospholipid synthesis enzyme / regulator of cell envelope turnover) and *rpoB* mutations respectively.

Mode of delivery and the pharmacokinetic properties of antimicrobials also vary between body sites, influencing which eco-evolutionary mode predominates and the wider impact of treatment on AMR within the patient microbiome more generally[150]. In accessible body sites, where drug can be delivered topically, orally, or by inhalation, it is likely that a high dose can readily be achieved, which could limit AMR emergence by spontaneous mutation[155], favouring other modes such as pre-existing variation, or immigration where resistant lineages are present at appreciable frequencies prior to treatment. By contrast, spontaneous mutation may be more favoured in body sites where it is challenging to achieve an effective dose[156] due to low drug penetrance. The systemic distribution of an antibiotic will also influence resistance selection in other body sites, and thus the risk of immigration of resistance to the infection site, or the distribution of resistance reservoirs within the host, relevant to future infections. For example, meropenem treatment for a urinary tract infection selected for meropenem resistant *P. aeruginosa* present in the gut, which subsequently immigrated to the lungs of an ICU patient[115].

3.3 - Variation with infection duration

Both acute and chronic infections can provide ample opportunity for spontaneous resistance mutations to arise, provided sufficiently large numbers of generations occur during population expansion. Chronic infections provide even greater potential for the accumulation of pre-existing resistant genotypes, because these infecting populations genetically diversify *in situ* and such high genetic diversity is typically stably maintained[157–159]. Moreover, hypermutator phenotypes are a common feature of many chronic infections[160–162], further potentiating genetic diversification and the

emergence of AMR. Chronic infections are likely to have already experienced rounds of treatment, leading to accumulation of resistant lineages within the patient that further complicate subsequent treatments[163].

Accordingly, chronic infections are expected to have a higher likelihood of resistance emergence[164]. For example, during persistent bacteraemia caused by *S. aureus*, significant standing diversity is generated during establishment of infection, with ~50% of patients acquiring resistance mutations prior to treatment[38], that were subsequently selected during the chronic stage of the infection. As a result, resistance against a wide range of antibiotics is observed in these chronic infections, including vancomycin, daptomycin, linezolid and trimethoprim/sulfamethoxazole and ciprofloxacin resistance[37,39,165]. Chronic infections may also offer greater opportunity for rare events to occur, such as HGT and immigration of resistant lineages. For instance, immigration of resistant strains is observed in ~14% of patients with persistent *S. aureus* bacteraemia[40].

3.4 – Variation with bacterial lifestyle

Both chronic[166], and acute[167] infections of some body sites, such as the lungs, commonly adopt a biofilm lifestyle, which could potentially influence the eco-evolutionary mode of resistance emergence. The biofilm matrix and multicellular structure can offer physical protection from antibiotics, which could reduce the effective dose and potentiate the emergence of resistance by spontaneous mutation if, for instance, this requires the accumulation of multiple small effect mutations[155]. Moreover, biofilms can promote genetic diversification and stabilise coexistence of multiple genotypes through spatial structure[168], potentially enabling accumulation of pre-existing standing genetic variation for resistance. Biofilm structures can additionally generate oxygen gradients[169], and be comprised of multiple microbial species, which can both further affect susceptibility to antimicrobials[170,171]. How such factors impact the eco-evolutionary mechanisms of AMR emergence is not well characterised within-patients, however hypoxia can induce elevated mutation rates and alter mutational spectra *in vitro* for *E. coli*[172] and alter the costs of resistance genes in *S. aureus*[173]. The effect of biofilms on HGT appears to be complex, in some cases enhancing rates of HGT[174], but their spatial structure can also inhibit the spread of mobile genetic elements reducing HGT[175].

3.5 – Variation with host immunity

Host immune responses have been shown to alter eco-evolutionary modes of AMR evolution. For example, effective host immunity suppresses diversification of *Acinetobacter baumanii,* which is reversed in immunosuppressed patients[176]. The immune system can also be vital for clearance of resistant infections once they are established. For example, meropenem resistant strains of *P. aeruginosa* that arose by spontaneous mutation were driven extinct by the host immune response in an acute lung infection[61]. Mathematical modelling also predicts that strong immune responses that persist even after bacterial population decline due to antibiotic treatment reduces the likelihood of evolution of resistance[177]. Additionally, pathogen immigration to an infection site is typically suppressed by the immune system, an effect that is reduced as immune function declines[178], potentially promoting immigration of resistant strains. Bacterial growth at an infection site can also alter immune function, for instance both *P. aeruginosa* and *S. aureus* generate microaerophilic or anaerobic infection microenvironments[179,180], affecting their sensitivity to antimicrobials[181,182] and impairing immune cell activity[183]. Available clinical data suggest that very ill patients with impaired immunity, such as those in intensive care units, are especially prone to secondary infection by resistant strains immigrating from other body sites. For example, in a study of 310 immunocompromised patients with bloodstream infections, 31% suffered from reinfection, which included 3 confirmed cases of reinfection with MDR bacterial strains[184]. Additionally, in a CF patient receiving a lung transplant, the allograft lungs were re-colonised by resistant *P. aeruginosa* from the patient's sinuses, during prophylactic antibiotic treatment with azithromycin, imipenem and tobramycin[185]. Despite its clear potential to impact within-patient AMR emergence, the role of the immune system remains relatively understudied. Further studies testing how host immunity shapes the mode of eco-evolutionary AMR emergence are urgently required.

4.0 – Future directions and Concluding remarks

Our review demonstrates that all four eco-evolutionary mechanisms of AMR emergence occur in the clinic, varying in their relative importance between infection sites and pathogen species. There are, however, important limitations of the current literature (Fig. 3A; explored in-depth in Box S1): First, it is likely that predominance of single-patient case reports biases the literature towards serious illness or unusual treatment outcomes. Moreover, this absence of replication at the patient-level limits our understanding of the unbiased real-world incidence of each mechanism, particularly those that occur more rarely. Secondly, bacterial population sampling per patient, especially prior to treatment, is very rarely adequate to confidently rule out alternate mechanisms, and it is therefore possible that reports of spontaneous mutations may instead be due to pre-existing resistance present at too low frequency to be sampled. Thirdly, clinical studies often lack control groups, and finally, lack access to patient metadata, particularly previous antimicrobial therapy, limiting our ability to understand what drives

variation in mechanisms of AMR emergence between patients. Together these limitations make identifying and distinguishing between eco-evolutionary mechanisms challenging. Many of these limitations could be overcome by integrating eco-evolutionary studies within future clinical trials of antimicrobial treatments (Box S1). Although such clinical trials often measure changes in pathogen load, it is much less common that resistance emergence or the associated genetic mechanisms are measured (Box 2). Whilst adding such analyses universally is unlikely to be cost-effective, routine biobanking of patient samples derived from antimicrobial clinical trials, as outlined in Fig. 3B, alongside consent for their re-use for eco-evolutionary analysis may provide an economical solution that would transform our understanding of AMR emergence.

Within-patient emergence of AMR is a significant clinical issue, and better understanding of the eco-evolutionary mechanisms through which emergence can occur will aid future therapeutic design and development. Our synthesis of the clinical literature reveals four eco-evolutionary mechanisms, each of which operates within human infections, but which vary in their importance among pathogens and between body sites and infection types. Despite this, significant gaps remain in our understanding, particularly around the frequency and importance of each eco-evolutionary mechanism in particular clinical settings. Better understanding of this would help to explain why resistance evolves in some scenarios and patients but not others. Eco-evolutionary theory provides a framework to understand and predict this variation in treatment outcome. Predicting within-patient AMR emergence on the basis of a deep mechanistic understanding of the eco-evolutionary processes has enormous potential to guide improved treatment decisions that limit AMR, extending the longevity of existing and new antimicrobial drugs. Such a personalised approach to treating individual infections, guided by eco-evolutionary principles could in future pave the way for the development of more robust and durable antimicrobial therapeutics, ultimately benefiting global health and improving patient outcomes. Translation of this body of eco-evolutionary theory requires that we embed this perspective within clinical studies and clinical trials. Achieving this will require the involvement of regulators and pharmaceutical companies to prioritise the study of AMR in clinical trials through improved trial designs and biobanking of samples for re-use in eco-evolutionary studies.

END OF MAIN TEXT

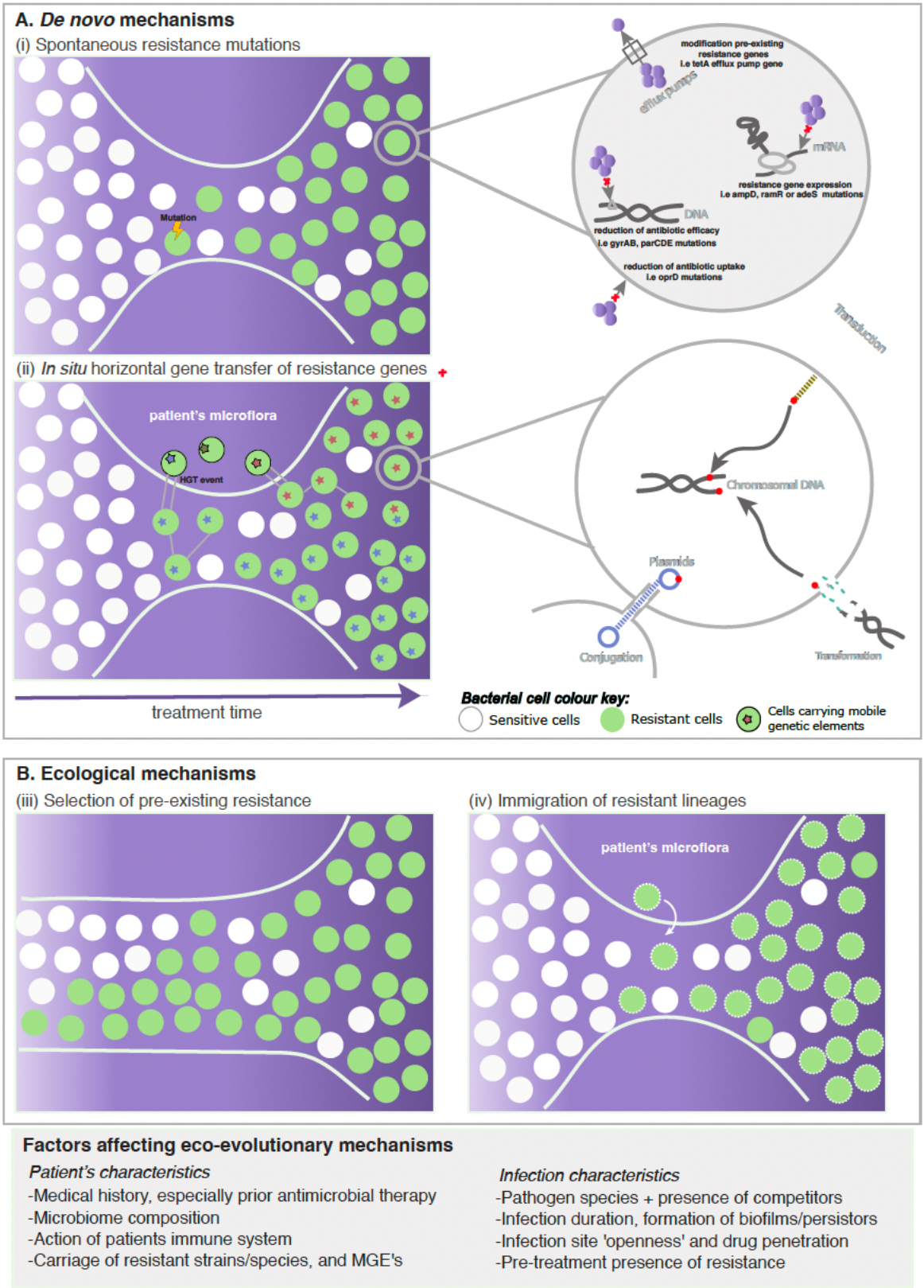


Figure 1: Four eco-evolutionary mechanisms of within-patient emergence of antimicrobial resistance. A) Resistance emergence through *de novo* evolutionary mechanisms: spontaneous mutation and horizontal gene transfer. Upon commencing antimicrobial treatment, the infecting bacterial population will decline (indicated in the left hand panels by the number of sensitive cells

(white) between the white borders, with treatment time running left to right) due to the negative effect of therapy and/or the immune system on bacterial growth. Spontaneous mutations arise continuously at random within a bacterial population, and if a mutation occurs that reduces susceptibility to the antibiotic treatment, these nascent resistant cells (green) will then increase in frequency along with an expansion of the infection population due to escape from the effects of therapy. These cells will have gained a resistance determinant - common mechanistic bases of these are indicated within the highlighted cells on the right hand side. Spontaneous mutations will typically act through i) reducing antibiotic efficacy, for example by reducing drug-binding or drug uptake ii) regulatory mutations affecting resistance gene expression or the activity of resistance determinants for example efflux pumps, and iii) modifying pre-existing resistance genes to enhance or modify the spectrum or level of resistance they confer, for example modification to drug-inactivating enzymes or export pumps. Spontaneous mutations often incur fitness costs negatively impacting growth of resistant cells in laboratory conditions, which may affect their survival within patients. Horizontal transfer of resistance genes within-patients occurs through three key mechanisms - conjugal transfer of plasmids, bacteriophage transduction, or uptake of DNA from the cell's environment (natural competence). B) Ecological mechanisms of resistance emergence. Selection of pre-existing resistance will occur immediately upon start of treatment, and may reduce the impact of treatment on bacterial population size. Resistant cells (green) will increase in frequency as sensitive cells (white) decline in frequency as treatment progresses, and the infecting population will continue to expand, escaping the inhibitory effect of the drug. Pre-existing resistant cells may be stably present at an appreciable frequency within an infecting population due to prior treatment with the same antibiotic, and due to their longer evolutionary history may already have undergone compensatory evolution to reduce fitness costs of resistance. In the case of immigration of resistant lineages, a resistant strain or species will be transferred to the infection site during therapy, which may occur from the host microflora or from elsewhere. This may occur at any time, however as the infection is cleared by the antimicrobial treatment, this reduces competition for an invading bacterial strain or species, which may aid its establishment at the infection site. The resistant lineage is then selected for, and the infecting population of this lineage may expand. Factors that can affect the probability and action of these eco-evolutionary mechanisms of within-patient AMR emergence are listed beneath the panels, broken down into patient and pathogen. A patient's medical history (in particular prior treatment with antimicrobials), and the nature of their infection will significantly impact likelihood of these eco-evolutionary modes of AMR emergence.

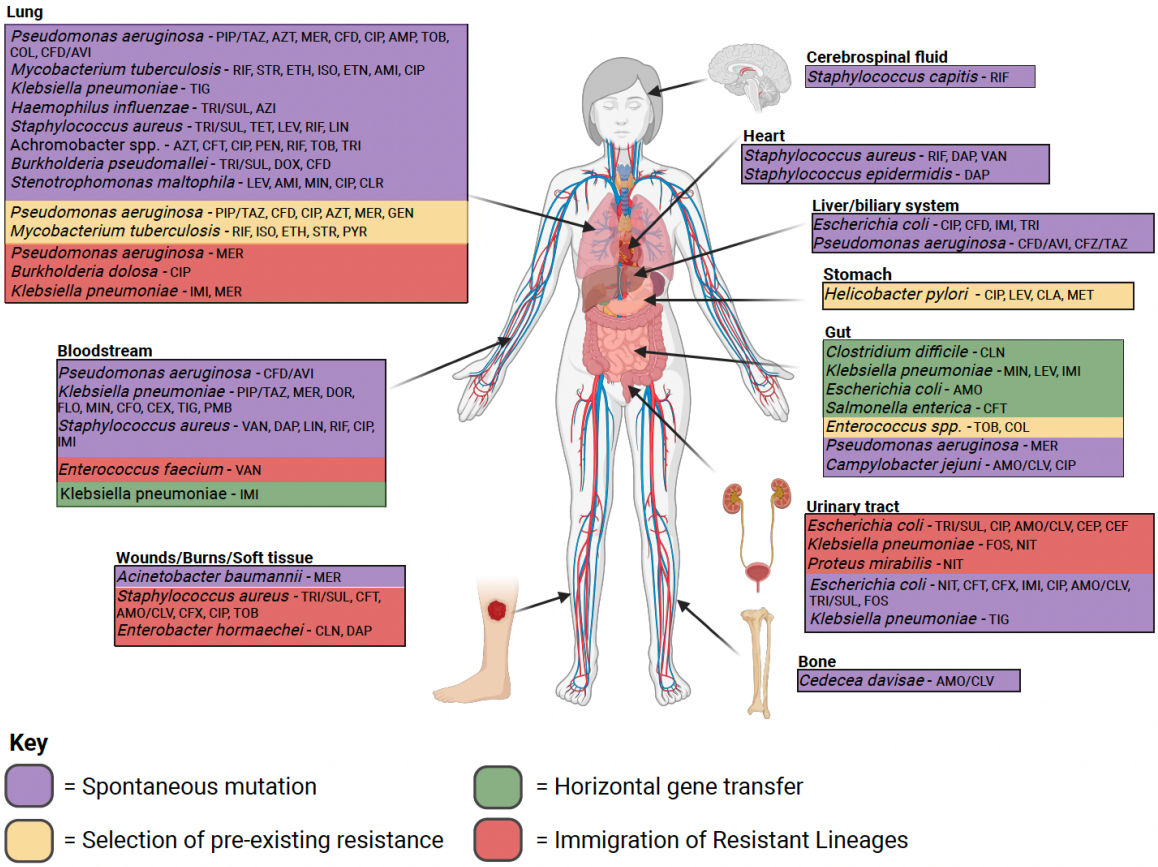


Figure 2: Reports of within-patient emergence of antimicrobial resistance across eco-evolutionary modes, body sites, organisms, and antibiotics. Studies of within-patient AMR emergence included in this review cover a wide range of body sites, antimicrobials and bacterial pathogens. Reports of spontaneous resistance evolution are the largest single group, and the lungs are a site where resistance emergence is most frequently documented. Reports for some host niches are dominated by particular eco-evolutionary mechanisms, for example HGT in the gut, and spontaneous mutation in more isolated infection sites including bone, cerebrospinal fluid, heart and liver infections. It should be noted however that the distributions of eco-evolutionary modes, sites and pathogens may reflect biases due to ease of study, methodologies used, or other factors. Antibiotics to which resistance evolved within-patient are detailed by 3 letter codes as follows: AMP = Ampicillin, AMI = Amikacin, AMO or AMO/CLV = Amoxicillin or Amoxicillin-clavulanate, AZI = Azithromycin, AZT = Aztreonam, CEF - Cefuroxime axetil, CEP = Cephalexin, CEX = Cefotaxime, CFO = Cefazolin, CFD or CFD/AVI- Ceftazidime or Ceftazidime-avibactam, CFZ/TAZ = Ceftolozane-tazobactam, CFX = Cefixime, CFT = Ceftriaxone, CIP = Ciprofloxacin, CLA = Clarithromycin, CLR = Chloramphenicol, CLN = Clindamycin, COL = Colistin, DAP = Daptomycin, DOX = Doxycycline, DOR = Doripenem, ETH = Ethambutol, ETN = Ethionamide, FLO = Flomoxef, FOS = Fosfomycin, GEN = Gentamicin, IMI = Imipenem, ISO = Isoniazid, LEV = Levofloxacin, LIN = Linezolid, MET = Metronidazole, MIN = Minocycline, NIT = Nitrofurantoin, PEN = Penicillin, PIP/TAZ = Piperacillin-tazobactam, PMB = Polymixin B, PYR = Pyrazinamide, RIF = Rifampicin, STR = Streptomycin, TET = Tetracycline, TIG = Tigecycline, TOB = Tobramycin, TRI/SUL = Trimethoprim-sulfamethoxazole, VAN = Vancomycin.

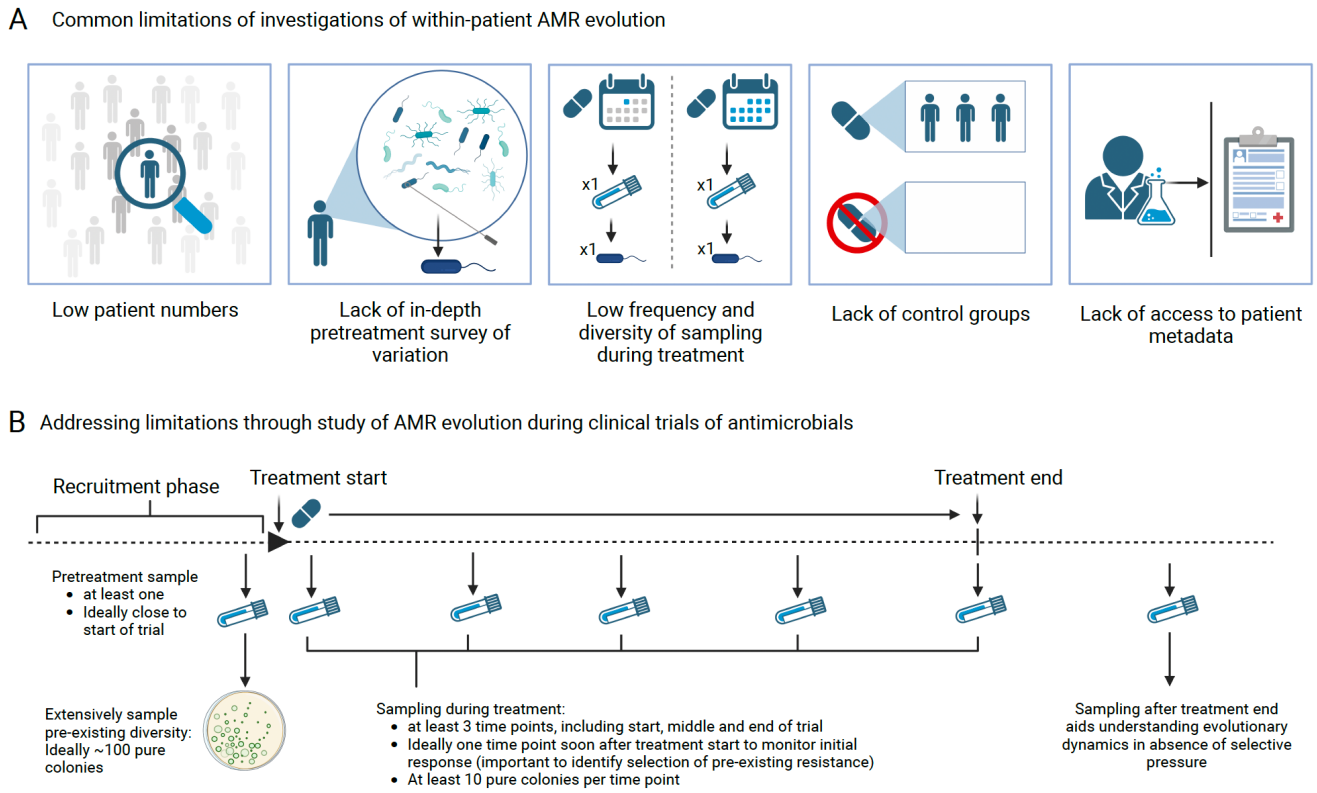


Figure 3: Addressing limitations of within-patient AMR emergence studies. A) Five key factors limit the power of studies investigating within-patient AMR emergence. Low patient numbers are a common feature of the literature on within-patient AMR, with single-patient case studies predominating. These are often unusual cases or those where treatment has failed, and can subsequently lack generalisability to the wider patient population. There is also a lack of sampling frequency and depth both prior to and during treatment. This risks missing key population and evolutionary dynamics during the course of infection, and can make it difficult to identify ecological eco-evolutionary mechanisms - particularly pre-existing resistance. Studies of within-patient resistance emergence typically also lack control groups - with good reason, as refusing safe and effective treatment would be unethical. However a lack of controls can render the disentanglement of adaptation to the host and adaptation to the antimicrobial more challenging. Finally, access to patient metadata which can provide important context can often be limited, for example information on prior antibiotic treatment history. B) How sampling regimens can be structured during a clinical trial of an antimicrobial to maximise power for investigating within-patient AMR emergence. Where possible, samples from the infection site taken prior to and during treatment should be bio-banked to facilitate later investigations. These investigations should include extensive surveying of bacterial diversity

675 from pre-treatment samples, which is essential for later identification of cases involving pre-existing
676 resistance as a mechanism. For samples taken during treatment, a sufficient number of bacterial
677 isolates should be investigated at each time point for evolutionary dynamics to be identified, as
678 identifying the timing of any *de novo* resistance or immigration of resistant lineages may provide key
679 insight to driving factors. Collection of samples after treatment can also provide useful insight,
680 particularly for chronic infections which may not have been cleared, but also for understanding the
681 aftereffects of antimicrobial therapy on host microflora at the infection site. We additionally provide
682 and in-depth discussion of these limitations and how they could be addressed in supplementary box
683 S1.
684
685 **Box 1: Towards predicting within-patient AMR emergence**
686
687 **Probabilities of AMR evolving within-patient**
688 The four eco-evolutionary mechanisms reviewed here - spontaneous mutation, selection of pre-
689 existing resistance, immigration of resistant lineages, and horizontal gene transfer - fundamentally
690 differ from one another through the process by which resistance is initially generated. Once a resistant
691 bacterial lineage is generated within an infection, it will be selected for by the relevant antimicrobial
692 therapy applied and sweep to high frequency or fixation[19]. The eco-evolutionary mode that generated
693 resistance will affect the probability that such resistance becomes established within-patient. This is
694 primarily through both the frequency and the prevalence at which resistant lineages originate within
695 the infection. Spontaneous mutation and horizontal gene transfer can both in theory begin with the
696 generation of a single resistant cell. This generates a degree of randomness as to whether the nascent
697 resistant cell survives or is killed before it can propagate itself, which will be affected by the
698 frequency of spontaneous mutant generation or horizontal transfer within the infection[186]. In contrast
699 is resistance evolution from pre-existing resistant lineages, or immigration of such lineages from
700 elsewhere. These mechanisms are far more likely to feature a larger population of resistant cells
701 which may accelerate resistance evolution[98], particularly as systemic antimicrobial therapy may
702 elevate the prevalence of resistant lineages in host microflora and reservoirs such as the gut[187],
703 making transfer of resistant lineages from host niches to the infection site more probable.
704
705 **Predicting within-host AMR evolution**
706 Predicting within-host emergence of AMR will require a variety of data on the patient and infection in
707 question. To predict the likelihood of pre-existing resistance or immigration of resistant lineages
708 occurring, an excellent starting point is information regarding the treatment history and past infections
709 of the same type that the patient may have experienced. Alongside more traditional microbiological
710 techniques and antibiotic susceptibility testing of isolates, this can be used to estimate an individuals

risk of resistance through these two mechanisms. Indeed, Stracy *et al.,* trained machine-learning models on such data, and found such a model could reduce the predicted risk of resistance emergence in UTIs (which occurs primarily through strain invasion) by 70%[108]. More challenging, are predicting likelihoods of spontaneous mutation and HGT in generating AMR within-patients. Mutational frequency, epistasis, and fitness cost trade-offs can each vary considerably between specific resistance genes[21,129] and resistance-transferring mobile genetic elements[188,189]. Despite recent advances in predicting HGT of resistance at a population level[190], the ability to predict risk of HGT mediated resistance evolution within a specific patient remains out of reach, as does prediction of a particular spontaneous mutation occurring. Efforts to do so will require complex knowledge of the bacterial fitness landscape within patients in the context of resistance[191], alongside mutational probabilities, which can vary across bacterial chromosomes[192] and with a variety of environmental and population factors[193].

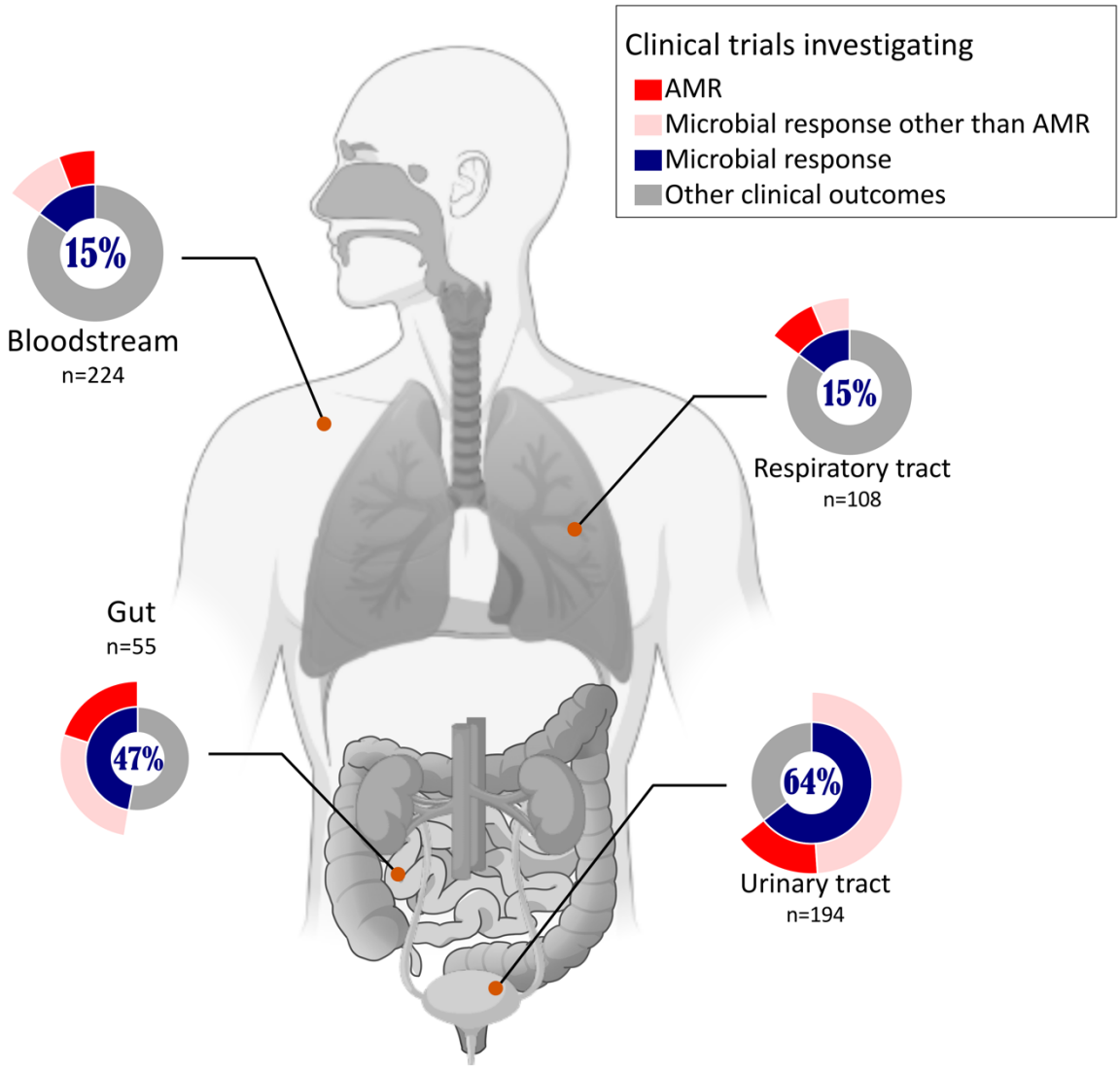
Box 2: Clinical trials of antimicrobials typically lack reporting on in-depth investigation of AMR development

To understand previous investigations in determining microbial response and AMR trials, we reviewed antibiotic clinical trials registered in the WHO International Clinical Trials Registry Platform (ICTRP) related to a range of body sites (The list of clinical trials and searching and binning methods are shown in Supplementary table 1). We found that many trials of antimicrobials did not report microbial responses (consisting of bacterial load and/or resistance related measures) in the primary or secondary outcomes registered on the platform. Respiratory tract and bloodstream infection trails did not report microbial responses for 85% and 82% of reports respectively, despite these two infection sites having the highest mortality statistics associated with and attributed to AMR in 2019[2]. Only 8% of respiratory infection trials and 6% of bloodstream infection trials of antibiotics reported AMR as an outcome. In contrast, trials of treatments for urinary tract infections and gut infections reported microbial response as an outcome in 64% and 47% of cases, and documented AMR outcome in 15% and 20% of cases respectively.

Clinical trials of antimicrobials are underutilised resources for the study of AMR evolution

Despite the numerous advantages that the clinical trial setting offers over clinical case-study reports for investigating within-patient AMR emergence, our analysis of clinical trial registered outcome measures indicated that resistance-related outcomes are rarely included. In this review, we suggest that such trials of antimicrobials should accommodate investigations of within-patient AMR emergence. As of December 2022, there are 62 novel antimicrobial therapies in the clinical trial

pipeline[194], for which no information is known about risks and likelihood of within-patient resistance evolution. Incorporating or spinning-off evolutionary investigations from clinical trials would allow information to be gathered on the risks of within-patient AMR emergence early for these novel antimicrobials and could support clinicians to develop initial guidelines for using these drugs in a manner that reduces risk of resistance. This would allow a pre-emptive strategy aimed at preventing AMR emergence in the clinic for new antimicrobials coming into use, alongside a more personalised approach to antimicrobial chemotherapy for existing therapeutics.



Exploration of microbial and AMR investigation reporting during clinical trials of antimicrobials: Proportions of surveyed registered clinical trials of antimicrobials (n=581) across four key infection sites, that list microbiological response as an reported primary or secondary

outcome (inner circles of plots, blue = yes, grey = no), and proportion of those that list AMR as a reported outcome (outer circles of plots, dark red = yes, light red = no).

Glossary:

Antibiotic/antimicrobial – Agents used to kill or inhibit the growth of microorganisms such as bacteria, fungi, viruses and parasites. Antibiotics refer to agents utilised against bacteria and can be classified as bactericidal (killing bacteria) or bacteriostatic (inhibiting bacterial growth).

Antimicrobial resistance (AMR) - The ability of microorganisms, such as bacteria, viruses, fungi, or parasites, to withstand the effects of drugs that would normally inhibit or kill them. This phenomenon occurs when these organisms adapt and develop resistance mechanisms against antimicrobial agents, rendering previously effective treatments ineffective. Clinically, AMR is defined as when the minimum inhibitor concentration (MIC) of the antimicrobial required to halt growth of a bacterium exceeds the clinical breakpoint – the highest concentration of that antimicrobial that can be given to a patient.

Antimicrobial tolerance – The ability of a population of micro-organisms to survive a transient exposure to a microbicidal agent. Differs from resistance in that the agent remains effective against the microbe as measured by MIC but requires a more prolonged treatment in order to successfully eliminate the infection.

Bacterial persistence – When a subpopulation of bacteria has a much higher tolerance to an antibiotic than the majority, that population described as persistent. When the pressure of the antibiotic is removed, this persistent community can re-emerge, creating a situation of recurrent infection despite antibiotic treatment.

Cross-resistance - Antimicrobial resistance that evolves through adaptation to another antimicrobial. This can occur when evolution of resistance to one antimicrobial confers resistance to another, typically due to a resistance mechanism that equally affects the action of all drugs within a class or has a non-specific mechanism such as multi-drug efflux pumps.

Collateral sensitivity - A situation where gain of resistance to one antimicrobial, results in increased sensitivity to another. This is a type of fitness trade-off.

Eco-evolutionary dynamics - The reciprocal interactions and feedback between ecological processes and evolutionary changes in populations over short time scales. Ecological shifts promote adaptation of populations to their changing environments, and the resulting evolutionary changes can in turn shape ecological interactions. In the context of infections – the within-patient niche ecology will shape the evolution of antibiotic resistance, which can then affect the ecology of the infection itself through failure to clear the infection, disease progression and loss of sensitive strains and microflora.

Fitness – A measure of reproductive success – the ability of an individual or population with the same genotype to survive, reproduce, and contribute that genotype to the next generation.

Fitness trade-off – A compromise in which the fitness advantage conferred by one trait comes at the expense of reducing the fitness effect of another trait. For example, resistance to an antibiotic may reduce growth rate when the antibiotic is absent.

Fixation – The state in which a genetic variant becomes the only variant present for that specific locus. All individuals within the population share that same allele.

Genetic drift - the change in frequency of an allele due to random chance. The impact of such random effects is stronger at smaller population sizes. Newly generated random mutations present at very low frequency must escape random loss due to genetic drift even if they provide a benefit before becoming established at a higher frequency in the population.

Horizontal gene transfer (HGT) – The process by which microorganisms may exchange genetic material that bypasses vertical transmission from parent to offspring.

Host microenvironment - The specific localised conditions and factors within a host organism such as its physical properties, local immune response, and neighbouring microbial communities, that influence the interactions between the host and its resident microorganisms at that site.

Host microflora – The community of microbes that reside in and on a host organism.

Hypermutators - A microbial strain with an unusually high mutation rate, often caused by deficiencies in DNA repair mechanisms. Under selection pressure from an antimicrobial agent, this rapid accumulation of spontaneous genetic mutations may accelerate the process of selection and thus evolution of AMR by increasing the likelihood of a mutation conferring resistance to occur.

Immigration of resistant lineages – Also referred to as strain invasion or replacement. The successful establishment of a novel microbial strain or species within a specific host environment. Certain strains possess traits that allow them to better adapt to the environment than pre-existing strains, and thus they are selected for and become the dominant strain. These novel invasive strains may derive from the host microflora or from the environment.

Population bottleneck – A sharp reduction in the size of a population usually due to a detrimental change in the environment. This significantly reduces genetic diversity and exaggerates the effect of genetic drift. In the context of bacterial infections, bottlenecks will be induced by the action of the immune system and antimicrobial therapy.

Pre-existing resistance – The presence of inherent or acquired antibiotic resistance genes within the infection population and/or microbiome prior to treatment with an antimicrobial.

Protected niche – A host microenvironment that offers microorganisms protection from external stresses, such as the host's immune system, antimicrobial therapy, competition from the host's microflora, or other adverse environmental conditions. Niches can promote the establishment of persistent microbial communities and may offer a safe haven for resistance evolution to occur within.

Selection – The process by which genetic variations within a population become more prevalent due to conferring traits that influence the fitness of the organisms in their environment. In the context of antimicrobial therapy, selection refers to the survival and growth of resistant strains and the loss of sensitive ones during treatment.

Selective Sweep – An evolutionary event where a highly advantageous mutation rapidly increases in frequency due to strong positive selective pressure. As it does, the genetic diversity in the region of the mutation decreases, creating a detectable signature of reduced allele frequency.

Spontaneous mutation – Also known as *de novo* mutation. Heritable alterations in the genome of a microbe that arise spontaneously during replication or repair, and were not previously present in the population or acquired from external sources of genetic material.

Supplementary Box S1 - Critical gaps in knowledge

Limited numbers of patients

The majority of the current literature are case reports of individual patients. These vary widely in their methodology and research focus, and are rarely designed for studying evolutionary dynamics.

Additionally, the available literature has a strong bias towards particular eco-evolutionary modes of AMR emergence, accessible host niches, and a handful of well-studied pathogens. Spontaneous resistance evolution is frequently studied as it can feasibly be evidenced from relatively low numbers of bacterial isolates, compared with the other eco-evolutionary modes, which require more extensive sampling of pathogen populations and/or the wider microbiota. Infections of the lungs and guts are commonly studied, likely due to the ease of the non-invasive sampling methods. Also, well-represented are chronic infections, where repeat sampling of individual patients is more probable. This bias, in turn, leads to an over-representation in the literature by pathogens common in these kinds of infections, including *P. aeruginosa*, *K. pneumoniae*, and *Staphylococcus* spp. There have only been very few larger-scale studies of within-patient AMR emergence that are designed to distinguish the eco-evolutionary mode of AMR emergence and involve more than ~30 patients. These target a limited range of infection contexts, including urinary tract and wound infections[108], lung infections[27,98,104] and bloodstream infections[40]).

Limited pathogen sampling

Low numbers of sampled bacterial colonies limits the resolution of many studies, and as such their power to distinguish between eco-evolutionary modes of resistance emergence. Many case studies report either a single or a small number of colonies, with the majority of studies focusing their work on <10 bacterial colonies isolated from patient samples[31,32,35,41,42,45,46,51–53,62,81,153]. This can limit the potential to capture pre-existing variation in resistance, and is likely to particularly limit the ability of studies to observe more complex or mixed modes of AMR emergence. For example, in a mathematical model, >38 samples were found to be required to accurately identify clonal lineages in a tumour with high mutational diversity[195]. More intensive sampling is unlikely to be feasible in all studies due to clinical considerations, but where possible should aim to gain a random sample of colonies such that this provides a sufficiently representative sample of genetic variation present in the infection.

Many studies lack samples collected prior to commencing antimicrobial treatment[43,47,48,51,53–55,81,115,190], but such pre-exposure samples are essential for determining the key features of the eco-evolutionary dynamics of AMR. For example, assessing whether resistant lineages were present at appreciable frequencies prior to treatment is required to distinguish spontaneous resistance mutation from other eco-evolutionary modes. This is evident from a study of acute *P. aeruginosa* lung infections where pre-existing resistance was present at 7-8% of the population[27], but would have likely been misclassified as spontaneous mutation without pre-treatment sampling. Pre-treatment sampling is unlikely to be possible in all cases, and indeed may be impossible for critical cases where immediate antibiotic treatment is required, but could feasibly be added where infections are not life-

907 threatening and/or before a new antibiotic is used against a chronic infection. Adding pre-treatment
908 sampling would greatly improve understanding of eco-evolutionary mechanisms and thus facilitate
909 AMR prediction and improve treatments.
910
911 The nature of clinical samples themselves can also pose a challenge. A clinical sample is effectively a
912 snapshot of the infecting population and therefore may not perfectly represent the population as a
913 whole. In many scenarios, this bias is unavoidable. However, partial sampling of the population
914 combined with low numbers of colonies may commonly exclude pre-existing resistant lineages,
915 potentially leading us to overestimate the role of spontaneous mutation. If indeed spontaneous
916 resistance mutation is less common than expected, this may limit the potential of adjuvant therapies
917 targeting spontaneous mutagenesis to prevent AMR evolution[196]. Although there are trade-offs
918 associated with sampling intensity and clinical considerations imposing limits on what is reasonable,
919 frequent in-depth sampling of populations before, during and after treatment, would provide enhanced
920 understanding of eco-evolutionary mechanisms at work within patients.
921
922 **Lack of control groups and patient metadata**
923 Existing studies on within-patient AMR emergence are predominantly lacking a control group. There
924 are obvious good reasons for this, primarily it being unethical to refuse treatment. However, it can
925 then be challenging to establish causality of the antibiotic treatment in driving the observed
926 evolutionary change without comparison to control groups. For example, adaptations to the host
927 environment can be conflated as adaptations to treatment: host antimicrobial peptides can select for
928 enhanced antibiotic resistance[197] and adaptation to macrophages *in vitro* has been shown to increase
929 *E. coli* resistance to aminoglycosides and fitness in murine infection models[198]. Nonetheless, often
930 clinical trials compare newer treatments to existing standards of care in a “non-inferiority" design,
931 where comparisons between two different treatment groups are possible. Here, even though there are
932 likely to be differences in bacterial targets and pharmacological properties between treatment arms of
933 the clinical trial, this design is powerful in enabling causal treatment-specific evolutionary responses
934 to be identified[199], partly overcoming the lack of untreated controls.
935
936 Lacking patient metadata may also complicate eco-evolutionary interpretation of clinical studies and
937 limit our understanding of the drivers of variation in outcomes among patients. A key factor is likely
938 to be antibiotic treatment history, however accessing such records can be challenging . Patients
939 previously exposed to higher levels of antibiotics are at greater risk of resistance emergence, in part
940 because their microflora is more likely to contain antibiotic resistant lineages[200], potentiating selection
941 of pre-existing resistance or immigration of resistant strains[108]. Additionally, individuals treated with
942 antibiotics could be receiving non-antibiotic treatments for a variety of other underlying health

conditions, which can impact AMR. For example, anti-inflammatory drugs (ibuprofen, naproxen, diclofenac) and β-blockers increase conjugative transfer rates of plasmids[201].

Lacking patient metadata is especially problematic for individual case reports, since these represent a biased sample of infections, typically including cases where treatment(s) failed, cases of unusual infections, or cases of unusual clinical outcomes[202]. While these unusual cases can provide important clinical experience for future treatment approaches, lacking patient metadata limits our ability to understand what features of these infections predicated them towards AMR emergence versus the majority of successful treatments. A wide variety of other host factors could influence AMR emergence, including the immune response, microflora, infection microenvironment, and treatment history, along with patient demographics and lifestyle factors. Without such metadata it will be challenging to link host and/or clinical characteristics to risks of particular eco-evolutionary modes to understand how better to target antibiotic treatments and other interventions to limit AMR emergence.

Making better use of clinical trials to understand eco-evolutionary mechanisms of AMR

Phase-III randomised controlled trials (RCT) for antimicrobials could be a powerful framework to study the eco-evolutionary modes of AMR emergence, solving several of the issues raised in this section. Typically, such RCTs include sufficiently large numbers of patients for statistical power and these patients are carefully selected to control for other sources of variation. Moreover, extensive patient metadata are usually collected. Multiple treatment groups are included enabling their comparison, and in some cases RCTs even include placebo groups that do not receive antimicrobial treatment. Indeed, RCTs have been used previously to infer the impact of treatment on AMR: Meta-analyses of clinical trial data for long-term azithromycin[203] and inhaled antibiotics in chronic lung infection[204] treatment show increased risk of resistance emergence in the treatment arms. Furthermore, longer durations and/or multiple-courses of antibiotics are associated with higher rates of resistance emergence[205,206]. In non-inferiority trials, increased resistance in both test treatment and next-best available therapy groups have been identified, primarily through phenotypic antimicrobial susceptibility testing[207–211], with some trials also including genetic causes of resistance[212,213]. However, although microbiological outcomes, such as pathogen abundance are often included in RCT designs, quantification of antibiotic resistance levels or genetic characterisation of resistance mutations is much less common (main text Box 2). Whilst adding such components to RCTs routinely may not be possible owing to limitations of costs and facilities, biobanking RCT samples for re-use can provide a practical, low-cost route to enhancing the utility of RCTs. For example, re-use of samples from a non-inferiority trial of ceftolozane/tazobactam versus meropenem for treating *P. aeruginosa* respiratory infections revealed a greater contribution of spontaneous mutation to AMR in

the meropenem treated patients (22% of patients compared to 0% in ceftolozane/tazobactam arm), but similar rates of strain immigration in both groups (~4% of patients)[213].

How then could RCT protocols be augmented to enable identification of the eco-evolutionary mechanisms of AMR? Distinguishing spontaneous mutation from pre-existing resistance requires samples to be taken before antibiotic treatment starts and for these pathogen populations to be sampled at sufficient depth to capture low-frequency pre-existing resistant variants. Tracking the dynamics of selection then requires dense longitudinal sampling of population diversity from multiple subsequent timepoints, ideally covering both during treatment and after cessation of treatment. For all these samples, testing multiple randomly selected bacterial colonies is crucial to gain an unbiased estimate of the genetic and phenotypic diversity present within the population. Sampling higher numbers of colonies will give a more accurate picture of the population, but after a certain point there are diminishing returns to sampling more colonies and use of power analyses can help to gauge the appropriate depth of sampling for the predicted level of genetic diversity (e.g., this is likely to be higher for chronic than acute infections, necessitating deeper sampling of the former). Attributing the source of immigrating resistant lineages or donors of resistance encoding MGEs requires analysis of the wider patient microbiota using metagenomics or selective culturing. Finally, understanding why patient infections vary in the eco-evolutionary mode of AMR emergence requires integration of these microbiological data with patient metadata, ideally including measures of host immune responses and their infection microenvironments. Although it is unlikely that all of these features can be included in every RCT, awareness of the samples required, collection of them during the trial (main text Fig. 3B) and biobanking for re-use would ensure that we maximise the utility of RCTs and gain the greatest insight possible from these valuable in-human eco-evolutionary experiments.

It is important to acknowledge the likely barriers to improving RCT design. Phase-III RCTs are already extremely expensive and mostly funded by pharmaceutical companies themselves, such that additional work and costs are unlikely to be included unless mandated by regulators. Commitment from trial funders and regulators to prioritise AMR studies as a core component of RCTs will therefore be necessary. Adding costs, however, carries inherent risks, potentially discouraging drug candidates from advancing through the pipeline. A potential alternative would be to ensure that RCT designs collect and biobank the necessary samples along with gaining appropriate ethical approval for follow-up eco-evolutionary analysis, even if not studied as part of the initial project. This would facilitate sample and data suitability and accessibility, creating a valuable resource for microbiologists and eco-evolutionary biologists to study the eco-evolutionary mechanisms of AMR through re-use of such biobanked samples whilst adding only limited costs to the RCT.

References (Max 140)

1. O'Neill, J. Tackling drug-resistant infections globally: final report and recommendations. (2016).
2. Murray, C. J. *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* **399**, 629–655 (2022).
3. Choudhury, R., Panda, S. & Singh, D. Emergence and dissemination of antibiotic resistance: A global problem. *Indian J. Med. Microbiol.* **30**, 384–390 (2012).
4. Bougnom, B. P. & Piddock, L. J. V. Wastewater for Urban Agriculture: A Significant Factor in Dissemination of Antibiotic Resistance. *Environ. Sci. Technol.* **51**, 5863–5864 (2017).
5. Goulas, A. *et al.* How effective are strategies to control the dissemination of antibiotic resistance in the environment? A systematic review. *Environ. Evid.* **9**, 4 (2020).
6. Zhu, G. *et al.* Air pollution could drive global dissemination of antibiotic resistance genes. *ISME J.* **15**, 270–281 (2021).
7. Ellabaan, M. M. H., Munck, C., Porse, A., Imamovic, L. & Sommer, M. O. A. Forecasting the dissemination of antibiotic resistance genes across bacterial genomes. *Nat. Commun.* **12**, 2435 (2021).
8. Grote, A. & Earl, A. M. Within-host evolution of bacterial pathogens during persistent infection of humans. *Curr. Opin. Microbiol.* **70**, 102197 (2022).
9. Didelot, X., Walker, A. S., Peto, T. E., Crook, D. W. & Wilson, D. J. Within-host evolution of bacterial pathogens. *Nat. Rev. Microbiol.* **14**, 150–162 (2016).
10. Castro, R. A. D., Borrell, S. & Gagneux, S. The within-host evolution of antimicrobial resistance in *Mycobacterium tuberculosis*. *FEMS Microbiol. Rev.* **45**, fuaa071 (2021).

11. Winstanley, C., O'Brien, S. & Brockhurst, M. A. *Pseudomonas aeruginosa* Evolutionary Adaptation and Diversification in Cystic Fibrosis Chronic Lung Infections. *Trends Microbiol.* **24**, 327–337 (2016).
12. Marvig, R. L., Sommer, L. M., Molin, S. & Johansen, H. K. Convergent evolution and adaptation of *Pseudomonas aeruginosa* within patients with cystic fibrosis. *Nat. Genet.* **47**, 57–64 (2015).
13. Giulieri, S. G. *et al.* Niche-specific genome degradation and convergent evolution shaping *Staphylococcus aureus* adaptation during severe infections. *eLife* **11**, e77195 (2022).
14. Azarian, T., Ridgway, J. P., Yin, Z. & David, M. Z. Long-Term Intrahost Evolution of Methicillin Resistant *Staphylococcus aureus* Among Cystic Fibrosis Patients With Respiratory Carriage. *Front. Genet.* **10**, 546 (2019).
15. Li, S., Feng, X., Li, M. & Shen, Z. In vivo adaptive antimicrobial resistance in *Klebsiella pneumoniae* during antibiotic therapy. *Front. Microbiol.* **14**, 1159912 (2023).
16. Friedman, N. D., Temkin, E. & Carmeli, Y. The negative impact of antibiotic resistance. *Clin. Microbiol. Infect.* **22**, 416–422 (2016).
17. Goossens, H., Ferech, M., Stichele, R. V. & Elseviers, M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. **365**, (2005).
18. Gustafsson, I. Bacteria with increased mutation frequency and antibiotic resistance are enriched in the commensal flora of patients with high antibiotic usage. *J. Antimicrob. Chemother.* **52**, 645–650 (2003).
19. Baquero, F. *et al.* Evolutionary Pathways and Trajectories in Antibiotic Resistance. *Clin. Microbiol. Rev.* **34**, e00050-19 (2021).

20. Vanacker, M., Lenuzza, N. & Rasigade, J.-P. The fitness cost of horizontally transferred and mutational antimicrobial resistance in *Escherichia coli*. *Front. Microbiol.* **14**, 1186920 (2023).
21. Melnyk, A. H., Wong, A. & Kassen, R. The fitness costs of antibiotic resistance mutations. *Evol. Appl.* **8**, 273–283 (2015).
22. Hendriksen, R. S. *et al.* Using Genomics to Track Global Antimicrobial Resistance. *Front. Public Health* **7**, 242 (2019).
23. Darby, E. M. *et al.* Molecular mechanisms of antibiotic resistance revisited. *Nat. Rev. Microbiol.* (2022) doi:10.1038/s41579-022-00820-y.
24. McEwen, S. A. & Collignon, P. J. Antimicrobial Resistance: a One Health Perspective. *Microbiol. Spectr.* **6**, 6.2.10 (2018).
25. Hiltunen, T., Virta, M. & Laine, A.-L. Antibiotic resistance in the wild: an eco-evolutionary perspective. *Philos. Trans. R. Soc. B Biol. Sci.* **372**, 20160039 (2017).
26. Larsson, D. G. J. & Flach, C.-F. Antibiotic resistance in the environment. *Nat. Rev. Microbiol.* **20**, 257–269 (2022).
27. Chung, H. *et al.* Rapid expansion and extinction of antibiotic resistance mutations during treatment of acute bacterial respiratory infections. *Nat. Commun.* **13**, 1231 (2022). **Captures dynamic fluctuations in resistance mutations across 420 *Pseudomonas aeruginosa* isolates during acute infection, capturing both spontaneous mutation and dynamic selection of pre-existing resistance start of antibiotic selection.**
28. Eklöf, J. *et al.* Persistence and genetic adaptation of *Pseudomonas aeruginosa* in patients with chronic obstructive pulmonary disease. *Clin. Microbiol. Infect.* **28**, 990–995 (2022). **Study of *P. aeruginosa* adaptation in COPD patients, covering 153 isolates**

from 23 patients across 1 year of infection, and identifying multiple spontaneous mutations conferring resistance to anti-pseudomonal drugs.

29. Hjort, K. *et al.* Dynamics of Extensive Drug Resistance Evolution of *Mycobacterium tuberculosis* in a Single Patient During 9 Years of Disease and Treatment. *J. Infect. Dis.* **225**, 1011–1020 (2022).
30. Khademi, S. M. H., Sazinas, P. & Jelsbak, L. Within-Host Adaptation Mediated by Intergenic Evolution in *Pseudomonas aeruginosa*. *Genome Biol. Evol.* **11**, 1385–1397 (2019).
31. Liao, W. *et al.* Evolution of tet(A) variant mediating tigecycline resistance in KPC-2-producing *Klebsiella pneumoniae* during tigecycline treatment. *J. Glob. Antimicrob. Resist.* **28**, 168–173 (2022).
32. Lindemann, P. C. *et al.* Case Report: Whole-Genome Sequencing of Serially Collected *Haemophilus influenzae* From a Patient With Common Variable Immunodeficiency Reveals Within-Host Evolution of Resistance to Trimethoprim-Sulfamethoxazole and Azithromycin After Prolonged Treatment With These Antibiotics. *Front. Cell. Infect. Microbiol.* **12**, 896823 (2022).
33. Long, D. R. *et al.* Polyclonality, Shared Strains, and Convergent Evolution in Chronic Cystic Fibrosis *Staphylococcus aureus* Airway Infection. *Am. J. Respir. Crit. Care Med.* **203**, 1127–1137 (2021).
34. Sommer, L. M. *et al.* Bacterial evolution in PCD and CF patients follows the same mutational steps. *Sci. Rep.* **6**, 28732 (2016).
35. Aihara, M. *et al.* Within-host evolution of a *Klebsiella pneumoniae* clone: selected mutations associated with the alteration of outer membrane protein expression conferred multidrug resistance. *J. Antimicrob. Chemother.* **76**, 362–369 (2021).

36. Boulant, T. *et al.* A 2.5-Year Within-Patient Evolution of *Pseudomonas aeruginosa* Isolates with *In Vivo* Acquisition of Ceftolozane-Tazobactam and Ceftazidime-Avibactam Resistance upon Treatment. *Antimicrob. Agents Chemother.* **63**, e01637-19 (2019).

37. Chen, C.-J., Huang, Y.-C. & Shie, S.-S. Evolution of Multi-Resistance to Vancomycin, Daptomycin, and Linezolid in Methicillin-Resistant *Staphylococcus aureus* Causing Persistent Bacteremia. *Front. Microbiol.* **11**, 1414 (2020).

38. Elgrail, M. M. *et al.* Convergent Evolution of Antibiotic Tolerance in Patients with Persistent Methicillin-Resistant *Staphylococcus aureus* Bacteremia. *Infect. Immun.* **90**, e00001-22 (2022).

39. Gao, W. *et al.* Large tandem chromosome expansions facilitate niche adaptation during persistent infection with drug-resistant *Staphylococcus aureus*. *Microb. Genomics* **1**, (2015).

40. Giulieri, S. G. *et al.* Genomic exploration of sequential clinical isolates reveals a distinctive molecular signature of persistent *Staphylococcus aureus* bacteraemia. *Genome Med.* **10**, 65 (2018).

41. van Hal, S. J. *et al.* In vivo evolution of antimicrobial resistance in a series of *Staphylococcus aureus* patient isolates: the entire picture or a cautionary tale? *J. Antimicrob. Chemother.* **69**, 363–367 (2014).

42. Mwangi, M. M. *et al.* Tracking the *in vivo* evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. *Proc. Natl. Acad. Sci.* **104**, 9451–9456 (2007).

43. Jin, X. *et al.* Resistance evolution of hypervirulent carbapenem-resistant *Klebsiella pneumoniae* ST11 during treatment with tigecycline and polymyxin. *Emerg. Microbes Infect.* **10**, 1129–1136 (2021).
44. Kao, C.-Y., Chen, J.-W., Liu, T.-L., Yan, J.-J. & Wu, J.-J. Comparative Genomics of *Escherichia coli* Sequence Type 219 Clones From the Same Patient: Evolution of the IncI1 blaCMY-Carrying Plasmid in Vivo. *Front. Microbiol.* **9**, 1518 (2018).
45. Vallée, M., Harding, C. & Hall, J. Exploring the in situ evolution of nitrofurantoin resistance in clinically derived uropathogenic *Escherichia coli* isolates. *J. Antimicrob. Chemother.* (2022) doi:10.1093/jac/dkac398. **Case of within-patient spontaneous evolution of nitrofurantoin resistance in *E. coli*, an antibiotic for which resistance in *E. coli* is rare. Resistance confers a 2%-10% reduction in doubling time in resistant isolates, indicating fitness trade-offs.**
46. Ye, M. *et al.* In vivo development of tigecycline resistance in *Klebsiella pneumoniae* owing to deletion of the *ramR* ribosomal binding site. *Int. J. Antimicrob. Agents* **50**, 523–528 (2017).
47. Hawkey, J. *et al.* Evolution of carbapenem resistance in *Acinetobacter baumannii* during a prolonged infection. *Microb. Genomics* **4**, (2018).
48. Dengler Haunreiter, V. *et al.* In-host evolution of *Staphylococcus epidermidis* in a pacemaker-associated endocarditis resulting in increased antibiotic tolerance. *Nat. Commun.* **10**, 1149 (2019).
49. Ji, S. *et al.* In-Host Evolution of Daptomycin Resistance and Heteroresistance in Methicillin-Resistant *Staphylococcus aureus* Strains From Three Endocarditis Patients. *J. Infect. Dis.* **221**, S243–S252 (2020).

50. Bloomfield, S. J. *et al.* Long-term Colonization by *Campylobacter jejuni* Within a Human Host: Evolution, Antimicrobial Resistance, and Adaptation. *J. Infect. Dis.* **217**, 103–111 (2018).

51. Low, A. S., MacKenzie, F. M., Gould, I. M. & Booth, I. R. Protected environments allow parallel evolution of a bacterial pathogen in a patient subjected to long-term antibiotic therapy: Evolution of a bacterial pathogen. *Mol. Microbiol.* **42**, 619–630 (2008). **Case of *E. coli* infection of liver cysts, studying the action of the protected cyst environment in facilitating resistance evolution. Spontaneous mutation to the promoter of *ampC* granted β-lactam resistance. Sheltered environment likely offered protection against action of antibiotic, alongside invasion or interference by other bacterial lineages.**

52. Yu, X. *et al.* Effect of Short-Term Antimicrobial Therapy on the Tolerance and Antibiotic Resistance of Multidrug-Resistant *Staphylococcus capitis*. *Infect. Drug Resist.* **Volume 13**, 2017–2026 (2020).

53. Notter, J. *et al.* AmpC hyperproduction in a *Cedecea davisae* implant-associated bone infection during treatment: a case report and therapeutic implications. *BMC Infect. Dis.* **22**, 33 (2022).

54. Colque, C. A. *et al.* Hypermutator *Pseudomonas aeruginosa* Exploits Multiple Genetic Pathways To Develop Multidrug Resistance during Long-Term Infections in the Airways of Cystic Fibrosis Patients. *Antimicrob. Agents Chemother.* **64**, e02142-19 (2020).

55. Diaz Caballero, J. *et al.* Selective Sweeps and Parallel Pathoadaptation Drive *Pseudomonas aeruginosa* Evolution in the Cystic Fibrosis Lung. *mBio* **6**, e00981-15 (2015).

56. Colque, C. A. *et al.* Longitudinal Evolution of the *Pseudomonas*-Derived Cephalosporinase (PDC) Structure and Activity in a Cystic Fibrosis Patient Treated with

β-Lactams. *mBio* **13**, e01663-22 (2022). **24-year study of *Pseudomonas aeruginosa* CF airway infection. Documents emergence of hypermutator lineage, parallel evolution of PDC cephalosporinase during ceftazidime therapy.**

57. Zhang, J. *et al.* Genomic and Phenotypic Evolution of Tigecycline-Resistant *Acinetobacter baumannii* in Critically Ill Patients. *Microbiol. Spectr.* **10**, e01593-21 (2022).

58. Sherrard, L. J. *et al.* Within-host whole genome analysis of an antibiotic resistant *Pseudomonas aeruginosa* strain sub-type in cystic fibrosis. *PLOS ONE* **12**, e0172179 (2017).

59. Alexander, H. K. & MacLean, R. C. Stochastic bacterial population dynamics restrict the establishment of antibiotic resistance from single cells. *Proc. Natl. Acad. Sci.* **117**, 19455–19464 (2020).

60. MacLean, R. C., Hall, A. R., Perron, G. G. & Buckling, A. The population genetics of antibiotic resistance: integrating molecular mechanisms and treatment contexts. *Nat. Rev. Genet.* **11**, 405–414 (2010).

61. Wheatley, R. *et al.* Rapid evolution and host immunity drive the rise and fall of carbapenem resistance during an acute *Pseudomonas aeruginosa* infection. *Nat. Commun.* **12**, 2460 (2021).

62. Mariam, S. H., Werngren, J., Aronsson, J., Hoffner, S. & Andersson, D. I. Dynamics of Antibiotic Resistant *Mycobacterium tuberculosis* during Long-Term Infection and Antibiotic Treatment. *PLoS ONE* **6**, e21147 (2011). **Investigation of *M. tuberculosis* evolution during a 9-year infection case study of one patient. Resistance mutations progressively accumulated through spontaneous mutation during this period, and evidence of clonal sweeps and co-existence of different resistance mutations**

reported.

63. Foster, P. L. Stress-Induced Mutagenesis in Bacteria. *Crit. Rev. Biochem. Mol. Biol.* **42**, 373–397 (2007).

64. Rodríguez-Rojas, A., Oliver, A. & Blázquez, J. Intrinsic and Environmental Mutagenesis Drive Diversification and Persistence of *Pseudomonas aeruginosa* in Chronic Lung Infections. *J. Infect. Dis.* **205**, 121–127 (2012).

65. Revitt-Mills, S. A. & Robinson, A. Antibiotic-Induced Mutagenesis: Under the Microscope. *Front. Microbiol.* **11**, 585175 (2020).

66. Wright, E. A., Fothergill, J. L., Paterson, S., Brockhurst, M. A. & Winstanley, C. Sub-inhibitory concentrations of some antibiotics can drive diversification of *Pseudomonas aeruginosa* populations in artificial sputum medium. *BMC Microbiol.* **13**, 170 (2013).

67. Linz, B. *et al.* A mutation burst during the acute phase of *Helicobacter pylori* infection in humans and rhesus macaques. *Nat. Commun.* **5**, 4165 (2014).

68. Estibariz, I. *et al. In Vivo* Genome and Methylome Adaptation of *cag* -Negative *Helicobacter pylori* during Experimental Human Infection. *mBio* **11**, e01803-20 (2020).

69. Mehta, H. H. *et al.* The Essential Role of Hypermutation in Rapid Adaptation to Antibiotic Stress. *Antimicrob. Agents Chemother.* **63**, e00744-19 (2019).

70. Rees, V. E. *et al.* Characterization of Hypermutator *Pseudomonas aeruginosa* Isolates from Patients with Cystic Fibrosis in Australia. *Antimicrob. Agents Chemother.* **63**, e02538-18 (2019).

71. Khil, P. P. *et al.* Dynamic Emergence of Mismatch Repair Deficiency Facilitates Rapid Evolution of Ceftazidime-Avibactam Resistance in *Pseudomonas aeruginosa* Acute Infection. *mBio* **10**, e01822-19 (2019).

72. Gabrielaite, M. *et al.* Transmission and Antibiotic Resistance of *Achromobacter* in Cystic Fibrosis. *J. Clin. Microbiol.* **59**, e02911-20 (2021).
73. Viberg, L. T. *et al.* Within-Host Evolution of *Burkholderia pseudomallei* during Chronic Infection of Seven Australasian Cystic Fibrosis Patients. *mBio* **8**, e00356-17 (2017).
74. Pompilio, A. *et al. Stenotrophomonas maltophilia* Phenotypic and Genotypic Diversity during a 10-year Colonization in the Lungs of a Cystic Fibrosis Patient. *Front. Microbiol.* **7**, (2016).
75. Turrientes, M. C. *et al.* Polymorphic Mutation Frequencies of Clinical and Environmental *Stenotrophomonas maltophilia* Populations. *Appl. Environ. Microbiol.* **76**, 1746–1758 (2010). **Epidemiological data from >9000 patients and genomes of 250 enterobacteria indicate highly frequent patient to patient transmission of pOXA-48 carbapenemase-encoding plasmid, and document frequent within-patient transfer of the plasmid horizontally between enterobacterial species.**
76. Iguchi, S., Mizutani, T., Hiramatsu, K. & Kikuchi, K. Rapid Acquisition of Linezolid Resistance in Methicillin-Resistant *Staphylococcus aureus*: Role of Hypermutation and Homologous Recombination. *PLOS ONE* **11**, e0155512 (2016).
77. Jolivet-Gougeon, A. *et al.* Bacterial hypermutation: clinical implications. *J. Med. Microbiol.* **60**, 563–573 (2011).
78. Gottig, S., Gruber, T. M., Stecher, B., Wichelhaus, T. A. & Kempf, V. A. J. In Vivo Horizontal Gene Transfer of the Carbapenemase OXA-48 During a Nosocomial Outbreak. *Clin. Infect. Dis.* **60**, 1808–1815 (2015).
79. Evans, D. R. *et al.* Systematic detection of horizontal gene transfer across genera among multidrug-resistant bacteria in a single hospital. *eLife* **9**, e53886 (2020).

80. Kociolek, L. K. *et al.* Whole-genome analysis reveals the evolution and transmission of an MDR DH/NAP11/106 Clostridium difficile clone in a paediatric hospital. *J. Antimicrob. Chemother.* **73**, 1222–1229 (2018).

81. Yoshino, M. *et al.* Stepwise Evolution of a *Klebsiella pneumoniae* Clone within a Host Leading to Increased Multidrug Resistance. *mSphere* **6**, e00734-21 (2021).

82. León-Sampedro, R. *et al.* Pervasive transmission of a carbapenem resistance plasmid in the gut microbiota of hospitalized patients. *Nat. Microbiol.* **6**, 606–616 (2021).

83. DelaFuente, J. *et al.* Within-patient evolution of plasmid-mediated antimicrobial resistance. *Nat. Ecol. Evol.* **6**, 1980–1991 (2022). **Landmark study of within-patient plasmid-mediated AMR evolution, including compensatory mutation and fitness trade-offs to enhance plasmid-borne AMR evolution.**

84. Arnold, B. J., Huang, I.-T. & Hanage, W. P. Horizontal gene transfer and adaptive evolution in bacteria. *Nat. Rev. Microbiol.* **20**, 206–218 (2022).

85. Gaibani, P. *et al.* Dynamic evolution of imipenem/relebactam resistance in a KPC-producing *Klebsiella pneumoniae* from a single patient during ceftazidime/avibactam-based treatments. *J. Antimicrob. Chemother.* **77**, 1570–1577 (2022).

86. Groussin, M. *et al.* Elevated rates of horizontal gene transfer in the industrialized human microbiome. *Cell* **184**, 2053-2067.e18 (2021).

87. Zeng, X. & Lin, J. Factors influencing horizontal gene transfer in the intestine. *Anim. Health Res. Rev.* **18**, 153–159 (2018).

88. Feasey, N. A. *et al.* Drug Resistance in *Salmonella enterica* ser. Typhimurium Bloodstream Infection, Malawi. *Emerg. Infect. Dis.* **20**, 1957–1959 (2014).

89. Weber, R. E. *et al.* IS26-Mediated Transfer of *bla*$_{NDM-1}$ as the Main Route of Resistance Transmission During a Polyclonal, Multispecies Outbreak in a German Hospital. *Front. Microbiol.* **10**, 2817 (2019).
90. Enault, F. *et al.* Phages rarely encode antibiotic resistance genes: a cautionary tale for virome analyses. *ISME J.* **11**, 237–247 (2017).
91. Billaud, M. *et al.* Analysis of viromes and microbiomes from pig fecal samples reveals that phages and prophages rarely carry antibiotic resistance genes. *ISME Commun.* **1**, 55 (2021).
92. Fernández-Orth, D. *et al.* Faecal phageome of healthy individuals: presence of antibiotic resistance genes and variations caused by ciprofloxacin treatment. *J. Antimicrob. Chemother.* **74**, 854–864 (2019).
93. Sutcliffe, S. G., Shamash, M., Hynes, A. P. & Maurice, C. F. Common Oral Medications Lead to Prophage Induction in Bacterial Isolates from the Human Gut. *Viruses* **13**, 455 (2021).
94. Haaber, J., Penadés, J. R. & Ingmer, H. Transfer of Antibiotic Resistance in *Staphylococcus aureus*. *Trends Microbiol.* **25**, 893–905 (2017).
95. Varga, M. *et al.* Efficient transfer of antibiotic resistance plasmids by transduction within methicillin-resistant *Staphylococcus aureus* USA300 clone. *FEMS Microbiol. Lett.* **332**, 146–152 (2012).
96. Barrett, R. & Schluter, D. Adaptation from standing genetic variation. *Trends Ecol. Evol.* **23**, 38–44 (2008).
97. Trindade, S. *et al.* Positive Epistasis Drives the Acquisition of Multidrug Resistance. *PLoS Genet.* **5**, e1000578 (2009).

98. Diaz Caballero, J. *et al.* Mixed strain pathogen populations accelerate the evolution of antibiotic resistance in patients. *Nat. Commun.* **14**, 4083 (2023). **Comparison of clonal origin and mixed-strain *P. aeruginosa* lung infections in evolution of AMR. Demonstrates that mixed-strain infections display accelerated evolution of AMR within-patient through selection of pre-existing resistant lineages.**
99. Trauner, A. *et al.* The within-host population dynamics of *Mycobacterium tuberculosis* vary with treatment efficacy. *Genome Biol.* **18**, 71 (2017).
100. Ailloud, F. *et al.* Within-host evolution of *Helicobacter pylori* shaped by niche-specific adaptation, intragastric migrations and selective sweeps. *Nat. Commun.* **10**, 2273 (2019).
101. Kao, C.-Y. *et al.* Heteroresistance of *Helicobacter pylori* from the same patient prior to antibiotic treatment. *Infect. Genet. Evol.* **23**, 196–202 (2014).
102. Bello Gonzalez, T. d. J. *et al.* Characterization of *Enterococcus* Isolates Colonizing the Intestinal Tract of Intensive Care Unit Patients Receiving Selective Digestive Decontamination. *Front. Microbiol.* **8**, 1596 (2017).
103. WHO. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022. https://www.who.int/publications/i/item/9789240062702 (2022).
104. Vargas, R. *et al.* In-host population dynamics of *Mycobacterium tuberculosis* complex during active disease. *eLife* **10**, e61805 (2021).
105. Roodgar, M. *et al.* Longitudinal linked-read sequencing reveals ecological and evolutionary responses of a human gut microbiome during antibiotic treatment. *Genome Res.* **31**, 1433–1446 (2021).
106. Li, J. *et al.* Antibiotic Treatment Drives the Diversification of the Human Gut Resistome. *Genomics Proteomics Bioinformatics* **17**, 39–51 (2019).

107. Malik, U. *et al.* Association between prior antibiotic therapy and subsequent risk of community-acquired infections: a systematic review. *J. Antimicrob. Chemother.* **73**, 287–296 (2018).

108. Stracy, M. *et al.* Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. *Science* **375**, 889–894 (2022). **A large scale study (>140,000 patients) indicating that the prevailing eco-evolutionary mode of in-host AMR for urinary tract and wound infections is immigration of resistant lineages, not spontaneous mutation**.

109. Lieberman, T. D. *et al.* Parallel bacterial evolution within multiple patients identifies candidate pathogenicity genes. *Nat. Genet.* **43**, 1275–1280 (2011).

110. Azarian, T. *et al.* Intrahost Evolution of Methicillin-Resistant *Staphylococcus aureus* USA300 Among Individuals With Reoccurring Skin and Soft-Tissue Infections. *J. Infect. Dis.* **214**, 895–905 (2016).

111. Woods, R. J. *et al.* The evolution of antibiotic resistance in an incurable and ultimately fatal infection. *Evol. Med. Public Health* **11**, 163–173 (2023).

112. Moradigaravand, D. *et al.* Within-host evolution of *Enterococcus faecium* during longitudinal carriage and transition to bloodstream infection in immunocompromised patients. *Genome Med.* **9**, 119 (2017).

113. Hayward, C., Brown, M. H. & Whiley, H. Hospital water as the source of healthcare-associated infection and antimicrobial-resistant organisms. *Curr. Opin. Infect. Dis.* **35**, 339–345 (2022).

114. Ding, B. *et al.* The Predominance of Strain Replacement Among *Enterobacteriaceae* Pairs With Emerging Carbapenem Resistance During Hospitalization. *J. Infect. Dis.* **221**, S215–S219 (2020). **Study identifying evolution of resistance in 41 patients treated**

with carbapenems, where a majority (90%) of *Klebsiella pneumoniae infections* developed resistance through immigration of strains of a different sequence type.

115. Wheatley, R. M. *et al.* Gut to lung translocation and antibiotic mediated selection shape the dynamics of *Pseudomonas aeruginosa* in an ICU patient. *Nat. Commun.* **13**, 6523 (2022). **Case study of meropenem resistance evolution by *Pseudomonas aeruginosa* spontaneously in both the lung and gut of a patient, along with translocation of resistant lineages from the gut to the lung.**
116. Dickson, R. P. *et al.* Enrichment of the lung microbiome with gut bacteria in sepsis and the acute respiratory distress syndrome. *Nat. Microbiol.* **1**, 16113 (2016).
117. Gillings, M. R., Paulsen, I. T. & Tetu, S. G. Genomics and the evolution of antibiotic resistance: Genomics and antibiotic resistance. *Ann. N. Y. Acad. Sci.* **1388**, 92–107 (2017).
118. Davies, J. & Davies, D. Origins and Evolution of Antibiotic Resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433 (2010).
119. Palmer, A. C. & Kishony, R. Understanding, predicting and manipulating the genotypic evolution of antibiotic resistance. *Nat. Rev. Genet.* **14**, 243–248 (2013).
120. MacLean, R. C. & San Millan, A. The evolution of antibiotic resistance. *Science* **365**, 1082–1083 (2019).
121. Baquero, F., Alvarez-Ortega, C. & Martinez, J. L. Ecology and evolution of antibiotic resistance. *Environ. Microbiol. Rep.* **1**, 469–476 (2009).
122. Eldholm, V. & Balloux, F. Antimicrobial Resistance in *Mycobacterium tuberculosis* : The Odd One Out. *Trends Microbiol.* **24**, 637–648 (2016).

123. Sun, G. *et al.* Dynamic Population Changes in *Mycobacterium tuberculosis* During Acquisition and Fixation of Drug Resistance in Patients. *J. Infect. Dis.* **206**, 1724–1733 (2012).
124. Eldholm, V. *et al.* Evolution of extensively drug-resistant *Mycobacterium tuberculosis* from a susceptible ancestor in a single patient. *Genome Biol.* **15**, 490 (2014).
125. Lehtinen, S. *et al.* Horizontal gene transfer rate is not the primary determinant of observed antibiotic resistance frequencies in *Streptococcus pneumoniae*. *Sci. Adv.* **6**, (2020).
126. Hall, R. J. *et al.* Gene-gene relationships in an *Escherichia coli* pangenome are linked to function and mobility. (2021) doi:10.17639/NOTT.7103.
127. Rocha, J., Henriques, I., Gomila, M. & Manaia, C. M. Common and distinctive genomic features of *Klebsiella pneumoniae* thriving in the natural environment or in clinical settings. *Sci. Rep.* **12**, 10441 (2022).
128. Matic, I. *et al.* Highly Variable Mutation Rates in Commensal and Pathogenic *Escherichia coli*. *Science* **277**, 1833–1834 (1997).
129. Kapel, N., Caballero, J. D. & MacLean, R. C. Localized *pmrB* hypermutation drives the evolution of colistin heteroresistance. *Cell Rep.* **39**, 110929 (2022).
130. Horton, J. S. & Taylor, T. B. Mutation bias and adaptation in bacteria. *Microbiology* **169**, (2023).
131. Boyce, K. J. Mutators Enhance Adaptive Micro-Evolution in Pathogenic Microbes. *Microorganisms* **10**, 442 (2022).
132. Luján, A. M. *et al.* Polymicrobial infections can select against *Pseudomonas aeruginosa* mutators because of quorum-sensing trade-offs. *Nat. Ecol. Evol.* **6**, 979–988 (2022).

133. Rojas, L. J. *et al.* Genomic heterogeneity underlies multidrug resistance in *Pseudomonas aeruginosa*: A population-level analysis beyond susceptibility testing. *PLOS ONE* **17**, e0265129 (2022).
134. Bianconi, I. *et al.* Persistence and Microevolution of *Pseudomonas aeruginosa* in the Cystic Fibrosis Lung: A Single-Patient Longitudinal Genomic Study. *Front. Microbiol.* **9**, 3242 (2019).
135. Tueffers, L. *et al. Pseudomonas aeruginosa* populations in the cystic fibrosis lung lose susceptibility to newly applied β-lactams within 3 days. *J. Antimicrob. Chemother.* **74**, 2916–2925 (2019).
136. Coluzzi, C. *et al.* Chance Favors the Prepared Genomes: Horizontal Transfer Shapes the Emergence of Antibiotic Resistance Mutations in Core Genes. *Mol. Biol. Evol.* **40**, msad217 (2023).
137. Papkou, A., Hedge, J., Kapel, N., Young, B. & MacLean, R. C. Efflux pump activity potentiates the evolution of antibiotic resistance across S. aureus isolates. *Nat. Commun.* **11**, 3970 (2020).
138. Lopatkin, A. J. *et al.* Clinically relevant mutations in core metabolic genes confer antibiotic resistance. *Science* **371**, eaba0862 (2021).
139. Gifford, D. R. *et al.* Identifying and exploiting genes that potentiate the evolution of antibiotic resistance. *Nat. Ecol. Evol.* **2**, 1033–1039 (2018).
140. Connor, C. H. *et al.* Multidrug-resistant *E. coli* encoding high genetic diversity in carbohydrate metabolism genes displace commensal *E. coli* from the intestinal tract. *PLOS Biol.* **21**, e3002329 (2023).
141. Bartell, J. A. *et al.* Bacterial persisters in long-term infection: Emergence and fitness in a complex host environment. *PLOS Pathog.* **16**, e1009112 (2020).

142. Reynoso-García, J. *et al.* A complete guide to human microbiomes: Body niches, transmission, development, dysbiosis, and restoration. *Front. Syst. Biol.* **2**, 951403 (2022).
143. Schenk, M. F. *et al.* Population size mediates the contribution of high-rate and large-benefit mutations to parallel evolution. *Nat. Ecol. Evol.* **6**, 439–447 (2022).
144. Andersson, D. I. Improving predictions of the risk of resistance development against new and old antibiotics. *Clin. Microbiol. Infect.* **21**, 894–898 (2015).
145. Dekaboruah, E., Suryavanshi, M. V., Chettri, D. & Verma, A. K. Human microbiome: an academic update on human body site specific surveillance and its possible role. *Arch. Microbiol.* **202**, 2147–2167 (2020).
146. Baron, S. A., Diene, S. M. & Rolain, J.-M. Human microbiomes and antibiotic resistance. *Hum. Microbiome J.* **10**, 43–52 (2018).
147. Moura De Sousa, J., Lourenço, M. & Gordo, I. Horizontal gene transfer among host-associated microbes. *Cell Host Microbe* **31**, 513–527 (2023).
148. Gumpert, H. *et al.* Transfer and Persistence of a Multi-Drug Resistance Plasmid in situ of the Infant Gut Microbiota in the Absence of Antibiotic Treatment. *Front. Microbiol.* **8**, 1852 (2017).
149. Baumgartner, M., Bayer, F., Pfrunder-Cardozo, K. R., Buckling, A. & Hall, A. R. Resident microbial communities inhibit growth and antibiotic-resistance evolution of *Escherichia coli* in human gut microbiome samples. *PLOS Biol.*
150. De Nies, L., Kobras, C. M. & Stracy, M. Antibiotic-induced collateral damage to the microbiota and associated infections. *Nat. Rev. Microbiol.* (2023) doi:10.1038/s41579-023-00936-9.

151. Bottery, M. J., Pitchford, J. W. & Friman, V.-P. Ecology and evolution of antimicrobial resistance in bacterial communities. *ISME J.* **15**, 939–948 (2021).
152. Flores-Mireles, A. L., Walker, J. N., Caparon, M. & Hultgren, S. J. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.* **13**, 269–284 (2015).
153. Linde, H.-J. *et al.* In Vivo Increase in Resistance to Ciprofloxacin in *Escherichia coli* Associated with Deletion of the C-Terminal Part of MarR. *Antimicrob. Agents Chemother.* **44**, 1865–1868 (2000).
154. Otani, S. & Coopersmith, C. M. Gut integrity in critical illness. *J. Intensive Care* **7**, 17 (2019).
155. Witzany, C., Rolff, J., Regoes, R. R. & Igler, C. The pharmacokinetic–pharmacodynamic modelling framework as a tool to predict drug resistance evolution: This article is part of the Microbial Evolution collection. *Microbiology* **169**, (2023).
156. Stanton, I. C., Murray, A. K., Zhang, L., Snape, J. & Gaze, W. H. Evolution of antibiotic resistance at low antibiotic concentrations including selection below the minimal selective concentration. *Commun. Biol.* **3**, 467 (2020).
157. Feliziani, S. *et al.* Coexistence and Within-Host Evolution of Diversified Lineages of Hypermutable *Pseudomonas aeruginosa* in Long-term Cystic Fibrosis Infections. *PLoS Genet.* **10**, e1004651 (2014).
158. Both, A. *et al.* Distinct clonal lineages and within-host diversification shape invasive *Staphylococcus epidermidis* populations. *PLOS Pathog.* **17**, e1009304 (2021).
159. Lewin, A. *et al.* Genetic diversification of persistent *Mycobacterium abscessus* within cystic fibrosis patients. *Virulence* **12**, 2415–2429 (2021).

160. Hall, K. M., Pursell, Z. F. & Morici, L. A. The role of the *Pseudomonas aeruginosa* hypermutator phenotype on the shift from acute to chronic virulence during respiratory infection. *Front. Cell. Infect. Microbiol.* **12**, 943346 (2022).
161. Maciá, M. D. *et al.* Hypermutation Is a Key Factor in Development of Multiple-Antimicrobial Resistance in *Pseudomonas aeruginosa* Strains Causing Chronic Lung Infections. *Antimicrob. Agents Chemother.* **49**, 3382–3386 (2005).
162. Prunier, A. *et al.* High Rate of Macrolide Resistance in *Staphylococcus aureus* Strains from Patients with Cystic Fibrosis Reveals High Proportions of Hypermutable Strains. *J. Infect. Dis.* **187**, 1709–1716 (2003).
163. Spellberg, B. & Rice, L. B. Duration of Antibiotic Therapy: Shorter Is Better. *Ann. Intern. Med.* **171**, 210 (2019).
164. Martínez, J. L., Baquero, F. & Andersson, D. I. Predicting antibiotic resistance. *Nat. Rev. Microbiol.* **5**, 958–965 (2007).
165. Miller, C. R., Monk, J. M., Szubin, R. & Berti, A. D. Rapid resistance development to three antistaphylococcal therapies in antibiotic-tolerant *Staphylococcus aureus* bacteremia. *PLOS ONE* **16**, e0258592 (2021).
166. Bjarnsholt, T. The role of bacterial biofilms in chronic infections. *APMIS* **121**, 1–58 (2013).
167. Kolpen, M. *et al.* Bacterial biofilms predominate in both acute and chronic human lung infections. *Thorax* **77**, 1015–1022 (2022).
168. Henriksen, N. N. S. E. *et al.* Biofilm cultivation facilitates coexistence and adaptive evolution in an industrial bacterial community. *Npj Biofilms Microbiomes* **8**, 59 (2022).
169. Jo, J., Price-Whelan, A. & Dietrich, L. E. P. Gradients and consequences of heterogeneity in biofilms. *Nat. Rev. Microbiol.* **20**, 593–607 (2022).

170. Gupta, S., Laskar, N. & Kadouri, D. E. Evaluating the Effect of Oxygen Concentrations on Antibiotic Sensitivity, Growth, and Biofilm Formation of Human Pathogens. *Microbiol. Insights* **9**, MBI.S40767 (2016).
171. Burmølle, M. *et al.* Enhanced Biofilm Formation and Increased Resistance to Antimicrobial Agents and Bacterial Invasion Are Caused by Synergistic Interactions in Multispecies Biofilms. *Appl. Environ. Microbiol.* **72**, 3916–3923 (2006).
172. Shewaramani, S. *et al.* Anaerobically Grown *Escherichia coli* Has an Enhanced Mutation Rate and Distinct Mutational Spectra. *PLOS Genet.* **13**, e1006570 (2017).
173. Hull, R. C. *et al.* Antibiotics Limit Adaptation of Drug-Resistant *Staphylococcus aureus* to Hypoxia. *Antimicrob. Agents Chemother.* **66**, e00926-22 (2022).
174. Madsen, J. S., Burmølle, M., Hansen, L. H. & Sørensen, S. J. The interconnection between biofilm formation and horizontal gene transfer. *FEMS Immunol. Med. Microbiol.* **65**, 183–195 (2012).
175. Stalder, T. & Top, E. Plasmid transfer in biofilms: a perspective on limitations and opportunities. *Biofilms Microbiomes* **2**, (2016).
176. Huo, W. *et al.* Immunosuppression broadens evolutionary pathways to drug resistance and treatment failure during *Acinetobacter baumannii* pneumonia in mice. *Nat. Microbiol.* **7**, 796–809 (2022).
177. Handel, A., Margolis, E. & Levin, B. R. Exploring the role of the immune response in preventing antibiotic resistance. *J. Theor. Biol.* **256**, 655–662 (2009).
178. Dropulic, L. K. & Lederman, H. M. Overview of Infections in the Immunocompromised Host. *Microbiol. Spectr.* **4**, 4.4.43 (2016).

179. Xu, Y. *et al.* In vivo gene expression in a *Staphylococcus aureus* prosthetic joint infection characterized by RNA sequencing and metabolomics: a pilot study. *BMC Microbiol.* **16**, 80 (2016).
180. Kolpen, M. *et al.* Nitric oxide production by polymorphonuclear leucocytes in infected cystic fibrosis sputum consumes oxygen. *Clin. Exp. Immunol.* **177**, 310–319 (2014).
181. Dwyer, D. J. *et al.* Antibiotics induce redox-related physiological alterations as part of their lethality. *Proc. Natl. Acad. Sci.* **111**, (2014).
182. Borriello, G. *et al.* Oxygen Limitation Contributes to Antibiotic Tolerance of *Pseudomonas aeruginosa* in Biofilms. *Antimicrob. Agents Chemother.* **48**, 2659–2664 (2004).
183. Hajdamowicz, N. H., Hull, R. C., Foster, S. J. & Condliffe, A. M. The Impact of Hypoxia on the Host-Pathogen Interaction between Neutrophils and *Staphylococcus aureus*. *Int. J. Mol. Sci.* **20**, 5561 (2019).
184. Raad, C. *et al.* Trends in bacterial bloodstream infections and resistance in immuno-compromised patients with febrile neutropenia: a retrospective analysis. *Eur. J. Pediatr.* **180**, 2921–2930 (2021).
185. Beaume, M. *et al.* Rapid adaptation drives invasion of airway donor microbiota by *Pseudomonas* after lung transplantation. *Sci. Rep.* **7**, 40309 (2017).
186. Hughes, D. & Andersson, D. I. Evolutionary Trajectories to Antibiotic Resistance. *Annu. Rev. Microbiol.* **71**, 579–596 (2017).
187. Luchen, C. C. *et al.* Impact of antibiotics on gut microbiome composition and resistome in the first years of life in low- to middle-income countries: A systematic review. *PLOS Med.* **20**, e1004235 (2023).

188. Fernández-Calvet, A. *et al.* The distribution of fitness effects of plasmid pOXA-48 in clinical *enterobacteria*: This article is part of the Microbial Evolution collection. *Microbiology* **169**, (2023).
189. Rodríguez-Beltrán, J. *et al.* Genetic dominance governs the evolution and spread of mobile genetic elements in bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 15755–15762 (2020).
190. Zhou, H., Beltrán, J. F. & Brito, I. L. Functions predict horizontal gene transfer and the emergence of antibiotic resistance. *Sci. Adv.* **7**, eabj5056 (2021).
191. Fragata, I., Blanckaert, A., Dias Louro, M. A., Liberles, D. A. & Bank, C. Evolution in the light of fitness landscape theory. *Trends Ecol. Evol.* **34**, 69–82 (2019).
192. Kivisaar, M. Mutation and recombination rates vary across bacterial chromosome. *Microorganisms* **8**, (2020).
193. Wei, W. *et al.* Rapid evolution of mutation rate and spectrum in response to environmental and population-genetic challenges. *Nat. Commun.* **13**, 4752 (2022).
194. Butler, M. S., Henderson, I. R., Capon, R. J. & Blaskovich, M. A. T. Antibiotics in the clinical pipeline as of December 2022. *J. Antibiot. (Tokyo)* **76**, 431–473 (2023).
195. Opasic, L., Zhou, D., Werner, B., Dingli, D. & Traulsen, A. How many samples are needed to infer truly clonal mutations from heterogenous tumours? *BMC Cancer* **19**, 403 (2019).
196. Merrikh, H. & Kohli, R. M. Targeting evolution to inhibit antibiotic resistance. *FEBS J.* **287**, 4341–4353 (2020).
197. Blanco, P., Hjort, K., Martínez, J. L. & Andersson, D. I. Antimicrobial Peptide Exposure Selects for Resistant and Fit *Stenotrophomonas maltophilia* Mutants That Show Cross-Resistance to Antibiotics. *mSphere* **5**, e00717-20 (2020).

198. Ramiro, R. S., Costa, H. & Gordo, I. Macrophage adaptation leads to parallel evolution of genetically diverse *Escherichia coli* small-colony variants with increased fitness in vivo and antibiotic collateral sensitivity. *Evol. Appl.* **9**, 994–1004 (2016).
199. Cisneros, J. M. *et al.* Colistin versus meropenem in the empirical treatment of ventilator-associated pneumonia (Magic Bullet study): an investigator-driven, open-label, randomized, noninferiority controlled trial. *Crit. Care* **23**, 383 (2019).
200. Hui, C., Lin, M.-C., Jao, M.-S., Liu, T.-C. & Wu, R.-G. Previous antibiotic exposure and evolution of antibiotic resistance in mechanically ventilated patients with nosocomial infections. *J. Crit. Care* **28**, 728–734 (2013).
201. Wang, Y. *et al.* Non-antibiotic pharmaceuticals promote the transmission of multidrug resistance plasmids through intra- and intergenera conjugation. *ISME J.* **15**, 2493–2508 (2021).
202. Nissen, T. & Wynn, R. The clinical case report: a review of its merits and limitations. *BMC Res. Notes* **7**, 264 (2014).
203. Li, H. *et al.* Meta-Analysis of the Adverse Effects of Long-Term Azithromycin Use in Patients with Chronic Lung Diseases. *Antimicrob. Agents Chemother.* **58**, 511–517 (2014).
204. Laska, I. F., Crichton, M. L., Shoemark, A. & Chalmers, J. D. The efficacy and safety of inhaled antibiotics for the treatment of bronchiectasis in adults: a systematic review and meta-analysis. *Lancet Respir. Med.* **7**, 855–869 (2019).
205. Costelloe, C., Metcalfe, C., Lovering, A., Mant, D. & Hay, A. D. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ* **340**, c2096–c2096 (2010).

206. Mo, Y., Oonsivilai, M., Lim, C., Niehus, R. & Cooper, B. S. Implications of reducing antibiotic treatment duration for antimicrobial resistance in hospital settings: A modelling study and meta-analysis. *PLOS Med.* **20**, e1004013 (2023).
207. Bassetti, M. *et al.* Efficacy and safety of cefiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria (CREDIBLE-CR): a randomised, open-label, multicentre, pathogen-focused, descriptive, phase 3 trial. *Lancet Infect. Dis.* **21**, 226–240 (2021).
208. Kaye, K. S. *et al.* Fosfomycin for Injection (ZTI-01) Versus Piperacillin-tazobactam for the Treatment of Complicated Urinary Tract Infection Including Acute Pyelonephritis: ZEUS, A Phase 2/3 Randomized Trial. *Clin. Infect. Dis.* **69**, 2045–2056 (2019).
209. Beigi, R. H., Austin, M. N., Meyn, L. A., Krohn, M. A. & Hillier, S. L. Antimicrobial resistance associated with the treatment of bacterial vaginosis. *Am. J. Obstet. Gynecol.* **191**, 1124–1129 (2004).
210. von Dach, E. *et al.* Effect of C-Reactive Protein–Guided Antibiotic Treatment Duration, 7-Day Treatment, or 14-Day Treatment on 30-Day Clinical Failure Rate in Patients With Uncomplicated Gram-Negative Bacteremia: A Randomized Clinical Trial. *JAMA* **323**, 2160 (2020).
211. Falcone, M. *et al.* Cefiderocol- Compared to Colistin-Based Regimens for the Treatment of Severe Infections Caused by Carbapenem-Resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **66**, e02142-21 (2022).
212. Skalet, A. H. *et al.* Antibiotic Selection Pressure and Macrolide Resistance in Nasopharyngeal *Streptococcus pneumoniae*: A Cluster-Randomized Clinical Trial. *PLoS Med.* **7**, e1000377 (2010).

213. Johnson, M. G. *et al.* Evaluating the emergence of nonsusceptibility among *Pseudomonas aeruginosa* respiratory isolates from a phase-3 clinical trial for treatment of nosocomial pneumonia (ASPECT-NP). *Int. J. Antimicrob. Agents* **57**, 106278 (2021). **Report of resistance evolution during a clinical trial of ceftolozane/tazobactam vs. meropenem for *P. aeruginosa* lung infection. Use of MLST to test for strain immigration/replacement events, alongside spontaneous resistance evolution. Excellent example of a study of within-patient AMR evolution based on a clinical trial of antimicrobials.**